NATURAL PRODUCTS CHEMISTRY AND CHEMICAL ECOLOGY OF TROPICAL MARINE ALGAE

A thesis submitted by
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in August 1991

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and the
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at
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Abstract

This thesis reports results from an investigation of the natural product chemistry and chemical ecology of tropical marine algae. The research has led to the discovery of novel metabolites in a number of algal species and demonstrated the ecological role of selected isolated metabolites.

Chapter one provides a general overview of the roles of secondary metabolites in the marine algae, including a review of all algal secondary metabolites previously investigated for their role in ecological interactions.

The algal Division Rhodophyta proved to be a rich source of secondary metabolites. Chapter two describes the structural elucidation of novel metabolites from the algae *Laurencia mariannensis, L. majuscula, Dasyphila plumerioides* and *Delisea pulchra* using chemical manipulations and high field nuclear magnetic resonance spectroscopy. The presence of previously reported metabolites, a number of which have now been fully characterised or reassigned, is also described.

The algal Divisions Chlorophyta, Phaeophyta and Cyanophyta proved to be less chemically rich than the Rhodophyta yielding only previously reported metabolites. Chapters three to five describe chemical investigations of the Chlorophyta *Microdictyon obscurum* and *Chlorodesmis fastigata*, the Phaeophyta *Sargassum* spp., and the Cyanophyta *Lyngbya* sp.

An opportunistic investigation of the natural product chemistry of the blue coral *Heliopora coerulea* (Alcyonacea) yielded interesting secondary metabolites of potential ecological significance. Chapter six describes the elucidation of novel metabolites from *H. coerulea.*

The ecological role of selected algal metabolites is described in the latter part of the thesis. Chapter seven treats the chemically mediated interaction between the red alga *Plocamium hamatum* and the alcyonacean soft coral *Sinularia cruciata.* Experimental studies have shown that the algal secondary metabolite chloromertensene plays a major role in allelopathic interactions with *S. cruciata.* The importance of natural irradiance levels on the distribution of the alga and its possible influence on the selection of secondary metabolites with allelopathic function is also discussed.

Chapter eight identifies the chemical defences of the temperate red alga *Delisea pulchra* against common herbivores. *In situ* feeding experiments were carried out to determine the deterrent effect of purified metabolites isolated from the alga on selected herbivore species.

Foreword

This thesis is presented in nine chapters. Chapter 1 is a general introduction to the natural products chemistry and chemical ecology of marine algae. The following chapters are divided into two major sections, the first on the isolation and structural elucidation of secondary metabolites from tropical marine algae (Chapters 2, 3, 4, 5) and a soft coral species (Chapter 6); and the second on chemical ecology studies of secondary metabolites isolated from tropical and temperate algae (Chapters 7 and 8). The final chapter (Chapter 9) contains the experimental data associated with the natural products chemistry section of this thesis.

Due to the combining of both chemical and ecological studies in this thesis, a composite literature referencing system has been adopted. This is designed to allow chemically and ecologically directed readers easier access to information referenced in this thesis. All information is referenced in the text in biological format (e.g. Bakus *et al.* 1986), and in the reference section in alphabetical order. However, for convenience all references of a chemical nature are presented in the format adopted by the journal, *Australian Journal of Chemistry*, while all those of a biological nature are presented in the format adopted by the journal, *Marine Biology*.

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Chapter 1

General Introduction

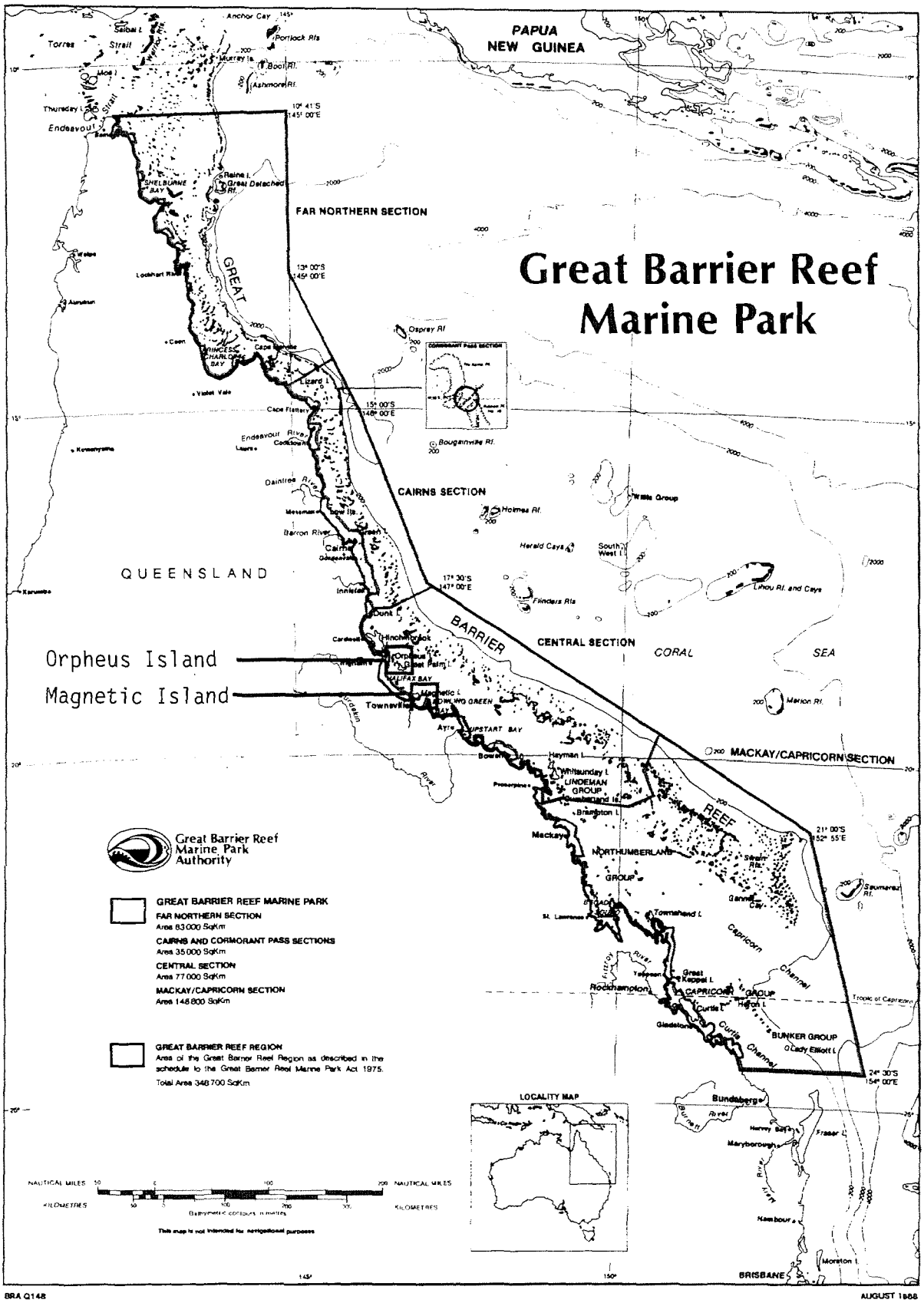


Figure 1 Map of the Great Barrier Reef and Coral Sea regions showing major sites of algal collections

Table 1. Algal species investigated for their secondary metabolite chemistry in this thesis

TAXON	SPECIES	COLLECTION NUMBER	LOCATION	DEPTH	ISOLATE
RHODOPHYTA					
Nemaliales					
Liagoraceae	*Liagora* sp.	HR892D	Holmes Reef	5-7 m	-----
Gigartinales					
Gracilariaceae	*Gracilaria edulis* (S.G. Gmelin) Silva	MI901A	Alma Bay, M.I.	1-3 m	-----
Halymeniaceae	*Halymenia durvillaei* Bory	CR891B	Chicken Reef	12 m	-----
		MI901B	Florence Bay, M.I.	4 m	-----
Hypneaceae	*Hypnea pannosa* J. Agardh	MI881A	Alma Bay, M.I.	3-5 m	-----
		OR881A	Pioneer Bay, O.I.	2-5 m	-----
		MI883E	Alma Bay, M.I.	3-5 m	-----
Bonnemaisoniales					
Bonnemaison-iaceae	*Delisea pulchra* (Greville) Montagne	SY891A	Cape Banks, N.S.W.	1-4 m	56-62
Ceramiales					
Rhodomelaceae	*Digenea simplex* (Wulfen) C. Agardh	P891A	Pandora Reef	2-4 m	-----
	Laurencia majuscula (Harvey) Lucas	MI884E	Geoffrey Bay, M.I.	1-3 m	40-48
	Laurencia marianensis Yamada	MI871F	Geoffrey Bay, M.I.	1-3 m	35-37

TAXON	SPECIES	COLLECTION NUMBER	LOCATION	DEPTH	ISOLATE
RHODOPHYTA					
Ceramiaceae	*Dasyphila plumarioides* Yendo	HR891I	Holmes Reef	10-20 m	51-53
CHLOROPHYTA					
Cladophorales,					
Anadyomonaceae	*Microdictyon obscurum* J. Agardh	HR892A	Holmes Reef	6 m	-----
		HR892J	Holmes Reef	20 m	-----
		HR891F	Holmes Reef	20 m	-----
Caulerpales					
Caulerpaceae	*Chlorodesmis fastigata* (C. Agardh) Ducker	JB871A	John Brewer Reef	------	63-64
PHAEOPHYTA					
Fucales,					
Cystoseiraceae	*Hormophysa cuneiformis* (J.F. Gmelin) P.C. Silva		Alma bay, M.I.	1-3 m	-----
Sargassaceae	*Sargassum* sp. cf. *bracteolosum* J. Agardh		Alma Bay, M.I.	1-3 m	-----
	Sargassum sp. cf. *linearifolium* (Turner) J. Agardh		Alma Bay, M.I.	1-3 m	65
	Turbinaria ornata (Turner) J. Agardh		Alma Bay, M.I.	2 m	-----
CYANOPHYTA					
Oscillatoriales					
Oscillatoriaceae,	*Lyngbya* sp.	BR891A	Bougainville Reef	0-20 m	-----

TAXON	SPECIES	COLLECTION NUMBER	LOCATION	DEPTH	ISOLATE
CYANOPHYTA Oscillatoriales Oscillatoriaceae,	*Lyngbya* sp.	BR881B	Britomart Reef	5-10 m	-----
(phycozoan)	*Ceratodictyon spongiosum* Zanardini symbiotic with *Sigmadocia symbiotica* Bergquist & Tizard	MI881B	Geoffrey Bay, M.I.	0-5 m	-----

Voucher specimens of all taxa listed are housed in the Herbarium, Botany Department, James Cook University of North Queensland (JCT). M.I.= Magnetic Island, ; O.I.=Orpheus Island, (see Fig. 1)

Classification follows that of Kraft and Woelkerling; In Clayton and King, 1990.

----- no secondary metabolites extractable in dichloromethane/methanol mixtures isolated.

1. INTRODUCTION

From a biologist's perspective "natural products chemistry has little meaning without a framework based on ecological or evolutionary theory" (Bakus *et al.* 1986). From a chemist's perspective one might argue that ecological and evolutionary theory have much to gain from natural products chemistry.

1.1 Marine natural products chemistry: algal secondary metabolites

Marine natural products chemistry, a field involving the isolation and structural elucidation of secondary metabolites from marine sources, has revealed a vast and varied array of novel chemical structures. The definition of a secondary metabolite adopted here is "a substance that has no known role in the internal economy of the producing organism" (Williams *et al.* 1989). These range from the very simple, such as simple haloforms (1), to the very complex such as palytoxin (2), but many have been shown to derive from or be present within a limited taxonomic range, often a single species or genus. A major impetus for research in this field has been the search for pharmacologically active compounds from marine sources (Munroe *et al.* 1987, Fautin 1988) . The rich resource of natural products already established has been comprehensively reviewed on a regular basis (Faulkner 1977, 1984, 1986, 1987, 1988, 1990, 1991). Between 1977 and 1985 alone, over 1,700 novel compounds from marine organisms have been described. The major taxa studied include microbes, bryozoans, tunicates, echinoderms, coelenterates, sponges and algae. Marine algae have afforded 48% of

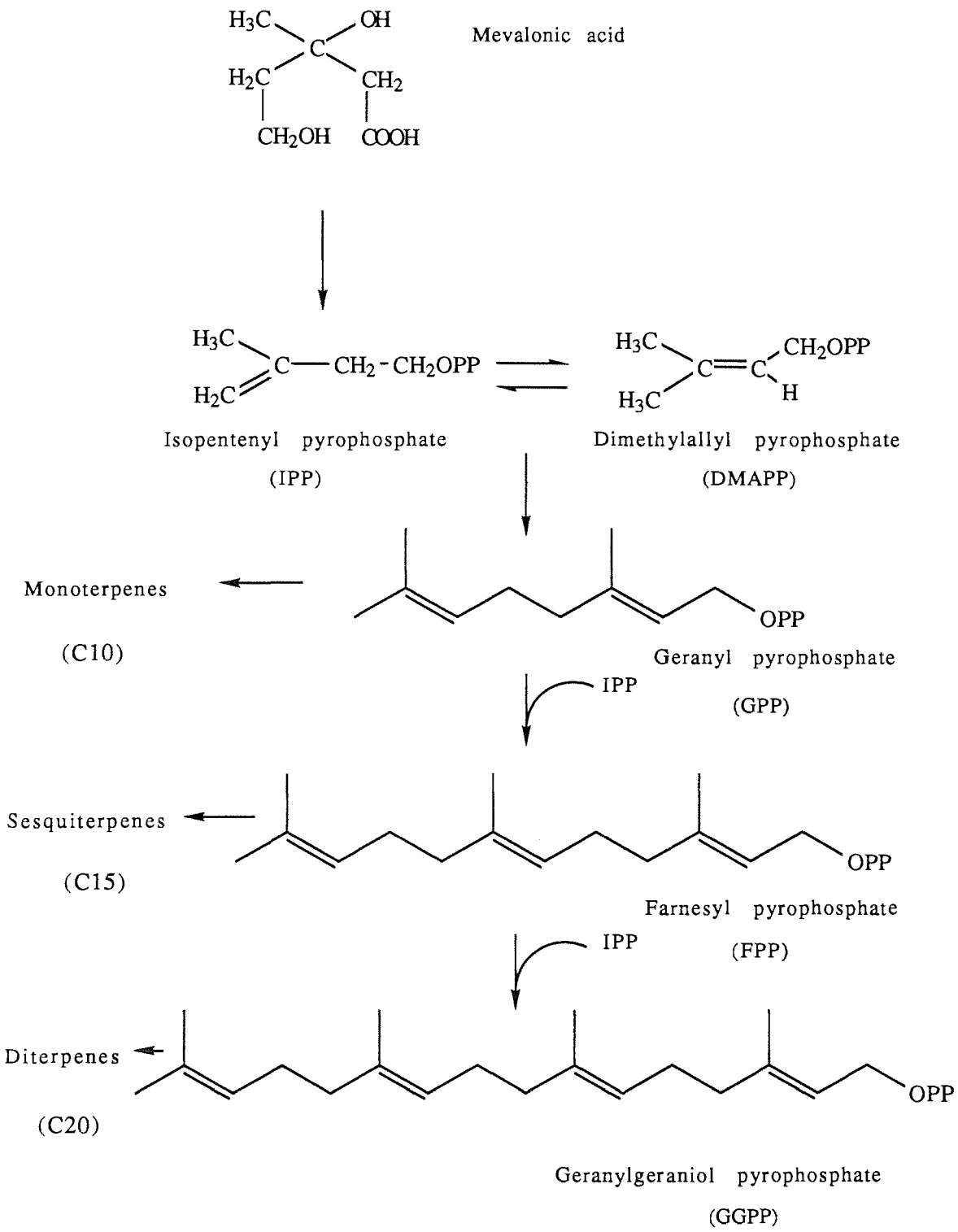
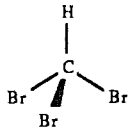

Mevalonic acid
Isopentenyl pyrophosphate
(IPP)
Dimethylallyl pyrophosphate
(DMAPP)
Monoterpenes
(C10)
Geranyl pyrophosphate
(GPP)
IPP
Sesquiterpenes
(C15)
Farnesyl pyrophosphate
(FPP)
IPP
Diterpenes
(C20)
Geranylgeraniol pyrophosphate
(GGPP)

Figure 2

the metabolites, by far the largest contributing taxon (Ireland *et al.* 1988). Further analysis of this data showed a predominance of non-nitrogenous metabolites isolated from red (Rhodophyta), green (Chlorophyta) and brown algae (Phaeophyta), while either nitrogenous or non-nitrogenous metabolites are recorded from phytoplankton and blue green algae (Cyanophyta) (Ireland *et al.* 1988). Of all the major groups of compounds isolated from algae, terpenes make up the bulk of secondary metabolites identified.

Terpenes are biosynthesised from the condensation of isopentenyl pyrophosphate and/or dimethylallyl pyrophosphate units derived from mevalonic acid (Fig. 2, adapted from Tapiolas 1985). These units may then undergo various cyclizations, oxidations, rearrangments, and halogenations to yield a plethora of compounds. Algal terpenes have been reviewed based on phylogenetic relations (Erickson 1983, Paul and Fenical 1987, Wright 1988), compound type (Martin and Darias 1978), and geographical distribution (Wright *et al.* 1990). Polyketides and compounds of mixed biosynthesis are the next most important groups after the terpenoids, with smaller proportions of aromatic amino acids, non-aromatic amino acids and other nitrogenous compounds (Ireland *et al.* 1988, van Alstyne and Paul 1989).
Other reviews of algal secondary metabolites include those concerned with spectroscopic properties of natural products (Sims *et al.* 1978) and other structural types (e.g. indoles, Christophersen, 1983).



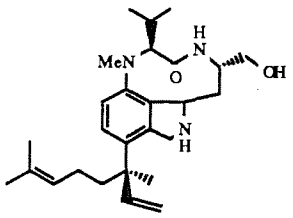
(1)

$C_{129}H_{223}N_3O_{54}$

(Moore and Bartolini 1981)

(Uemura *et al.* 1981)

(2)



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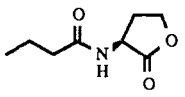

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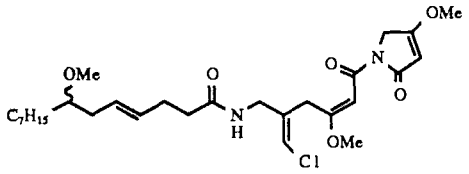

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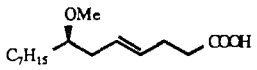

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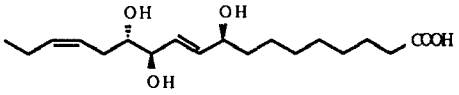

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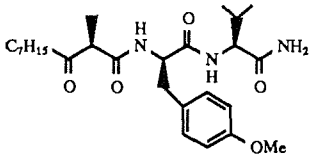

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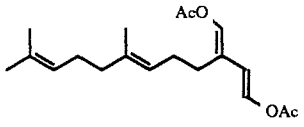
CYANOPHYTA



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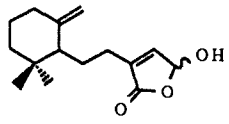

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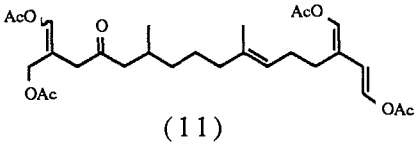

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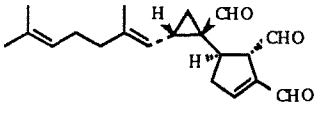

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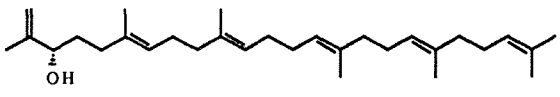

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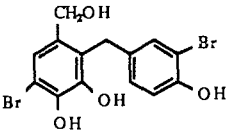

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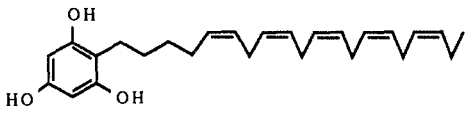
CHLOROPHYTA

The Cyanophyta (blue green algae) produce a wide variety of secondary metabolites, including indole alcohols (3), amides (4,5), long chain fatty acids (6,7), lipodipeptides (8) and various combinations of these classes of compounds. The Chlorophyta (green algae) predominantly produce isoprenoid-derived compounds, such as sesquiterpenes (9, 10) and diterpenes (11, 12), and squalene derivatives (13) as well as other types of compounds such as phenolic acetogenins (14) which are occasional exceptions to this pattern (Wright 1988).

The Phaeophyta (brown algae) produce phlorotannins (15), prenylated aromatics (16), sesquiterpenes containing two fused six-membered rings (17) and diterpenes based on a xenicane (18) or dolabellane (19) ring system (Wright 1988).

The Rhodophyta (red algae) produce polyhalogenated monoterpenes (20), halogenated sesquiterpene (21) and diterpene (22) derived compounds, pyrones (23), halogenated phloroglucinol derivatives (23a) and compounds of varied biosynthetic origins (Wright 1988).

This brief overview of the source and diversity of algal secondary metabolites illustrates the achievement of natural product chemists to date, i.e., the contribution to scientific knowledge of the structural diversity in nature. However, it also raises the question as to why such a diverse range of compounds occurs in nature. Williams *et al.* (1989) argue that secondary metabolites serve the producing organism by improving its survival fitness (the structure-function hypothesis). This leads one to the second major area in which natural product chemists contribute to knowledge - the field of chemical ecology. This is the investigation of the influence on survival fitness of an organism resulting from the



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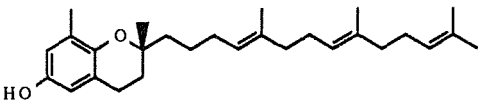

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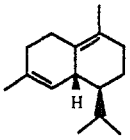

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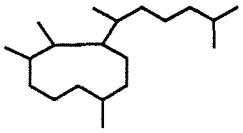

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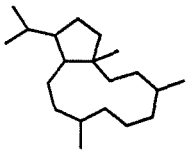

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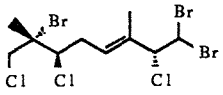
PHAEOPHYTA



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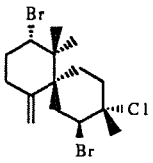

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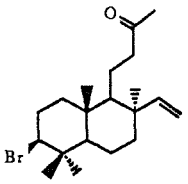

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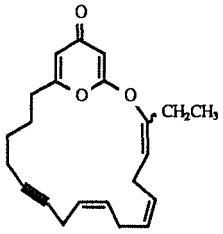

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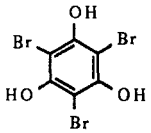


(23a)

RHODOPHYTA

production of specific secondary metabolites. It is on this aspect of marine natural products chemistry that the remainder of this review will focus.

1.2. Marine chemical ecology

1.2.1. Chemically mediated algal interactions:

The goal-directed nature of pharmacologically based and drug company funded research, and the general lack of opportunities or willingness of researchers to engage in projects involving chemists and ecologists, has meant that only a small fraction of the secondary metabolites isolated have been investigated for possible ecological roles. Similarly, information important to the ecologist or chemical ecologist regarding the presence, concentration, and distribution of compounds is often neglected by chemists due to their myopic interest in isolating and identifying only novel metabolites. As collaborative research between chemists and ecologists increases, and observational and experimental science is recognised as providing clues to the role of natural products in the marine environment, the field of marine chemical ecology appears poised to reveal further knowledge of the complexity of chemically-mediated interactions between organisms.

As with natural products chemistry, the fields of chemical ecology (Harborne 1986, 1989) and marine chemical ecology (Bakus *et al.* 1986, Van Alstyne and Paul 1989) have been reviewed on a regular basis. These reviews are a good indication of the recent origins of marine chemical ecology. In the latest review of chemical ecology, marine organisms occupy only one of the twenty seven review sections (Harborne 1989). A comprehensive review of marine

chemical ecology in preparation (Paul 1991 in prep.) should add new information to the literature and stimulate further research. Algal chemical ecology to date has focused on four major fields: chemical defences, antifouling agents or properties, allelopathy and pheromones.

1.2.1.1. **Chemical defences**

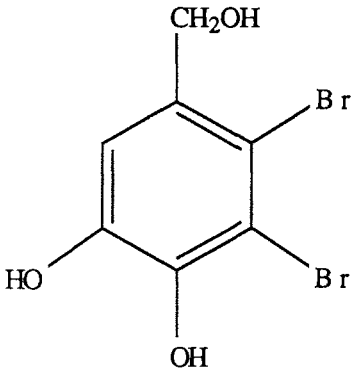
Of the various aspects of chemical ecology, the chemical defence of an organism by the production of noxious or toxic chemicals is one of the most thoroughly investigated and reviewed. The presence and role of chemical defences has been well documented for terrestrial organisms (Rosenthal and Jansen 1979) and for the major groups of marine benthic flora and fauna. The role of secondary metabolites as feeding deterrents has been documented for fish (Tachibana 1988), sponges (Green 1977, Thompson *et al.* 1985), soft corals (Sammarco and Coll 1988), bryozoans (Christophersen 1985), holothurians (Bakus 1974, Bakus and Green 1974, Lucas *et al.* 1979), ascidians (Stoecker 1980a, b) and molluscs (Faulkner and Ghiselin 1983, Karuso 1987). Chemical defences in algae have been thoroughly reviewed (Hay and Fenical 1988; van Alstyne and Paul 1989; Duffy and Hay 1990). A major review by Hay and Fenical (1988) covers all aspects of chemically mediated marine algae-herbivore interactions. This includes a general review of the number and structural types of compounds isolated from marine algae, the ecological role and physiological effects of these compounds, their spatial and temporal distribution, and the effect that these defences have on structuring marine communities. A

comparison is also made between chemical defences in marine and terrestrial communities.

Since this review was published, research on chemical defences has focused on the effect on herbivory of qualitative and quantitative variation of specific secondary metabolites (e.g. Hay *et al.* 1988a, 1989b, 1990; van Alstyne 1988, Steinberg and Paul 1990, Paul *et al.* 1990, Renaud *et al.* 1990). A comprehensive listing of compounds which have so far been investigated for their role as feeding deterrents in controlled ecological experiments, together with quantitative data on natural variation in the concentration of these metabolites, is included in Table 2. The main thrust of this area of research has again been reviewed recently by Duffy and Hay (1990). This latter review includes a discussion of spatial, associational, temporal and size-related refuges from predation, the importance of morphological and structural defences, the levels of tolerance of herbivory, and the role of chemical defences.

1.2.1.2. **Antifouling**

An understanding of the role of marine natural products as anti-fouling agents has improved considerably in recent times as a result of studies carried out to investigate the potential of natural products as replacements for the ecologically unacceptable heavy metal-based antifouling agents (Alzieu *et al.* 1986). However, unlike the testing of marine natural products for pharmacological activity, the testing of natural products for antifouling properties is inextricably linked to an examination of the ecological role of these compounds. The dual mechanisms of fouling and antifouling, and



(24)

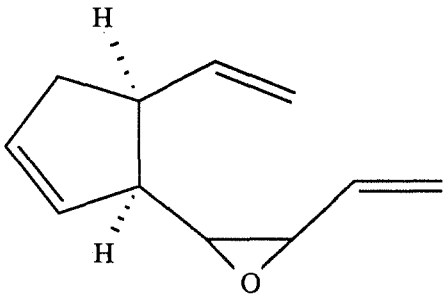
the chemical and physical means by which marine invertebrates and marine algae prevent the fouling of their surfaces, have recently been reviewed (Davis *et al.* 1989, Wahl 1989). Secondary metabolites with specific antifouling properties have been isolated from ascidians (Davis and Wright 1990), sponges (Nakatsu *et al.* 1983, Thompson *et al.* 1985, Walker *et al.* 1985), gorgonians (Targett *et al.* 1983, Bundurraga and Fenical 1985, Gerhart *et al.* 1988) and soft corals (Coll *et al.* 1987). Tannin-like substances isolated from *Sargassum natans* and *S. fluitans* (Sieburth and Conover 1965), *Fucus vesiculosus* (McLachlan and Craigie 1966), and *Laminaria digitata* and *Himanthalia elongata* (Al-Ogily and Knight Jones 1977) have been implicated in antifouling. Khfaji and Boney (1979) suggest an antifouling compound is exuded by *Chondrus crispus* germlings, while Phillips and Towers (1982a, b) suggest that lanosol (24), a brominated phenol exuded by *Rhodomela larix*, may act as an antifoulant. There is, however, still no direct evidence that specific algal secondary metabolites play a role in antifouling.

1.2.1.3. **Allelopathy**

Marine natural products have been postulated to play a role in competitive interactions including allelopathy (Hellebust 1975, Fletcher 1975, Vadas 1979, Harlin 1987). Allelopathic interactions, "the reputed baneful influence of one living plant upon another due to secretion of toxic substances" (Webster's Third International Dictionary; see also McNoughton and Wolf 1979) are well documented in the terrestrial environment (Rosenthal and Jansen 1979, Fox 1981, Rice 1984). In the marine environment, allelopathy has been experimentally demonstrated in soft corals

(Sammarco *et al.* 1983) and implicated in a number of interactions between invertebrates and other biota, including anemones (Sebens 1976, Bak and Borsboom 1984), sponges (Jackson and Buss 1975, Sullivan *et al.* 1983, Thompson 1985, Porter and Targett 1988), ascidians (Bak *et al.* 1981) and scleractinian corals (Sammarco *et al.* 1985, De Ruyter van Stevenick *et al.* 1988). There is, however, no experimental evidence to date that marine algae exert allelopathic effects on neighbouring organisms.

Competitive interactions between microscopic planktonic algae have been described for a number of species (see reviews by Fogg 1966 and Hellebust 1975), and the concept of a "chemical claw" mechanism of interspecific competition between macroscopic marine algae was proposed as early as 1948 by Walker and Smith. This mechanism was invoked to explain the effect of the exudates from *Ascophyllum nodosum* on zoospores of *Laminaria cloustoni.* Fletcher (1975) observed that growth of the crustose red algae *Porphyrodiscus simulans* and *Rhodophysema elegans* was inhibited in culture by ectocrines of *Ralfsia spongiocarpa,* a species known to liberate considerable quantities of tannins into tide pools (Conover and Sieburth 1966). Khfaji and Boney (1979) demonstrated that diatom growth was inhibited in the vicinity of *Chondrus crispus* germlings grown in culture. Secondary metabolites have been shown to be exuded into the water column by a number of algae (Carlson and Carlson 1984, Gschwend *et al.* 1985), but there is no direct evidence to date that secondary metabolites are released from the algae involved in these interspecific interactions, or that these algal secondary metabolites are the cause of the observed deleterious effects in these competitive situations.

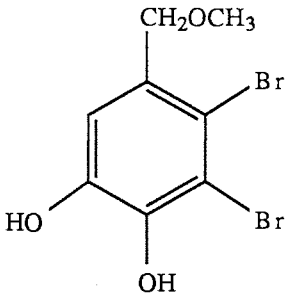


(25)

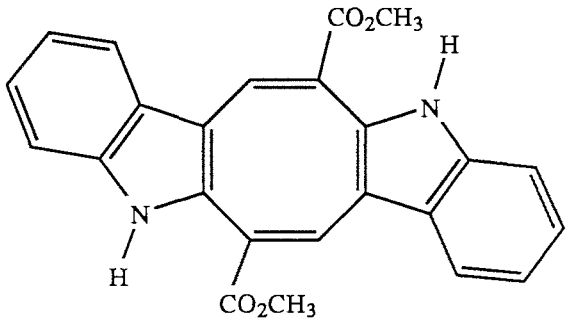
1.2.1.4. Chemotaxis

The role of small chemical molecules as sexual pheromones has been described for several marine taxa. These include the hydromedusae (Miller 1979), siphonophores (Carre and Sardet 1981), the phylum metazoa (Miller 1985) and more recently scleractinian corals (Coll *et al.* 1990a, b). However, the best studied group of organisms is the marine algae, in particular the Phaeophyta. Maier and Müller comprehensively reviewed the field of sex pheromones in algae in 1986. The methodology developed by the pioneering work of Müller (1976) has been adapted for use in identifying sperm attraction in scleractinian and alcyonacean coral species (Coll *et al.* 1990b). Research in the algal field continues to focus on the Phaeophyta and continues to yield significant results. Müller *et al.* (1988) identified a further spermatozoid releasing and attracting factor caudoxine (25) from *Perithalia caudata* (Phaeophyta). Previously identified algal pheromones have also been isolated from species from which they had not been reported previously, and their roles as pheromones (Müller *et al.* 1990, Phillips *et al.* 1990a, b), their mechanism of action (Maier and Müller 1990), their specificity (Maier *et al.* 1988) and their quantitative determination (Müller and Schmid 1988) have been investigated.

A listing of the algal secondary metabolites identified as chemotactic agents is included in Table 2.



(26)

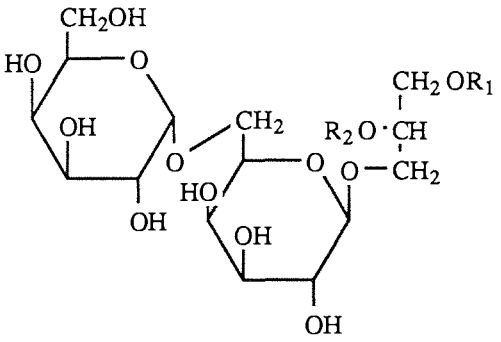


(27)

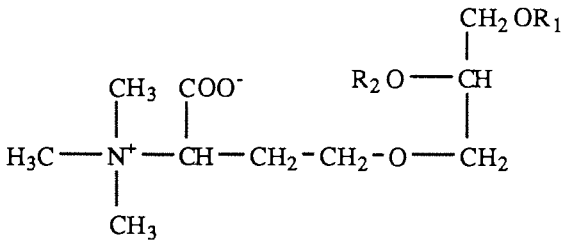
1.2.2. Other roles

1.2.2.1. Plant growth regulators

The presence of plant growth regulators in algae has been well documented in the literature. Auxins (Jacobs *et al.* 1985) and cytokinins (de Nys *et al.* 1990) have been isolated from algae or algal extracts (Tay *et al.* 1985, 1987); while gibberellin-like activity has been demonstrated in algal extracts (Kubo *et al.* 1990). All three groups of plant growth regulators have been shown to play a role in the regulation of growth in marine algal species (Kentzer *et al.* 1975, Bradley and Cheney 1990, de Nys *et al.* 1991b). Plant growth regulator-like activity has been associated with the presence of natural products in two marine algae. The gibberellin-like activity of α-O-methyllanosol (26) isolated from *Odonthalia washingtoniensis* and *O. floccosa* (Rhodophyta) (Kubo *et al.* 1990), and the auxin-like activity of the pigment caulerpin (27) and its hydrolysis product isolated from *Caulerpa sertulariodes* and *C. racemosa* (Raub *et al.* 1987) suggest a growth regulator role for these natural products. There is, however, no evidence to show that these metabolites act as endogenous growth regulators in the respective species, although compounds related to both metabolites are known plant growth regulators. Caulerpin is a dimer of the known auxin indole acrylic acid (Hofinger 1969) while the free benzyl alcohol lanosol stimulates red algal growth (Kamisaka 1973). These metabolites have been included in Table 2 as compounds investigated for their ecological role on account of the strength of evidence regarding their activity in common plant growth regulator bioassays (Letham *et al.* 1978).

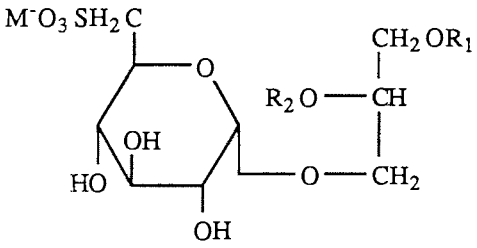


(28)

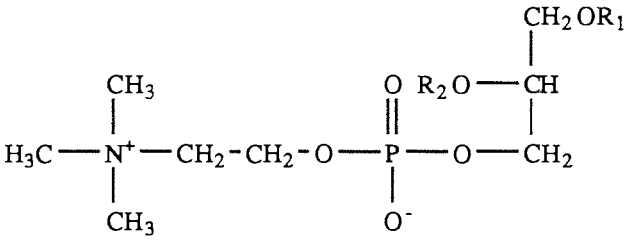


(29) (R_1= R_2= acyl)

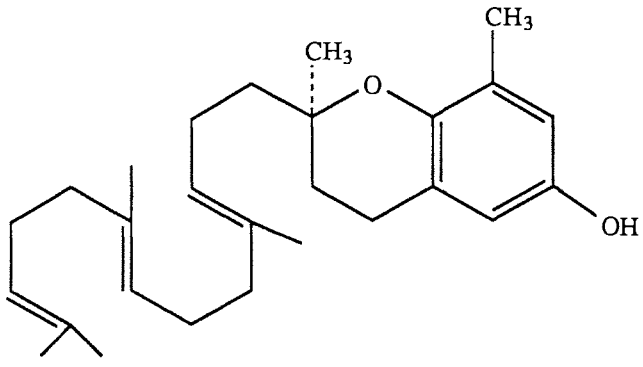
(30) (R_1= acyl, R_2= OH)



(31)



(32)



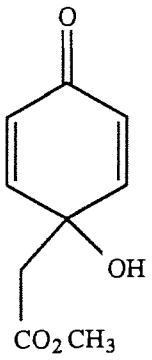
(33)

1.2.2.2. Phagostimulants

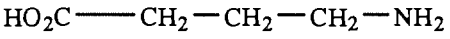
As well as having demonstrated roles in suppressing herbivory, certain secondary metabolites have also been shown to stimulate the feeding of herbivores (phagostimulants). Phagostimulants have been isolated from the algae *Ulva pertusa* (Chlorophyta) and *Undaria pinnatifida* (Phaeophyta). A series of glycolipids, digalactosyldiacyl-glycerol (DGDG) (28), 1,2-diacylglyceryl-4-O-(N,N,N-trimethyl)-hormoserine (DGTH) (29), 1-monoacylglyceryl-4-O-(N,N,N-trimethyl)-hormoserine (MGTH) (30), 6-sulphoquinovosyl-diacyl-glycerol (SQDG) (31) and phosphatidylcholine (32), and have been shown to act as specific phagostimulants for the seahare *Aplysia juliana* (Sakata *et al.* 1986), the mollusc *Haliotis discus,* and the gastropods *Turbo cornutus* and *Omphalius pfeifferi* (Sakata *et al.* 1988).

1.2.2.3. Induction of larval settlement and metamorphosis

Chemical mediation has been implicated in the induction of settlement and of metamorphosis in various taxa (Hadfield 1986, Morse 1990). These groups include cnidarians (Chia and Bickwell 1977), polychaetes (Pawlik and Faulkner 1986, Pawlik 1988, Jensen *et al.* 1990) and molluscs (Hadfield 1977, 1986; Morse (D.E.) *et al.* 1979, Rumrill and Cameron 1983, Morse (A.N.C.) *et al.* 1984). Algal secondary metabolites have been shown to act as settlement inducers (Kato *et al.* 1975a, Yvin *et al.* 1985) and metamorphosis inducers (Yvin *et al.* 1985) for marine invertebrate larvae. The compound δ-tocotrienol (33), isolated from *Sargassum tortile*,



(34)



(34a)

induces settlement of swimming larvae of a hydrozoan, while jacaranone (34) isolated from *Delesseria sanguinea* induces the settlement and metamorphosis of the larvae of *Pecten maximus* (Yvin *et al.* 1985). Abalone larvae and the larvae of a number of other groups settle and metamorphose rapidly in the presence of crustose coralline red algae, an effect which has been attributed to the presence of γ-amino-butyric acid (GABA) (34a) and related analogues in the red algae (see review by Morse 1990).

1.2.2.4. Secondary metabolites as taxonomic indicators (Chemosystematics)

The distribution of natural products in marine organisms provides the basis for their use as indicators of phylogenetic relationships between different species. The use and potential of a biochemical approach to plant systematics has been reviewed (Harborne and Turner 1984, Waterman and Grey 1987), and current work in the terrestrial field continues to support the use of secondary metabolites as chemosystematic indicators (e.g. Wolter-Filho *et al.* 1989). The principles of chemosystematics based on secondary metabolites have been applied to the sponges (Berquist and Wells 1983), echinoderms (Stonik and Elyakov 1988), gorgonians (Gerhardt 1983) and soft corals (Kashman 1980, Sammarco and Coll 1988). Sterols and lipids have been used as phylogenetic indicators for some microalgae (Volkmann and Hallegraff 1988, Billard *et al.* 1990) and macroalgae (Khotimchenko *et al.* 1990), while agar composition has been inferred as a taxonomic indicator in the genus *Gracilaria* (Bird *et al.* 1987, Miller and Furneaux 1987). Secondary metabolites can be used as chemotaxonomic indicators at the family

level, e.g. halogenated monoterpenes are restricted to the families Plocamiaceae, Rhizophyllidaceae and Cermiaceae of the Rhodophyta (Naylor *et al.* 1983), while bis-enol acetates are restricted to the families Caulerpaceae and Udoteaceae of the Chlorophyta (Paul and Fenical 1987). The presence or absence of secondary metabolites has also been of limited use at the level of genus and species. Fenical and Norris (1975) used the presence of secondary metabolites as a means of distinguishing three forms of *Laurencia pacifica* as separate species. The distribution of the green pigment caulerpin has been suggested as a chemotaxonomic indicator in the genus *Caulerpa* (Schwede *et al.* 1987). The most wide ranging use of secondary metabolites as taxonomic indicators in the algae has been the use of diterpenes as taxonomic markers for species of the genus *Dictyota* (Teixeira and Kelecom 1987, 1988, Teixeira *et al.* 1990), while differences in the types of halogenated monoterpenes detected in *Plocamium violaceum* and *P. cartilagineum* have been used to designate chemotypes of the species from varying localities (Crews *et al.* 1977, Rovirosa *et al.* 1988). The possibility of using chemotaxonomy in conjunction with more classical taxonomic methods has not yet been fully explored. The application of cladistic analysis to marine secondary metabolites has been shown to be a useful tool (Gerhardt 1983), and application of similar techniques to algal metabolites may prove fruitful. However, wider application will depend upon further information regarding the temporal and spatial distribution of algal natural products, including reports of the presence of metabolites which have been previously identified in newly investigated organisms, becoming available.

1.2.3. Objectives

There are an estimated 400 species of marine algae in the Northern Australian region (Womersley 1990). For the Townsville area in North East Queensland, 144 intertidal algal taxa have been recorded (Ngan and Price 1980). Very little information is available on the distribution of most other algal species in northern Australia. Of the estimated 400 species in the region, the secondary metabolite chemistry of only a few has been investigated. With the exception of the opportunistic collections of algal species from the Great Barrier Reef, only seven species of red algae, four species of brown algae, two species of blue-green algae and six species of green algae have so far been investigated in any detail with respect to their secondary metabolite content (Wright *et al.* 1990). Reflecting the situation elsewhere, even fewer species have been studied with regard to the possible ecological role of the metabolites in controlled ecological experiments. The exceptions to this rule are the assaying of a number of secondary metabolites as feeding deterrents (Hay *et al.* 1989a), the investigation of interactions between the alga *Chlorodesmis fastigata* (Chlorophyta), specialist marine invertebrate herbivores, and their predators (Hay *et al.* 1989b), and an investigation of the role of polyphenolics as feeding deterrents in tropical brown algae (Steinberg *et al.* 1991 in press).

This thesis extends earlier phytochemical studies of tropical marine algae, and investigates the role of particular secondary metabolites amenable to ecological study.

Table 2. Algal secondary metabolites which have been investigated for their role in ecologicial interactions. The metabolites are ordered under the genus from which they occur within the four algal Divisions. The functions for which they were investigated are as listed below.

defence = assayed as a feeding deterrent, i.e. a possible defensive compound. This does not prove a defensive function, only that this possibilty has been investigated.

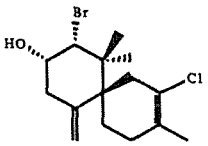
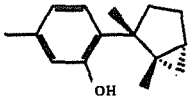
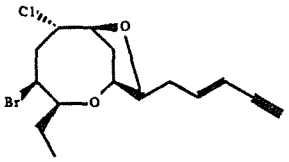
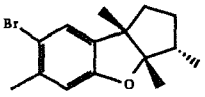
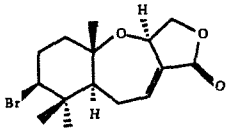
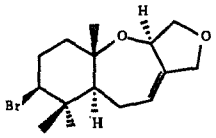
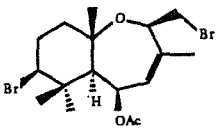
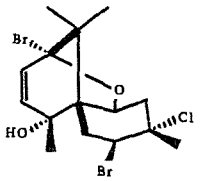
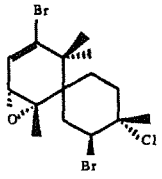
pheromone = compounds shown to function as gamete attractants.

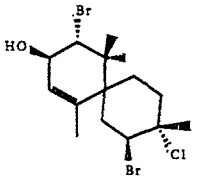
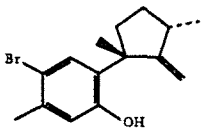
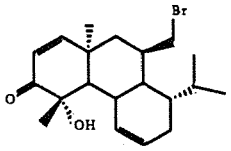
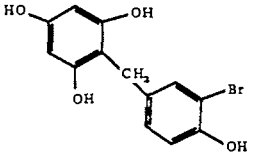
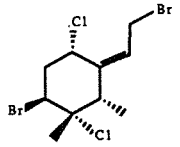
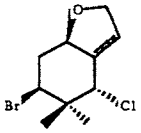
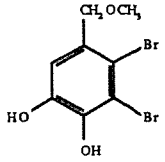
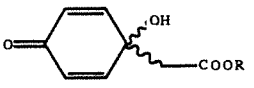
plant GR = compounds shown to have plant growth regulator-like properties in standard bioassays for plant growth regulator activity.

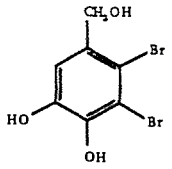
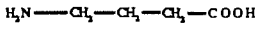
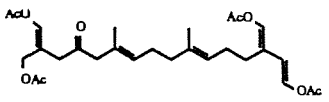
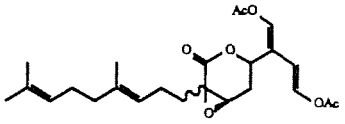
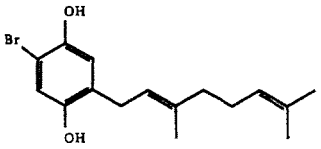
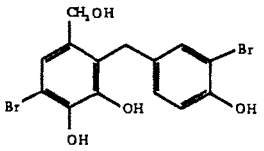
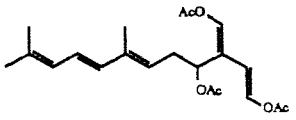
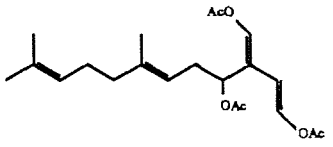
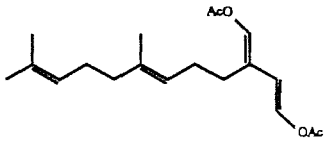
inducer = compounds shown to act as larval settlement and/or metamorphosis inducers.

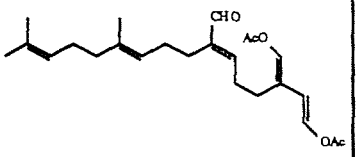
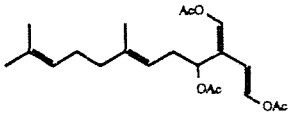
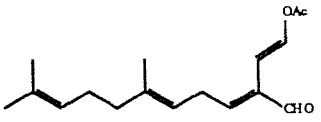
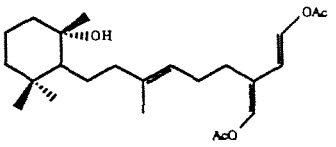
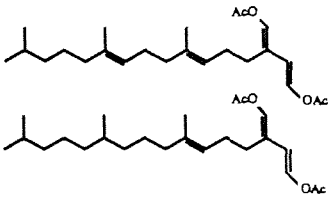
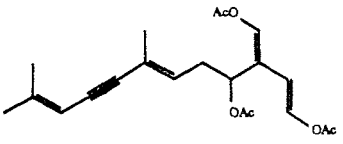
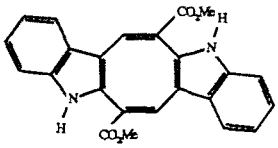
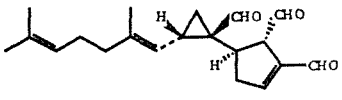
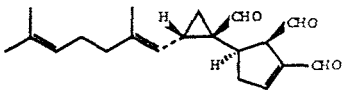
variation = compounds which have been analysed for quantitative variation with respect to ecological parameters.

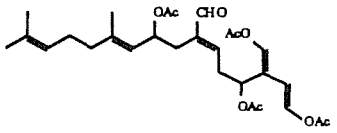
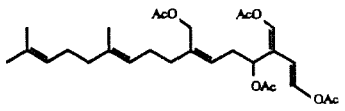
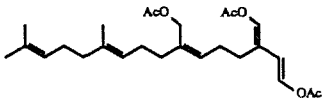
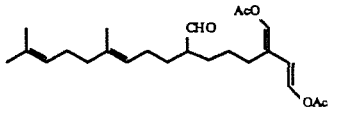
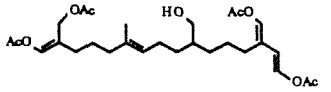
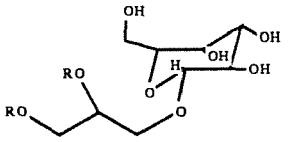
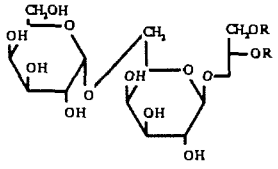
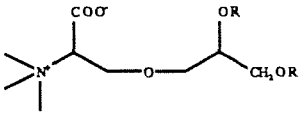
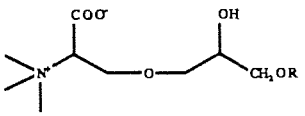
phagostim.= compounds shown to act as phagostimulants (increase feeding responses) in herbivores.

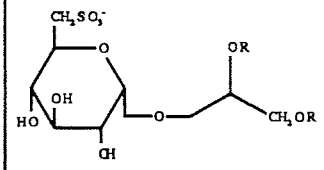
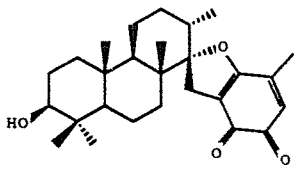
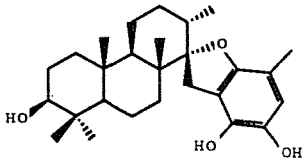
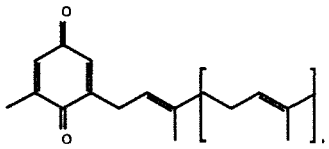
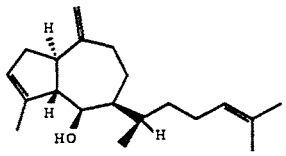
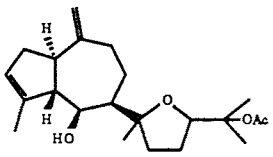
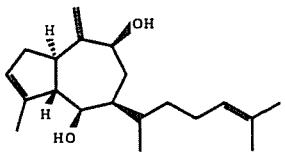
Taxon	Species	Metabolite & references	Structure	Function
Division <u>Rhodophyta</u> *Laurencia*	*Laurencia* spp.	elatol Hay et al. 1987b, 1989a Paul et al. 1989		defence
	Laurencia spp.	debromolaurin-terol Hay et al. 1989a		defence
	L. snyderae	chlorofucin Hay et al. 1989a		defence
	Laurencia spp.	aplysin Hay et al. 1987b, 1989a		defence
	L. cf. palisada	aplysistatin Paul et al. 1989		defence
	L. cf. palisada	palisadin A Hay et al. 1989a Paul et al. 1989		defence
	L. cf. palisada	5-acetoxy-palisadin B Paul et al. 1989		defence
	L. pacifica	pacifenol Hay et al. 1989a		defence
	L. pacifica	prepacifenol Hay et al. 1989a		defence

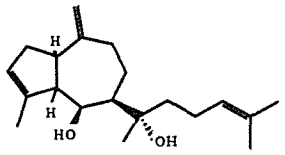
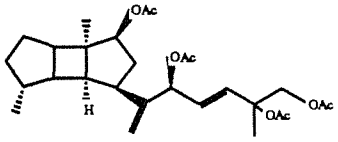
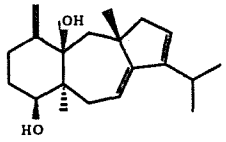
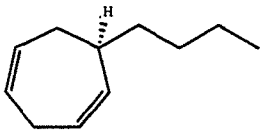
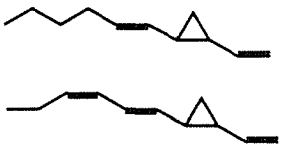
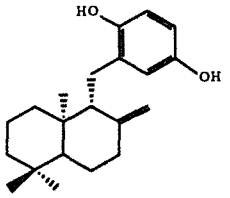
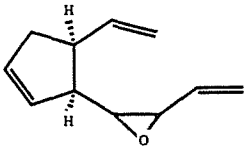
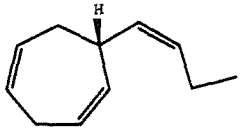
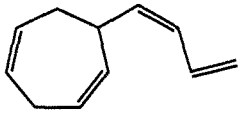
Taxon	Species	Metabolite & references	Structure	Function
	L. pacifica	unnamed chamigrene Hay et al. 1989a		defence
	Laurencia spp.	isolaurinterol Hay et al. 1987b		defence
Sphaerococcus	*S.coronopifolius*	sphaerococc-enol A Hay et al. 1989a		defence
Vidalia	*V. obtusiloba*	vidalol A Hay et al. 1989a		defence
Ochtodes *Desmia (Portieria)*	*O. secundiramea* *D. hornemannii*	ochtodene Paul et al. 1987 Wylie and Paul 1988 Paul et al. 1990		defence
Ochtodes	*O. secundiramea*	chondrocole C Paul et al. 1987		defence
Odonthalia	*O. floccosa* *O. washington-iensis*	α-O-methyllan-osol Kubo et al. 1990		plant GR
Liagora	*L. farinosa*	acetogenin Wylie and Paul 1988 Paul et al. 1990		defence
Delesseria	*D. sanguinea*	jacaranone Yvin et al. 1985		inducer

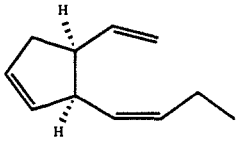
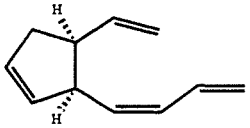
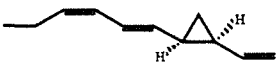
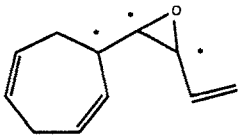
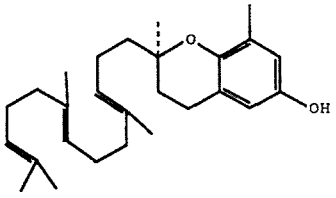
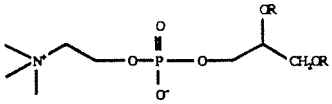
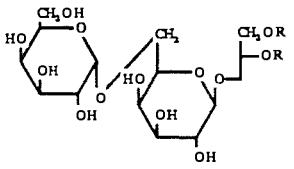
Taxon	Species	Metabolite & references	Structure	Function
Rhodomelea	*R. larix*	Ianosol Phillips and Towers 1982a,b		variation
Coralline algae Family (Corallina-ceae)		γ- amino butyric acid see Morse 1990		inducer
Division Chlorophyta *Chlorodesmis*	*C. fastigiata*	chlorodesmin Paul 1987 Wylie and Paul 1988 Hay et al.1989a,b Paul et al. 1990		defence
Pseudochloro-desmis	*P. furcellata*	epoxylactone Paul 1987		defence
Cymopolia	*C. barbata*	cymopol Hay et al. 1987b, 1989a		defence
Arainvillea	*A. obscura*	avrainvilleol Wylie and Paul 1988 Paul et al. 1990		defence
Rhipocephalus	*R. phoenix*	rhipocephalin Paul and Fenical 1986, 1987		variation
Rhipocephalus *Udotea*	*R. phoenix* *U. cyathiformis*	dihydrorhipo-cephalin Paul and Fenical 1986		variation
Udotea	*U. geppii* *U. conglutinata*	flexilin Paul et al. 1989, 1990		defence variation

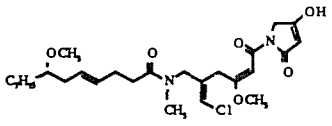
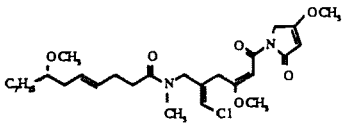
Taxon	Species	Metabolite & references	Structure	Function
Udotea	*U. argentea* *U. flabellum*	udoteal Paul and Fenical 1986 Paul 1987 Paul et al.1989, 1990		variation
Udotea	*U. cyathiformis*	sesquiterpene Paul and Fenical 1987		variation
Udotea	*U. cyathiformis*	aldehyde Paul and Fenical 1986		variation
Caulerpa	*C. brownii*	diacetate Paul et al. 1989		defence
Caulerpa	*C. brownii*	trifarin/ dihydrotrifarin mixture Paul et al. 1989		defence
Caulerpa	*C. sertulariodes*	caulerpenyne Paul and Fenical 1986 Targett et al. 1986 Wylie and Paul 1988 Paul et al. 1990		defence variation
Caulerpa	*Caulerpa* sp.	caulerpin Raub et al. 1987 Wylie and Paul 1988 Paul et al. 1990		defence plant GR
Halimeda	*H. incrassata*	halimedatrial Hay et al. 1988b Paul and van Alstyne 1988		defence variation
Halimeda	*H. incrassata*	epihalimeda-trial Hay et al. 1988b		variation

Taxon	Species	Metabolite & references	Structure	Function
Halimeda	*H. incrassata*	halimedatetra-acetate Paul and Fenical 1986 Targett et al. 1986 Paul 1987 Hay et al. 1988b Paul and van Alstyne 1988		defence variation
Halimeda	*H. incrassata*	halimedin Targett et al. 1986		defence
Penicillus	*P. dumetosus*	triacetate Paul and Fenical 1986, 1987		variation
Penicillus	*P. pyriformis* *P. dumetosus*	dihydroudoteal Paul and Fenical 1986		variation
Tydemania	*T. expeditionis*	alcohol Paul et al. 1990		defence
Bryopsis	*B. pennata*	bryopsin Paul et al. 1990		defence
Ulva	*U. pertusa*	digalactosyldi-acylglycerol Sakata et al. 1988		phagostim.
Ulva	*U. pertusa*	diacylglyceryl-trimethylhormo serine Sakata et al. 1988		phagostim.
Ulva	*U. pertusa*	monoacylgly-ceroltrimethyl-hormoserine Sakata et al. 1988		phagostim.

Taxon	Species	Metabolite & references	Structure	Function
Ulva	*U. pertusa*	sulphoquinovo-syldiacyl-glycerol Sakata et al. 1988		phagostim.
Division Phaeophyta *Stypopodium*	*S. zonale*	stypoldione Hay et al. 1989a		defence
Stypopodium	*S. zonale*	stypotriol Hay et al. 1987b, 1989a		defence
Stypopodium	*S. zonale*	geranylgeranyl methyl quinone Hay et al. 1989a		defence
Glossophora *Dictyota* *Dilophus* *Pachydictyon*	*Glossophora* spp. *Dictyota* spp. *Dilophus* spp. *Pachydictyon* spp.	pachydictyol A Hay et al.1987a, b, 1988c, 1989a, 1990 Wylie and Paul 1988 Paul et al. 1989, 1990		defence
Glossophora *Dictyota* *Dilophus* *Pachydictyon*	*Glossophora* spp. *Dictyota* spp. *Dilophus* spp. *Pachydictyon* spp.	dictyol H Hay et al. 1989a		defence
Glossophora *Dictyota* *Dilophus* *Pachydictyon*	*Glossophora* spp. *Dictyota* spp. *Dilophus* spp. *Pachydictyon* spp.	dictyol B Hay et al. 1989a		defence

Taxon	Species	Metabolite & references	Structure	Function
Glossophora *Dictyota* *Dilophus* *Pachydictyon*	*Glossophora* spp. *Dictyota* spp. *Dilophus* spp. *Pachydictyon* spp.	dictyol E Hay et al. 1987a, 1988c, 1989a		defence
Dictyota *Stoechosper-* *mum*	*S. marginatum*	spatane diterpene Hay et al. 1989a		defence
Dictyota	*D. divaricata*	dolastane diterpene Hay et al. 1989a		defence
Dictyota	*D. dichotoma*	(-) dictyopterene C' (dictyotene) in Maier and Müller 1986		pheromone
Dictyopteris	*Dictyopteris* spp.	dictyopterene A mix2:1 dictyopterene B (also see - hormosirene) Hay et al. 1988a		defence
Dictyopteris	*D. undulata* (=*D. zonarioides*)	zonarol Hay et al. 1989a		defence
Perithalia	*P.caudata*	(+-)caudoxirene Müller et al. 1988		pheromone
Ectocarpus *Sphacelaria* *Adenocystis*	*E. siliculosus* *E. fasciculatus* *S. rigidula* *A. utricularis*	(+) ectocarpene dictyopterene D' in Maier and Müller 1986		pheromone
Desmarestia	*D. aculeata* *D. viridis*	(+-) desmarestene in Maier and Müller 1986		pheromone

Taxon	Species	Metabolite & references	Structure	Function
Cutleria *Chorda*	*C. multifida* *C. tomentosa*	(+-) multifidene in Maier and Müller 1986, Phillips et al. 1990		pheromone
Syringoderma	*S. phinneyi*	(+-) viridiene in Maier and Müller 1986		pheromone
Fucus	*F. serratus* *F. vesiculosus* *F. spiralis*	fucoserratene in Maier and Müller 1986		pheromone
Ascophyllum *Dictyosiphon*	*A. nodosum* *D. foeniculaceus*	finaverrene in Maier and Müller 1986		pheromone
Cystophora	*C. siliquosa*	cystophorene in Maier and Müller 1986		pheromone
Hormosira *Xiphophora* *Durvillea* *Scytosiphon* *Colpomenia*	*H. banksii* *X.chondrophyla* *X. gladiata* *D. potatorum* *D. antarctica* *D. willana* *S. lomentaria* *C. peregrina*	(+-) hormosirene in Maier and Müller 1986 (see also dictyopterene B)		pheromone
Laminariaceae *Alariaceae* *Lessoniaceae*	29 species (studied)	(+-) lamoxirene in Maier and Müller 1986 * no stereochem assigned		pheromone
Sargassum	*S. tortile*	δ-tocotrienol Kato et al. 1975		inducer
Undaria	*U. pinnatifida*	phosphatidyl-choline Sakata et al. 1988		phagostim
Undaria	*U. pinnatifida*	digalactosyl-diacylglycerol Sakata et al. 1988		phagostim

Taxon	Species	Metabolite & references	Structure	Function
Division Cyanophyta *Microcoleus*	*M. lyngbyaceus*	malyngamide B Paul et al. 1990		defence
Microcoleus	*M. lyngbyaceus*	malyngamide A Wylie and Paul 1988		defence

Chapters 2-6

Natural Products Chemistry

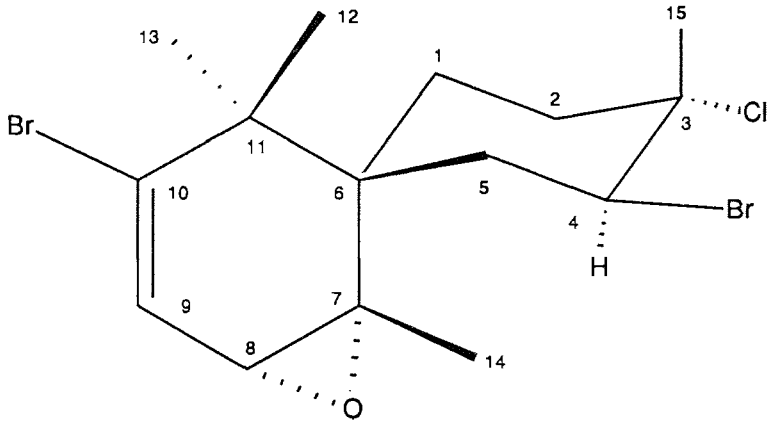
2. INVESTIGATION OF THE NATURAL PRODUCTS CHEMISTRY OF SELECTED RHODOPYHTA (RED ALGAE)

2.1. *Laurencia* spp.

Although the genus *Laurencia* and the family Rhodomelaceae have been investigated with respect to their natural products chemistry (Erickson 1983, Wright *et al.* 1990), these investigations have by no means been exhaustive. This is in part due to the variation in the natural products between species (Erickson 1983), and within a species exposed to differing environments (Capon *et al.* 1988, Wright *et al.* 1990, Suzuki and Kurosawa 1985, Suzuki *et al.* 1985). It is this richness and variety of secondary metabolites that led to the current investigation; namely the collection and extraction of *Laurencia marianensis*, a species not previously investigated, and a reinvestigation of *Laurencia majuscula*. *L. majuscula* has been previously investigated from two other locations in Great Barrier Reef waters (Wright *et al.* 1990): Zoe Bay, Hinchinbrook Island, and Florence Bay, Magnetic Island.

2.1.1. *Laurencia marianensis* MI871F

A sample of *L. marianensis* (Yamada) was collected from the subtidal reef flat of Geoffrey Bay, Magnetic Island at a depth of 1-3 m. The sample (MI871F) was frozen on collection, freeze dried and subsequently exhaustively extracted with DCM followed by MeOH. Rapid chromatography of the combined extracts on a silica gel column using mixtures of light petroleum and ethyl acetate as eluents afforded 16 fractions. HPLC separation of fractions selected



deoxyprepacifenol (35)

Table 3. ^{13}C and ^{1}H n.m.r. data for deoxyprepacifenol (35). Assignments are based on short range (*J* 135 Hz, XHCORR) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ) ($CDCl_3$)	^{1}H (δ) (C_6D_6)	Carbon number
23.5* (q)	1.63 (s)	1.01 (s)	14
23.6* (q)	1.69 (s)	1.50 (s)	15
24.3* (q)	1.20 (s)	0.78 (s)	13
24.7* (q)	1.17 (s)	0.64 (s)	12
25.8 (t)	1.33 (td, *J* 4.0, 13.8, 13.9 Hz)	0.89 (m)	1
	1.77 (dq, *J* 3.3, 3.3, 3,6, 13.9 Hz)	1.59 (m)	
38.9 (t)	2.21 (dt, *J* 3.6, 4.0, 13.8 Hz)	1.87 (dd, *J*13.0, 13.9 Hz)	2
	2.30 (td, *J* 3.3, 13.8, 13.8 Hz)	2.13 (dt, *J* 3.5, 4.0, 13.9 Hz)	
39.5 (t)	2.16 (dd, *J* 13.0, 14.5 Hz)	2.05 (dt, *J* 3.5, 3.5, 13.9 Hz)	5
	2.43 (dd, *J* 3.3, 4.4, 14.5 Hz)	2.42 (td, *J* 3.5, 13.9, 13.9 Hz)	
45.8 (s)			11
49.1 (s)			6
56.2 (d)	2.94 (d, *J* 2.8 Hz)	2.21 (d, *J* 2.8 Hz)	8
58.2 (s)			7
63.1 (d)	4.69 (dd, *J* 4.4, 13.0 Hz)	4.74 (dd, *J* 4.0, 13.0 Hz)	4
71.2 (s)			3
124.1 (d)	6.24 (d, *J* 2.8 Hz)	5.85 (d, *J* 2.8 Hz)	9
143.7 (s)			10

* Resonances which have been reassigned or confirmed.

on the basis of their tlc profile yielded three sesquiterpene metabolites, (35), (36) and (37).

The least polar of these three compounds (35) had identical ^{13}C n.m.r. and mass spectral data to those of the previously reported sesquiterpene deoxyprepacifenol (Ireland *et al.* 1976, Sims *et al.* 1978). Deoxyprepacifenol has been isolated from *Laurencia* species from a wide geographic range (Erickson 1983, Hall and Reiss 1986, Kukuchi *et al.* 1985, Ojika *et al.* 1982, Suzuki and Kurosawa 1985, Suzuki *et al.* 1985, Watanbe *et al.* 1989). Although the absolute configuration of deoxyprepacifenol has been determined by X-ray crystallography (Fronczek and Caccamese 1989, Ireland *et al.* 1976), the ^{1}H and ^{13}C n.m.r. characterisation of this compound remained incomplete. High field 2D n.m.r. experiments and n.O.e. difference spectroscopy were used to assign the data for this compound completely. This resulted in the identification of four carbon signals previously unassigned (Sims *et al.* 1978) and the detailing of ^{1}H n.m.r. chemical shifts and coupling constants not previously reported (Ojika *et al.* 1982).

Proton double resonance and 2D homonuclear (COSY) experiments allowed assignment of ^{1}H n.m.r. chemical shifts and coupling constants (Table 3). Protons were unambiguously assigned to the signals for the carbons to which they were attached using a 2D short range or directly bonded heteronuclear correlation experiment ($CDCl_3$, XHCORR J=135 Hz), with the exception of the two methyl groups resonating at δ1.17 and δ1.20. When the experiment was repeated using C_6D_6 as solvent, unambiguous assignment of these methyl protons and the carbons to which they were attached

Table 4. Long range (J 10 Hz, COLOC) ^{13}C-^{1}H correlations for deoxyprepacifenol (35).

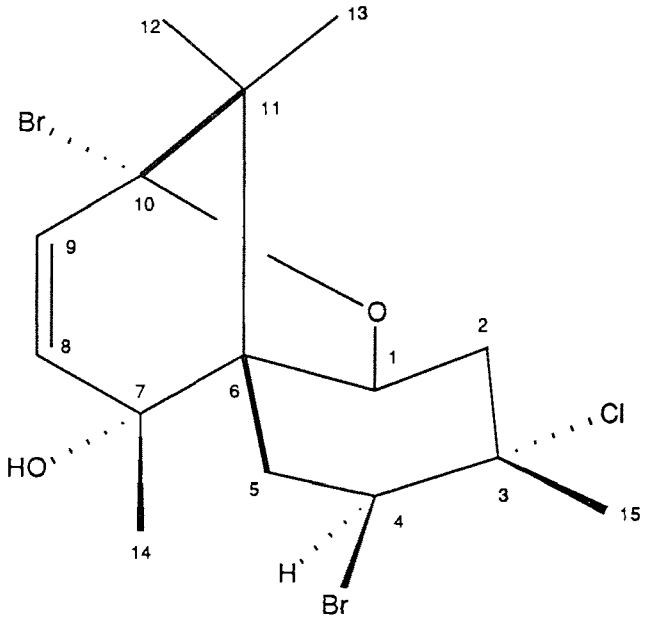
^{1}H (δ) ($CDCl_3$)	^{13}C-^{1}H long range correlations
1.17	45.8, 49.1, 143.7
1.20	45.8, 49.1, 143.7
1.63	23.5, 49.1, 58.2
1.69	38.9, 63.1, 71.2
1.77	39.5, 49.1
2.16	49.1, 63.1
2.43	25.8, 49.1, 63.1, 71.2
2.94	124.1, 143.7
6.24	45.8

became possible (Table 4). This also confirmed all other short range correlations.

The long range correlations ($CDCl_3$, C_6D_6, COLOC J=10 Hz, Table 4) permitted further confirmation of the assignments of the carbon signals. The methyl signals δ1.17 (24.7 ppm) and δ1.20 (24.3 ppm) showed correlations to the carbon signals at 24.3, 24.7, 45.8, 49.1 and 143.7 ppm, while those of the methyl signal δ1.63 (23.5 ppm) showed correlations to 23.5, 49.1 and 58.2 ppm. This confirms the assignment of C6 as 49.1 ppm, C11 as 45.8 ppm and C14 as 23.5ppm. The methyl signal δ1.69 (23.6 ppm) showed connectivity to the carbon signals 23.6, 38.9, 63.1 and 71.2 ppm; C15 was therefore 23.6 ppm. Long range correlations from the methylene signals δ1.77, δ2.16 and δ2.43 and the methine signals δ2.94 and δ6.24 further confirm the carbon assignments (Table 4).

The assignment of C12 and C13 was resolved using an n.O.e. difference experiment in C_6D_6. Irradiation of the methyl signal δ0.64 (24.7 ppm) caused an n.O.e. enhancement of the methyl signal δ1.01 (23.5 ppm, 1.2%), the methylene signal δ2.05 (39.5 ppm, 2.9%) and the methine signal δ2.21 (56.2 ppm, 1.1%). Irradiation of the methyl signal δ0.78 (24.3 ppm) caused an n.O.e. enhancement of the signal at δ0.64 (24.7 ppm, 1.2%) and the methylene signal δ1.87 (38.9 ppm, 3.8%). The assignment of C12 was therefore 24.7 ppm and C13, 24.3 ppm completing the full characterisation of deoxyprepacifenol.

The most polar of the three compounds (36) had identical ^1H and ^{13}C n.m.r. spectral data to that of the previously reported sesquiterpene pacifenol (Caccamese *et al.* 1986, Sims *et al.* 1971, Stallard and Faulkner 1974). Pacifenol is related to deoxypre-



pacifenol (36)

Table 5. ^{13}C and ^{1}H n.m.r. data for pacifenol (36). Assignments are based on short range (J 135 Hz, XHCORR) and long range (J 10 Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
23.5* (q)	1.10 (s)	24.6, 52.0, 53.3, 99.8	12
24.6* (q)	1.28 (s)	23.5, 52.0, 53.3 99.8	13
25.1* (q)	1.50 (s)	53.3, 77.0, 134.3	14
33.6* (q)	1.77 (s)	46.1, 59.4, 69.0	15
34.2* (t)	2.16 (t, J 10.8, 13.1 Hz) 2.30 (dd, J 4.0, 10.8 Hz)	59.4, 77.0	5
46.1* (t)	2.33 (dd, J 12.6, 14.8 Hz) 2.68 (dd, J 5.1, 14.8 Hz)	69.0, 74.1	2
52.0* (s)			11
53.3* (s)			6
59.4 (d)	5.43 (dd, J 4.0, 13.1 Hz)		4
69.0 (s)			3
74.1 (d)	4.67 (dd, J 5.1, 12.6 Hz)		1
77.0 (s)			7
99.8 (s)			10
132.4 (d)	6.04 (d, J 9.8 Hz)	77.0	8
134.3 (d)	5.38 (d, J 9.8 Hz)	99.8	9

* Resonances which have been reassigned or confirmed.

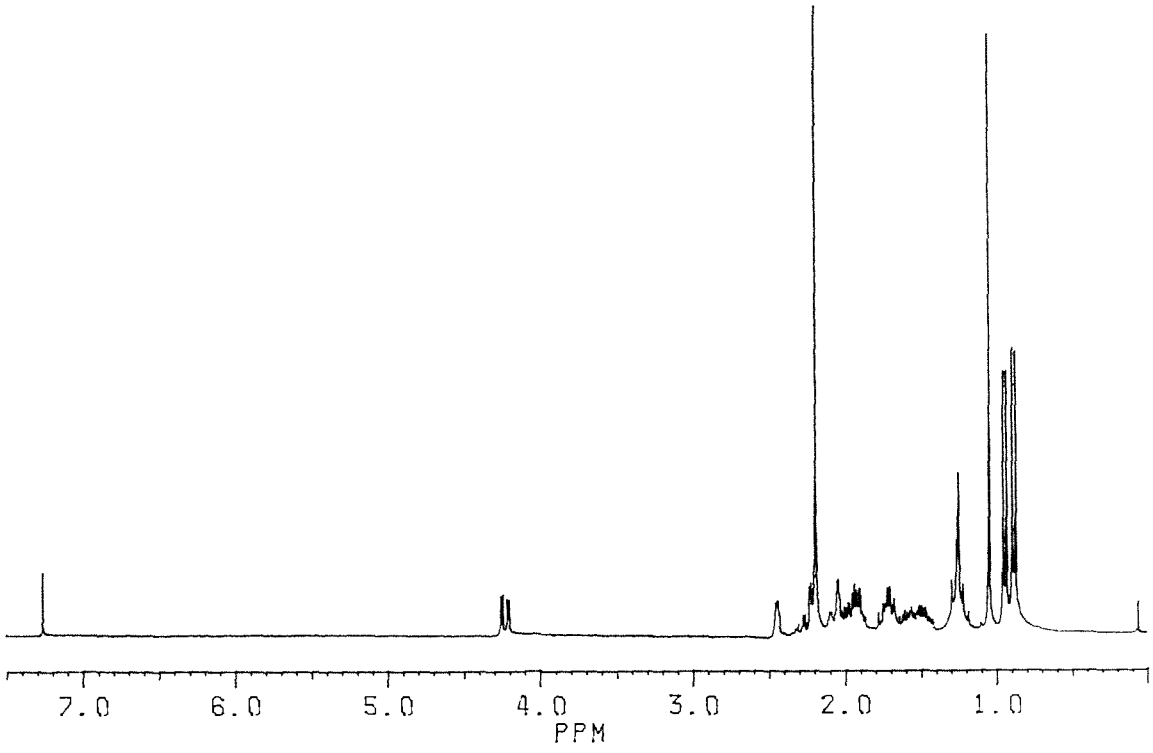
pacifenol and like the latter has previously been isolated from a number of *Laurencia* species from the Pacific (Selover and Crews 1980, Suzuki 1980, Sims *et al.* 1971, Sims *et al.* 1973) and Mediterranean (Caccamese *et al.* 1986) regions and from the herbivorous opisthobranch mollusc *Aplysia californica* (Stallard and Faulkner 1974). One of these species, *L. elata* also yielded deoxyprepacifenol (Hall and Reiss 1986). Pacifenol, when assayed *in-situ,* did not deter grazing by the herbivorous rabbitfish *Siganus doliatus* (Table 2).

The absolute configuration of pacifenol has been determined (Fronczek and Caccamese 1986), although the characteristion of pacifenol by ^{1}H and ^{13}C n.m.r. in the literature is incomplete. High field 2D heteronuclear experiments and n.O.e difference spectroscopy have now been used to characterise the compound fully. This resulted in the reassignment of four carbon signals and the confirmation of the definitive assignment of four carbon resonances not previously assigned to specific carbon atoms (Sims *et al.* 1978).

(36) had identical ^{13}C, ^{1}H n.m.r., and mass spectral data to those previously reported (Table 5). Selective proton decoupling experiments confirmed the previous assignment of proton resonances and coupling constants (Caccamese *et al.* 1986). A short range 2D experiment (XHCORR, J= 135 Hz) associated signals for all protons with the signals for the carbons to which they are attached (Table 5). The long range correlation (COLOC J=10 Hz) especially for CH_3 resonances permitted connectivities to be established two and three bonds from the methyl protons permitting confirmation of all the carbon assignments (Table 5).

Table 6. ^{13}C and ^{1}H n.m.r. data for (37). Assignments are based on short range (J 135 Hz, XHCORR) and long range (J 7 Hz, INAPT) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
16.3 (q)	0.86 (d, J 6.7 Hz)	22.3, 29.2, 47.4	3''
21.7 (t)	1.49 (m)		2'
	1.71 (m)		
22.3 (q)	0.96 (d, J 6.7 Hz)		2''
22.3 (q)	1.05 (s)	38.8, 47.6, 50.3, 61.3	4''
23.6 (t)	1.55 (m)		6'
	2.08 (m)		
27.8 (q)	2.19 (s)	210.6	2
29.2 (d)	1.73 (m)		1"
30.7 (t)	2.01 (m)		5'
	2.15 (m)		
38.8 (t)	1.28 (m)		3'
	1.96 (m)		
47.4 (d)	1.92 (m)		1'
47.6 (q)			3'a
47.8 (d)	2.44 (ddd, J 2.5, 2.5, 2.5 Hz)	23.6, 30.8, 47.4, 47.6, 50.3, 210.6	7'
50.3 (d)	2.22 (dd, J 2.5, 12.0 Hz)		7'a
61.3 (t)	4.22 (dd, J 4.3, 12.7 Hz)		4'
210.6 (q)			1



^{1}H n.m.r. spectrum of (37)

The assignment of C12 and C13 was resolved on the basis of n.O.e. experiments. Irradiation of the methyl signal δ1.10 (23.5 ppm) caused n.O.e. enhancement of the methyl signals δ1.28 (24.6 ppm, 1.3%), δ1.50 (25.1 ppm, 4.3%), the equatorial methylene proton δ2.30 (34.2 ppm, 1.0%) and the methine protons δ5.38 (134.3 ppm, 0.6%) and δ6.04 (132.4 ppm, 0.8%). Similarly irradiation of the methyl signal δ1.50 (25.1 ppm) caused an n.O.e. enhancement of the methyl signal δ1.10 (23.5 ppm, 1.0%), the equatorial methylene proton δ2.30 (34.2 ppm, 3.6%) and the methine proton δ5.38 (134.3 ppm, 3.1%). The irradiation of the methyl signal δ1.28 (24.6 ppm) caused an n.O.e. enhancement of the axial methylene protons δ2.16 (34.2 ppm, 3.0%) and δ2.33 (46.1, 3.1%)

These data are consistent with the assignment of the C12 methyl resonance at 23.5 ppm and the C13 methyl resonance at 24.6 ppm completing the full characterisation of pacifenol.

Of the three metabolites isolated, the compound with intermediate polarity, (37), was an as yet unidentified sesquiterpene. (37) had a molecular formula $C_{15}H_{25}BrO$ by HREIMS and contained one signal in the ^{13}C n.m.r. spectrum for a carbonyl carbon (210 ppm, ketone) dictating that the compound must be bicyclic. The ^{1}H n.m.r. spectrum revealed the presence of four methyl groups (an isopropyl group: δ0.86, d, J= 6.7 Hz; δ0.96, d, J= 6.7 Hz; an aliphatic methyl signal: δ1.05, s; and a methyl ketone: δ2.19, s). Also present in the ^{1}H n.m.r. spectrum were signals for eight methylene protons and four methine protons (Table 6). Unambiguous assignment of protons to their attached carbons was established using a short range 2D heteronuclear experiment (XHCORR, J=135 Hz) (Table 6).

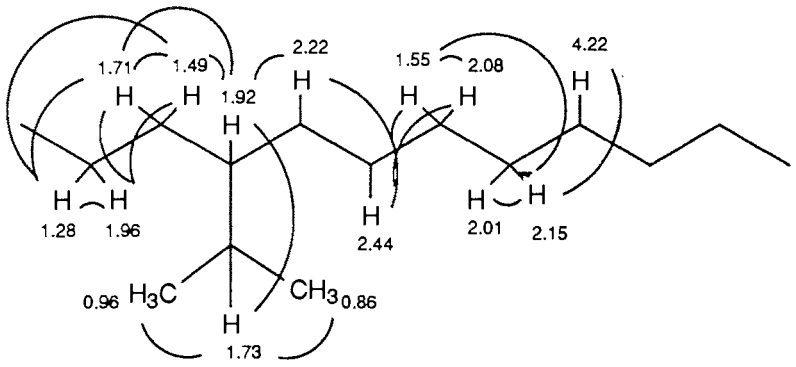


Figure 3

Cross peaks between proton signals from COSYDQF spectra of (37)

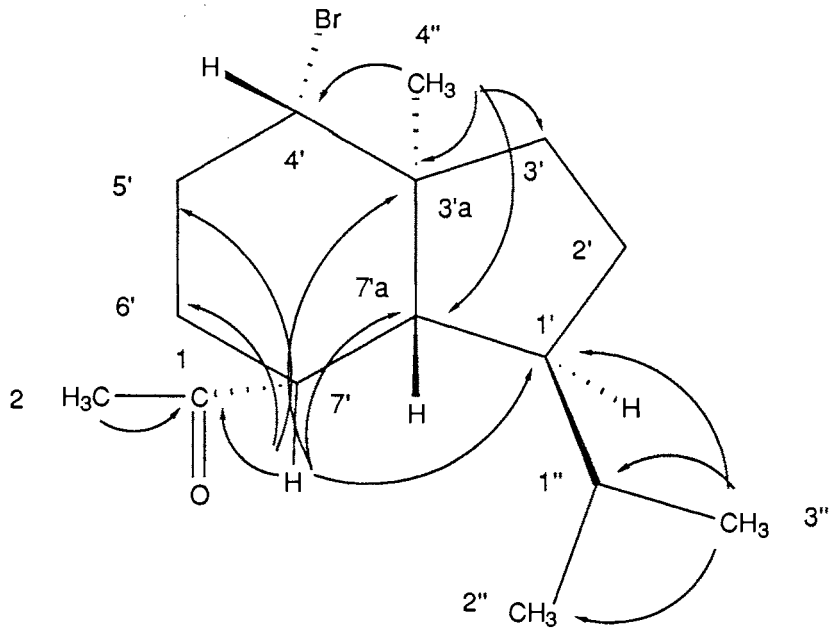
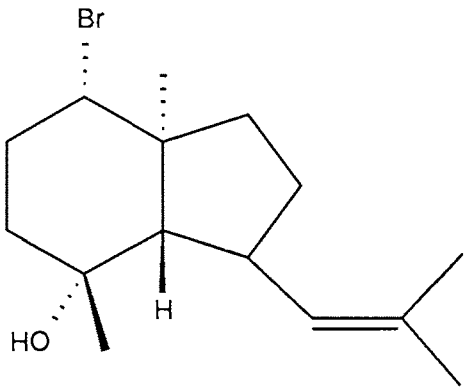


Figure 4

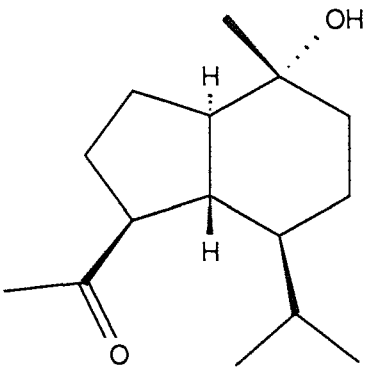
Long range ^{13}C-^{1}H correlations (37)

Proton double resonance experiments and a 2D homonuclear (COSYDQF) experiment allowed the establishment of a major fragment of the molecule (Fig. 3). The presence of cross peaks in the COSYDQF spectrum was used to distinguish couplings between adjacent protons (Fig. 3).

The presence of a methyl ketone, an isopropyl group and one other methyl group leaves nine carbons in a bicyclic ring system. This suggests a carbon skeleton similar to that of the oppositanes (Erickson 1983, Martin and Darias 1978, Wratten and Faulkner 1977) (38) and oplopanone (Wratten and Faulkner 1977) (39) based on a fused five-six membered (substituted indene) ring system. The structure of the molecule was established using selective long range correlation experiments (INAPT, J=7 Hz) (Table 6). The methyl signal δ0.86 (16.3 ppm, (C3")) showed correlations to the carbon signals 22.3 (C2"), 29.2 (C1"), and 47.4 ppm (C1'), confirming the position of the isopropyl group. The methyl signal δ1.05 (22.3 ppm, (C4")) showed correlations to the carbon signals 38.8 (C3'), 47.6 (C3'a), 50.3 (C7'a) and 61.3 ppm (C4'), confirming the assignment of carbons around the quaternary bridgehead position (47.6 ppm (C3'a)). The methyl signal δ2.19 (27.8 ppm (C2)) (INAPT, J=7 Hz) showed a correlation to the carbonyl carbon (210.6 ppm (C1)) further confirming the presence of a methyl ketone while the methine signal δ2.44 (47.8 ppm (C7')) showed correlations to the carbon signals 23.6 (C6'), 30.7 (C5'), 47.4 (C1'), 47.6 (C3'a), 50.3 (C7'a) and 210.6 ppm (C1).



oppositol (38)



oplopanone (39)

These correlations confirmed the ring structure and the placement of the methyl, isopropyl and methyl ketone groups in the ring system (Fig. 4).

The stereochemistry of the molecule was determined using n.O.e. difference spectroscopy and the coupling constants of proton signals in the ^1H n.m.r. spectra. The configuration and conformation of the molecule which was consistent with these data is shown in Fig. 5. Irradiation of the methyl signal at δ0.86 (16.3 ppm) caused an n.O.e. enhancement of the methine signal δ2.22 (50.3 ppm, 0.93%) while irradiation of the methyl signal δ0.96 (22.3 ppm) caused an n.O.e. enhancement of the signals δ1.49 (21.7 ppm, 1.64%), δ1.71 (and or δ1.73) (21.7, 29.2 ppm, 3.00%), δ1.92 (47.4 ppm, 1.94%), δ2.22 (50.3 ppm, 1.49%) and δ2.44 (47.8 ppm, 1.01%). Irradiation of the methyl signal δ1.05 (22.3 ppm) caused an n.O.e. of the methylene signal δ1.96 (38.8 ppm, 1.27%), and the overlapping methylene signal δ2.15 (30.7 ppm) and methyl signal δ2.19 (27.8 ppm) (3.49%). The signals for the methyl and methylene protons were able to be distinguished due to their multiplicity. Similarly, irradiation of the methyl signal δ2.19 (27.8 ppm) caused an n.O.e. enhancement of the methyl signals δ0.96 (22.3 ppm, 1.04%) and δ1.05 (22.3 ppm, 1.52%). Irradiation of the methine proton signal δ2.44 (47.8 ppm) caused an n.O.e. enhancement of the methylene signal δ1.55 (23.6 ppm, 1.89%) and the methine proton δ1.73 (29.2 ppm, 3.97%). Irradiation of the methine signal δ4.22 (61.3 ppm) caused an n.O.e. enhancement of signals δ2.15 (30.7 ppm, 1.17%) and δ2.22 (50.3 ppm, 1.49%).

These data suggest a *trans*-ring junction and a 1-3 diaxial positioning of the bridgehead methyl and the methyl ketone groups with the six membered ring in a chair conformation. Similarly this

conformation agrees with the coupling constants observed for the methine proton signals δ2.44 (bd, *J*=2.5 Hz) and δ4.22 (dd, *J*=4.3, 12.7 Hz). (37) is therefore 1-[(1'*S**,3'a*S**,4'*S**,7'*R**,7'a*R**)-4'-bromo-1'-isopropyl-3'a-methyl-octahydro-1*H*-inden-7-yl]ethanone.

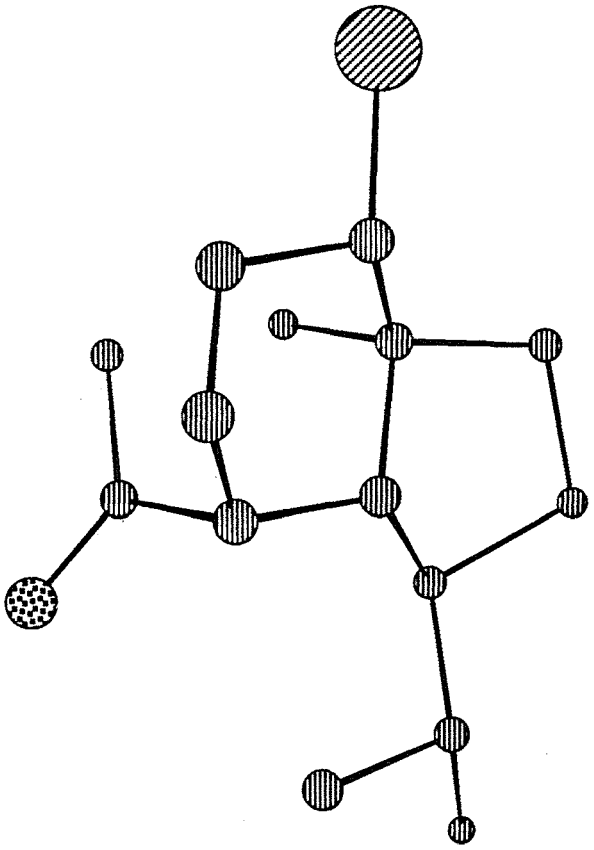


Figure 5

Three-dimensional representation of (37)

2.1.2. *Laurencia majuscula* MI884E

Due to the range of secondary metabolites previously isolated by members of our research group from *L. majuscula,* a further collection of this alga was made from the subtidal reef flat of Geoffrey Bay, Magnetic Island (28/ix/88). The sample was frozen on collection, freeze dried and subsequently exhaustively extracted with DCM followed by MeOH to yield a crude extract. Rapid chromatography of the extract on a silica gel column using light petroleum and ethyl acetate mixtures as eluents afforded 24 fractions.

HPLC separation of selected fractions yielded the previously reported sesquiterpenes, the triquinane (40) and the chamigranes (41), (42), (43), (44) and (45).

Comparison of the ^{1}H n.m.r. spectra of sesquiterpenes (40)-(44) with authentic compounds confirmed the identity of (40)-(44) in order of increasing polarity as follows:

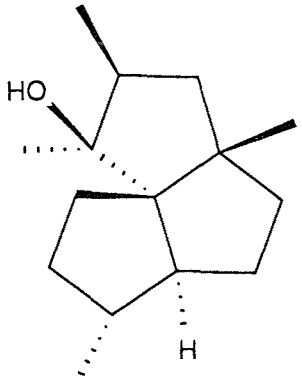
(40) (1*S**,2*S**,3*S**,5*S**,8*S**,9*S**)-tricyclo[6.3.0.$0^{1,5}$] undecan-2-ol (Coll and Wright 1989a)

(41) *iso*-obtusadiene (Coll and Wright 1989a, Gerwick *et al.* 1987)

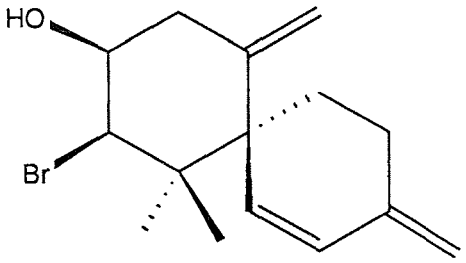
(42) (1*Z*,8*R**,9*R**)-8-bromochamigra-1,11(12)-dien-9-ol (Wright *et al.* 1990)

(43) (1(15)*E*,2*Z*,4*S*,8*R*,9*S*)-8,15-dibromochamagra-1(15),2,11(12)-trien-9-ol (Coll and Wright 1989a)

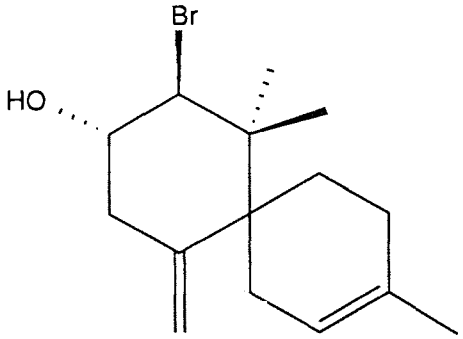
(44) (1(15)*Z*,2*Z*,4*S*,8*R*,9*S*)-8,15-dibromochamigra-1(15),2,11(12)-trien-9-ol (Coll and Wright 1989a, Suzuki and Kurosawa 1978, Suzuki *et al.* 1979)



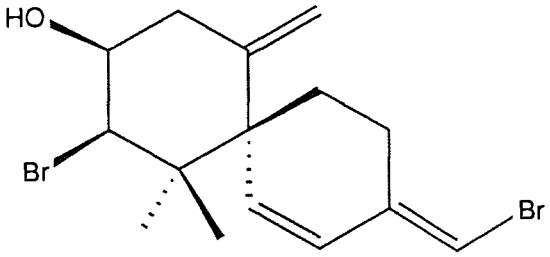
(40)



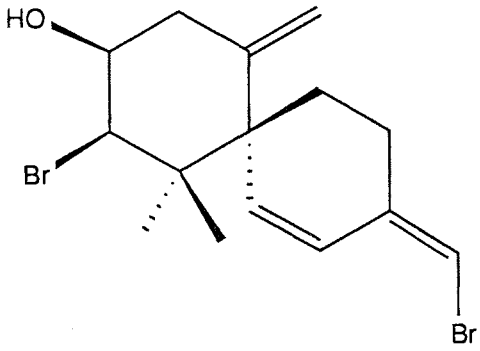
(41)



(42)



(43)

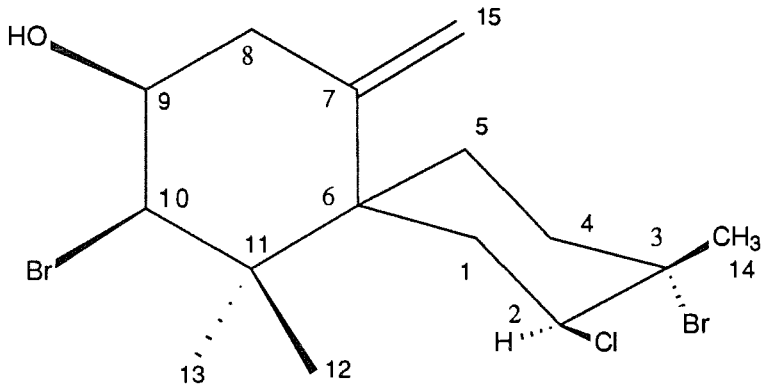


(44)

* stereochemistry not assigned

The most polar compound (45) had similar ^{1}H and ^{13}C n.m.r. spectral data, and optical rotation and melting point to the previously reported compound *iso*-obtusol (Gonzalez *et al.* 1979b, Kennedy *et al.* 1988). Full characterisation of the compound by n.m.r. remained incomplete in the literature. High field 2D n.m.r. and n.O.e. experiments were used to fully characterise the compound. This resulted in the reassignment of two carbon signals, the confirmation of four carbon signals previously deemed interchangeable, and the assignment of ^{1}H n.m.r. chemical shifts and coupling constants in addition to those previously reported (Kennedy *et al.* 1988).

Iso-obtusol has been isolated from two species of *Laurencia* , *L. obtusa* (Gonzalez *et al.* 1976, 1979a, b; Kennedy *et al.* 1988; Martin *et al.* 1989) and *L. majuscula* (Capon *et al.* 1988)
The absolute configuration of *iso*-obtusol has been determined by X-ray crystallography (Gonzalez *et al.* 1979a) and resulted in the reassignment of the original structure of the compound (Gonzalez *et al.* 1976). (45) had identical ^{13}C and ^{1}H n.m.r. spectral data to that previously reported (Table 7). The multiplicities of the carbon signals were established using a DEPT experiment. This showed the carbon signals 25.5 ppm and 33.0 ppm to be CH_2 and CH_3 respectively rather than CH_3 and CH_2 as previously reported (Kennedy *et al.* 1988). The carbon shift 33.0 ppm (CH_3) is consistent with similar compounds bearing an equatorial methyl group on a chlorinated carbon (c.f. pacifenol, see Table 5). A short range 2D heteronuclear experiment (XHCORR J= 135 Hz) associated signals for all protons with the signals for the carbons to which they are attached (Table 7) while a 2D proton homonuclear (COSY)



iso-obtusol (45)

Table 7. ^{13}C and ^{1}H n.m.r. data for iso-obtusol (45). Assignments are based on short range (*J* 135 Hz, XHCORR) and long range (*J* 7 Hz, INAPT) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
24.8* (q)	1.32 (s)	25.2, 43.5	12
25.2* (q)	1.04 (s)	24.8, 43.5	13
25.5* (t)	1.85 (dt, *J* 3.4, 3.4, 12.3 Hz)		5
	2.03 (td, *J* 3.4, 12.3, 12.3 Hz)		
33.0* (q)	1.90 (s)	33.2, 65.0, 71.1	14
33.2* (t)	1.80 (dt, *J* 3.4, 3.4, 12.3 Hz)		4
	2.21 (td, *J* 3.5, 12.3, 12.3 Hz)		
33.7* (t)	2.77 (dd, *J* 3.8, 15.7 Hz)		1
	3.06 (bd, *J* 15.7 Hz)		
39.2 (t)	2.41 (dd, *J* 3.9, 11.9Hz)		8
	2.69 (t, *J* 11.9 Hz)		
43.5 (s)			11
43.7 (s)			6
65.0 (d)	4.42 (bs)		2
69.6 (d)	3.65 (bd)		9
71.1 (s)			3
76.2 (d)	4.40 (m)		10
113.8 (t)	4.90 (s)		15
	5.15 (s)	39.2, 147.4	
147.4 (s)			7

* Resonances which have been reassigned or confirmed.

experiment confirmed the previous assignments of proton resonances and coupling constants (Kennedy *et al.* 1988). These experiments allowed the assignment of C5 as 25.5 ppm, C4 as 33.2 ppm and C1 as 33.7 ppm. Long range correlations (INAPT J=7 Hz) allowed the confirmation of the carbon assignments. The methyl proton signals at δ1.90 (33.0 ppm) showed correlations to the carbon signals 33.2, 65.0 and 71.1 ppm. This confirms the assignment of C4 as 33.2 ppm and C14 as 33.0 ppm. The methyl proton signals δ1.04 (25.2 ppm) and δ1.32 (24.8 ppm) showed connectivities to the carbon signals 24.8, 25.2 and 43.5 ppm confirming their assignments as either C12 or C13. The assignment of C12 and C13 was resolved using an n.O.e. difference experiment. Irradiation of the methyl signal δ1.04 (25.2 ppm) caused an n.O.e. enhancement of the methine signals δ4.40 (76.2 ppm, 3.9%) and δ3.65 (69.6 ppm, 3.6%), the methylene signal δ1.85 (25.5 ppm, 2.8 %) and the methyl signal δ1.32 (24.8 ppm, 1.4%). Irradiation of the methyl signal δ1.32 (24.8 ppm) caused an n.O.e. enhancement of the the methine signal δ4.40 (76.2 ppm, 2.3%), the methylene signals δ2.77 (33.7 ppm, 3.0%) and δ2.03 (25.5 ppm, 2.3%), and the methyl signal δ1.04 (25.2 ppm, 1.4%).

The assignment of C12 was therefore 24.8 ppm and C13, 25.2 ppm completing the characterisation of *iso*-obtusol. The structure which corresponds to these data agrees with the X-ray crystallographic structure of Gonzalez *et al.* (1979a) . However, it calls into question the structure of *iso*-obtusol assigned by Kennedy *et al.* (1988), which appears to have the opposite stereochemistry at the spiro-centre of the molecule, resulting in the misplacement of the Cl in the B ring.

As well as the isolation of these previously reported compounds, the collection of *L. majuscula* yielded three novel metabolites possessing a skeletal type not previously reported in the literature.

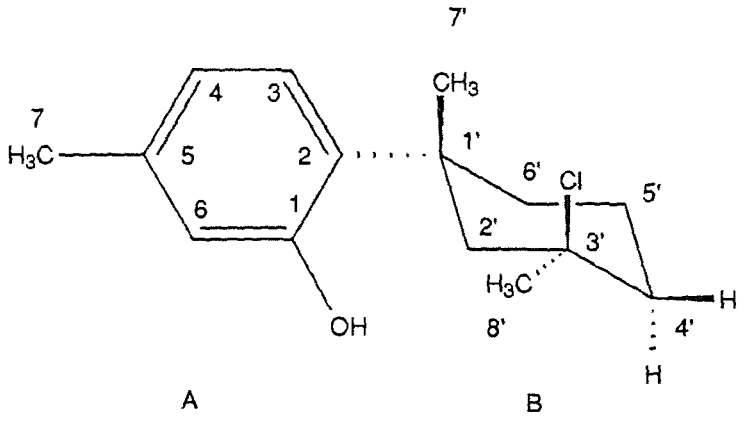
The least polar of the three novel compounds, (46) had the molecular formula $C_{15}H_{21}ClO$ by HREIMS. The compound thus had 5 double bond equivalents. The presence of signals for 6 sp^2 carbons (115.5 (d), 120.1 (d), 124.5 (d), 126.1 (s), 136.9 (s), 155.8 (s) ppm) suggested that the compound possessed an aromatic ring and thus (46) must be bicyclic with a cycloalkane B ring. The presence of signals in the ^1H n.m.r. spectrum for three aromatic protons δ6.68 (s), 6.70 (dd, *J* 2.0, 7.7 Hz) and 7.10 (d, *J* 7.7 Hz) and absorbances in the i.r. spectrum (ν_{max} 860, 710 cm^{-1}) were consistent with the presence of a 1,2,4-trisubstituted benzene ring. Substituents on the aromatic (A) ring were a methyl group (^1H δ 2.32, (s)) a hydroxyl group (^{13}C 155.8 ppm, (s)) and the second ring of the molecule.
A 2D homonuclear correlation (COSYDQF) experiment allowed part of the remaining fragment to be established, while unambiguous assignment of protons to the carbons to which they are attached was established using an XHCORRD experiment (*J*= 135 Hz). Due to the presence of two overlapping methyl signals in the spectrum (δ 1.41, 1.41, $CDCl_3$), the sample was dissolved in C_6D_6 and the experiments repeated. This allowed sufficient separation of the signals to unambiguously assign the methyl protons to their respective carbons (Table 8).
Cross peaks in the COSYDQF ($CDCl_3$) spectrum confirmed the presence of a 1,2,4-trisubstituted aromatic ring. However, due to the overlapping of a large number of signals in the ^1H n.m.r.

Table 8. ^{13}C and ^{1}H n.m.r. data for (46). Assignments are based on short range (*J* 135 Hz, XHCORRD) ^{13}C-^{1}H correlations.

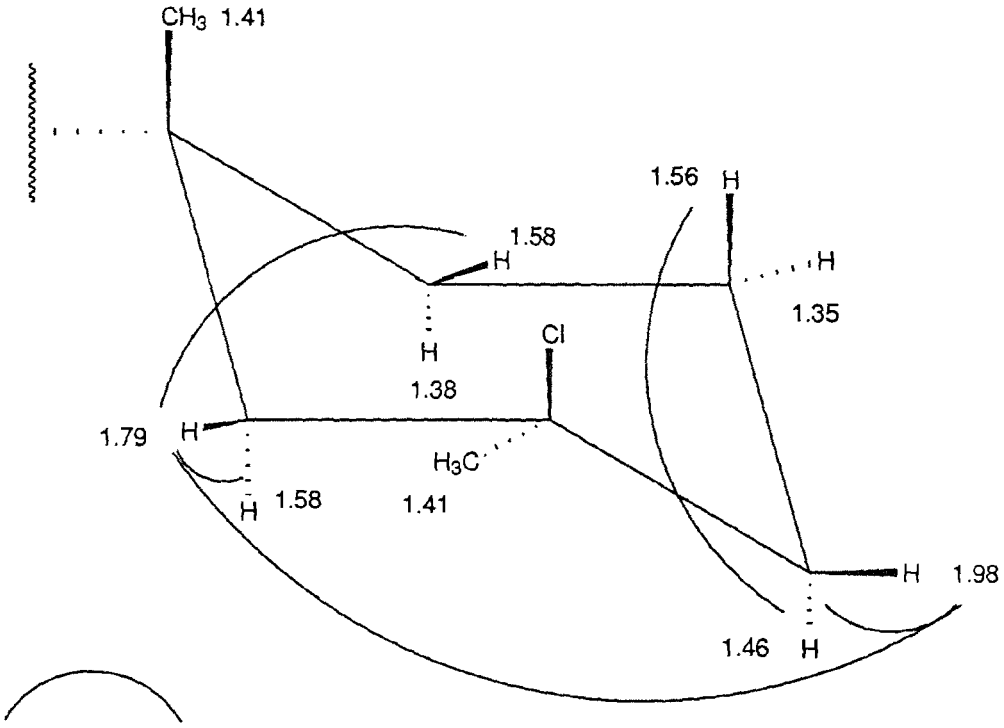
^{13}C (ppm)	^{1}H (δ) $CDCl_3$	^{1}H (δ) C_6D_6	Carbon number
19.9 (t)	1.35 (m)	1.26 (m)	5'
	1.56 (m)	1.49 (m)	
20.9 (q)	2.32 (s)	2.14 (s)	7
27.1 (q)	1.41 (s)	1.19 (s)	7'
29.2 (q)	1.41 (s)	1.24 (s)	8'
32.8 (s)			1'
38.8 (t)	1.46 (m)	1.16 (m)	4'
	1.98 (dm, *J* 10.9 Hz)	1.80 (dm, *J* 13.1 Hz)	
40.5 (t)	1.38 (m)	1.25 (m)	6'
	1.58 (d, *J* 12.9 Hz)	1.40 (m)	
44.1 (t)	1.58 (m)	1.15 (m)	2'
	1.79 (dt, *J* 2.4, 2.4, 13.0 Hz)	1.53 (dt, *J* 2.3, 2.3, 12.7 Hz)	
74.8 (s)			3'
115.5 (d)	6.68 (s)	6.79 (s)	6
120.1 (d)	6.70 (dd, *J* 1.0, 7.7 Hz)	6.64 (d, *J* 7.7 Hz)	4
124.5 (d)	7.10 (d, *J* 7.7 Hz)	6.98 (d, *J* 7.7 Hz)	3
126.1 (s)			2
136.9 (s)			5
155.8 (s)			1

Table 9. ^{13}C and ^{1}H n.m.r. data for (46). Assignments are based on long range (*J* 10 Hz, COLOC, C_6D_6*, COLOC, $CDCl_3$**)^{13}C-^{1}H correlation and a 1D INAPT (*J* 7 Hz, $CDCl_3$+) experiment.

^{1}H	^{13}C-^{1}H long range correlations
1.19*	32.8, 40.5, 44.1, 126.1
1.24*	38.8, 44.1, 74.8
1.79+	27.1, 29.2, 32.8, 38.8, 40.5, 74.8, 126.1
1.98+	19.9, 29.2, 40.5, 44.1, 74.8
2.32**	115.5, 120.1, 136.9
6.70**	20.9, 115.5, 126.1
7.10**	136.9, 155.8



(46)



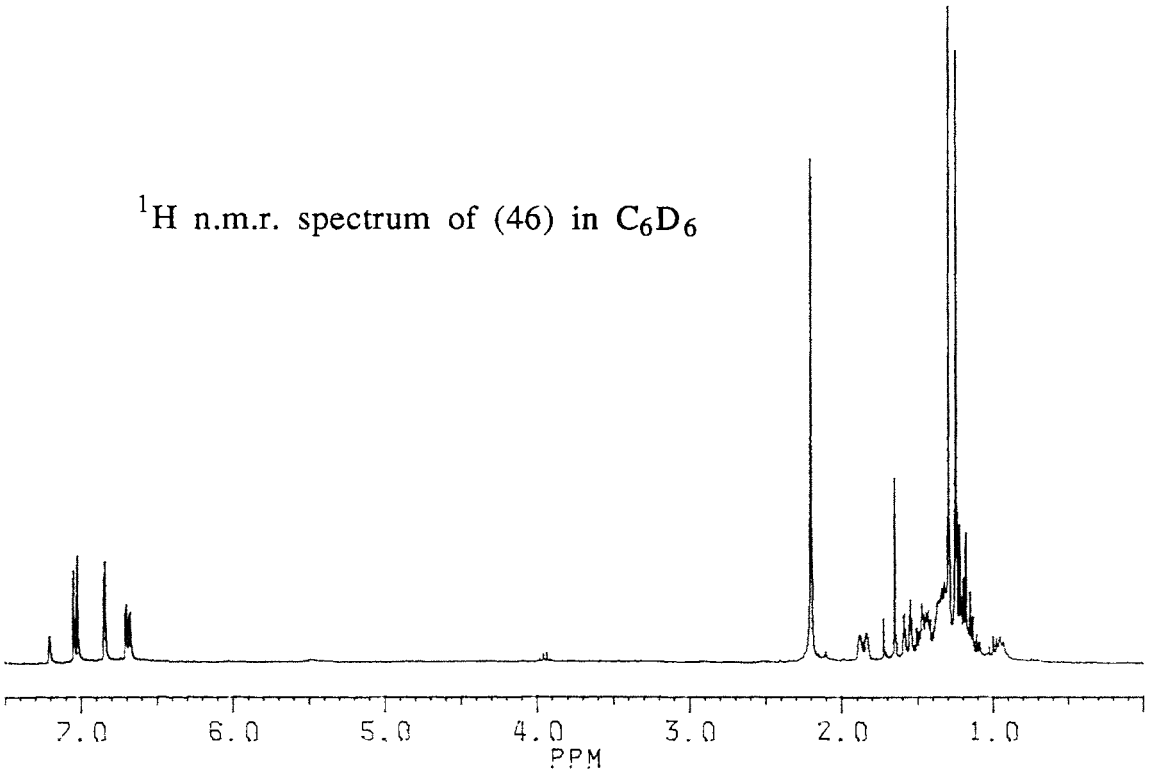
Crosspeaks from COSYDQF spectra of (46)

Figure 6.

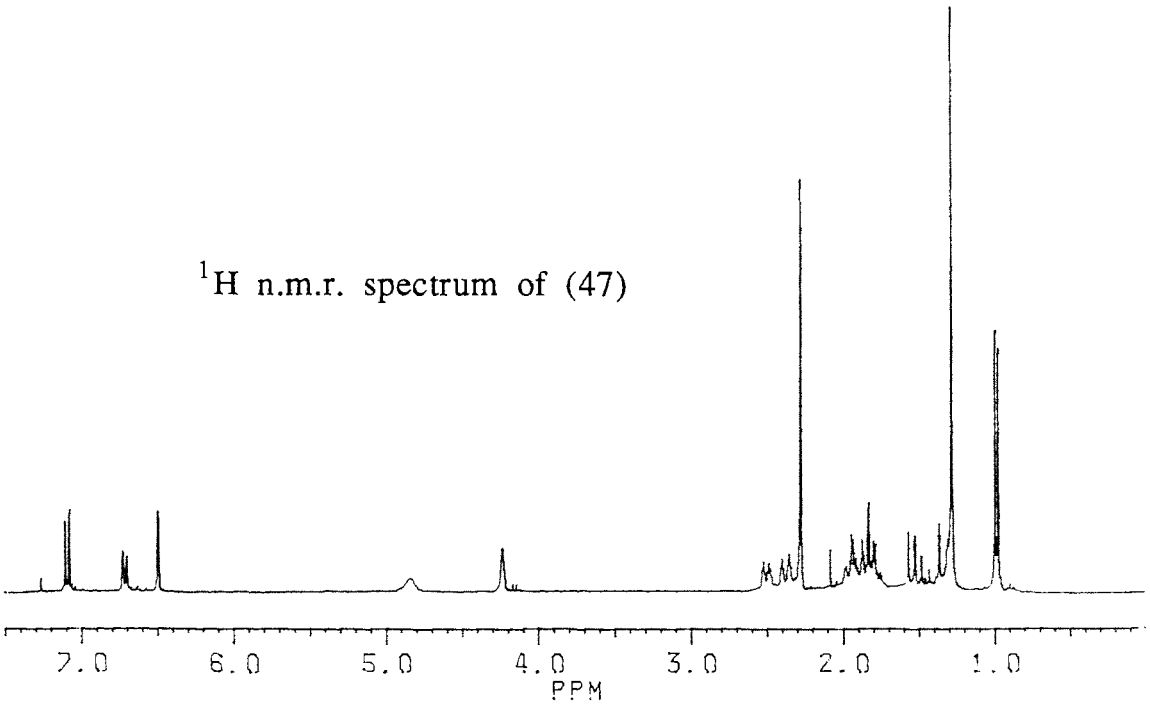
spectrum, the remaining fragment of (46) was not able to be established using only ^1H-^1H correlations. Cross peaks showed a correlation between the methylene proton signal δ1.98 (38.8 ppm) and the geminal proton signal δ1.46 (38.8 ppm), and the methylene proton signals δ1.56 (19.9 ppm) and δ1.79 (44.1 ppm). The methylene proton signal δ1.79 showed crosspeaks to δ1.58 (44.1, 40.5 ppm) and δ1.98 (38.8 ppm) (Fig. 6).

Long range correlations using a 2D heteronuclear experiment (COLOC J= 10 Hz) and selective 1D heteronuclear experiments (INAPT J= 7 Hz) in $CDCl_3$ and C_6D_6 were used to confirm the assignment of the carbon skeleton. Correlations from the aromatic proton signals δ6.70 (120.1 ppm) and δ7.10 (124.5 ppm) ($CDCl_3$) and the aromatic methyl signal δ2.32 (20.9 ppm) ($CDCl_3$) confirmed the proposed aromatic system while correlations from the aliphatic proton signals established the assignment of carbons in the B ring (Table 9). The methyl signal δ1.19 (27.1 ppm) (C_6D_6) showed correlations to the carbon signals 27.1, 32.8, 40.5, 44.1 and 126.1 ppm. The methyl signal δ1.24 (29.2 ppm) (C_6D_6) showed correlations to the carbon signals 29.2, 38.8, 44.1 and 74.8 ppm. The methylene signal δ1.79 (44.1 ppm) ($CDCl_3$) showed correlations to the carbon signals 27.1, 29.2, 32.8, 38.8, 40.5, 74.8 and 126.1 ppm while the methylene signal δ1.98 (38.8 ppm) ($CDCl_3$) showed correlations to the carbon signals 19.9, 29.2, 40.5, 44.1 and 74.8 ppm. The strong two and three bond correlations from these methyl and methylene proton signals dictate the assignment of the carbon signals for the B ring (Fig. 6). The relative stereochemistry of the molecule was determined using n.O.e. difference spectroscopy (C_6D_6) and proton coupling data. Irradiation of the methyl signal δ1.19 (C_6D_6) caused an n.O.e. enhancement of the signals δ1.40 (2.5%),

^{1}H n.m.r. spectrum of (46) in C_6D_6



^{1}H n.m.r. spectrum of (47)



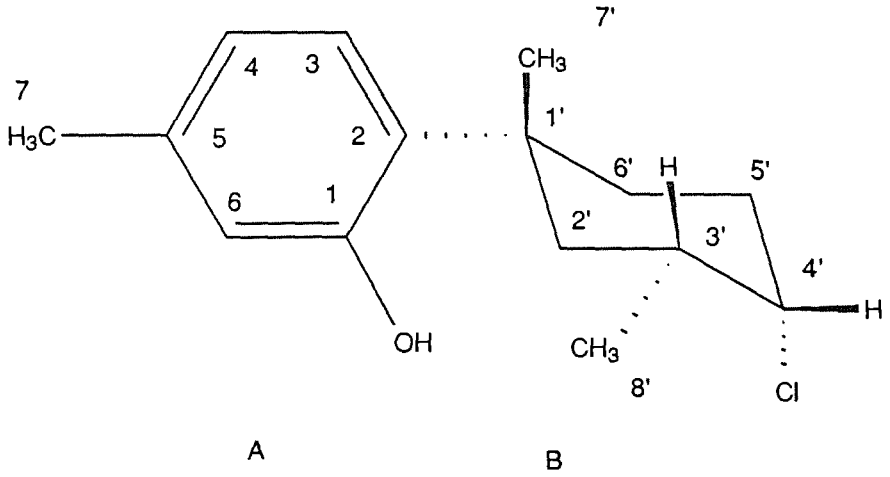
δ1.53 (0.9%) and δ6.98 (4.5%). Irradiation of the methyl signal δ1.24 (C_6D_6) caused an n.O.e. enhancement of the signals δ1.53 (2.4%) and δ1.80 (2.5%). Irradiation of the methylene proton signal δ1.53 (C_6D_6) caused an n.O.e. enhancement of the geminal proton signal δ1.15 (6.7%) and the methyl signal δ1.19 (1.8%). Finally irradiation of the methylene proton signal δ1.80 (C_6D_6) caused an n.O.e. enhancement of the geminal proton signal δ1.16 (8.31%) and the overlapping methyl signal δ1.24 and methylene signal δ1.26 (5.33%). These data confirm the relative stereochemistry of the molecule and confirm the $^3J_{H-H}$ couplings observed in the COSYDQF spectrum (Fig. 6).

(46) is therefore (1'*R**,3'*R**)-2-(3'-chloro-1',3'-dimethylcyclohexyl)-5-methyl-phenol.

The next more polar of the three novel compounds, (47), had the same molecular formula $C_{15}H_{21}ClO$ by HREIMS as (46). The compound had 5 double bond equivalents by ^{13}C n.m.r., and the presence of 6 sp^2 carbons (117.8 (d), 121.0 (d), 128.8 (d), 128.8 (s), 136.8 (s), 154.6 (s) ppm) suggested that the structure was similar to (46). The presence of two overlapping carbon signals at 128.8 ppm was demonstrated using a carbon off-resonance n.m.r. experiment. The presence of signals in the ^1H n.m.r. spectrum for three aromatic protons δ6.54 (s), 6.73 (d, *J* 7.4 Hz) and 7.12 (d, *J* 7.4 Hz) and absorbances in the i.r. spectrum again indicated the presence of a 1,2,4-trisubstituted benzene ring and suggested that the compounds differed only in the substitution pattern of the B ring. Substituents on the aromatic ring were a methyl group (^1H δ 2.30, (s)) a hydroxyl

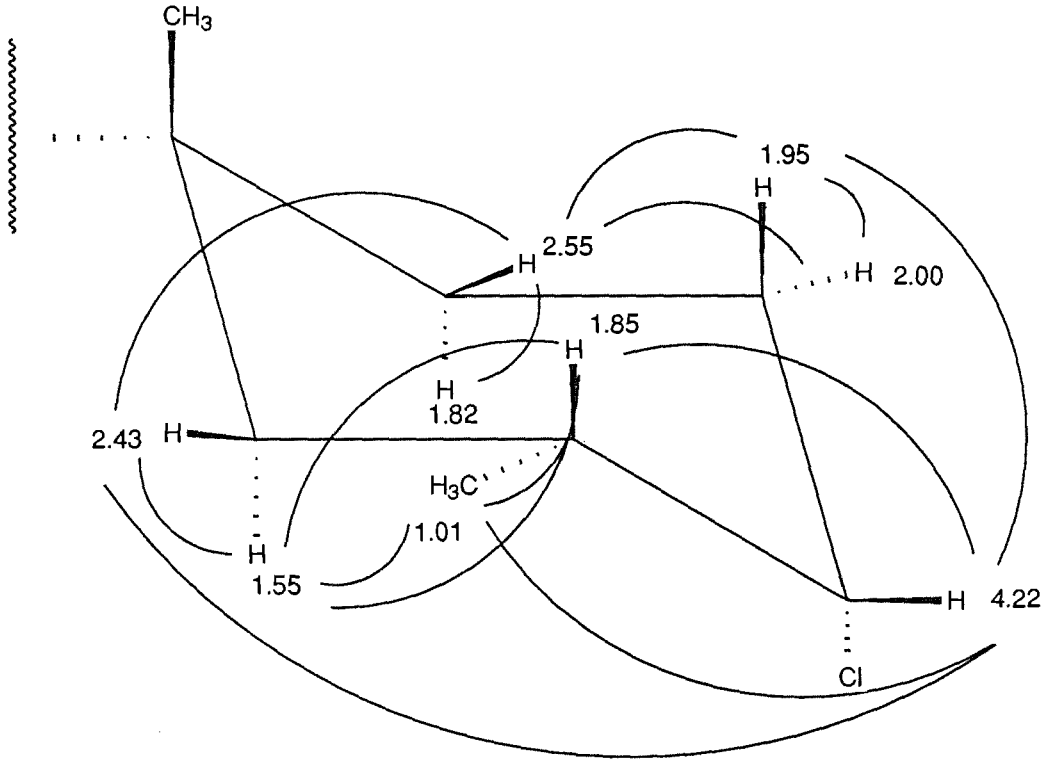
Table 10. ^{13}C and ^{1}H n.m.r. data for (47). Assignments are based on short range (*J* 135 Hz, XHCORRD), and long range (*J* **7 Hz, INAPT**; *J* 10Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
19.9 (q)	1.01 (d, *J* 6.6 Hz)	33.6, 38.5, 67.2	8'
20.5 (q)	2.30 (s)	117.8, 121.0, 136.8	7
30.2 (t)	1.82 (m)		6'
	2.55 (m)	**30.3, 32.0, 38.5, 38.9, 67.2, 128.8**	
30.3 (q)	1.32 (s)	30.2, 38.5, 128.8	7'
32.0 (t)	1.95 (m)	33.6, 38.9	5'
	2.00 (m)		
33.6 (d)	1.85 (ddd, *J* 6.6, 13.3, 14.1 Hz)		3'
38.5 (t)	1.55 (t, *J* 13.0, 13.3 Hz)	**38.9, 67.2, 128.8**	2'
	2.43 (brd, *J* 14.1 Hz)	**19.9, 30.3, 33.6, 38.9, 67.2, 128.8**	
38.9 (s)			1'
67.2 (d)	4.22 (brs)	**19.9, 30.2, 32.0, 33.6, 38.5**	4'
117.8 (d)	6.54 (bs)	20.5, 121.0	6
121.0 (d)	6.73 (bd, *J* 7.4 Hz)	117.8, 128.8	4
128.8 (s)			2
128.8 (d)	7.12 (d, *J* 7.4 Hz)	136.8, 154.6	3
136.8 (s)			5
154.6 (s)			1



A B

(47)



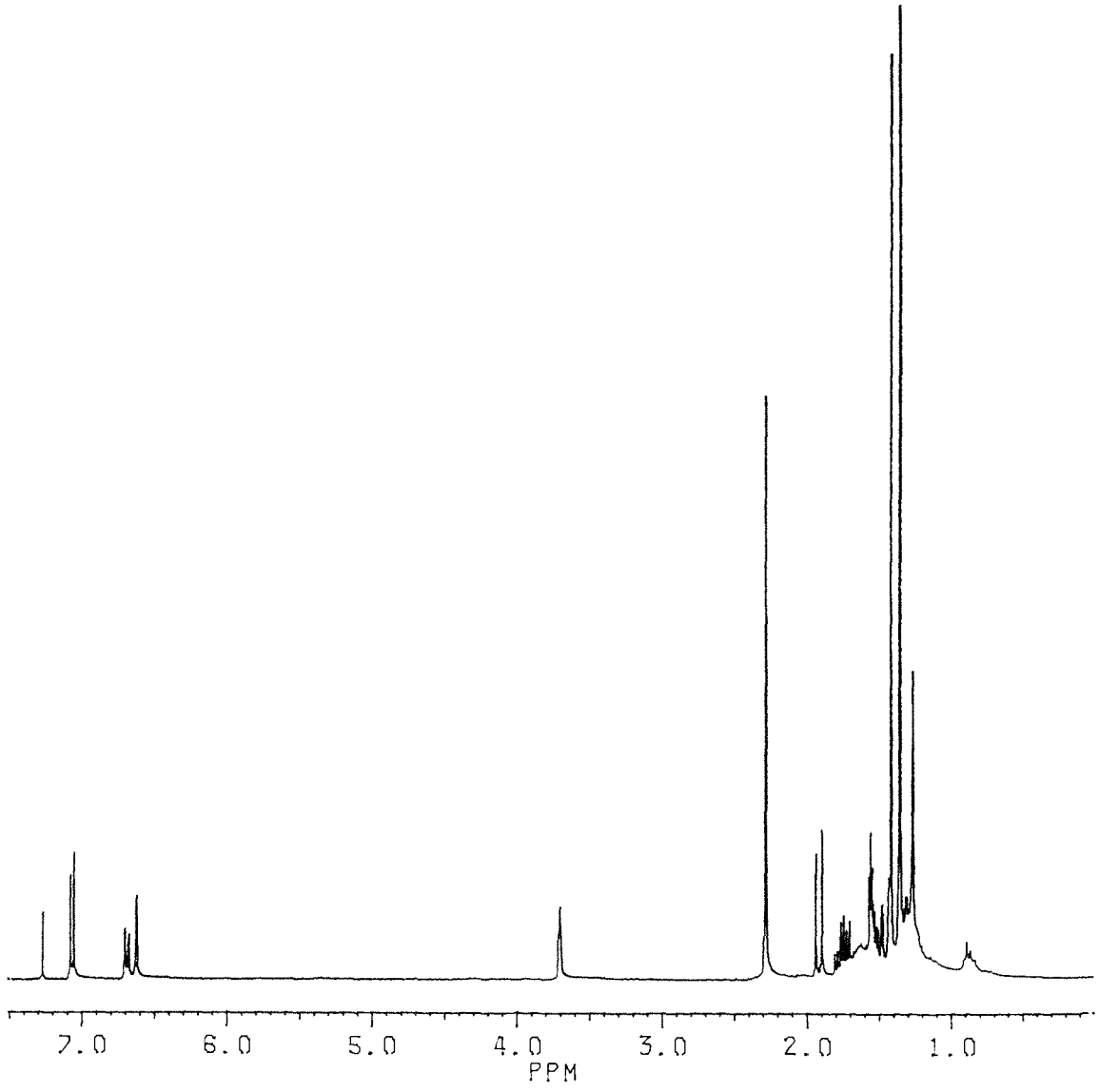
Crosspeaks from COSYDQF spectra of (47)

Figure 7.

group (^{13}C, 154.6 ppm, (s)) and the single attachment to the B ring of the molecule.

Proton double resonance experiments and a 2D homonuclear correlation (COSYDQF) experiment allowed the remaining fragment to be established. Irradiation at δ1.55 collapsed the signal at δ2.43 to a doublet and the signal at δ1.85. Similarly irradiation at δ1.85 collapsed the signals at δ1.55 and δ2.43 and the doublet at δ1.01, while irradiation at δ2.43 collapsed the signals at δ1.55 and δ1.85. Irradiation at δ1.01 collapsed the signal at δ1.85. Irradiation at δ1.82 collapsed the signal at δ2.55 while irradiation at δ2.55 effected the signals at δ2.00, δ1.95, and δ1.82. These correlations were confirmed by crosspeaks between these signals in the COSYDQF experiment. Further crosspeaks in this spectrum allowed the establishment of the remaining portion of the B ring (Fig. 7).

Unambiguous assignment of protons to their attached carbons was established using an XHCORRD experiment (J=135 Hz) (Table 10). Long range correlations using a heteronuclear 2D experiment (COLOC J=10 Hz) and selective INAPT experiments (J=7 Hz) confirmed the assignment of the carbon skeleton (Table 2).
The methyl signal δ1.01 (19.9 ppm) showed correlations to the carbon signals 19.9, 33.6, 38.5 and 67.2 ppm. The methyl signal δ1.32 (30.3 ppm) showed correlations to the carbon signals 30.2, 30.3, 38.5 and 128.8 ppm. The correlation between this methyl proton signal and the carbon signal at 128.8 ppm is confirmed by the long range correlations of the proton signals δ1.55, δ2.43 and δ2.55 with the same carbon signal (INAPT J= 7 Hz) (Table 10). All the remaining long range correlations further confirmed the proposed structure (Table 10).



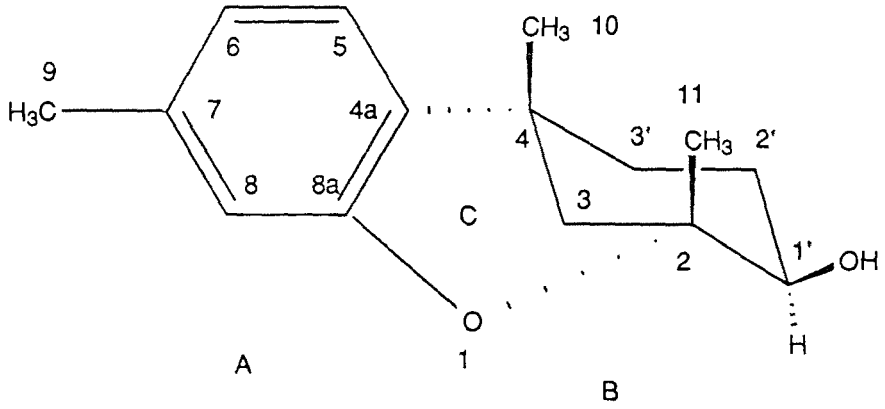
^{1}H n.m.r. spectrum of (48)

The relative stereochemistry of the molecule was determined using n.O.e. experiments and proton coupling data. Irradiation of the methyl signal δ1.01 (19.9 ppm) enhanced signals of the protons at δ1.55 (1.43 %), δ1.85 (2.83 %), δ2.43 (1.18 %) and δ4.22 (1.56 %), while irradiation of the methyl signal at δ1.32 (30.3 ppm) enhanced the signals at δ1.85 (1.56 %), δ2.43 (0.98 %) and δ2.55 (0.90 %). Similarly irradiation at δ4.22 caused enhancement of the signals at δ1.01 (1.76 %), 1.85 (6.85 %) and δ1.95 (3.73 %). This data establishes the relative configuration of the molecule (Fig. 7). (47) is therefore (1'*R**,3'*R**,4'*S**)-2-(4' chloro-1',3'-dimethylcyclo hexyl)-5-methyl-phenol.

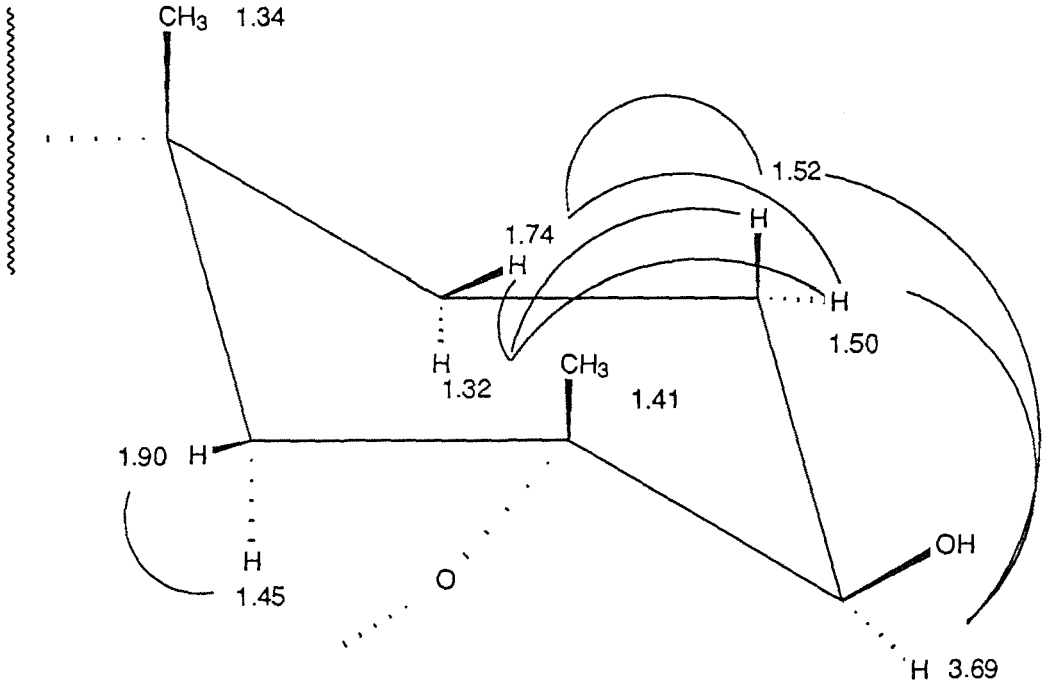
The most polar of the three compounds, (48), had the molecular formula $C_{15}H_{20}O_2$ by HREIMS. Due to the presence of only fourteen signals in the ^{13}C n.m.r. spectrum ($CDCl_3$), the sample was dissolved in C_6D_6 and the experiment repeated. This established the presence of the quaternary carbon 76.8 ppm. The compound thus had 6 double bond equivalents. The presence of six sp^2 carbons and three aromatic protons again suggested the presence of a 1,2,4-trisubstituted aromatic ring and thus the molecule must be tricyclic. Substitutents on the aromatic ring were a methyl group δ2.27 (21.0 ppm) and an oxygen function (^{13}C, 154.6 ppm, (s)).
A 2D homonuclear n.m.r. correlation (COSYDQF) experiment confirmed the presence of a 1,2,4-trisubstituted aromatic ring and allowed a major portion of the remaining fragment to be established (Fig. 8). The methylene proton signals δ1.50 and δ1.52 showed cross peaks to the proton signals δ1.32, δ1.74 and δ3.69. The methylene proton signal δ1.74 showed cross peaks to the proton signals δ1.32, δ1.50 and δ1.52. The methylene proton signal δ1.90

Table 11. ^{13}C and ^{1}H n.m.r. data for (48). Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (*J* **7 Hz or 9 Hz* INAPT**; *J* 10Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
21.0 (q)	2.27 (t, *J* 1.8 Hz)	**115.8, 120.6, 137.3**	9
25.7 (q)	1.41 (s)	**38.3, 72.2, 76.8**	11
26.7 (q)	1.34 (s)	**32.4, 34.8, 38.3, 126.1**	10
27.6 (t)	1.50 (m)		2'
	1.52 (m)		
32.4 (s)			4
34.8 (t)	1.32 (m)		3'
	1.74 (dt, *J* 2.5, 2.5, 13.8 Hz)	**32.4, 126.1**	
38.3 (t)	1.45 (bd, *J* 13.2 Hz)		3
	1.90 (d, *J* 13.2 Hz)	**32.4, 126.1**	
72.2 (d)	3.69 (brs)*	**25.7, 34.8, 38.3, 76.8**	1'
76.8 (s)			2
115.8 (d)	6.62 (s)		8
120.6 (d)	6.67 (dd, *J* 1.8, 7.8 Hz)	115.8	6
124.6 (d)	7.05 (d, *J* 7.8 Hz)	137.3, 154.6	5
126.1 (s)			4a
137.3 (s)			7
154.6 (s)			8a



(48)



Crosspeaks from COSYDQF spectra of (48)

Figure 8.

showed a cross peak to δ1.45 while the methine proton signal δ3.69 showed cross peaks to the proton signals δ1.50 and δ1.52.
Unambiguous assignment of protons to their attached carbons was established using an XHCORRD heteronuclear n.m.r. experiment (J=135 Hz) (Table 11).
Long range correlations using a 2D heteronuclear n.m.r. experiment (COLOC, J=10 Hz) and 1D selective INAPT experiments (J=7 Hz) were used to confirm the assignment of the carbon skeleton. The connectivities established from the aromatic protons and the aromatic methyl group further substantiate the assignment of the aromatic ring, while correlations from the aliphatic proton signals confirmed the assignment of the B ring (Table 11).
The methyl signal δ1.34 (26.7 ppm) showed correlations to the carbon signals 32.4, 34.8, 38.3 and 126.1 ppm. A residual signal to carbon 72.2 ppm was also observed, most probably from the overlapping methylene proton signal δ1.32. The methyl signal δ1.41 (25.7 ppm) showed correlations to the carbon signals 38.3, 72.2 and 76.8 ppm. The methylene proton signals δ1.74 (34.8 ppm) and δ1.90 (38.3 ppm) both showed correlations to the carbon signals 32.4 and 126.1 ppm. The methine proton signal δ3.69 (72.2 ppm) showed correlations to the carbons 25.7, 34.8, 38.3 and 76.8 ppm. These correlations confirm the assignment of the carbon positions in the B ring (Fig. 8) as a substituted cyclohexane as in (46) and (47). The sole exception was that of the connectivity between the oxygenated carbons 76.8 ppm in ring B and 154.6 ppm in the aromatic ring. The only possible connection between these two rings is an ether linkage establishing the C ring (Fig. 8). This is substantiated by the presence of a C-O-C absorption band (1056

cm^{-1}) in the i.r. spectrum and the HREIMS which requires an additional double bond equivalent.

The relative stereochemistry of the molecule was determined using n.O.e. experiments and proton coupling data. Irradiation of the methyl signal δ1.34 (26.7 ppm) enhanced signals at the protons δ1.52 (1.3%), δ1.74 (4.4%), δ1.90 (1.0%) and δ7.05 (4.1%). Irradiation at the methyl signal δ1.41 (25.7 ppm) enhanced the signals at δ1.90 (1.4 %) and δ3.69 (2.1%). Irradiation at δ1.74 (34.8 ppm) caused an enhancement of the signals δ1.32 (6.5%) and 1.34 (14.0%). Irradiation at δ1.90 caused enhancement of the signals δ1.34 (2.2%), 1.41 (4.4%) and1.45 (2.0%). Irradiation at δ3.69 caused an enhancement of the signals at δ1.41 (3.3%), δ1.50 (1.25%) and δ1.52 (4.4%). (48) is therefore (1'*R**,2*R**,4*R**)-2,4-(1'-hydroxypropano)-2,4,7-trimethylchroman.

Due to the novel nature of the carbon skeleton of these compounds further chemical studies were carried out to confirm the structure of these molecules.

The presence of free hydroxyl groups in the compounds was confirmed by the acetylation of (47) and (48). The presence of a methyl singlet δ2.32, the downfield shift of the aromatic protons, and no significant change in the shifts of the protons in the B ring in the ^1H n.m.r. spectrum of the acetate of (47) were consistent with the fact that (47) was a phenol. Similarly the presence of a methyl singlet δ2.10, a downfield shift of the proton signal δ3.69 to δ4.85 in the B ring, and the unchanged shifts of the aromatic proton shifts in the ^1H n.m.r. spectrum of the acetate of (48) were consistent with the proposed structure of (48) as as a tricyclic compound containing

an ether and a secondary alcohol functional group. The tertiary alcohol function as an alternative possibility to (48) was thus ruled out.

To further investigate the relationship between the three compounds, (47) was converted to (48). (47) underwent dehydrohalogenation when heated with KOH in MeOH under reflux to yield a single product (49).

(49) had the molecular formula $C_{15}H_{20}O$ by LREIMS and ^{13}C n.m.r.. The compound thus had 6 double bond equivalents and the presence of 8 sp^2 carbons, six of which were in an aromatic ring dictated that the compound must be bicyclic as expected. The presence of signals in the ^1H n.m.r. spectrum for three aromatic protons δ6.50 (s), δ6.68 (dd, J= 0.9, 7.6 Hz) and δ6.96 (d, J= 7.6 Hz) and an aromatic methyl group δ2.26 (s) confirms the presence of the 1,2,4-trisubstituted aromatic ring. Similarly the presence of a methyl signal δ1.74 (s) and a methine proton signal δ5.35 (bs) confirms the presence of a cycloalkene B ring.

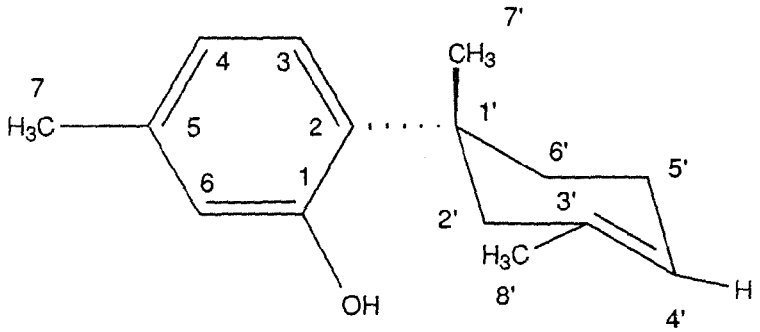
A 2D homonuclear correlation n.m.r. experiment (COSYDQF) and 1D (INAPT, J=7 Hz) and 2D (XHCORRD, J= 10 Hz, COLOC, J=10 Hz) heteronuclear correlation n.m.r. experiments allowed assignment of the proton and carbon signals in the molecule (Table 12, Fig. 9). This established the position of the double bond and the allylic methyl group in the B ring.

The relative stereochemistry of the molecule was established using n.O.e. experiments and proton coupling data.

Irradiation of the methyl signal δ1.37 (25.7 ppm) enhanced the signals δ2.05 (1.6%), δ2.14 (1.4%) and δ6.96 (1.1%). Irradiation of the

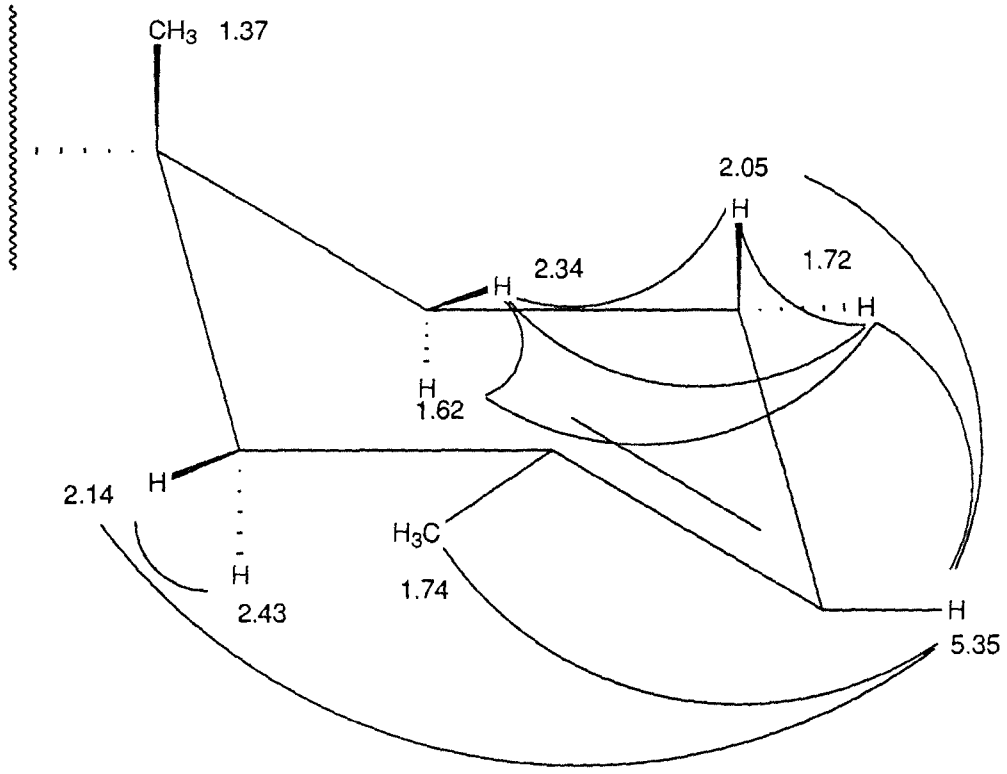
Table 12. ^{13}C and ^{1}H n.m.r. data for (49). Assignments are based on short range (J 135 Hz, XHCORRD) and long range (J **7 Hz, INAPT**; J 10Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
20.5 (q)	2.26 (s)	117.4, 121.0, 136.8	7
23.5 (t)	1.72 (m)		5'
	2.05 (m)		
23.7 (q)	1.74 (s)	**42.4, 120.6, 132.6**	8'
25.7 (q)	1.37 (s)	32.2, 36.7, 42.4, 130.9	7'
32.2 (t)	1.62 (m)		6'
	2.34 (m)		
36.7 (s)			2'
42.4 (t)	2.14 (bd, J 17.0 Hz)		6'
	2.43 (bd, J 17.0 Hz)		
117.4 (d)	6.50 (s)	121.0	6
120.6 (d)	5.35 (bs)	**23.5, 23.7, 32.2, 42.4**	4'
121.0 (d)	6.68 (dd, J 0.9, 7.7 Hz)		4
127.8 (d)	6.96 (d, J 7.7 Hz)	136.8, 154.0	3
130.9 (s)			2
132.6 (s)			3'
136.8 (s)			5
154.0 (s)			1



A B

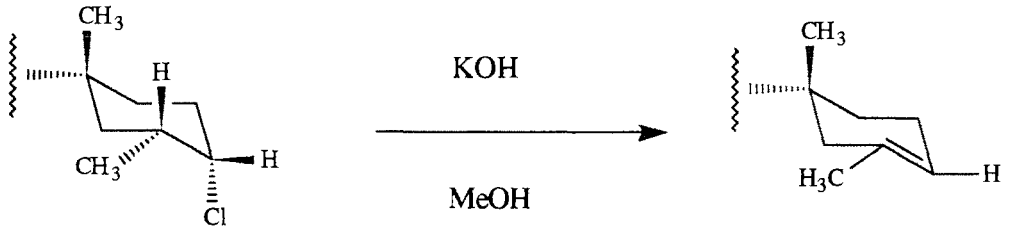
(49)



Crosspeaks from COSYDQF spectra of (49)

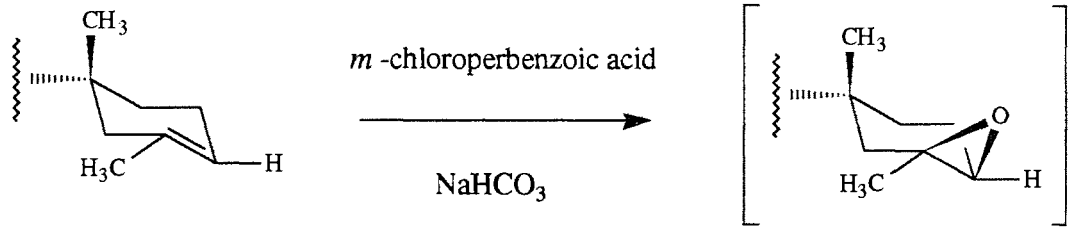
Figure 9.

1.) dehydrohalogenation: 1,2-elimination

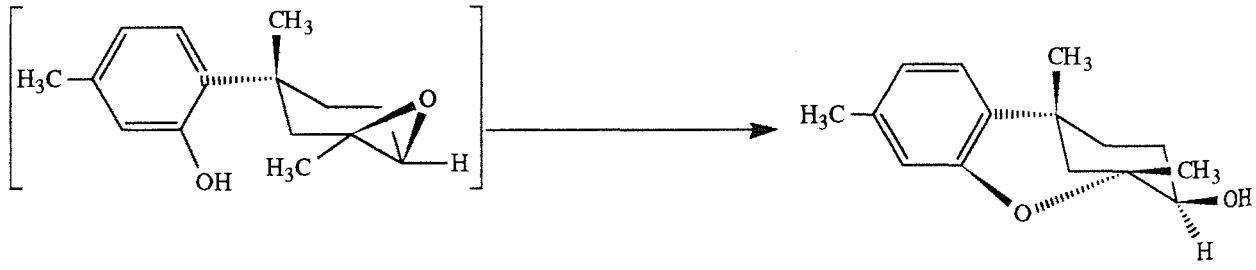


(47) (49)

2.) epoxidation



base catalysed cleavage



(48)

Figure 10

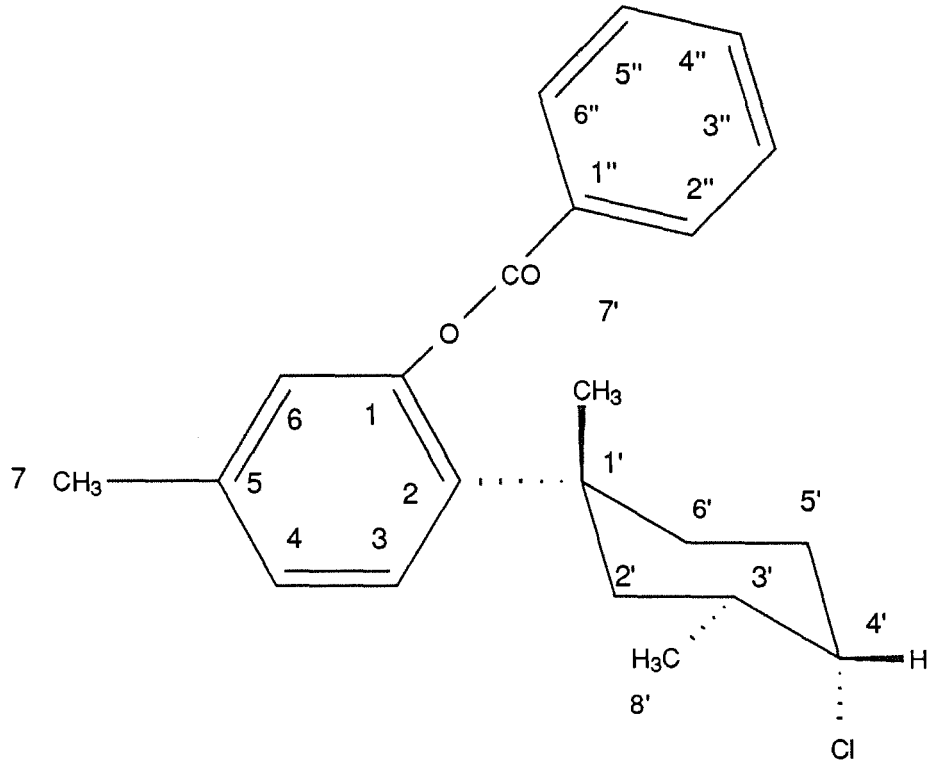
methyl signal δ1.74 enhanced the signals δ2.14 (6.1%), δ2.43 (3.3%) and δ5.35 (3.9%). Irradiation of the methylene proton signal δ2.43 (42.4 ppm) enhanced the signals δ1.62 (9.1%), δ1.74 (3.7%) and δ2.14 (9.1%). Irradiation of the methine proton signal δ5.35 (120.6 ppm) caused an enhancement of the signal δ1.74 (5.3%). This data establishes the relative stereochemistry of the molecule (Fig. 9). (49) is therefore (1'*R**)-2-(1',3'-dimethyl-3'-cyclohexenyl)-5-methyl-phenol.

Epoxidation of (49) was then carried out using *m*-chloroperbenzoic acid (*m*CPBA) in the presence of $NaHCO_3$. This reaction yielded a single product, the previously isolated (48), confirming the relationship between the two metabolites (Fig. 10).

Due to the difficulty of obtaining a suitable crystalline sample of any of the three isolated metabolites, (47) was reacted with benzoyl chloride to obtain a crystalline product (50) which was recrystallised from light petroleum ether and has subsequently been submitted for analysis by X-ray crytallography. (50) was characterised by extensive 1D and 2D ^{1}H and ^{13}C n.m.r.. This resulted in the assignment of all proton and carbon resonances in agreement with the expected product as (1'*R**,3*R**,4'*S**)-2-(4'-chloro-1',3'-dimethyl-cyclohexyl)-5-methyl-phenyl-benzoate (Table 13).

Table 13. ^{13}C and ^{1}H n.m.r. data for (50). Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (*J* 7Hz, INAPT) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
19.8 (q)	0.89 (d)		
20.5 (q)	2.34 (s)	125.4, 126.9, 137.3	7
31.1 (t)	1.83 (m)		6'
	2.28 (ddd, *J* 2.6, 2.7, 13.7 Hz)		
31.6 (q)	1.26 (s)	31.1, 39.1, 39.3, 134.0	7'
31.9 (t)	1.94 (m)		5'
	1.95 (m)		
33.7 (d)	1.84 (m)		3'
39.1 (t)	1.56 (dd, *J* 12.6, 13.8 Hz)		2'
	2.11 (bd, *J* 13.8 Hz)		
39.3 (s)			1'
66.7 (d)	4.20 (brs)		4'
125.4 (d)	6.90 (s)	20.5, 126.9, 134.1, 149.5	6
126.9 (d)	7.05 (d, *J* 8.2 Hz)		4
128.7 (d)	7.57 (dd, *J* 7.7, 8.0 Hz)	130.2	2''
128.7 (d)	7.57 (dd, *J* 7.7, 8.0 Hz)		2''
128.8 (d)	7.27 (d, *J* 8.2 Hz)		3
130.2 (d)	8.20 (d, *J* 8.0 Hz)	130.2, 133.7, 165.5	3''
130.2 (d)	8.20 (d, *J* 8.0 Hz)		3''
131.7 (s)			1''
133.7 (d)	7.67 (dd, *J* 7.7, 7.7 Hz)		4''
134.1 (s)			2
137.3 (s)			5
149.5 (s)			1
165.5 (s)			7''



(50)

The genus *Laurencia* has been reported to produce twenty-three sesquiterpenoid skeletons (Erickson 1983). Of these only the cuparane, laurane and cyclolaurane skeletal types consist of a non-fused bicyclic ring system possessing an aromatic ring, but none contain metabolites similar to those isolated. A survey of the relevant literature on marine natural products (Erickson 1983, Faulkner 1977, 1984, 1986, 1987, 1988, 1990) and terrestrial and marine sesquiterpeniods (Roberts and Bryson 1984, Fraga 1985, 1986, 1987, 1988, 1990a, b) also failed to identify a cyclohexylphenol ring system isolated as a natural product. A further computer assisted search of Chemical Abstracts (CAS on-line) identified a number of substituted cyclohexylphenols as products of chemical syntheses (e.g. Burnell *et al.* 1984, 1987) but none as natural products. It therefore appears that the isolated compounds constitute a regular isoprenoid sesquiterpene carbon skeleton not previous isolated as a natural product.

The presence of a new skeletal type from an algal species which has not only been investigated in the Great Barrier Reef environs (Wright *et al.* 1990) but through out a large part of its range of distribution (Erickson 1983, Caccamesse *et al.* 1986), illustrates that the further investigation of previously investigated algae may be of interest from the perspective of natural products chemistry.

2.2. *Dasyphila plumarioides* HR891I

The algae *Dasyphila plumarioides* was collected as part of a collaborative Russian-Australian investigation of the pharmacological activity of tropical marine organisms from the greater Great Barrier Reef area. The collection was carried out on the Russian research vessel, R.V. Akademik Oparin and spanned the region of the coral sea from Flinder's Reef in the south to Osprey Reef in the north. Pharmacological screening of samples was carried out on-board. The privilege of taking part in this expedition allowed the survey and collection of a number of algal species not previously reported from this area and a phytochemical study of some of the species collected.

D. plumarioides was collected from Holmes Reef (Lat 147° 52', Long 16° 30') in the Coral Sea at a depth of 10 metres. The alga occurred in mono-specific patches in crevices and on cave walls and shaded areas. A subsample of the alga was taken on collection for pharmacological screening while the remainder of the sample was frozen for later analysis. Pharmacological screening of the alga showed no activity in any of the assays performed. Due to the abundance of *D. plumarioides* in the collection area, the natural product chemistry of the alga was investigated. The sample was freeze dried and exhaustively extracted in DCM followed by MeOH to yield a crude extract. Rapid chromatography of the extract on a silica gel column using mixtures of light petroleum and ethyl acetate as eluents afforded 21 fractions. HPLC of selected fractions yielded the previously reported compound (51), the previously unreported but related vinyl acetylene (52), and the novel sesquiterpene (53).

The least polar of the three compounds had identical ^{13}C and similar ^{1}H n.m.r. spectral data identical to those of the previously reported compound chlorofucin (51) (Howard *et al.* 1980) isolated from the Californian *Laurencia* species *Laurencia snyderae* . Chlorofucin has been shown to reduce grazing by the herbivorous fish *Siganus doliatus* (rabbitfish) even though chlorofucin and *S. doliatus* have not been shown to co-occur previously (Hay *et al.* 1989a).

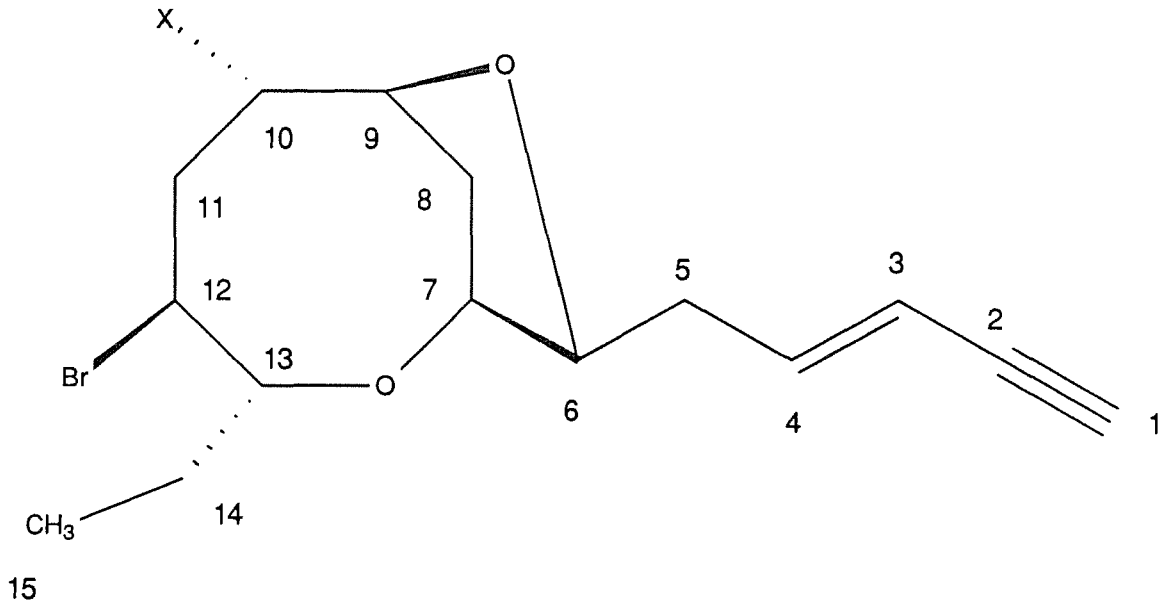
The absolute configuration of chlorofucin has been determined by X-ray crystallography (Howard *et al.* 1980). The reassignment of two carbon signals and the assignment of four carbon signals previously assigned as interchangeable was made based on high field 2D heteronuclear n.m.r. experiments used to elucidate the structure of chlorofucin (51). This study also suggests that a similar reassignment of carbon resonances be made to the related compound bromofucin (54) (Coll and Wright 1989b). Chlorofucin had identical ^{13}C and similar ^{1}H n.m.r. spectral data to those previously reported. A homonuclear 2D experiment (COSYDQF) allowed all fifteen carbon atoms to be linked, while a short range 2D heteronuclear experiment (XHCORRD, J=135 Hz) associated signals for all protons with the signals for the carbons to which they are attached (Table 14). This resulted in the reassignment of the carbon signals C5 and C11 as 32.5 ppm and 38.3 ppm respectively and the assignment of the carbon signals C6 (84.0), C7 (69.7), C9 (79.2) and C13 (83.0) previously assigned as interchangeable (Howard *et al.* 1980).

Due to the spectral similarity between chlorofucin and bromofucin, it was assumed that all assignments and stereochemical features of bromofucin were the same as those of chlorofucin, with the

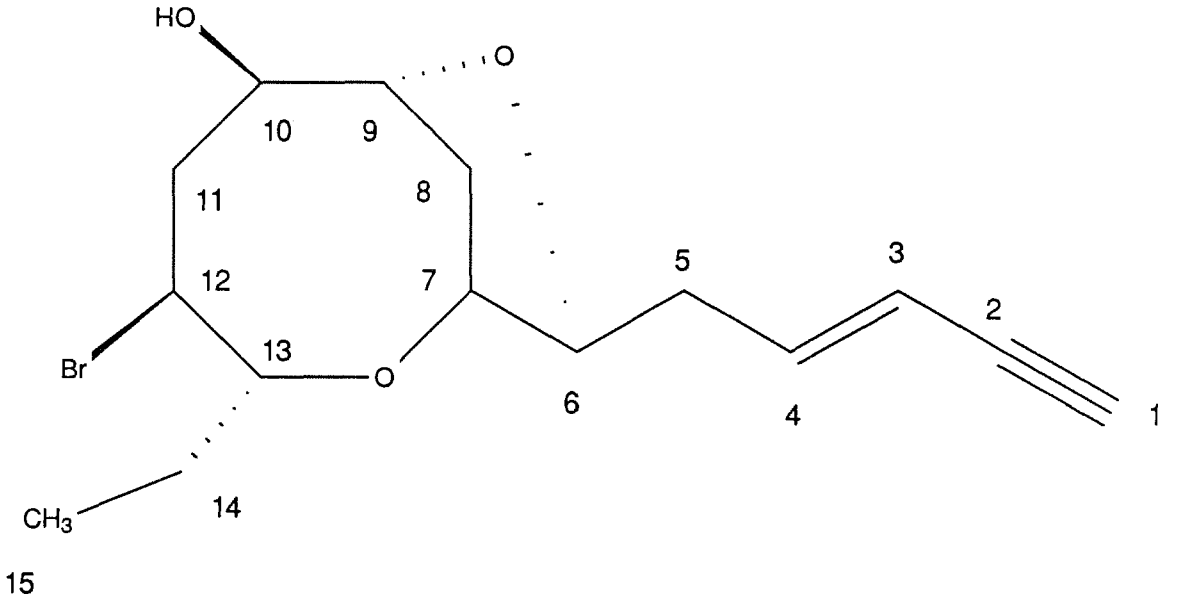
Table 14. ^{13}C and ^{1}H n.m.r. data for chlorofucin (51), bromofucin (54)** and (52). Assignments are based on short range (*J* 135 Hz, XHCORRD) correlations.

Carbon	chlorofucin (51)		bromofucin (54)**		compound (52)	
	^{13}C (ppm)	$^{1}H(\delta)$ *J* (Hz)	^{13}C (ppm)	^{1}H (δ) *J* (Hz)	^{13}C (ppm)	^{1}H (δ) *J* (Hz)
C15	11.7 (q)	1.05 (t, 7.4)	11.8 (q)	1.06 (t, 7.4)	11.8 (q)	1.05 (t, 7.4)
C14	22.9 (t)	1.65 (ddq, 7.4, 11.7, 14.7)	23.0 (t)	1.68 (ddq, 7.4, 11.7, 14.7)	23.1 (t)	1.65 (ddq, 7.4, 11.7, 14.7)
		2.00 (ddq, 2.6, 7.4, 14.7)		1.99 (ddq, 2.6, 7.4, 14.7)		2.00 (ddq, 2.6, 7.4, 14.7)
C13	83.0# (d)	3.89 (ddd, 2.6, 9.7, 11.7)	82.8# (d)	3.89 (ddd, 2.6, 9.7, 11.7)	83.2*(d)	3.90 (ddd, 2.6, 9.7, 11.7)
C12	51.4 (d)	4.50 (t, 9.7, 9.7)	52.7 (d)	4.50 (dd, 9.0. 9.7)	51.8 (d)	4.43 (t, 9.6, 9.6)
C11	38.3# (t)	2.50 (m)	38.5# (t)	2.53 (m)	33.6 (t)	2.50 (m)
		3.27 (ddd, 2.3, 9.0, 15.8)		3.34 (ddd, 2.3, 9.0, 15.8)		2.90 (ddd, 2.3, 9.0, 15.8)
C10	61.0 (d)	4.20 (brs)	55.2 (d)	4.33 (brs)	79.4 (d)	3.45 (brs)
C9	79.2 (d)	4.28 (dd, 3.5, 9.5)	79.6 (d)	4.40 (dd, 3.5, 9.5)	76.4 (d)	4.20 (dd, 3.5, 9.5)
C8	33.2 (t)	2.05 (ddd, 3.4, 9.5, 15.6)	33.5 (t)	2.06 (ddd, 3.4, 9.5, 15.6)	33.2 (t)	1.95 (ddd, 3.4, 9.5, 15.6)
		2.35 (d, 15.6)		2.36 (d, 15.6)		2.28 (d, 15.6)
C7	69.7# (d)	3.99 (brs)	69.8# (d)	4.00 (brs)	70.0 (d)	3.99 (brs)
C6	84.0 (d)	3.75 (ddd, 1.7, 7.1, 7.2)	84.3 (d)	3.74 (ddd, 1.7, 7.1, 7.2)	83.3* (d)	3.68 (ddd, 1.7, 7.1, 7.2)
C5	32.5# (t)	2.55 (m)	32.5# (t)	2.53 (m)	32.5 (t)	2.50 (m)
		2.58 (m)		2.53 (m)		2.50 (m)
C4	141.8 (d)	6.20 (ddd, 7.4, 7.9, 15.5)	141.8 (d)	6.20 (ddd, 7.4, 7.9, 16.0)	141.2 (d)	6.22 (ddd, 7.5, 7.5, 15.5)
C3	111.3 (d)	5.58 (ddd, 2.0, 3.5, 15.5)	111.4 (d)	5.58 (ddd, 2.0, 3.5, 16.0)	111.3 (d)	5.58 (ddd, 2.0, 3.5, 15.5)
C2	82.2 (s)		82.1 (s)		82.8 (s)	
C1	76.4 (d)	2.80 (d, 2.0)	76.4 (d)	2.81 (d, 2.0)	76.4 (d)	2.80 (d, 2.0)

resonances which have been reassigned or confirmed.
* assignments which are reversable.
** Coll and Wright 1989b



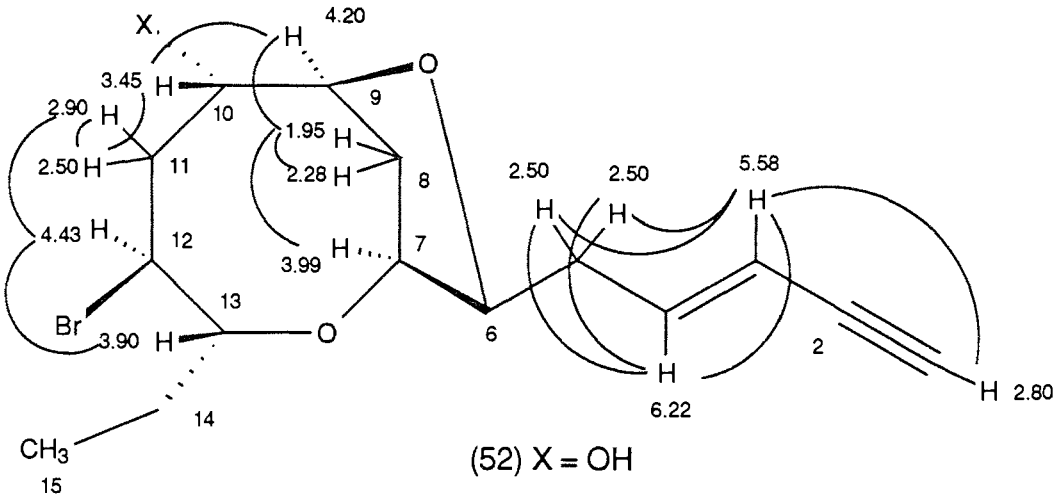
(51) X = Cl, chlorofucin
(54) X = Br, bromofucin
(52) X= OH



(55) X=OH, laurefucin

exception of the resonance of the carbon position C10 where bromine is substituted for chlorine [61.2 in (51) against 55.2 in (54)] (Coll and Wright 1989b) (Table 14). It is therefore suggested that a similar change in the assignment of carbon resonances of bromofucin be made (54).

The most polar compound (52) had very similar ^{1}H and ^{13}C n.m.r. spectral data to that of chlorofucin and bromofucin (Table 14). Comparison of ^{1}H and ^{13}C n.m.r. data of (52) with those of chlorofucin revealed that the methine proton signals at δ4.20 (C10), δ3.27 (C11) and δ4.28 (C9) were deshielded to δ3.45, δ2.90 and δ4.20 respectively (Table 14). There were no other significant differences in the remaining proton signals and the coupling constants for all proton signals were virtually identical to those of chlorofucin and bromofucin. The carbon spectrum had similar resonances for all carbons with the exception of the carbon signals 79.4, 76.4 and 33.6 ppm in (52) which occurred at 61.0, 79.2 and 38.3 ppm in (51, Cl) and 55.2, 79.6 and 38.5 ppm in (54, Br) (Table 14). These altered chemical shifts are consistent with the presence of a hydroxyl group at the C10 position of the molecule, this position being substituted by chlorine in chlorofucin (51) and bromine for bromofucin (54). This was confirmed by a molecular formula $C_{15}H_{21}O_3Br$ as determined by HREIMS. A 2D heteronuclear n.m.r. (COSYDQF) experiment allowed two major fragments of the molecule to be established. Crosspeaks between proton signals are shown in Fig. 11. Due to the paucity of sample an inverse heteronuclear 2D short range (HMQC J=135 Hz) experiment was used to unambiguously assign some proton signals to the carbons to which they were attached. This was particularly useful in assigning proton



(52) X = OH

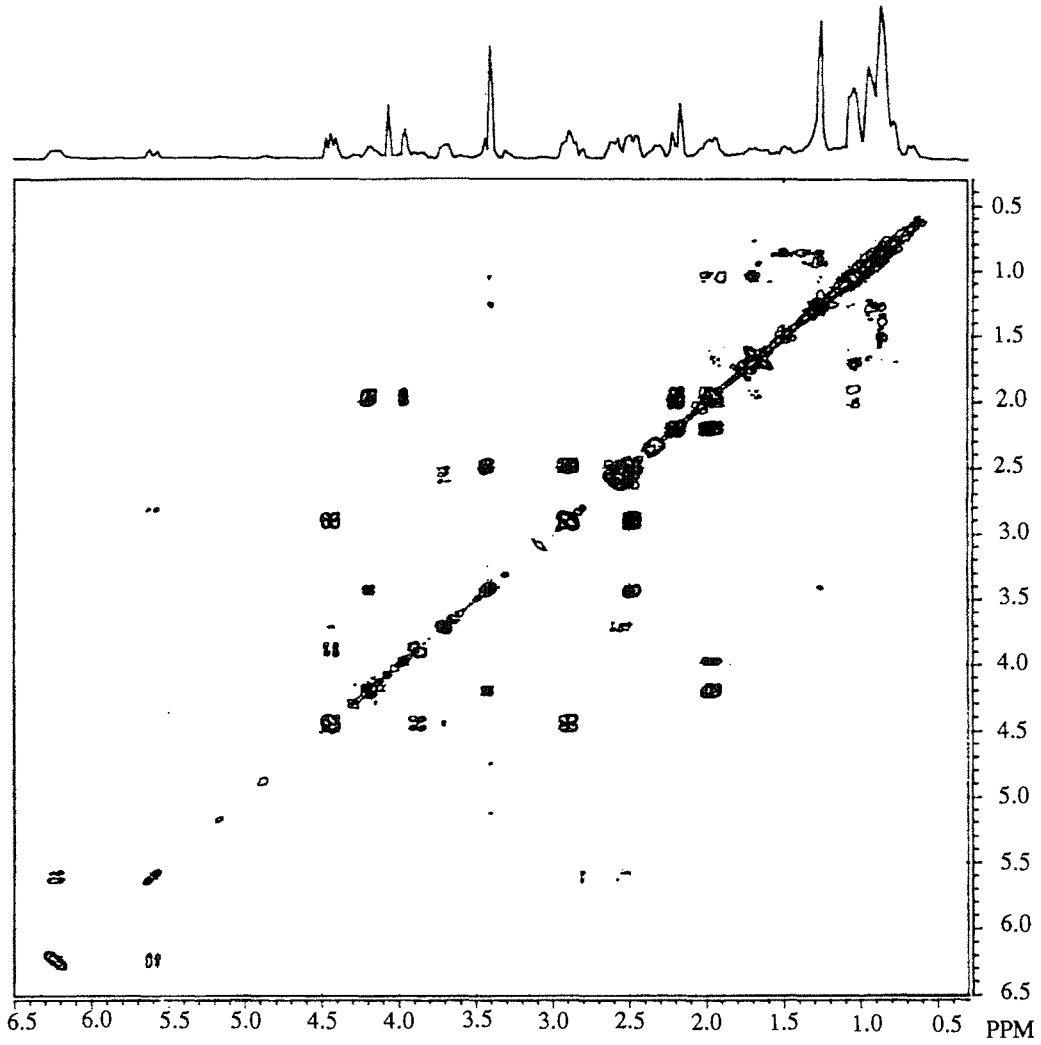


Figure 11

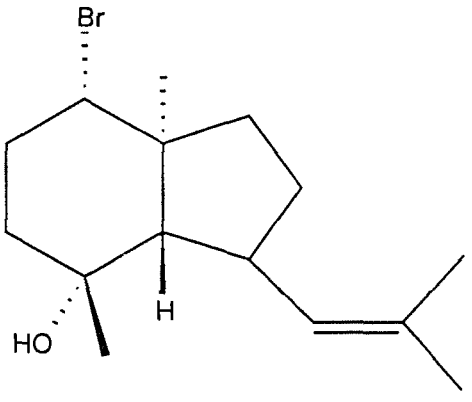
COSYDQF spectrum of (52)

signals to their attached carbons in the 50 - 90 ppm section of the carbon spectrum allowing the assignment of the carbon signals C7 (70.0 ppm), C9 (76.4 ppm), C10 (79.4 ppm), C12 (51.8 ppm) and C13 (83.2 or 83.3 ppm). The remaining signals were assigned based on the assignments of chlorofucin and bromofucin (Table 14). The relative stereochemistry of the molecule is assumed to be the same as that of chlorofucin and bromofucin due to the remarkable similarity between the proton spectra and coupling constants of the three compounds. (52) is therefore (3*E*,6*S*,7*S*,9*S*,10*S*,12*S*,13*R*)-10-hydroxy-12-bromo-6,9;7,13-bisepoxypentadec-3-en-1-yne. This molecule has a similar structure to that of laurefucin (55) (Fukuzawa *et al.* 1972, 1990; Furusaki *et al.* 1973) with the exception of the configuration of the hydroxyl group at C10 and the epoxide carbons at positions C6, C7, and C9. Based on the X-ray crystallography data for (55) (Furusaki *et al.* 1973) and the similar nature of the proton signals in the ^{1}H n.m.r. spectrum of (51), (52) and (54), the configuration of the hydroxyl group (C10) is designated as *S* in (52) as opposed to *R* in laurefucin (55). Similarly C6, C7 and C9 are all designated *S* in (52) as opposed to *R* in laurefucin (55).

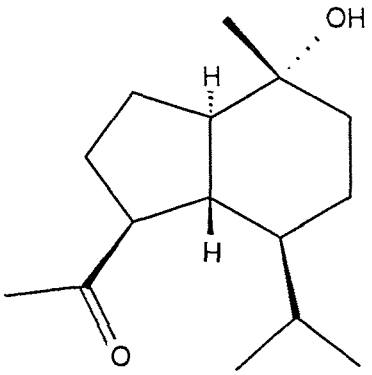
The compound with intermediate polarity, (53), had a partial molecular formula $C_{15}H_{25}$ as indicated by ^{13}C n.m.r. The ^{13}C n.m.r. spectrum for (53) contained carbon resonances for two sp^2 carbons (121.0 (d), and 134.5 (s) ppm) and one quaternary oxygenated carbon (84.0 ppm (s)). Assuming this to be correct, the compound should have a molecular formula $C_{15}H_{26}O$, with three double bond equivalents and thus must be bicyclic. The ^{1}H n.m.r. spectrum contained resonances for four methyl groups, four methylene

Table 15. ^{13}C and ^{1}H n.m.r. data for (53). Assignments are based on short range (J 135 Hz, XHCORRD) and long range (***J*=7Hz, INAPT** ; J=10 Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
18.4 (q)	1.60 (d, J 1.2 Hz)	26.3, 121.0, 134.5	4'
19.1 (q)	0.96 (d, J 6.5 Hz)	**30.2, 34.9, 49.2**	5'
19.5 (q)	0.76 (d, J 6.9 Hz)	**29.4, 30.3, 47.6**	6'
24.0 (t)	1.26 (m)		7
	1.63 (m)		
26.3 (q)	1.73 (d, J 1.2 Hz)	18.4, 121.0, 134.5	3'
29.4 (t)	1.22 (m)		6
	1.45 (m)		
30.2 (t)	1.12 (m)		2
	1.95 (m)		
30.3 (d)	2.06 (m)		5
34.7 (t)	1.36 (m)		3
	1.60 (m)		
34.9 (d)	1.75 (m)		1
47.6 (d)	2.52 (dd, J 4.4, 10.6 Hz)	**29.4, 30.3, 49.2, 84.0, 121.0,134.5**	4
49.2 (d)	1.05 (dd, J 4.0, 11.0 Hz)		7a
84.0 (s)			3a
121.0 (d)	5.06 (ddd, J 1.2, 1.2, 10.6 Hz)		1'
134.5 (s)			2'

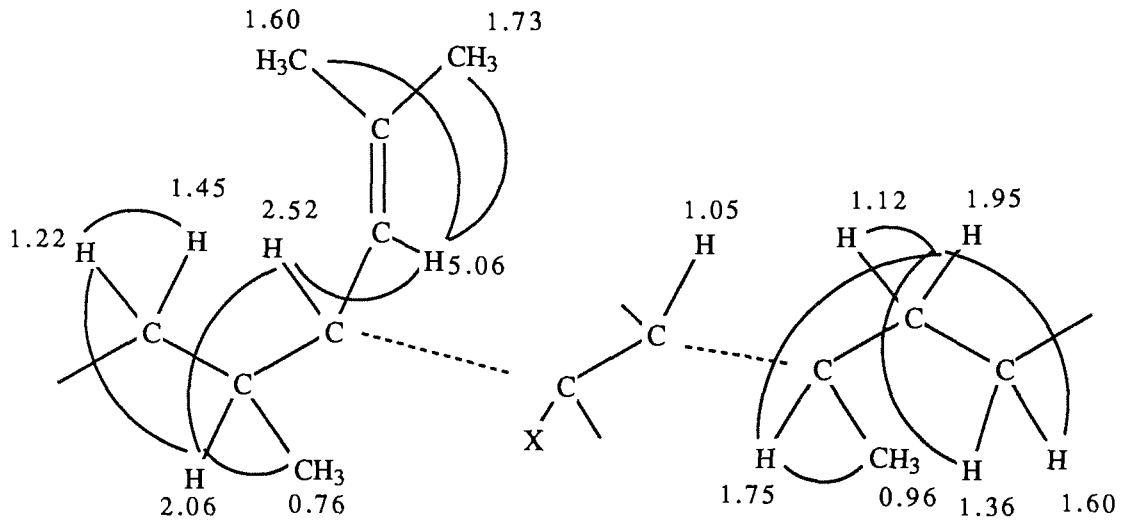


oppositol (38)



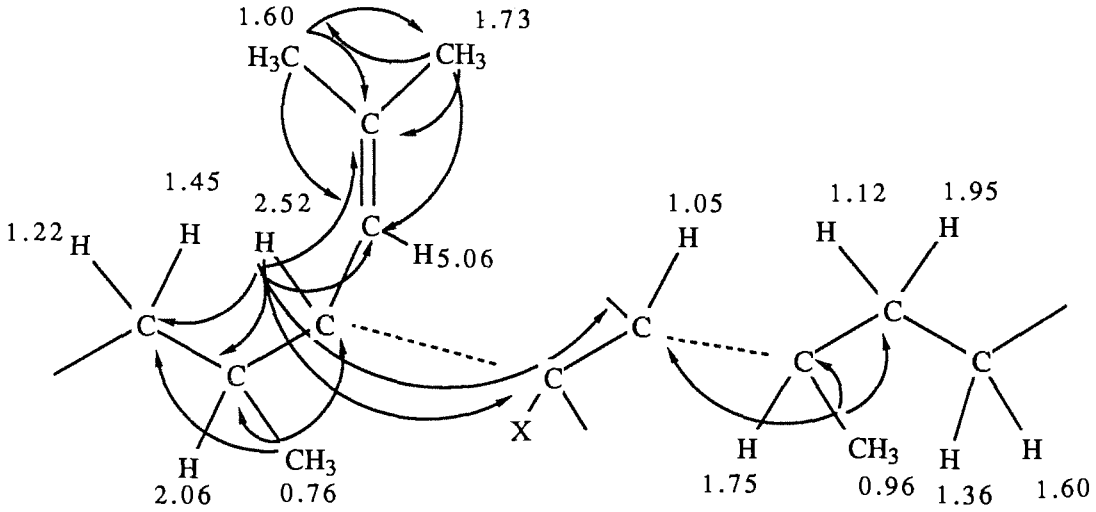
oplopanone (39)

groups and five methine groups (Table 15). The two most downfield methyl signals δ1.60 (d, *J* 1.2 Hz) and δ1.73 (d, *J* 1.2 Hz) were adjacent to the quaternary carbon signal 134.5 (s) ppm dictating the presence of a 2-methyl-1-propenyl unit. The nine remaining carbon signals thus formed a bicyclic ring system. The only possible structure fitting this data is that of a substituted indene ring system (fused five-six membered ring) which is represented by the known metabolite (37) and the previously reported compounds oppositol (38) and oplopanone (39). Oppositol (38) contains a similar 2-methyl-1-propenyl unit. The elucidation of the ring system and the assignment of resonances was carried out using 2D n.m.r. experiments. A short range (D2=0.3 μs) and long range (D2=0.2 s) COSYDQF experiment and selective proton decoupling experiments were used to establish two fragments of the molecule. However, due to the overlapping of a large number of signals in the ^1H n.m.r. spectrum, the complete structure was not able to be established. The cross peaks established by the COSYDQF experiments are shown in Fig. 12. These correlations are in agreement with data obtained from individual decoupling experiments (see experimental). In a further attempt to establish the remaining fragment, (53) was dissolved in C_6D_6 and the experiment repeated without any improvement in the separation of the proton signals. Similarly a relayed coherrence transfer experiment (RCT) failed to provide any further information. In a further attempt to resolve the overlapping proton signals $Eu(fod)_3$ was added to (53). Although causing a downfield shift of some proton signals, no significant improvement in the resolution of proton signals was obtained prior to a substantial decrease in signal resolution. Unambiguous assignment of protons to their attached carbons was established using an



Fragment 1 Fragment 2

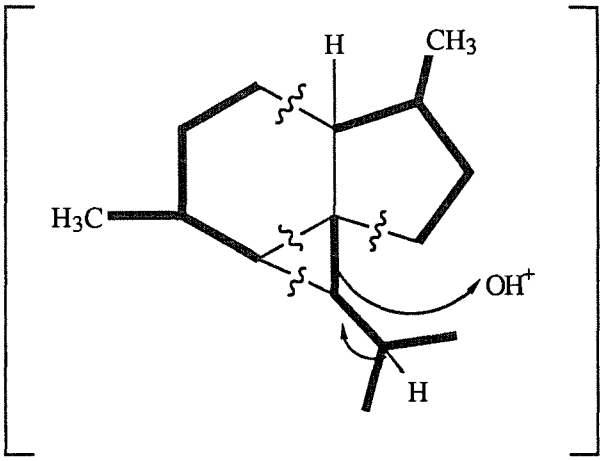
Figure 12. Crosspeaks from COSYDQF (D2=0.3 μs) spectra of (53)



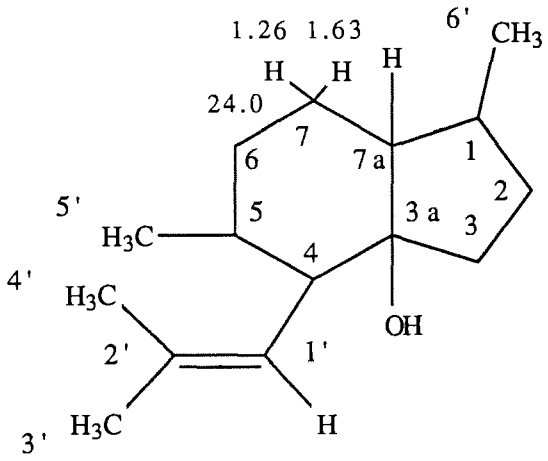
Fragment 1 Fragment 2

Figure 13. Long range correlations from COLOC (J=10 Hz) and INAPT (J=7 Hz) experiments

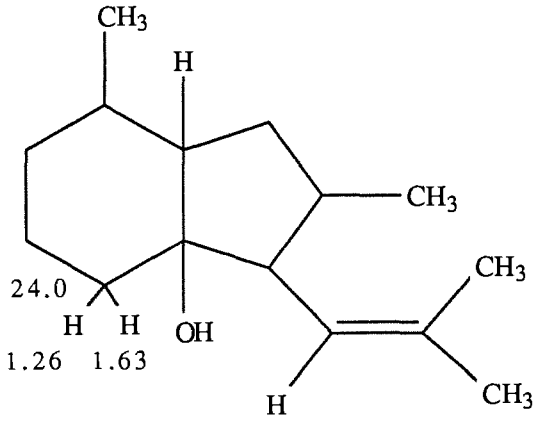
XHCORRD experiment (J=135 Hz) (Table 15). Long range correlations using a 2D heteronuclear experiments (COLOC, J=10 Hz) and selective 1D heteronuclear experiments (INAPT, J=7 Hz) were used to confirm the assignment of parts of the carbon skeleton (Table 15). The methyl signals δ1.60 (18.4 ppm) and δ1.73 (26.3 ppm) showed correlations to the carbon signals 18.4, 26.3, 121.0 and 134.5 ppm confirming the presence of a 2-methyl-1-propenyl unit. The methyl signal δ0.76 (19.5 ppm) showed correlations to the carbon signals 19.5, 29.4, 30.3 and 47.6 ppm. The methyl signal δ0.96 (19.1 ppm) showed correlations to the carbon signals 19.1, 30.2, 34.9 and 49.2 ppm while the methine signal δ2.52 (47.6 ppm) showed correlations to the carbon signals 29.4, 30.3, 49.2, 84.0, 121.0 and 134.5. These correlations established the connectivity between fragments 1 and 2 (Figs 12 and 13). The compound decomposed before further spectroscopic data could be obtained resulting in only tentative identification of the compound based on n.m.r. data. Due to our inability to unambiguously assign the position of the carbon signal at 24.0 ppm, there are two possible structures which fit the data either (53) or (53a). There does not appear to be a regular isoprenoid precursor which suggests a biosynthetic origin for (53a). However, rearrangement of the regular isoprenoid structure (53b) does suggest a possible biosynthetic origin for (53). Based on these considerations, (53) appears to be the more probable structure. (53) is therefore tenatively identified as 4-(2'-methyl-1'-propenyl)-1,5-dimethyl-octahydro-1*H*-inden-3a-ol.



(53b), possible precursor of (53)



(53)

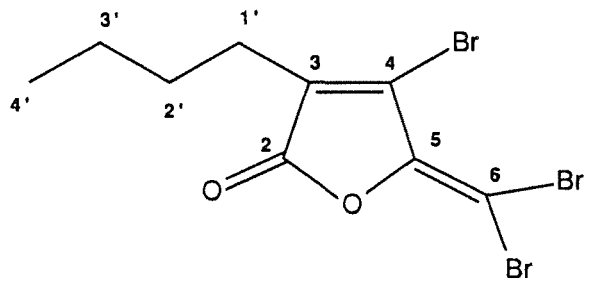


(53a)

2.3. *Delisea pulchra* SY891A

The secondary metabolite content of a collection of *Delisea pulchra* (Greville) Montagne, from the University of Sydney Marine Research Area, Cape Banks, Sydney, N.S.W. was investigated. *Delisea pulchra* (also previously reported as *Delisea fimbriata*, see Miller 1990 for a review of taxonomy) has previously afforded a number of secondary metabolites from collections covering a wide geographic range (Kazlaukas *et al.* 1977, Sydney, Rose *et al.* 1977, Antarctic peninsula, Pettus *et al.* 1977, Antarctic peninsula). Our re-investigation of this alga was motivated by observations that it was not preyed upon by herbivores in the area and that it remained free of the normal fouling epiphytes. Crude extracts of *Delisea pulchra* and the purified secondary metabolites isolated from the alga were tested in an accompanying ecological study in an attempt to explain the above field observations. (see Chapter 7).

The frozen alga was freeze dried and exhaustively extracted with dichloromethane followed by ethyl acetate. The combined extract was fractionated by rapid chromatography on a silica gel column (Coll and Bowden 1986) using light petroleum and ethyl acetate mixtures as eluent. Fractions from this chromatography afforded seven compounds which were purified and characterised. Four of these compounds, the halogenated lactones (56), (57), (58), (59) had been isolated previously, of which three remained to be fully characterised by n.m.r. The remaining three metabolites (60), (61), (62) were polyhalogenated alkenes, two of which were as yet unreported in the literature.



(56)

Table 16. ^{13}C and ^{1}H n.m.r. data for (56). Assignments are based on short range (J 165 Hz, XHCORRD) and long range (J 7 Hz, INAPT) ^{13}C-^{1}H correlations.

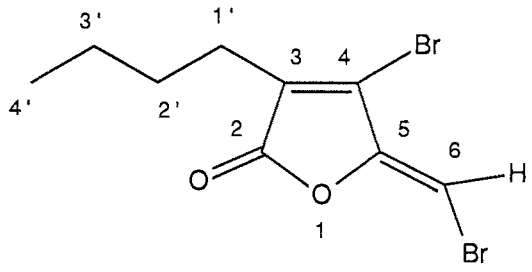
^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
13.7 (q)	0.90 (t, J 7.2 Hz)		4'
22.4 (t)	1.30 (dq, J 7.0, 7.0, 7.2 Hz)		3'
25.8 (t)	2.40 (t, J 7.5 Hz)	22.4, 28.8, 128.4, 138.0, 164.8	1'
28.8 (t)	1.55 (dq, J 7.0, 7.0, 7.5 Hz)	13.7, 22.4, 25.8, 138.0	2'
81.4 (s)			6
128.3 (s)			4
138.0 (s)			3
144.8 (s)			5
164.8 (s)			2

The least polar of the halogenated lactones, (56), was identified as fimbrolide (4c) on the basis of its ^{1}H and ^{13}C n.m.r. spectral data (Kazlaukas *et al.* 1977, Norton 1977). Kazlaukas *et al.* (1977) reported ^{1}H n.m.r. and low resolution mass spectral data for this compound, deriving its structure from data for similar acetoxyfimbrolides, while Norton (1977) reported and assigned the carbon data of the compound based on chemical shift considerations, off-resonance proton decoupling experiments and spin-lattice relaxation time measurements.

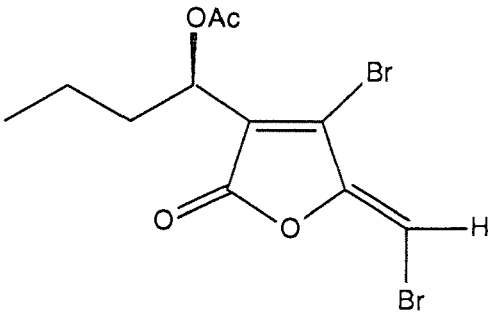
High field n.m.r. techniques including short range 2D (XHCORRD J=165 Hz) and selective long range 1D (INAPT J=7Hz) correlation experiments were used to confirm and complete assignment of this molecule which agreed with previous assignments (Table 16) (Norton 1977). (56) is therefore 3-*n*-butyl-4-bromo-5-(dibromo-methylidene)-2(5*H*)-furanone.

Although attempts to synthesise this compound have been unsuccessful (Caine and Ukachukwu 1985), a large number of related structures with strong antimicrobial and antifungal properties have been synthesised (Larock *et al.* 1978, Beecham and Sims 1979, Caine and Ukachukwu 1985, Calderon *et al.* 1987, Jefford *et al.* 1989).

The next more polar halogenated lactone (57) had ^{1}H and ^{13}C n.m.r. spectral properties similar to those reported for the natural and synthetic fimbrolide (4b) (Kazlaukas *et al.* 1977, Norton 1977, Beecham and Sims 1979, Caine and Ukachukwu 1985). Full ^{1}H and



(57)



(58)

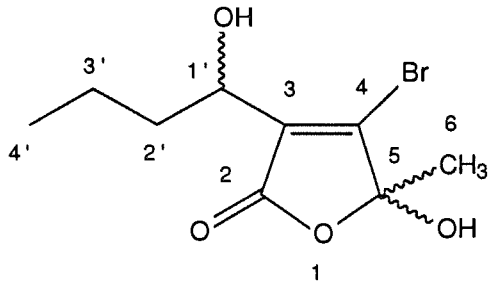
Table 17. ^{13}C and ^{1}H n.m.r. data for (57). Assignments are based on short range (J 165 Hz, XHCORRD) and long range (J 7 Hz, INAPT) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
13.6 (q)	0.93 (t, J 7.2 Hz)		4'
22.4 (t)	1.34 (dq, J 7.0, 7.0, 7.2 Hz)		3'
25.0 (t)	2.37 (t, J 7.0 Hz)	22.4, 29.0, 130.1, 133.9, 165.4	1'
29.0 (t)	1.56 (dq, J 7.5 Hz)		2'
90.8 (d)	6.25 (s)	130.1	6
130.1 (s)			4
133.9 (s)			3
150.0 (s)			5
165.4 (s)			2

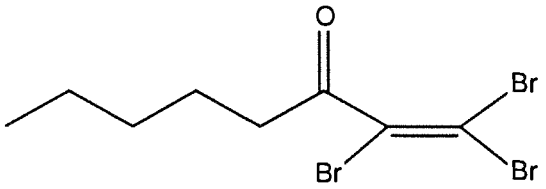
^{13}C n.m.r. characterisation of such a well studied compound had not yet been reported, and so high field n.m.r. techniques including short (XHCORRD J=165 Hz) and long range (INAPT J=7Hz) correlation experiments were used to complete its assignment (Table 17). (57) is therefore the dibromobutenolide (fimbrolide) (5Z)-3-*n*-butyl-4-bromo-5-(bromomethylidene)-2(5*H*)-furanone.

The next most polar halogenated lactone (58) had identical ^{1}H n.m.r. and i.r. data to those reported for compound (2b) (Kazlaukas *et al.* 1977) and ^{1}H and ^{13}C n.m.r. and i.r. and u.v. data reported for compound (1b) (Pettus *et al.* 1977). It is therefore (1*R*, 5Z)-3-(1-acetoxybutyl)-4-bromo-5-(bromomethylidene)-2(5*H*)-furanone.

The most polar halogenated lactone (59), had most n.m.r. data, m.s., i.r. and u.v. spectral data identical to those of the previously reported compound bromobeckerlide which was first isolated as a natural product of the alga *Beckerella subcostatum* (Ohta 1977) and has since been synthesised (Jefford *et al.* 1989) because of its potent antifungal and antimicrobial properties. Bromobeckerlide occurred as an optically inactive product, identical in reported properties with the synthetic product. The natural product as isolated by Ohta (1977) occurred as an inseparable (10:1) mixture of bromobeckerlide and chlorobeckerlide. There is no evidence to suggest the co-occurrence of chlorobeckerlide and bromobeckerlide in this sample. As the full ^{1}H and ^{13}C n.m.r. assignment of bromobeckerlide has not yet been reported, high field n.m.r. techniques including short (XHCORRD J=135 Hz) and long range (COLOC J=10Hz, INAPT J=7Hz) correlation experiments were used to make these unambiguous



(59)



(60)

Table 18 . ^{13}C and ^{1}H n.m.r. data for (59). Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (***J* 7 Hz, INAPT**, *J* 10 Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
13.7 (q)	0.94 (t, *J* 7.3 Hz)	18.6, 36.9	4'
18.6 (t)	1.33 (m)		3'
	1.43 (m)		
23.6 (q)	1.70 (s)	106.3, 146.3	6
36.9 (t)	1.64 (m)		2'
	1.82 (m)		
67.2 (d)	4.48 (t, *J* 7.2 Hz)	**18.6, 36.9, 132.9, 146.3**	1'
106.3 (s)			5
132.9 (s)			3
146.3 (s)			4
168.3 (s)			2

assignments (Table 18). (59) is therefore (*R*,*S*) 3-(1'-hydroxybutyl)-4-bromo-5-hydroxy-5-methyl-2(5*H*)-furanone.

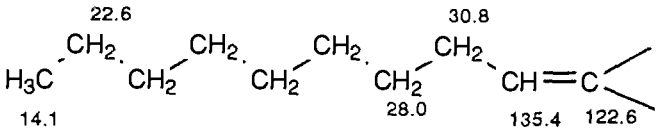
The least polar of the polyhalogenated alkenes, (60), had ^{1}H and ^{13}C n.m.r. and i.r. data identical to those of the pentyl tribromovinyl ketone (1a) reported by Rose *et al.* (1977) save that the carbonyl carbon at 196.8 ppm was not observed due to the small sample size. The presence of a signal in the i.r. spectrum at 1720 cm^{-1} however confirms the presence of the carbonyl group. (60) is therefore 1,1,2-tribromo-oct-1-en-3-one.

The less polar of the two new compounds (61) had a molecular formula $C_{12}H_{21}Br_3O$, contained one carbon-carbon double bond (^{13}C nmr 122.6 (s), 135.4 (d) ppm), and was thus acyclic (Table 19). The ^{1}H n.m.r. spectrum showed the presence of one methyl group δ0.88 (t), a methylene envelope δ1.26 (8H, bs), two methylene groups (δ1.43, 2H, quin, *J*= 7.2 Hz), (δ2.22, 2H, quart, *J*= 7.1 Hz), a hydroxyl proton (δ2.81, d, *J*= 5.7 Hz) and three methine protons (δ4.46, t, *J*=5.7 Hz), (δ5.94, d, *J*=5.7), (δ6.27, t, *J*=7.1 Hz). Proton homonuclear decoupling experiments allowed the establishment of two fragments. Irradiation of the methyl signal δ0.88 showed a coupling to the methylene group at δ1.26. The proton signal at δ1.43 showed a coupling to the methylene group δ1.26 and irradiation at δ1.43 collapsed the quartet at δ2.22 to a doublet. Similarly irradiation at δ1.26 collapsed the triplet at δ 0.88 to a singlet and the quintet at δ1.43 to a triplet. Irradiation at δ2.22 collapsed the same quintet at δ1.43 to a triplet and the triplet at δ6.27 to a singlet, while

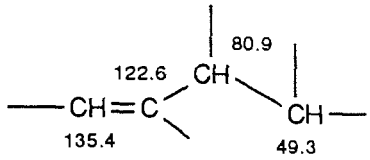
Table 19. ^{13}C and ^{1}H n.m.r. data for (61). Assignments are based on short range (J 135 Hz, XHCORRD) and long range (J 7 Hz, INAPT) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
14.1 (q)	0.88 (t, J 6.8 Hz)		12
22.6 (t)	1.26 (brs)		11
28.0 (t)	1.43 (quin, J 7.2 Hz)		6
29.1 (t)	1.26 (brs)		*
29.2 (t)	1.26 (brs)		*
29.3 (t)	1.26 (brs)		*
30.8 (t)	2.22 (q, J 7.2 Hz)		5
31.8 (t)	1.26 (brs)		*
49.3 (d)	5.94 (d, J 5.7 Hz)		1
80.9 (d)	4.46 (t, J 5.7 Hz)		2
122.6 (s)			3
135.4 (d)	6.27 (t, J 7.1 Hz)	80.9, 122.6	4
OH	2.81 (d, J 5.7 Hz)		

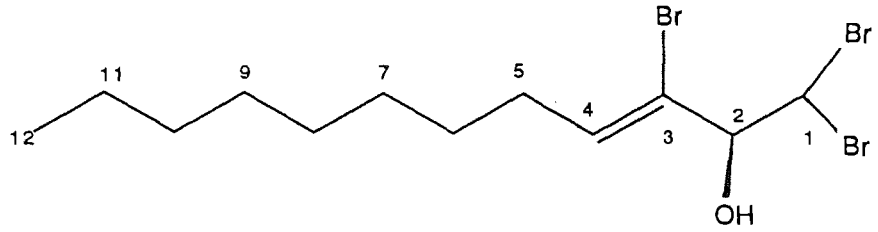
* methylene envelope carbon numbers 7-10 unassigned.



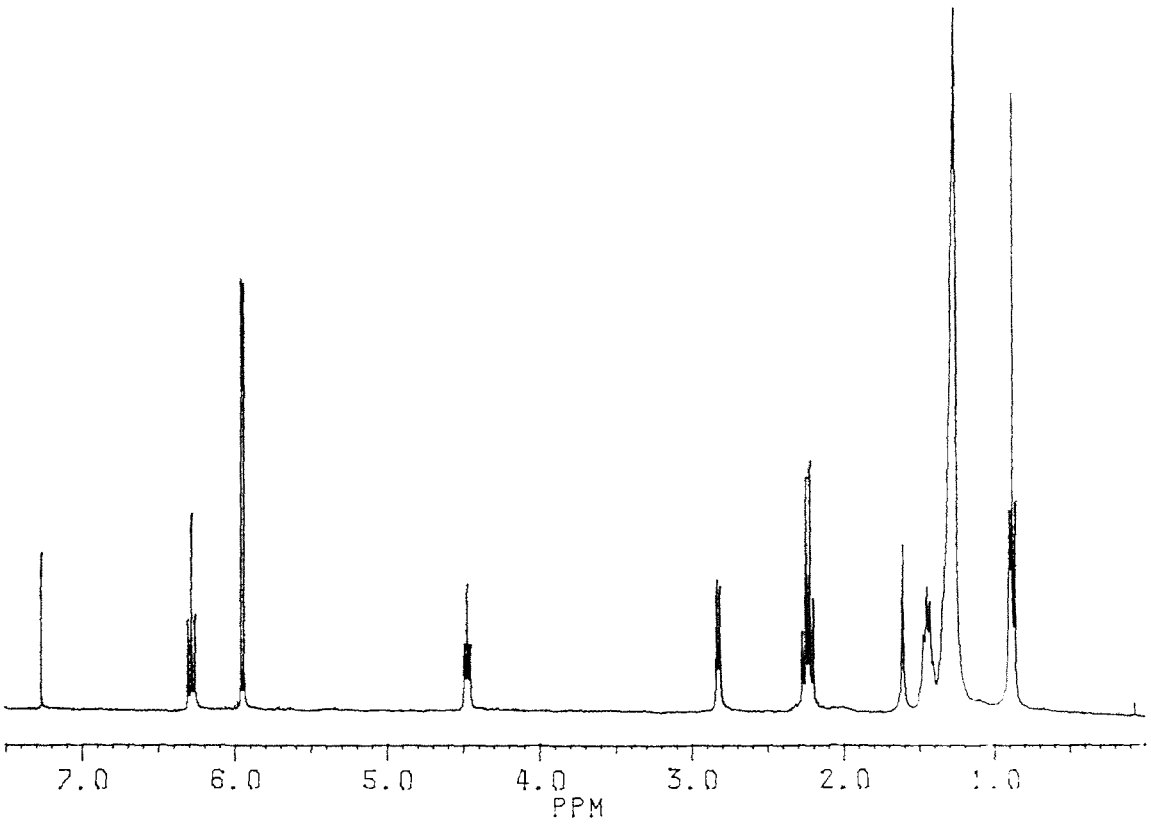
Fragment 1



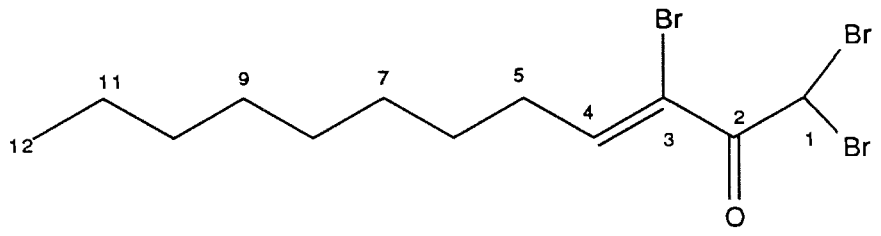
Fragment 2



(61)



^{1}H n.m.r. spectrum of (61)



(61a)

irradiation at δ6.27 collapsed the quartet at δ2.22 to a triplet. This allowed the establishment of fragment 1.

The second fragment (fragment 2) was established as follows. Irradiation of δ2.81 collapsed the triplet at δ4.46 to a doublet. Similarly irradiation at δ4.46 collapsed the doublet at δ2.81 to a singlet and the doublet at δ5.94 to a singlet. Irradiation at δ5.94 collapsed the triplet at δ4.46 to a doublet.

Unambiguous assignment of protons to their attached carbons was established using an XHCORRD experiment (J=135 Hz) (Table 19). The only possible connection of the fragments is through the carbon-carbon double bond resulting in the assignment of the carbon skeleton. An INAPT experiment (J=7 Hz) allowed confirmation of the assignments of the skeleton.

Irradiation of the methine proton signal δ6.27 showed correlations to carbon signals 80.9 and 122.6 ppm confirming the assignment of the carbon 80.9 ppm adjacent to the double bond.

The assignment of a Z configuration of the double bond in the structure was established using n.O.e experiments and chemical studies on the molecule.

An n.O.e. was observed at the 10% level between the vinyl proton δ6.27 and the methine proton δ4.46 on irradiation of either of the two signals. Similarly a 2% n.O.e was observed between δ6.27 and the OH signal at δ2.81. In order to further confirm these observations the molecule was oxidised using pyridinium chlorochromate (PCC) (Corey and Suggs 1975) to yield the ketone (61a). This caused a downfield shift of the methine proton signal of (63) (δ5.94, d, J 5.7 Hz) of 0.75 ppm (δ6.68, s) and of the vinyl proton signal (δ6.27, t, J 7.1 Hz) of 1.18 ppm (δ 7.45, t, J 7.1 Hz) as would be expected for a Z configuration of the double bond. Horeau

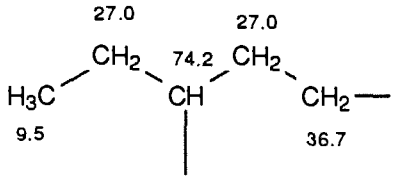
determination of the absolute configuration of the secondary alcohol at C2 indicated it had the *S*-configuration. (61) is therefore (2*S*)-1,1,3,-tribromo-2-hydroxydodec-3-ene

The more polar of the two new metabolites (62) had the molecular formula $C_{10}H_{13}Br_3O_3$ and incorporated one carbon-carbon double bond. The sp^2 carbons of the double bond comprised one monobrominated carbon and a terminal dibrominated carbon (^{13}C nmr, 121.4 (s), 91.7 (s) ppm). The molecule also contained two carbonyl carbons (170.8 (s), 196.4 (s) ppm) and was thus acyclic. The ^1H spectrum showed the presence of two methyl groups (δ0.89(t), δ2.00 (s)) the latter being adjacent to a carbonyl carbon; four methylene groups (δ1.63, 2H, quin, *J*=7.1 Hz), (δ1.84, 1H, q, *J*= 7.4 Hz), (δ1.97, 1H, q, J=7.4 Hz), (δ2.80, 2H, t, *J*=7.4 Hz) and one methine proton on an oxygenated carbon (δ4.83, m) (Table 20). Proton double resonance experiments allowed the establishment of the major portion of the molecule (fragment 1). Irradiation of the methyl signal δ0.89 simplified the quintet at δ1.63 to a doublet while irradiation at δ1.63 collapsed the triplet at δ0.89 to a singlet and simplified the multiplet at δ4.83. Similarly irradiation at δ4.83 collapsed the quintet at δ1.63 to a quartet and simplified the signals at δ1.84 and δ1.97. Irradiation of the signal at δ1.84 simplified the signals at δ1.97 and δ4.83 and collapsed the triplet at δ2.80 to a doublet, while a similar effect occurred on irradiation of the signal at δ1.97 simplifying the signals at δ1.84 and δ4.83 while collapsing the triplet at δ2.80 to a doublet.

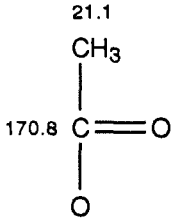
Unambiguous assignment of protons to their attached carbons was established by a 2D heteronuclear n.m.r. experiment (XHCORRD, *J*= 135 Hz) (Table 20).

Table 20. ^{13}C and ^{1}H n.m.r. data for (62). Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (***J* 7 Hz, INAPT**, *J* 10 Hz, COLOC) range ^{13}C-^{1}H correlations.

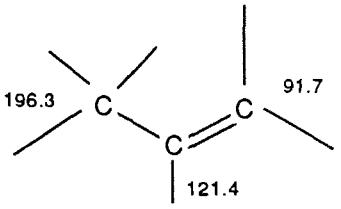
^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
9.5 (q)	0.89 (t, *J* 7.1 Hz)	27.0, 74.2	8
21.1 (q)	2.00 (s)	170.8	10
27.0 (t)	1.63 (quin, *J* 7.1 Hz)		7
27.0 (t)	1.84 (q, *J* 7.4 Hz) 1.97 (q, *J* 7.4 Hz)		5
36.7 (t)	2.80 (t, *J* 7.4 Hz)	**121.4, 196.4**	4
74.2 (d)	4.83 (m)	**9.5, 27.0, 36.7, 170.8**	6
91.7 (s)			1
121.4 (s)			2
170.8 (s)			9
196.4 (s)			3



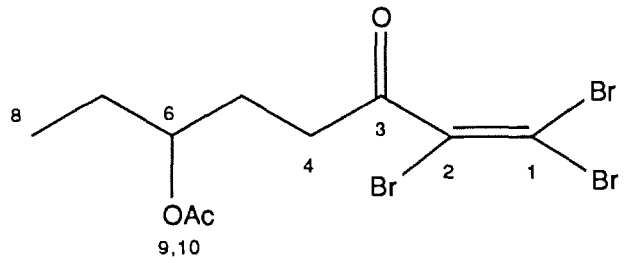
Fragment 1



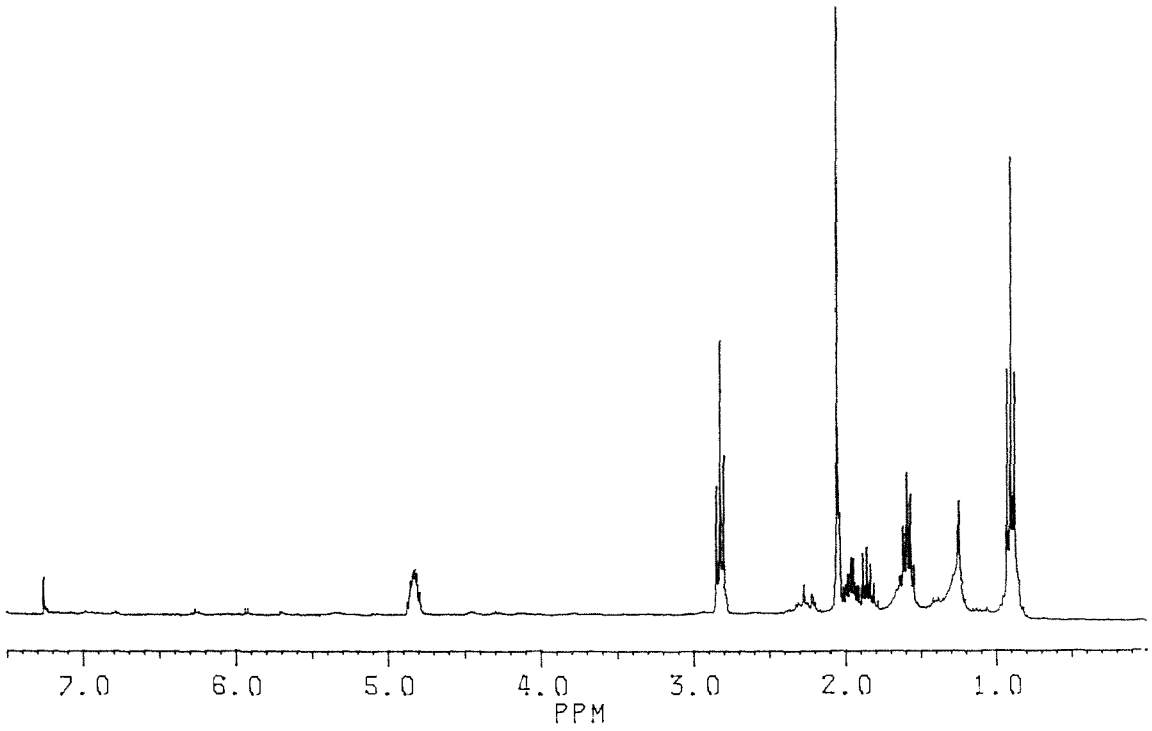
Fragment 2



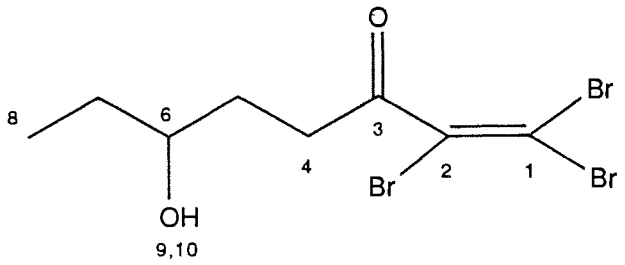
Fragment 3



(62)



^{1}H n.m.r. spectrum of (62)



(62a)

The remainder of the molecule was established using ^{13}C-^{1}H long range n.m.r. correlation experiments (COLOC J=10Hz, INAPT J=7Hz). The methyl signal δ0.89 showed correlations to the carbon signals 9.5, 27.0 and 74.2 ppm further confirming the previous assignments. The methyl signal δ2.00 showed correlations to the carbonyl carbon signal 170.8 ppm further confirming the presence of an acetate unit (fragment 1) connected to the oxygenated carbon (74.2 ppm). This is further confirmed by the absence of a hydroxyl signal and the presence of a carbonyl (C=O) stretch (1739 cm^{-1}) in the i.r. spectrum. The methylene proton signal δ2.80 showed correlations to the carbon signals 36.7, 121.4 and 196.4 ppm. This confirms the presence of the carbonyl carbon (196.4 ppm) adjacent to the methylene group (δ2.80) and the presence of a terminal tribrominated double bond (fragment 2) completing the structure. In order to establish the relative configuration of the molecule at C3 the molecule was converted to a secondary alcohol using an enantio-selective enzymatic hydrolysis (Laumen and Schneider 1984, Turner 1989). The reaction yielded the secondary alcohol (62a) as 50% yield from the original starting material. In comparison with the ester an upfield shift of the proton signal δ4.83 to δ3.56 and a downfield shift of the methylene proton signal δ2.83 to δ2.97 was observed in the ^{1}H n.m.r. spectrum of the alcohol. Similarly the methyl proton signal was shifted downfield from δ0.89 to δ0.96. The alcohol decomposed before further spectroscopic data could be obtained. The optical rotation of the remaining starting material confirmed that the compound occurred as a racemic mixture. (62) is therefore (*R*,*S*)(1*Z*) 6-acetoxy-1,1,2-tribromodec-1-en-3-one.

3. INVESTIGATION OF THE NATURAL PRODUCTS CHEMISTRY OF SELECTED CHLOROPHYTA (GREEN ALGAE)

Two species of algae from the division Chlorophyta were examined during the course of this study *Microdictyon obscurum* and *Chlorodesmis fastigata.*

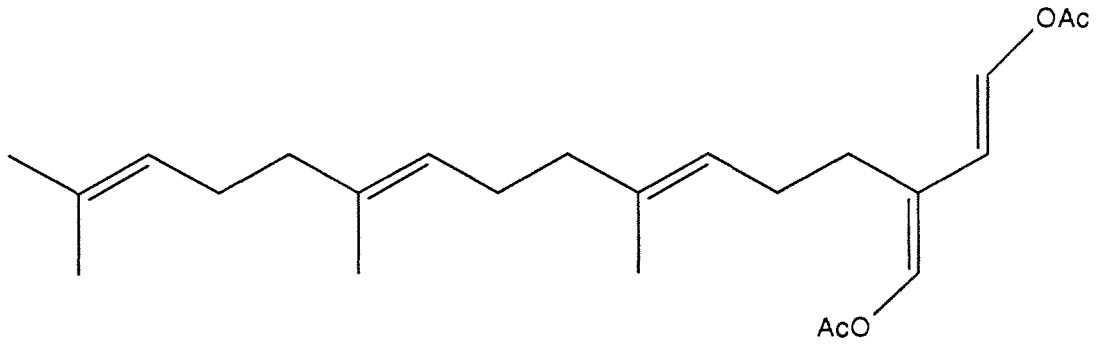
3.1. *Microdictyon obscurum* HR892A, HR891F

Microdictyon obscurum a species not previously investigated in the literature was particularly abundant on the offshore reefs of the Coral Sea where it was collected, growing in an exposed habitat which offered no physical refuge from predation. These offshore reef areas have large populations of herbivores (Scott and Russ 1987) yet the alga was not observed to be grazed upon, and no signs of grazing were seen in the populations investigated. A thorough investigation of two separate collections of this alga failed to isolate any secondary metabolites which were extractable with dichloromethane/methanol mixtures. More polar compounds were not investigated.

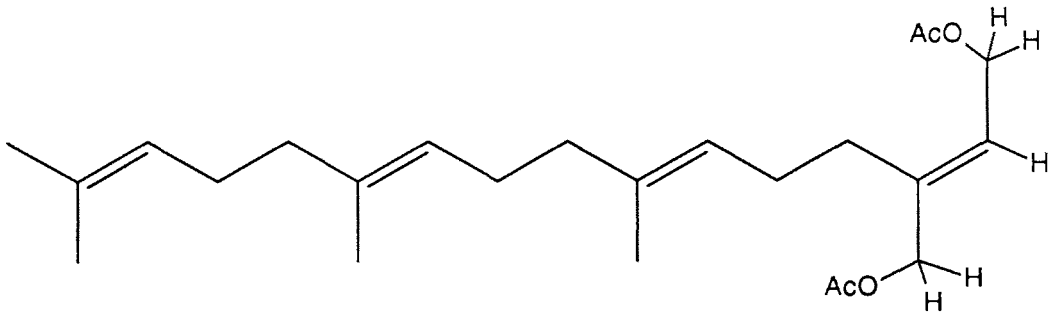
3.2. *Chlorodesmis fastigata* JB871A

Chlorodesmis fastigata is one of the most common macroalgae encountered on the outer reef area of the Great Barrier.
A sample of the alga collected from John Brewer Reef was freeze dried and exhaustively extracted with DCM. From this extract two diterpenes (63) and (64) were purified and characterised.

Comparison of the ^{1}H n.m.r. spectra of (63) and (64) with authentic spectra of previously isolated and reported compounds from *C. fastigata* showed (63) and (64) to be 14,15-didehydrotrifarin (Wells and Barrow 1979) and (2*E**,6*E**,10*E**)-1-acetoxy-3-acetoxymethyl-7,11,15-trimethylhexadeca-2,6,10,14-tetraene (Wright *et al.* 1990) respectively.



(63)



(64)

4. INVESTIGATION OF THE NATURAL PRODUCTS CHEMISTRY OF SELECTED PHAEOPHYTA (BROWN ALGAE)

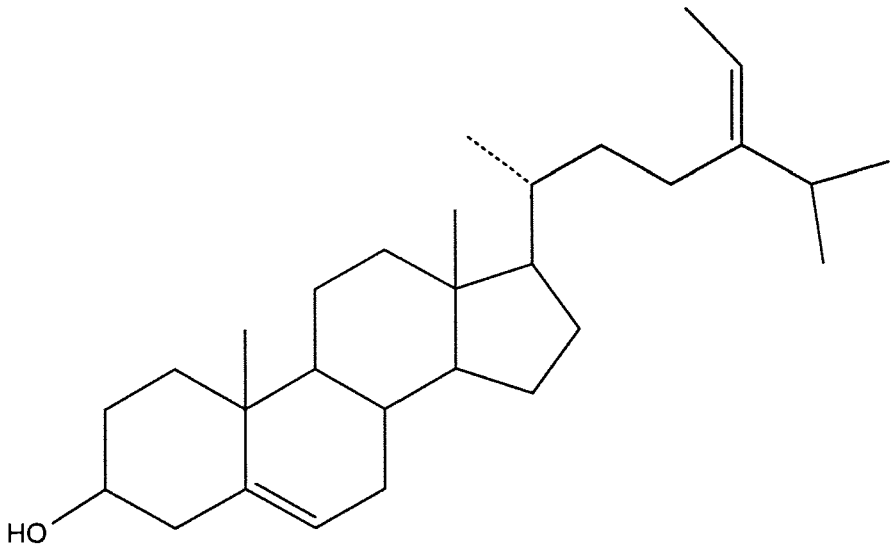
4.1. *Sargassum* spp.

An investigation of the non-polar secondary metabolite chemistry of seven tropical species of *Sargassum* was carried out as part of collaborative research into the comparative chemical defences of temperate and tropical *Sargassum* spp. (Steinberg *et al.* 1991 in press).

Seven distinct species of *Sargassum* (K. Edyvane, pers. comm.) were collected from Alma Bay, Magnetic Island for chemical investigation. Four of these species are well described, *S. decurrens, S. polycystum S. Oligocystum and S. linearifolium* based on a revision of the taxonomy of tropical *Sargassum* spp. of eastern Australia (Edyvane in prep.). The other three tropical *Sargassum* species investigated, *Sargassum* sp. A, sp. B, and sp. D have not been described, but appear to be distinct species.

A sample (100-200 g) of each species of *Sargassum* was freeze dried and extracted in DCM and examined by tlc. The chromatogram showed no difference in the chemical characteristics of all seven species.

A more detailed investigation was carried out on a larger sample of *Sargassum* c.f. *linearifolium* from the same collection at Alma Bay. The sample of fresh material (1.1 kg) was freeze dried and extracted with DCM to yield a crude extract. The extract (1.0 g) was partitioned by rapid chromatography on a silica gel column using light petroleum and ethyl acetate mixtures as eluents to afford 20



(65) fucosterol

fractions. Fractions which had similar chromatograms were combined and further column chromatography was carried out on selected fractions. All fractions were examined by tlc and ^{1}H n.m.r.. The only isolated compound was the ubiquitous brown algal sterol, fucosterol (65). No secondary metabolites related to those previously isolated from temperate *Sargassum* species (Kato *et al.* 1975b, Kusumi *et al.* 1981, Shizuru *et al.* 1982) were detected in any of the tropical *Sargassum* species.

Concurrent analyses of the levels of polyphenolic compounds in relation to the feeding deterrent properties of selected algal species, demonstrated for the first time that variation in algal polyphenolic levels had no affect on their susceptibility to herbivory (Steinberg *et al.* 1991). Furthermore, it was found that unpalatable tropical brown algae did not consistently contain deterrent non-polar secondary metabolites as had earlier been proposed by Steinberg and Paul (1990). The ecological implications of the lack of non-polar secondary metabolites in some species, and the fact that feeding deterrence is not related to polyphenolic levels in the *Sargassum* species studied, is discussed as part of other collaborative research in Steinberg *et al.* 1991 (in press).

5. INVESTIGATION OF THE NATURAL PRODUCTS CHEMISTRY OF SELECTED CYANOPHYTA (BLUE GREEN ALGAE)

5.1. *Lyngbya* sp. BR891A

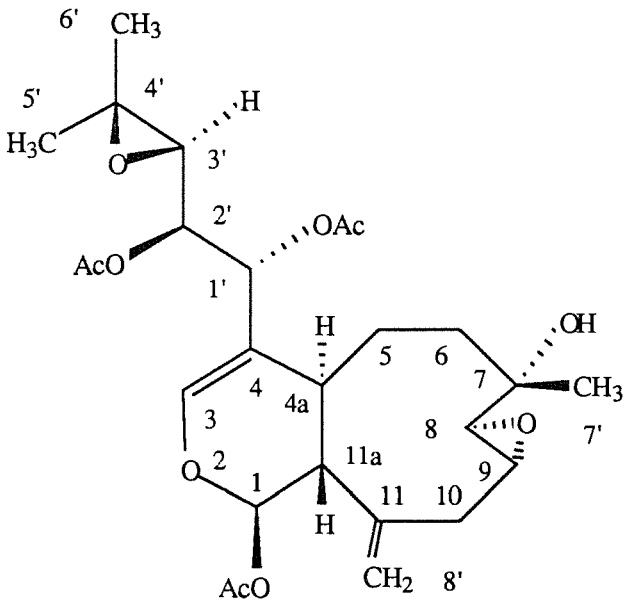
The alga *Lyngba* sp. was of particular interest due to its relationship with the shrimp *Alpheus frontalis* which weaves the alga into a tube in which it lives. Upon collection it was thought that there may be some type of chemically mediated interaction between the alga and the shrimp based on either a defensive or settlement interaction. An extensive investigation of the alga did not detect any secondary metabolites.

6. INVESTIGATION OF THE NATURAL PRODUCTS CHEMISTRY OF *HELIOPORA COERULEA* (ALCYONARIA)

6.1. *Heliopora coerulea* OR892B

The alcyonarian coral *Heliopora coerulea* commonly known as the blue coral was collected from Cattle Bay, Orpheus Island GBR, an area adjacent to the Pelorus-Orpheus channel where ecological studies were carried out (see Chapter 7). *H. coerulea* is one of the most abundant species in this area but seldom seen in other fringing reef areas of the Palm Island group. It is the sole extant member of the alcyonarian order Coenothecalia and is unique from other alcyonarian corals in that it possesses a solid tubular skeleton composed of aragonite resembling scleractinian corals in its gross morphology (Babcock 1990). Field observations of *H. coerulea* suggest that it is a less preferred food source of the crown of thorns starfish *Acanthaster plancii* (Babcock pers. comm.). Due to the unique nature of this organism, its relationship to the Gorgonaceae and Alcyonacea which are rich in secondary metabolites, and its proximity to research sites, *H. coerulea* was investigated for its secondary metabolite chemistry.

An initial collection of *H. coerulea* was made (14/xi/1989). The sample was frozen on collection, freeze dried and subsequently exhaustively extracted with DCM followed by MeOH to yield a crude extract. Rapid chromatography of the extract on silica gel using light petroleum and ethyl acetate as eluents afforded 15 fractions. HPLC separation of selected fractions yielded two metabolites not previously reported in the literature.



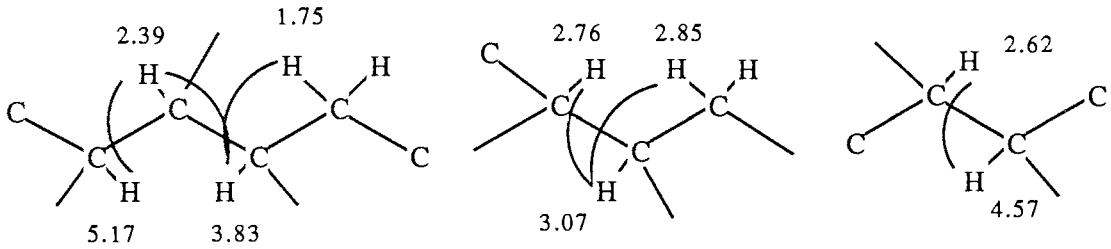
(66) helioxenicane A

Table 21. ^{13}C and ^{1}H n.m.r. data for (66). Assignments are based on short range (J 135 Hz, XHCORRD) and long range (J 10 Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
19.1 (q)	1.29 (s)	19.1, 24.2, 58.2, 61.4	6'
20.7 (q)	2.05 (s)		
20.9 (q)	2.04 (s)		
21.1 (q)	2.01 (s)		
24.2 (q)	1.26 (m)	19.1, 24.2, 58.2, 61.4	5'
26.4 (t)	1.72 (m)		5
	2.00 (m)		
31.2 (d)	3.20 (brd, J 5.6 Hz)		4a
32.3 (t)	1.95 (m)		10
	2.73 (m)	62.5	
32.9 (t)	1.95 (m)		6
	2.73 (m)		
33.2 (q)	1.38 (m)	33.2, 70.2, 62.5, 32.9	7'
43.9 (d)	2.66 (t, J 5.6 Hz)		11
58.2 (s)	-----		4'
59.1 (d)	3.00 (dt, J 4.8, 4.8, 9.7 Hz)		9
61.4 (d)	2.80 (d, J 8.2 Hz)		3'
62.5 (d)	2.74 (d, J 7.9 Hz)		8
70.2 (s)	-----		7
70.8 (d)	4.94 (dd, J 6.6, 8.2 Hz)		2'
73.5 (d)	5.47 (d, J 6.6 Hz)	73.5, 141.8	1'
91.7 (d)	6.10 (d, J 5.6 Hz)		1
111.5 (s)	-----		4
116.0 (t)	5.04 (s)	32.3	8'
	5.07 (s)	32.3	
141.8 (d)	6.54 (d, J 1.5 Hz)	91.7, 111.5, 141.8	3
142.2 (s)	-----		11
169.1 (s)	-----		
169.3 (s)	-----		
170.0 (s)	-----		

The least polar of the metabolites (66) had a molecular formula $C_{26}H_{36}O_{10}$ by HREIMS. The ^{13}C spectrum contained signals for twelve oxygenated carbons including one oxygenated sp^2 carbon (141.8 (d) ppm) and three carbonyl carbons (169.1 (s), 169.3 (s), 170.0 (s) ppm) (Table 21). It also contained signals for an exocyclic methylene group (116.0 (t), 142.2 (s) ppm). The presence of three downfield methyl groups in the ^1H n.m.r. spectrum of (66) at δ2.01 (s), 2.04 (s), and 2.05 (s) was consistent with the presence of three acetate groups. The ^1H n.m.r. spectrum also contained signals for three more shielded methyl groups at δ1.26, 1.29 and 1.38. The compound thus had nine double bond equivalents which included three acetate groups and two double bonds. The compound thus possessed four rings. Comparison of spectral data with those of previously reported compounds, suggested that the compound was a xenicane diterpene closely related to previously reported marine natural products (Groweiss and Kashmann 1978, 1983; Kashman and Groweiss 1978, 1980; Bowden *et al.* 1982, Coval *et al.* 1984, Lelong *et al.* 1987, Almourabit *et al.* 1990).

High field 2D n.m.r. experiments were used to assign the data of (66) and elucidate the structure. Proton double resonance and a 2D homonuclear (COSY) experiment (see experimental) allowed assignment of ^1H chemical shifts and couplings (Table 21). Proton were unambiguously assigned to their associated carbon signals using a 2D short range experiment (XHCORRD, J=135 Hz), while a long range correlation experiment (COLOC, J= 10Hz) permitted confirmation of the relationships between the carbon and proton signals (Table 21). These data permitted the deduction of the structure of (66). Data for two xenicane diterpenes, helioxenicanes A and B, isolated from an Okinawan collection of *Heliopora coerulea*



Fragment 1 Fragment 2 Fragment 3

Figure 14. Crosspeaks from COSYDQF spectra of (67)

Table 22. ^{13}C and ^{1}H n.m.r. data for Helioxenicin C. Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (*J* 7 Hz, HMBC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
18.6 (q)	1.39 (s)	24.4, 59.4, 64.9	4'
24.4 (q)	1.33 (s)	18.6, 59.4, 64.9	3'
25.5 (t)	1.75 (m)		4
	n.r.		
29.8 (t)	n.r.		9
	2.85 (m)	61.0, 61.8, 119.0, 143.5	
30.0 (d)	3.83 (t, *J* 10.7 Hz)		3b
32.0 (q)	1.36 (s)	34.1, 61.8, 72.1	5'
34.1 (t)	n.r.		5
	n.r.		
57.1 (d)	2.39 (dd, *J* 3.3, 10.4 Hz)		10a
59.4 (s)	-----		2'
61.0 (d)	3.07 (dd, *J* 4.4, 4.4 Hz)		8
61.8 (d)	2.76 (m)		7
64.9 (d)	2.62 (d, *J* 8.7 Hz)	24.4, 84.7, 118.9	1'
72.1 (s)			6
84.7 (d)	4.57 (brd, *J* 8.7 Hz)		2
98.0 (d)	5.17 (s)		11
103.6 (d)	6.08 (s)	64.9, 84.7, 118.9	12a
118.9 (d)	5.73 (s)	84.7, 103.6	3
119.0 (t)	5.15 (s)	29.8, 57.1	6'
	5.16 (s)		
143.5 (s)*	-----		3a
143.8 (s)*	-----		10

* signals interchangeable
n.r. not resolved

was simultaneously reported at the Pharmamar conference, Madrid (Higa, unpublished data 1990). This report included X-ray crystallographic studies which determined the relative stereochemistry of these molecules. Comparison of authentic ^{1}H and ^{13}C n.m.r. spectra of helioxenican A and (66) confirm that the two compounds are identical. (66) is therefore the previously unpublished xenicane diterpene (1*R*,1'*R*,2'*S*,3'*S*,4a*S*,7*S*,8*S*,9*R*,11a*S*)-1-acetoxy-4(1',2'-diacetoxy-3',4'-epoxy-4'-methylpentyl)-8,9-epoxy-7-hydroxy-7-methyl-11-methylene-1,4a,5,6,7,8,9,10,11a-decahydrocyclonona[c]pyran, which has been given the trivial name helioxenicane A.

The most polar of the two metabolites (67) had a molecular formula $C_{20}H_{28}O_6$ by HREIMS. A comparison of the ^{1}H and ^{13}C n.m.r. spectra of (67) with that of (66) and other related metabolites (e.g. Almourabit *et al.* 1990) suggested it was a xenicane diterpene similar to (66). The ^{13}C n.m.r. spectrum contained signals for seven oxygenated and one doubly oxygenated carbons. It also contained signals for four sp^2 carbons (103.6 (d), 119.0 (t), 143.5 (s), 143.8 (s) ppm), while the ^{1}H n.m.r. spectrum contained signals for three aliphatic methyl groups (Table 22). The compound thus had seven double bond equivalents suggesting that the compound possessed five rings. Unambiguous assignment of proton signals to the carbon signals to which they are attached was established using a 2D short range experiment (XHCORRD, J= 135 Hz) (Table 22). A homonuclear correlation (COSYDQF) experiment allowed a number of fragments of the molecule to be established. Cross peaks in the COSYDQF spectrum allowed correlations to be made between the methine proton signals δ5.17 and δ2.39. Similarly the signal δ2.39 showed

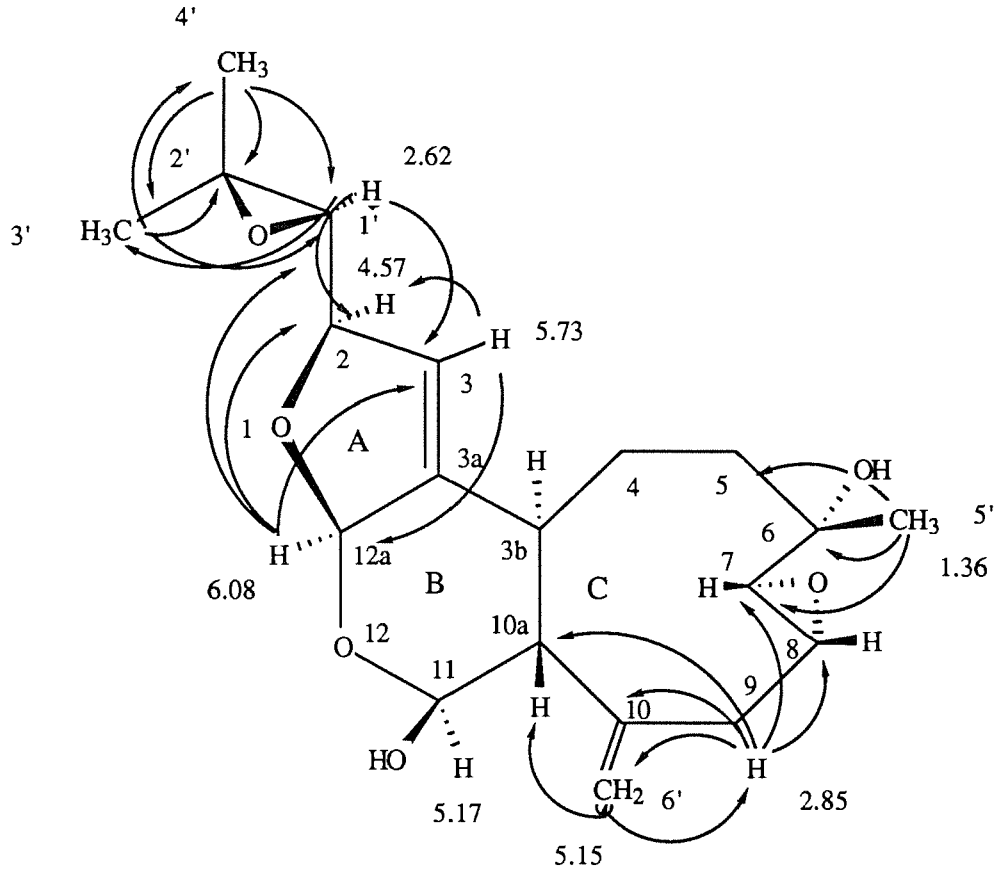
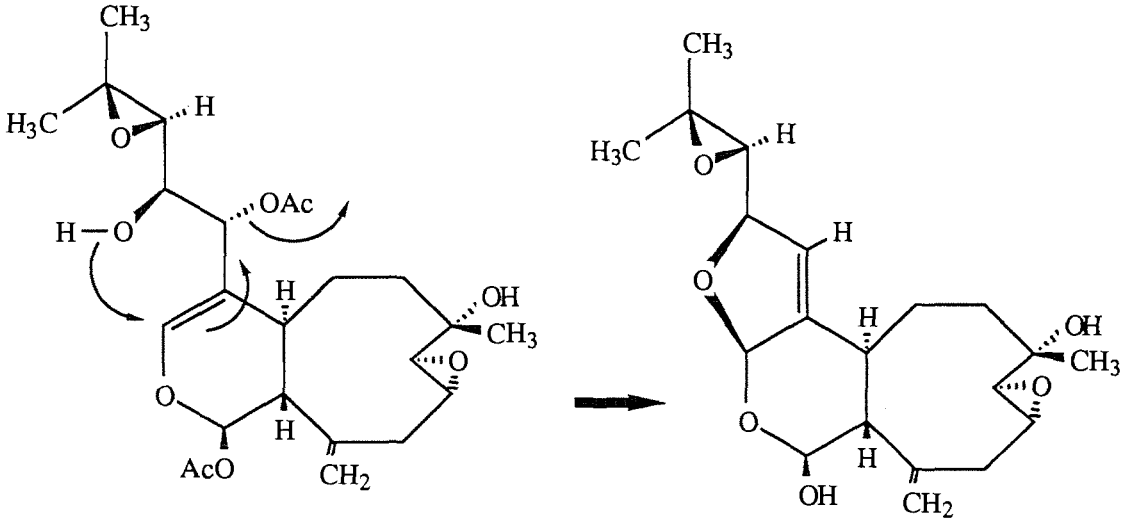


Figure 15. Long range correlations (HMBC, J= 7Hz) for (67)

cross peaks to δ5.17, and to the methine proton signal δ3.83, while the methine signal δ3.83 showed cross peaks to the signal δ2.39 and the methylene signal δ1.75. The methylene proton signal δ1.75 showed a cross peak to the signal δ3.83. (Fragment 1, Fig. 14). The methine proton signals δ2.76 and δ3.07 showed a cross peak to each other while δ3.07 also showed a cross peak to the methylene signal δ2.85. Similarly the proton signal δ2.85 showed a cross peak to the signal δ3.07 (Fragment 2. Fig. 14). The methine proton signals δ2.62 and δ4.57 showed cross peaks to each other (Fragment 3, Fig. 14). An inverse heteronuclear long range correlation experiment (HMBC, J= 7 Hz) was used to connect the fragments of the molecule and confirm the assignment of the carbon skeleton (Table 22). The methyl signals δ1.33 (24.4 ppm) and δ1.39 (18.6 ppm) showed correlations to the carbon signals 59.4 and 64.9 ppm confirming the presence of a terminal epoxide (C1'-C2') and the assignment of the C1' and C2' positions in the molecule (Fig. 15, Table 22).
The methyl signal δ1.36 (32.0 ppm) showed correlations to the carbon signals 34.1, 61.8 and 72.1 ppm. The methylene signal δ2.85 (29.8 ppm) showed correlations to the carbon signals 61.0, 61.8, 119.0 and 143.5 ppm while the signal for the methylene protons δ5.15 and δ5.16 (119.0 ppm) showed correlations to the carbon signals 29.8 and 57.1 ppm. These correlation confirmed the position of the epoxide (C7-C8) and hydroxyl (C6) groups, and the assignment of carbon signals in the C ring of the molecule (Fig. 15, Table 22).

The long range correlations from the methine signals δ2.62, 5.73 and 6.08 allowed the assignment of the final fragment of the molecule. The proton signal δ2.62 (64.9 ppm) showed correlations

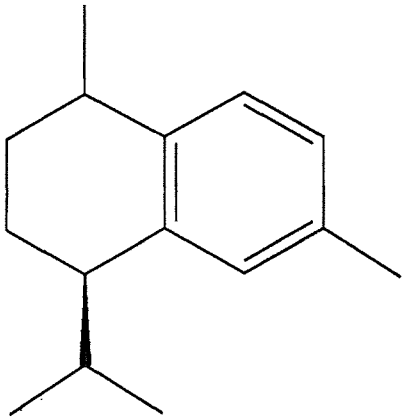


C2' alcohol of (66) (67)

Figure 16

to the carbon signals 24.4, 84.7 and 118.9 ppm. The proton signal δ5.73 (118.9 ppm) showed correlations to the carbon signals 84.7 and 103.6 ppm while the signal δ6.08 (103.6 ppm) showed correlations to the carbon signals 64.9 and 84.7 and 118.9 ppm. These correlations confirm the presence of an ether linkage between the C2 (84.7 ppm) and C12a (103.6 ppm) positions in the molecule. They also allow the assignment of the olefinic carbon signals 118.9 and 143.5 ppm to the C3 and C3a positions respectively, and the doubly oxygenated carbon signal 103.6 ppm to the C12a position of the molecule (Fig. 15, Table 22). The presence in the literature of large five bond couplings in molecules containing similar partial structures ($^5J_{C-H}$ ~ 5.8 Hz, pp 491, Kalinowski *et al.* 1984), suggests that the correlation between the proton signal δ6.08 (C12a, 103.6 ppm) and the carbon signal 64.9 ppm (C1') in the HMBC experiment is most probably due to a strong five bond coupling between the two signals in the *cis* conformation.

The similarity of ^1H and ^{13}C n.m.r. spectral data for signals in the C5, C6, C7 and C8 positions of the molecule with those of (66) suggest the same relative stereochemistry as that for (66). This is supported by the likelihood that (67) is a product derived from the C2' alcohol of (66) as represented in Fig. 16. Compound (67) is therefore the new xenicane diterpene (1'*S**,2*R**,3b*S**6*S**,7*S**,8*R**, 10a*S**,11*S**,12a*R**)-2-(1',2'-epoxy-2'-methylpropyl)-7,8-epoxy-6,11-dihydroxy-6-methyl-10-methylene-2,3b,4,5,6,7,8,9,10,10a, 11,12a-dodecahydrocyclonona[4,5-e]furo[2,3-b]pyran, which we call helioxenicane C.

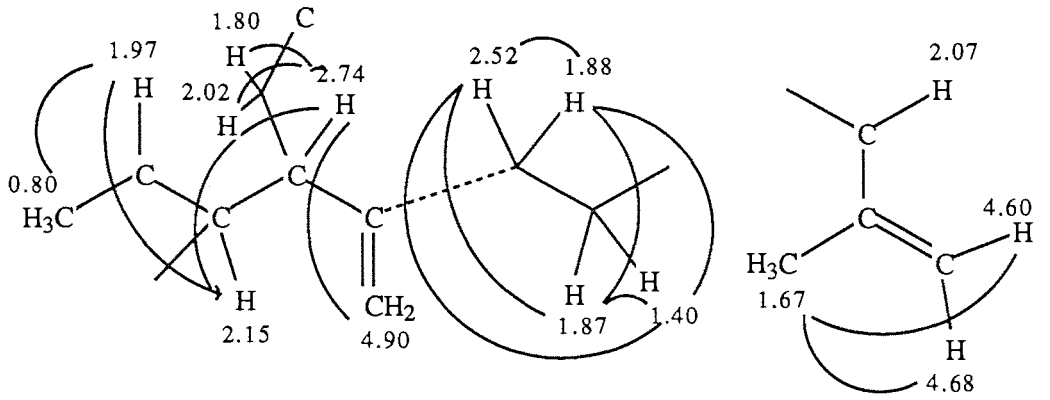


calamenene (68)

Due to the presence of novel secondary metabolites in the initial collection of *Heliopora coerulea*, a study was undertaken to investigate the chemical ecology of this species. *H. coerulea* has separate male and female colonies, the female colony producing brooding larvae which are fertilised and brooded in the tip of the female polyp (Babcock 1990). Using the characteristic of eggs at the surface of the colony to distinguish between male and female, a second collection of *H. coerulea* was made at the time of spawning (22/xii/91). Female colonies containing eggs were collected as were colonies without eggs in their polyps or in the coenenchymal tissue; these were deemed male. A small number of eggs were also collected to investigate their natural product chemistry.

The sample of female *H. coerulea* was frozen on collection, freeze dried and subsequently extracted with DCM followed by MeOH to yield a crude extract. Rapid chromatography of the extract on a silica gel column using light petroleum and ethyl acetate mixtures as eluents afforded 14 fractions. Further column chromatography and HPLC of selected fractions yielded the previously reported sesquiterpene (68) and two metabolites (69) and (70) not previously reported in the literature.

The compound (68) was identified as the commonly occurring sesquiterpene calamenene on the basis of its ^{1}H n.m.r. spectrum (de Mayo *et al.* 1965) Calamenene and related sesquiterpenes have been isolated from terrestrial sources (de Mayo *et al.* 1965), the gorgonian *Pseudoplexaura porosa* (Weinheimer *et al.* 1968) and the alcyonarian *Sinularia mayi* (Beechan *et al.* 1978).



Fragment 1 Fragment 2 Fragment 3

Figure 17.

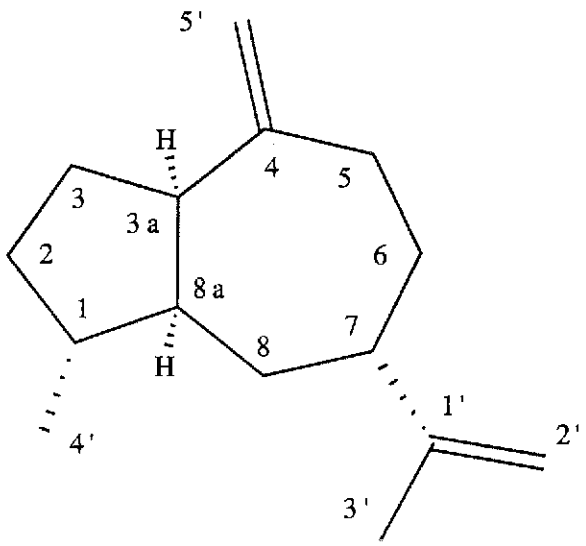
Crosspeaks between proton signals from COSYDQF spectra of (69)

Table 23. ^{13}C and ^{1}H n.m.r. data for (69). Assignments are based on short range (*J* 135 Hz, XHCORRD, HMQC) and long range (*J* 7 Hz, HMBC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
14.5 (q)	0.80 (d, *J* 7.1 Hz)	34.3, 36.9, 49.4	4'
19.3 (q)	1.67 (s)	47.3, 108.9, 150.8	3'
28.3 (t)	1.80 (m)		3
	2.02 (m)		
30.6 (t)	1.40 (m)		6
	1.87 (m)		
34.3 (t)	1.48 (m)	14.5, 36.9, 45.7, 49.4	2
	1.51 (m)		
36.0 (t)	1.65 (m)		8
	1.90 (m)		
36.9 (d)	1.97 (m)		1
41.7 (t)	1.88 (m)		5
	2.52 (dt, *J* 2.9, 2.9, 12.8 Hz)	30.6, 45.7, 109.0, 152.6	
45.7 (d)	2.74 (ddd, *J* 9.1, 9.1, 9.2 Hz)	28.3, 36.9, 41.7, 49.4, 109.0, 152.6	3a
47.3 (d)	2.07 (m)	19.3, 30.6, 36.0, 49.4, 109.0, 150.8	7
49.4 (d)	2.15 (m)	14.5, 36.9, 45.7, 47.6, 152.6 8a	8a
108.9 (t)	4.60 (t, *J* 1.5 Hz)	19.3, 47.3, 150.8	2'
	4.68 (t, *J* 1.5 Hz)	19.3, 47.3, 150.8	
109.0 (t)	4.90 (d, *J* 1.3 Hz)	41.7, 45.7, 152.6	5'
150.8 (s)			1'
152.6 (s)			4

The less polar of the two previously unreported metabolites (69) had a molecular formula $C_{15}H_{24}$ by HREIMS. The compound thus had four double bond equivalents. The ^{13}C spectrum contained signals for four sp^2 carbons (108.9 (t), 109.0 (t), 150.8 (s), 152.6 (s) ppm) while the ^1H n.m.r. spectrum contained signals for two methyl groups (δ 0.80 (s), 1.67 (s)) the most downfield of which was adjacent to an sp^2 carbon. This indicated the presence of an exocyclic methylene and isopropylene unit, with the remaining ten carbons in a bicyclic ring sysytem.

Unambiguous assignment of protons to the carbon signals to which they are attached was established using an XHCORRD (J= 135 Hz) and the HMQC (J= 135 Hz) experiment (Table 23). Proton double resonance and a homonuclear correlation (COSYDQF) experiment allowed only part of the molecule composed of two fragments, to be established. This was due to the overlapping of a large number of signals in the ^1H n.m.r. spectrum. Cross peaks in the COSYDQF spectrum allowed a correlation to be made between the methyl proton signal δ0.80 and the methine proton signal δ1.97. Similarly the methine proton signal δ1.97 showed cross peaks to the methyl signal δ0.80 and the methine signal δ2.15. The methine proton signal δ2.15 showed cross peaks to the methine proton signals δ1.97 and δ2.74. The methine signal δ2.74 showed cross peaks to the methine signal δ2.15, the methylene proton signals δ1.80 and 2.02, and the methylene proton signal δ4.90, while the signal δ4.90 showed a cross peak to the methine signal δ2.74 (Fig 17. Fragment 1). The geminal methylene proton signals δ1.88 and 2.52 showed cross peaks to each other and to the methylene proton signals δ1.40 and 1.87. Similarly the geminal methylene proton signals δ1.40 and 1.87 showed cross peaks to the signals δ1.88 and 2.52 (Fig 17.



(69)

Fragment 2). Cross peaks were observed between the methyl signal δ1.67 and the methylene proton signals δ4.60 and δ4.68 while the methylene signals δ4.60 and δ4.68 showed cross peaks to each other and to the methyl signal δ1.67 (Fig 17. Fragment 3).

Long range correlations using an inverse heteronuclear multiple bond correlation experiment (HMBC, J= 7 Hz) were used to connect the fragments of the molecule and confirm the assignment of the carbon skeleton (Table 23). The methyl signal δ0.80 (14.5 ppm) showed correlations to the carbon signals 34.3, 36.9 and 49.4 ppm, while the methylene proton signal δ1.51 (34.3 ppm) showed correlations to the carbon signals 14.5, 28.3, 36.9, 45.7 and 49.4 ppm (Table 23). This confirmed the presence of a five membered ring and suggested that the compound was a guainane sesquiterpene. Further long range correlations (Table 23) confirmed the presence of a guaiane ring system (a fused five-seven bicyclic ring system) and the placement of the methylene and exocyclic isopropylene groups in the C4 and C7 positions in the molecule respectively (Table 23).

The relative stereochemistry of the molecule was established using a 2D homonuclear phase sensitive n.O.e. experiment (NOESYPH) and proton coupling data. The methyl signal δ0.80 showed cross peaks to the methylene proton signal δ1.48, the methyl signal δ1.67, the methine signals δ1.97 and 2.15 and the exocyclic isopropylene proton signal δ4.68. The methyl proton signal δ1.67 showed cross peaks to methyl signal δ0.80, a number of overlapping signals in the δ1.85-1.95 region of the spectrum, the methine signals δ2.07 and 2.15 and the exocyclic isopropylene signal δ4.60. The methine signal

δ2.15 showed cross peaks to the methyl signals δ0.80 and 1.67, a signal in the δ1.85-1.95 region of the spectrum and to the adjacent methine signal δ2.74. The above correlations and a cross peak from the methine proton signal δ2.74 to the adjacent methine signal δ2.15 confirmed the presence of *cis*-ring junction at the C3a and C8a positions of the molecule, the *cis*-conformation of δ2.15 (C8a) and the methyl signal δ0.80 (C4'), and the axial positioning of the exocyclic isopropylene group. The methine signal δ2.74 showed further cross peaks to the methylene signals δ1.48, 1.80 and 1.88. The methylene signal δ2.52 showed cross peaks to the methylene signals δ1.40 and 1.88 and to the exocyclic methylene signal δ4.90. The methylene proton signal δ4.60 showed cross peaks to the methyl signals δ0.80 and 1.67 while its geminal partner δ4.68 showed cross peaks to the methine signals δ2.07 and 2.15. These correlations and consideration of the chemical shift of the proton signals further confirmed the relative stereochemical of the molecule. This stereochemistry also agrees with the coupling constants observed for the methylene proton signal δ2.52 (dt, 2.9, 2.9, 12.8 Hz) and the methine signal δ2.74 (ddd, 9.1, 9.1, 9.2 Hz).

Guaiane sesquiterpenes have been previously reported from terrestrial sources (Fraga 1985, 1986, 1987, 1988, 1990a, b) and from gorgonians, algae and alcyonarians (Faulkner 1977, 1984, 1987). A computer-assisted search of the literature (CAS on-line) and an investigation of more dated literature (Stevens 1965, Chemical Abstracts to 1951) showed that this compound has not yet been reported. (69) is therefore the novel secondary metabolite (1*R**,3a*R**,7*S**,8a*S**)-7-isopropylene-1-methyl-4-methylene-1,2,3,3a,4,5,6,7,8,8a-decahydroazulene.

Table 24. ^{13}C and ^{1}H n.m.r. data for Helioporene A (70). Assignments are based on short range (*J* 135 Hz, XHCORRD) and long range (***J* 7 Hz, INAPT**, *J* 10 Hz, COLOC) ^{13}C-^{1}H correlations.

^{13}C (ppm)	^{1}H (δ)	^{13}C-^{1}H Long Range Correlations	Carbon number
10.9 (q)	2.01 (s)	115.4, 131.2, 142.9	8'
17.7 (q)	1.74 (d, *J* 1.1 Hz)	25.6, 129.7, 130.2	4'
20.8 (q)	1.03 (d, *J* 5.9 Hz)	39.7, 44.8	5'
22.3 (q)	1.27 (d, *J* 7.0 Hz)	32.3, 121.2	6'
25.6 (q)	1.67 (d, *J* 1.1 Hz)	17.7, 129.7, 130.2	3'
28.3 (t)	1.15 (brd, *J* 4.7, 11.7 Hz) 2.15 (m)		9
28.9 (d)	3.07 (ddd, *J* 7.0, 7.4, 7.4 Hz)		7
29.6 (d)	1.59 (m)	35.2, 39.7	1
32.3 (t)	1.40 (m) 2.17 (m)		8
35.2 (d)	3.58 (dt)	**29.6, 39.7, 115.4, 129.7 130.2, 130.6, 131.2**	3
39.7 (t)	1.65 (m) 1.65 (m)		2
44.8 (d)	2.01 (m)		9a
99.9 (t)	5.84 (d, *J* 1.3 Hz) 5.90 (d, *J* 1.3 Hz)		7'
115.4 (s)			4
121.2 (s)			6a
129.7 (s)			2'
130.3 (d)	5.10 (ddd, *J* 1.1, 1.1, 9.3 Hz)	**17.7, 25.6, 35.3, 131.2**	1'
130.6 (s)			6b
131.2 (s)			3a
142.9 (s)			6
142.9 (s)			5

The most polar of the three metabolites was the previously unreported compound (70). The compound had a molecular formula $C_{21}H_{28}O_2$ by HREIMS and thus contained 8 double bond equivalents. The ^{13}C n.m.r. spectrum contained signals for 8 sp^2 carbons (115.4 (s), 121.2 (s), 129.7 (s), 130.3 (d), 130.6 (s), 131.2 (s), 142.9 (s), 142.9 (s) ppm) while the ^1H n.m.r. spectrum contained signals for an aromatic methyl group (δ2.01 (s)), two allylic methyl groups (δ1.67 (d, *J*1.1 Hz), δ1.74 (d, *J*1.1 Hz)) and two aliphatic methyl groups (δ1.03 (d, *J*5.9 Hz), δ1.27 (d, *J*7.0 Hz)) (Table 24). This indicated that the compound was tetracyclic. The presence of a 2-methyl-1-propenyl group, and six quaternary sp^2 carbon signals, two of which were oxygenated (142.9 (s), 142.9 (s) ppm) suggested a fused ring system based on an aromatic ring, while the presence of a doubly oxygenated carbon signal (99.9 ppm (t)) and absorbances in the i.r. spectrum (υ_{max} 1089 cm^{-1}) indicated the presence of a cyclic ether (Fragment 1, Fig. 18). A 2D homonuclear correlation (COSYDQF) experiment allowed the remaining part of the molecule to be established (Fragment 2, Fig. 18), while unambiguous assignment of protons to the carbons to which they are attached was established using an XHCORRD experiment (*J*= 135 Hz) (Table 24).

Long range correlations using a 2D heteronuclear experiment (COLOC, *J*= 10Hz) and selective 1D heteronuclear experiments (INAPT *J*= 7 Hz) allowed connection of the fragments and confirmed the assignment of the carbon skeleton (Table 24). The methyl signal δ1.03 (20.8 ppm) showed correlations to the carbon signals 39.7 (C2) and 44.8 ppm (C9a), while the methyl signal δ1.27 (22.3 ppm) showed correlations to the carbon signals 32.3 (C8) and 121.2 ppm

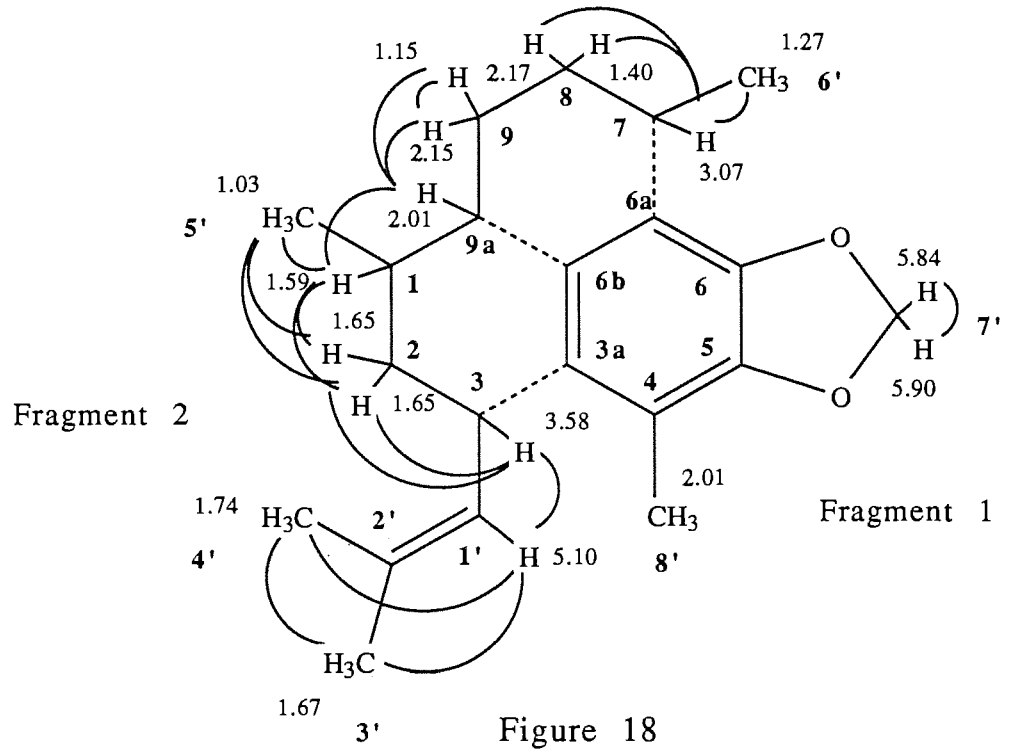
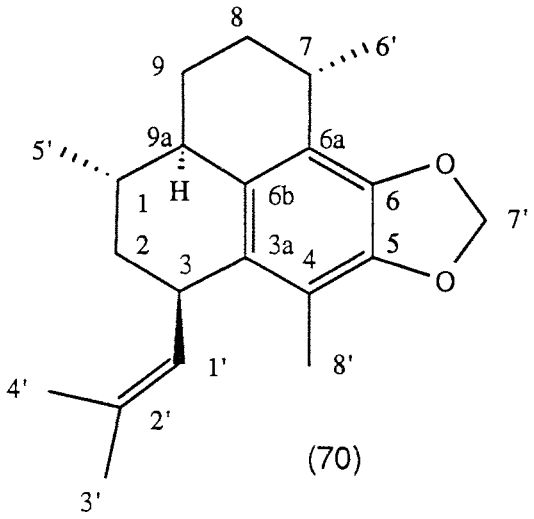


Figure 18

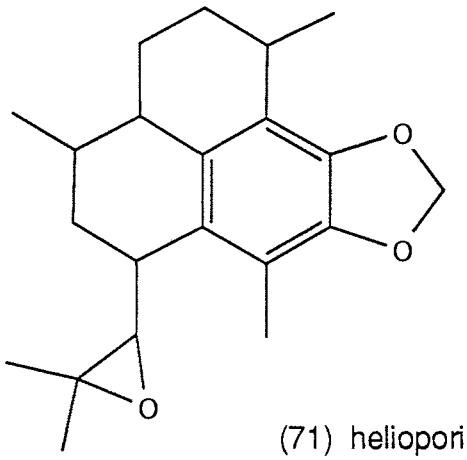
Crosspeaks between proton signals from COSYDQF spectra of (70)

(C6a). The methyl signals δ1.67 (25.6 ppm) and δ1.74 (17.7 ppm) showed correlations to the carbon signals 17.7 (C4'), 25.6 (C3'), 129.7 (C2') and 130.3 ppm (C1') confirming the presence of a 2-methyl-1-propenyl group. The methine signal δ1.59 (29.6 ppm) showed correlations to the carbon signals 35.2 (C3) and 39.7 ppm (C2). The connectivity between the carbon signals 35.2 (C3) and 131.2 ppm (C3a), and the assignment of carbon signals for the aromatic ring was established by long range correlations from the aromatic methyl proton signal δ2.01 and the methine proton signals δ3.58 and δ5.10. The methyl signal δ2.01 (10.9 ppm) showed correlations to the carbon signals 115.4 (C4), 131.2 (C3a) and 142.9 ppm (C5); the methine signal δ3.58 (35.2 ppm) showed correlations to the carbon signals 29.6 (C1), 39.7 (C2), 115.4 (C4), 129.7 (C2'), 130.3 (C1') , 130.6 (C6b) and 131.2 (C3a) ppm, while the methine signal δ5.10 (130.3 ppm) showed correlations to the carbon signals 17.7 (C4'), 25.6 (C3'), 35.2 (C3) and 131.2 ppm (C3a) (Table 24).

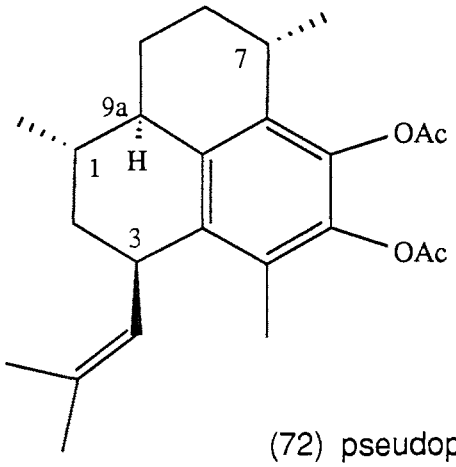
The compound (70) is related to the previously reported pseudopterosins (Look *et al.* 1986, Harvis *et al.* 1988), and seco pseudopterosins (Look and Fenical 1987), and to a similar unpublished metabolite helioporin A (71) isolated from an Okinawan collection of *Heliopora coerulea* and reported at the Pharmamar conference, Madrid (Higa, unpublished data 1990). The relative stereochemistry of (70) was determined by comparison of spectral data with those of related metabolites and was confirmed by a 2D homonuclear phase sensitive n.O.e. experiment (NOESYPH). (70) had very similar ^{1}H and ^{13}C n.m.r. data at the C1, C3, C7 and C9a chiral centres to that of the pseudopterosin diacetate (72) and other similarly related metabolites (Harvis *et al.* 1988). This suggested that (70) possessed



(70)



(71) helioporin A



(72) pseudopterosin diacetate

the same 1*S**,3*R**, 7*S** ,9a*R** configuration as (72) and other isolated pseudopterosins and seco-pseudopterosins. Correlations from the NOESYPH experiment confirmed this relative stereochemistry. (70) is therefore the novel secondary metabolite (1*S**,3*R**,7*S**,9a*R**)-3-(2'-methyl-1'-propenyl)-1,4,7-trimethyl-5,6-methylenedioxy-2,3,7,8,9,9a-hexahydrophenalene, which has been given the name helioporene A.

Chapters 7-8

Chemical Ecology

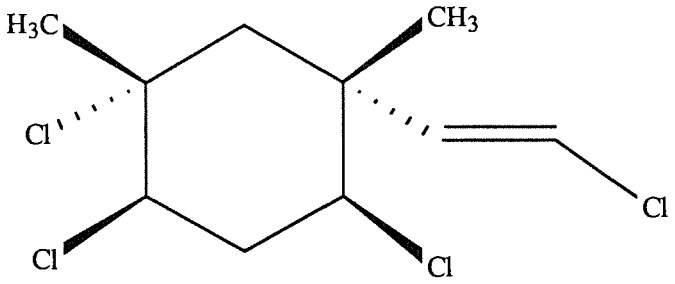
7. CHEMICALLY MEDIATED INTERACTIONS OF THE RED ALGA *PLOCAMIUM HAMATUM*

7.1. Introduction

Secondary metabolites have been shown to act as allelopathic agents in soft corals (Sammarco *et al.* 1983) and have been implicated as such in anemones (Sebens 1976, Bak and Borsboom 1984), sponges (Jackson and Buss 1975, Sullivan *et al.* 1983, Thompson 1985), ascidians (Bak *et al.* 1981) and scleractinian corals (Sammarco *et al.* 1985, De Ruyter van Stevenick *et al.* 1988). Algal secondary metabolites have also been implicated in allelopathy (Hellebust 1975, Fletcher 1975, Vadas 1979, Harlin 1987, see Chapter 1.2.1.3). There is however no direct evidence that secondary metabolites are the cause of any deleterious effects in the situations where allelopathy has been reported.

In the present study interactions between the red alga *Plocamium hamatum* J. Agardh (Rhodophyta) and other benthic organisms, especially soft corals, were investigated on an inshore fringing coral reef in the GBR. *P. hamatum* is one of the most abundant large fleshy algae in the area, and produces a number of secondary metabolites (Coll *et al.* 1988), of which chloromertensene, a tetrachlorinated monoterpene (73), is the predominant compound. The latter compound has been shown to be associated with very low feeding preference as assessed using herbivorous fishes (M. Hay pers. comm.).

Field observations of sessile reef organisms, including soft corals, sponges and gorgonians, living in close proximity to *P. hamatum*, revealed that wherever contact occurred, the invertebrates



chloromertensene (73)

suffered varying degrees of tissue necrosis. Accordingly the possible role of *P. hamatum* and of chemical constituents, especially chloromertensene, in the observed tissue necrosis was investigated in a series of manipulative field experiments. The results of an initial series of such experiments showed that all *P. hamatum* transplanted to sites with high levels of irradiance died back. In order to determine if light was an important contributing factor in the cryptic habit of *P. hamatum* and hence the need to compete for space in low light situations, a further field study was carried out to investigate the effect of light levels on the distribution of the alga.

7.2. Methods

7.2.1. Study Site and Organisms

Field experiments were conducted in the Pelorus Channel between Orpheus Island and Pelorus Island (18° 34'S, 146° 29'E), in the Palm Island group, 80 km north west of Townsville in the central region of the Great Barrier Reef during Nov-Dec, 1988. This area was also the collection site for the algal samples from which chloromertensene was first isolated (Coll *et al.* 1988). The study area is a fringing coral reef community dominated by soft corals, among which *Sinularia cruciata* (Tixier-Durivault), *S. flexibilis* (Quoy and Gaimard), *S. polydactyla* (Ehrenberg) and *Clavularia inflata* (Schenk) are the major species. *Plocamium hamatum* is the dominant large fleshy alga in this area and was most commonly found growing in rocky crevices or around and between large individuals of the soft corals listed above. Herbivorous fishes are also common in the area (Russ 1984).

7.2.2. Chemical Procedures

Details of the isolation and structural elucidation of chloromertensene have been described previously (Coll *et al.* 1988). In the present study the purity of chloromertensene was established by ^{1}H n.m.r. spectroscopy prior to use.

7.2.3. Field Observations of Interactions

After the initial observation of tissue necrosis in a number of soft coral species apparently caused by contact with the red alga *Plocamium hamatum* (Bowden *et al.* 1989), a field survey was carried out in the same region to determine the generality of this phenomenon. The survey recorded incidences of necrosis whenever *P. hamatum* was in contact with invertebrates.

7.2.4. Manipulative Experiments

7.2.4.1. Effects of *Plocamium hamatum* on *Sinularia cruciata*.

In order to test the effect of *Plocamium hamatum* on *Sinularia cruciata*, healthy intact plants of *P. hamatum* (7-10 cm tall) with attached substratum were carefully collected and transferred to three galvanised wire grids (2 m^2 in area, of mesh size 250 mm x 50 mm) anchored to the reef. An equal number of colonies of the soft coral *Sinularia cruciata* were collected and relocated to each of the grids. The grids were sited within 5 m of the collection site and at the same depth (3 m). Two treatments were used, one in which *P. hamatum* was placed in direct physical contact with *S. cruciata* (n=6), and one in which *P. hamatum* was placed so that it was unable to make contact with the soft coral under any current

conditions, i.e., in close proximity to (<5 cm) but not in direct contact with the coral (n=6). Relocated controls of *P. hamatum* (n=6) and *S. cruciata* (n=6) were also attached to the grid so that each individual was at least 30 cm from any other. Other control colonies of *S. cruciata* were labelled and left in their natural environment as field controls either in direct contact with *P. hamatum* (n=6) or not in direct contact with *P. hamatum* (n=6). *S. cruciata* colonies were observed after 24 hr, 48 hr and 14 days and tissue necrosis recorded. Necrosis was considered to have occurred when the tissue darkened and sloughed from the surface of the colony. Further observations were made after six weeks. The results were analyzed using a two-tailed Fischer exact test (Zar 1984).

7.2.4.2. Effects of Chloromertensene on *Sinularia cruciata*.

In order to determine whether the necrotic effects of the alga on the soft coral tissue were chemically induced, fronds of synthetic aquarium "plants " [dried and dyed colonies of the bryozoan *Sertularia argentea*, supplied by Aquafern, England], were coated with a solution of chloromertensene dissolved in diethyl ether. The diethyl ether evaporates leaving the chloromertensene adhering to the fronds. The final concentration of chloromertensene on the "plants" was 2%. This is approximately the average concentration of chloromertensene recorded in natural populations of *Plocamium hamatum*, which varies between 0-5 % of the freeze dried weight of the alga (Wright 1988). Other specimens were coated with diethyl ether only to act as controls. Lipid soluble metabolites such as chloromertensene have been shown to adhere to surfaces after the diethyl ether evaporates, and only slowly diffuse into aqueous

media such as seawater (McConnell *et al.* 1982, Targett *et al.* 1986, Hay *et al.* 1987b, Paul *et al.* 1987). Four replicates of chloromertensene coated and diethyl ether treated control "plants" were attached as previously described to grids in the field so that they were in contact with *Sinularia cruciata* . The *S. cruciata* colonies were examined for tissue necrosis or other deleterious effects after 24 hr, 48 hr and 14 days. Further observations were made after six weeks. The results were analyzed using a two-tailed Fisher exact test (Zar 1984).

7.2.5. The Relationship between Irradiance levels and the Distribution of *Plocamium hamatum*

7.2.5.1. Transect Studies

To further investigate the habitat requirements of *P. hamatum* studies were carried out using three 20m x 1m belt transects parallel to the depth gradient on the coral reef flat adjacent to the study site. The percentage area covered by soft corals, hard corals, gorgonians other marine organisms, coral rubble and sand was recorded. The number of intact *P. hamatum* plants was recorded and calculated as the density of *P. hamatum* in number of plants per square metre of substrate category. The data was transformed using a logarithmic transformation (y: log (1+x)) and analysed using a one factor analysis of variance (ANOVA) followed by a multiple range (Student-Newmann-Keuls (SNK)) test (Zar 1984).

7.2.5.2. Effects of Light on Algal Distribution

In order to determine if light was an important factor in determining the cryptic habit of *Plocamium hamatum* and hence the need to compete for space in low light situations, a series of *in-*

situ experiments was carried out to determine the tolerance of *P. hamatum* to different levels of irradiance. Light measurements were carried out at fifteen sites on the transplant grids where *P. hamatum* was placed, at the sites of the field controls of *P. hamatum* and at the sea surface prior to and after every set of measurements. The irradiance recorded at different sites was expressed as a percentage of the surface irradiation measured at the beginning of each series of measurements. The light levels were measured using a LI-COR Inc model LI188B quantum meter, and the irradiance ($\mu E\ m^{-2}\ s^{-1}$) integrated over a 10 second period. Measurements were taken from 1300-1400 hr on three cloudless days during summer, a period of high irradiance levels (06/x/88, 20/x/88, 28/xi/88). The apparent deleterious effect of excess light on *P. hamatum* was measured as the presence or absence of bleaching of the alga. No effort was made to quantify the extent of bleaching. The results were analysed using a two-tailed Fischers exact test (Zar 1984).

7.2.6. **Pharmacological Testing**

Due to the ecological observations associated with *Plocamium hamatum*, chloromertensene was tested in the following pharmacological assays. The compound was tested against P338 murine leukemia cells, the *Polio* and *Herpes simplex* virues, the bacteria *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, the fungus *Candida albicans*, and for mammalian cell cytotoxicity.

7.3. Results

7.3.1. General Field Observations of Interactions

Field observation of a number of soft coral species [*Sinularia flexibilis* (n=12), *S. cruciata* (n=14), *S. polydactyla* (n=4), *and Clavularia sp.* (n=2)], a sponge [*Dictyoceratida sp.* (n=1)] and a gorgonian [*Isis* sp. (n=5)] revealed that the invertebrates suffered tissue necrosis whenever they were in contact with the alga *Plocamium hamatum*. In contrast close association between soft coral species (*S. flexibilis* and *S. cruciata*) and the green alga *Chlorodesmis fastigata* (n=9) did not result in necrosis.

7.3.2. Manipulative Experiments

7.3.2.1. Effects of *Plocamium hamatum* on *Sinularia cruciata*

Tissue necrosis was recorded in all cases where *Plocamium hamatum* was in contact with *Sinularia cruciata* (Fig. 19) (Fischer exact test, $p < 0.01$) (Fig. 20). The initial response of *S. cruciata* to contact with *P. hamatum* was polyp withdrawal, followed by thinning of the surface mucus layer after 24 hours. After 48 hours, tissue hardening and darkening was evident, which eventually led to localised tissue death in all treatments in which *S. cruciata* was in direct contact with the alga (Fig. 19a). After six weeks, necrosis in some *S. cruciata* colonies had occurred to such an extent that spicules were exposed (e.g. Fig. 19b). Spicule exposure was only observed when larger *P. hamatum* plants were in contact with *S. cruciata* colonies. None of the relocated colonies of *S. cruciata* which were out of direct contact with *P. hamatum* (5 cm or 30 cm separation) suffered any type of tissue necrosis (Fischer exact test,

Figure 19. Allelopathic effects on *Sinularia cruciata* (polyp diameter approximately 2 mm)

(A) Polyp withdrawal and tissue necrosis in lobe of *S. cruciata* after 48 h contact with *Plocamium hamatum* ;

(B) Exposure of spicules of *S.cruciata* resulting from tissue necrosis after prolonged contact with *P. hamatum*;

(C) Tissue necrosis (arrowed) in parts of a colony of *S. cruciata* after 48 h contact with Aquafern^{R} coated with chloromertensene

(A)

(B)

(C)

p<0.01) (Fig. 20). In two cases, *P. hamatum* plants which were originally out of direct contact with *S. cruciata* (>5 cm) grew into contact with the coral after about six weeks and necrosis occurred. Control colonies of the soft coral were unchanged throughout the course of the experiment.

7.3.2.2. **Effects of Chloromertensene on *Sinularia cruciata***

In all cases, portions of colonies of *Sinularia cruciata* in contact with aquarium "plants" coated with chloromertensene showed tissue necrosis after 48 hours (Fig. 19c) (Fischer exact test, $p < 0.05$) (Fig 21). No tissue necrosis was induced by the controls treated with diethyl ether alone (Fischer exact test, $p < 0.05$) (Fig. 21), although mucus loss occurred in some areas of the soft coral in contact with the "plants", possibly due to abrasion. The severity of tissue necrosis in *S. cruciata* in direct contact with the aquarium "plants" coated with chloromertensene was greater after 14 days than after 48 hours, although it was less severe than that resulting from contact with *Plocamium hamatum* for the same period. After 48 hours, two of the four diethyl ether control "plants" had decreased in size, apparently due to grazing, and the remaining parts had become epiphytized by algae and small sessile invertebrates. After two weeks, all the diethyl ether controls had either entirely disappeared, or consisted only of the lower parts of the thicker central axes. In contrast, all "plants" coated with chloromertensene were intact after two weeks with few epiphytes present. From field observations, the only commonly occurring fouling organism on healthy *P. hamatum* is the foraminifera *Calcarina sponglera*.(Carpenter) L.

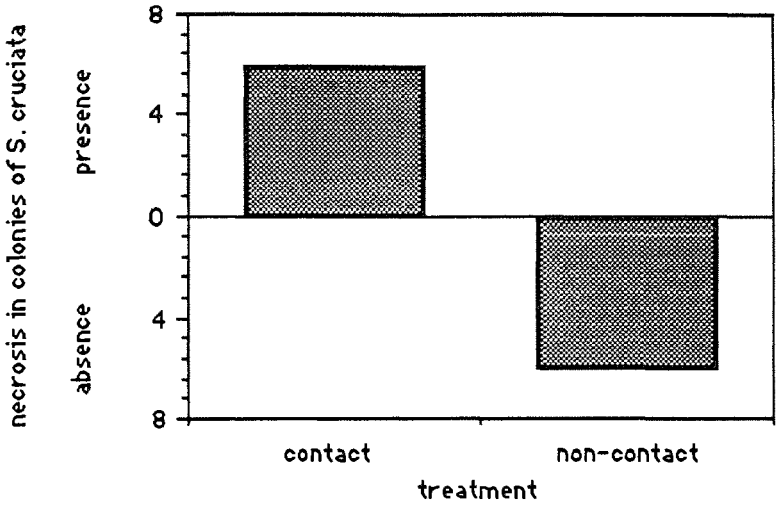


Figure 20. The effect of intact individuals of *Plocamium hamatum* in contact and non-contact (control) relationships with *Sinularia cruciata*, recorded as the presence and absence of necrosis of soft coral tissue (n=6 in both cases, Fischer exact test, $p<0.01$).

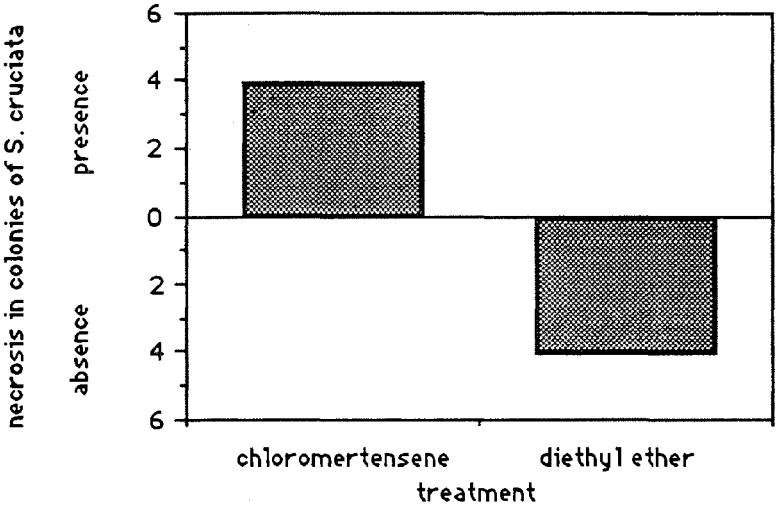


Figure 21. The effect of chloromertensene and diethyl ether (control) coated "plants" in contact with *Sinularia cruciata*, recorded as the presence and absence of necrosis of soft coral tissue (n=4 in both cases, Fischer exact test, $p<0.05$).

7.3.3. The Relationship between Irradiance levels and the Distribution of *Plocamium hamatum*

7.3.3.1. Transect Results

The distribution of *Plocamium hamatum* is dependent on substrate type (ANOVA, $p<0.001$, Figs 22a and b). *P. hamatum* was found to occur only on coral rubble and attached to the sterile basal regions of the soft coral species *Sinularia cruciata, S. flexibilis, S. conferoides, and S. polydactyla*, and the hard coral *Acropora formosa* (Fig. 22a). *P. hamatum* was not recorded in association with the soft coral *Clavularia* sp., the hard corals *Montipora digitata, Stylophora* sp., *Pectinia* sp., an unidentified species of hard coral, the gorgonian *Isis* sp., or where the substrate was sand (Fig. 22a). The density of *P. hamatum* was significantly different on different substrate categories (ANOVA, $p< 0.05$). The substrate categories coral rubble, *S. flexibilis*, *S. polydactyla* and *S. conferoides* supported a high density of *P. hamatum* (e.g. were significantly different from those which did not support *P. hamatum*). However, the substrate categories *S. conferoides* and *A. formosa* were not significantly different from those which did not support the alga (ANOVA, $p< 0.05$, Fig. 22a). *P. hamatum* only occurred in association with *A. formosa* in one small patch of coral on one of the three transects.

Due to the morphological similarity of three of the four species of the soft coral genus *Sinularia*, *S. cruciata, S. conferoides* and *S. polydactyla,* the data for these three species was combined and the data set re-analysed. This resulted in four substrate categories which suppported *P. hamatum*, namely rubble, *S. flexibilis,* the remaining *Sinularia* spp. combined, and *Acropora formosa* (Fig. 22b). The substrate categories rubble, *S. flexibilis* and *Sinularia* spp.

Figure 22 (a and b). The density of *Plocamium hamatum* on different categories of substrata (expressed as the number of individuals per m^2 of substrate). Mean (log 1+x) density values (bars) and standard errors (vertical lines) are shown. Horizontal lines link substratum types for which occurrence of *P. hamatum* was not significantly different (single factor ANOVA followed by SNK multiple range test, p=0.05; see text for further details)

Figure 22a.1= coral rubble, 2= *S. flexibilis*, 3= *S. polydactyla*, 4= *S. cruciata*, 5= *S. conferoides*, 6= *Acropora formosa*, 7= sand, 8= *Clavularia* sp., 9= *Montipora digitata*, 10= hardcoral x, 11= *Pectina* sp., 12= *Isis* sp., 13= *Stylophora* sp.

Figure 22b. 1= coral rubble, 2= *S. flexibilis*, 3= *S. polydactyla*, *S. cruciata* and *S. conferoides* combined, 4= *Acropora formosa*, 5= sand, 6= *Clavularia* sp., 7= *Montipora digitata*, 8= hardcoral x, 9= *Pectina* sp., 10= *Isis* sp., 11= *Stylophora* sp.

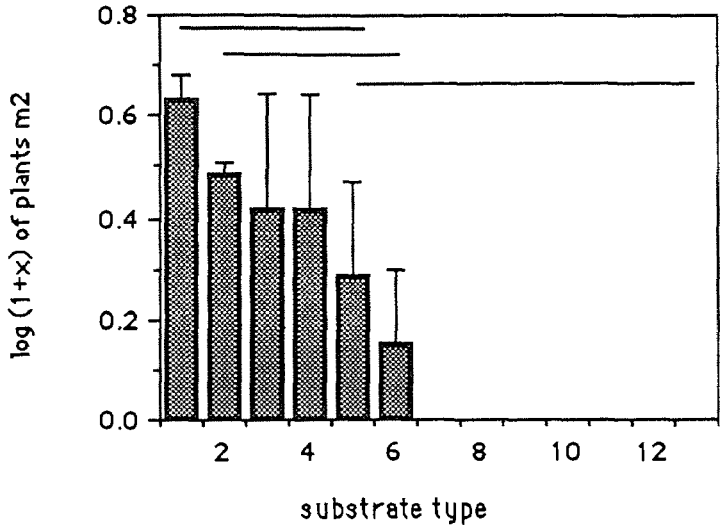


Figure 22a.

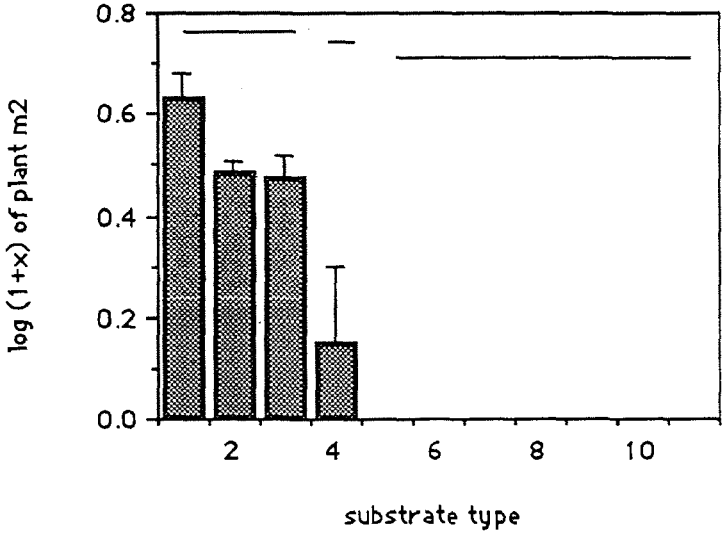


Figure 22b.

combined were significantly different from *A. formosa* and all other substrate categories, while *A. formosa* was significantly different from all other substrate categories (ANOVA, $p < 0.05$, Fig. 22b). This resulted in three distinct substrate groupings, the first which supported a high density of *P. hamatum*, the second with a lower density and the third which did not support the alga (Fig. 22b).

7.3.3.2. Light Measurements

Bleaching of *Plocamium hamatum* tissue occurred in all individuals where irradiance was recorded as greater than 17% of the incident surface value (n=8) (Fischers exact test, $p < 0.01$). At lower light levels no bleaching was observed in individuals at any of the measurement sites (n=8) (Fischers exact test, $p < 0.01$) (Fig. 23). Irradiance levels at the field controls were consistently below 10% of surface incident values. Although no attempt was made to quantitatively assess the degree of bleaching by higher light, it was observed that at all sites where *P. hamatum* was reduced to basal tissue, the light levels recorded were between 43% and 55 % of the surface incident values. The highest light level recorded at any of the study sites was 55% of the surface incidence value. Further observations of *P. hamatum* at moderate light levels (between 17% and 30 % of the surface incident values) showed that although bleaching occurred, the algae was still capable of causing tissue necrosis in associated soft corals. Similar observations were made on *P. hamatum* in the field.

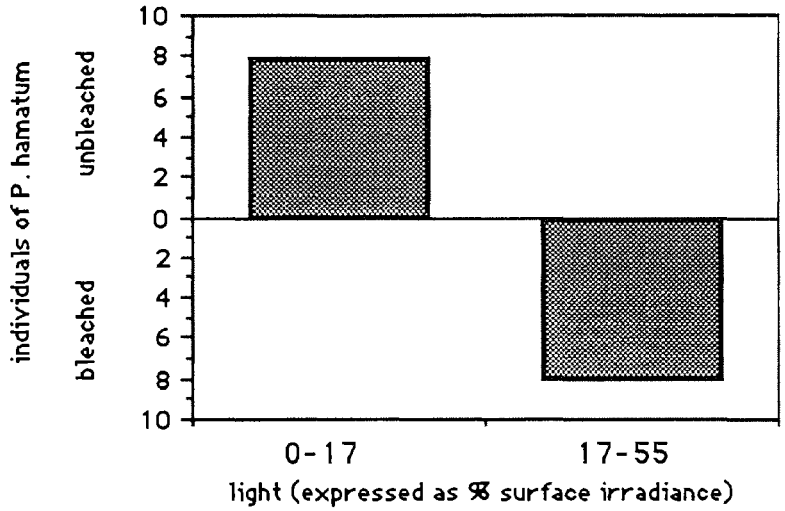


Figure 23. The relationship between light and the presence and absence of bleaching in *P. hamatum*.

7.3.4. Pharmacological Testing

The results of testing chloromertensene in pharmacological bioassays are as listed below:

P338 Murine Leukaemia cell test: the lethal dose was > 25 μg/ml and the compound is therefore considered inactive by the standards of the National Cancer Institute, U.S.A.

Antiviral assays: No activity was recorded against *Herpes simplex* virus or *Polio* virus at a concentration of 40 μg/disk and no cytotoxicity against host mammalian cells was evident.

Antibacterial and antifungal assays: No activity was recorded against the bacteria *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and the fungus *Candida albicans* at a concentration of 60 μg/disk.

7.4. Discussion

The high species diversity and population densities on coral reefs suggests that space can be a limiting resource for benthic reef organisms (Glynn 1973, Porter 1974, Jackson 1977, Sheppard 1980, Benayahu and Loya 1981). Consequently a number of mechanisms have evolved which enable particular species to compete successfully for space (see reviews by Sheppard 1982, Sammarco and Coll 1988), one of which is allelopathy (Sammarco and Coll 1988). Macroalgae are generally considered important competitors for space on reefs, often being among the first organisms to occupy newly available space, and some reef algal species have been shown

to limit the settlement of coral larvae (Dart 1972, Birkeland 1977), or to kill juvenile corals by overgrowing them (Potts 1977; Sammarco 1980, 1982; Bak and Engel 1979; Van Moorsel 1985), or by entrapping sediment (Sammarco 1980). Overgrowth of larger coral colonies by algae has also been observed (Connell 1973, Glynn 1973, Dahl 1974, Coll *et al.* 1987) and demonstrated experimentally (Potts 1977, Sammarco 1982, Lewis 1986). Competitive interactions between microscopic planktonic algae have been described for a number of species (see reviews by Fogg 1966 and Hellebust 1975), and the concept of a "chemical claw" mechanism of interspecific competition between macroscopic marine algae was proposed as early as 1948 by Walker and Smith. This mechanism was invoked to explain the effect of the exudates from *Ascophyllum nodosum* on zoospores of *Laminaria cloustoni*. Fletcher (1975) observed that growth of the crustose red algae *Porphyrodiscus simulans* and *Rhodophysema elegans* was inhibited in culture by ectocrines of *Ralfsia spongiocarpa*, a species known to liberate considerable quantities of tannins into tide pools (Conover and Sieburth 1966). Khfaji and Boney (1979) demonstrated that diatom growth was inhibited in the vicinity of *Chondrus crispus* germlings grown in culture. Algal secondary metabolites have been shown to be exuded into the water column by a number of algae (Carlson and Carlson 1984, Gschwend *et al.* 1985), but there is no direct evidence to date that secondary metabolites are released from the algal species involved in these interspecific interactions, or that algal secondary metabolites are the cause of the observed deleterious effects in these competitive situations.

In the present study, field observations and manipulative experiments have established that contact between the alga

Plocamium hamatum and the soft coral *Sinularia cruciata* is necessary for tissue necrosis to occur, and that the necrosis is largely caused by the major secondary metabolite produced by the alga, chloromertensene. Although the observed necrosis in *S. cruciata* caused by chloromertensene coated on artificial fronds was less severe than that caused by *P. hamatum*, this was not unexpected, as the living alga continues to synthesise the compound (Wright 1988). After prolonged (six weeks) exposure to *P. hamatum*, spicules of *S. cruciata* were visible, and secondary algal infection of the dead tissue was observed, indicating a loss of the normal resistance of the soft coral to algal overgrowth (Coll *et al.* 1987). Our experiments show that chloromertensene is not released into the water column in sufficient quantities to effect organisms in close proximity, but that direct contact is necessary for necrosis to occur ($p<0.0025$). Chloromertensene may be localised in cytoplasmic vesicles in the surface cells of the alga (see Young *et al.* 1980), which may be excised or burst when these cells are abraded.

It appears that chloromertensene may also function in the alga as an antifouling agent. In the experiment involving chloromertensene coated test "plants", it was found after 48 hr that a number of the uncoated controls became colonized by epiphytes and grazed by fish, while those coated with chloromertensene were not colonised by epiphytes and remained intact. After 14 days the "plant" controls had been completely consumed while the chloromertensene coated "plants" were still intact. It is not known if the fish were avoiding the coated "plants" in our experiment due to the absence of epiphytes as a food source, or due to presence of chloromertensene as a feeding deterrent, although chloromertensene has been

shown in certain circumstances to be a feeding deterrent (M. Hay pers. comm.).

Studies of other macroalgae associated with soft corals, such as *Chlorodesmis fastigata,* an alga known to produce secondary metabolites (Wells and Barrow 1979, Paul and Fenical 1985), did not reveal any comparable contact-induced allelopathic interaction between the alga and associated biota, suggesting that the allelopathic effects observed in *Plocamium hamatum* may be species-specific in a particular environmental niche. This is consistent with the hypothesis of Whittaker and Feeney (1971) that chemical defenses play an important role in niche differentiation and control of community structure in numerous ecosystems. Chloromertensene thus appears to play a multifunctional role in the success of the red alga *Plocamium hamatum* (de Nys *et al.* 1991a). It plays an allelopathic role by preventing overgrowth by the locally dominant soft corals, and appears to act as an antifouling and antifeedant agent further ensuring success of the alga in suitable habitats.

Although the results of this study show that chloromertensene has a number of ecological roles, it was not active in any of the pharmacological assays. Hay *et al.* (1989a) showed a similar lack of correlation between compounds which play an ecological role in natural communities and those which are pharmacologically active.

In the second series of field experiments relating habitat to light levels it is apparent that *P. hamatum* is adversely effected by levels of irradiance above approximately 20% of surface incidence

values. This became evident when a preliminary field experiment was carried out. With the algae set up on a grid fully exposed to surface irradiation, the algae (and not the corals) died back. The experiment was relocated to a "shaded site" beneath a large coral overhang in order to reduce irradiance below 20% of surface levels. Under these conditions the algae thrived and necrosis was observed. While there is some information regarding the photosynthetic adaptation of algae to decreased light at depth (Drew 1983, Kirk 1983, Larkum and Barrett 1983, Dayton 1985), less is known of the role of irradiance levels in restricting the upper limits of algal distributions. The inhibition of photosynthesis by high irradiance has been well documented for phytoplankton (Harris 1980) and has been demonstrated in certain rhodophytes (Mathieson and Dawes 1974, King and Schramm 1976, Ramus and Rosenberg 1980). The inhibition of growth due to high irradiance levels has also been reported for a number of red algal species. Bieble (1957) reported extensive cytomorphological damage and death of sublittoral red algae at high irradiance within two hours, while intertidal species survived the same light level without detectable effect after 24 hours. Inhibition of growth by high irradiance has been reported for the sporelings of red algal species (Boney and Corner 1962, 1963; Jones and Dent 1971) and adult *Eucheuma* sp. (Polne *et al.* 1981). The presence of "shade loving" (sciaphilic) or obligate understory species (Larkum *et al.* 1967, Sears and Cooper 1978) which are susceptible to high levels of irradiance has been experimentally demonstrated (e.g. Dayton 1975). The genus *Plocamium* has been described as a "shade lover" from observations of its distribution (Boudouresque 1969, 1970). Dayton (1975) included *Plocamium coccineum* as part of the subtidal obligate understory species, which

die completely or die back to the holdfast due to increased light levels when the algal overstory (canopy) is removed. Similarly Kain (1987) experimentally demonstrated the limitation of growth of *Plocamium cartilagineum* by high levels of irradiance when removed from the canopy understory to more exposed locations.

This study shows *Plocamium hamatum* to be a "shade loving" species. Exposure to irradiance levels above 20% of surface irradiation values led to bleaching of the alga and in some cases breakdown of all but the basal tissue. The species is thus restricted to habitats which provide a refuge from high irradiance levels. These conclusions are confirmed by the results of the transect studies. *P. hamatum* was most commonly found amongst coral rubble substrate or beneath soft corals of the genus *Sinularia* (Figs. 22a and b). These organisms have massive morphologies and thus provide more shading (i.e. lower light levels) than do organisms which have more open bush-like (e.g. *Montipora digitata*) and plate-like morphologies (e.g. *Pectinia* sp.), or less irregular substrates (e.g. sand). These shaded situations thus provide the habitat in which the continued growth of *P. hamatum* can take place. There is no evidence to suggest that *P. hamatum* preferentially settles or grows in association with the genus *Sinularia* for any other reason than as a refuge from high levels of irradiance. *P. hamatum* was recorded in contact with, and causing necrosis in, a range of invertebrates during preliminary field studies carried out to determine the generality of necrosis produced by *P. hamatum*. A number of these interactions were recorded under large overhangs and other shaded areas as opposed to the open reef flat where the transect studies were later undertaken. *P. hamatum* was sometimes observed to

occur on sand in some of these locations although it is possible that the instability of this substrate as well as high levels of irradiance may affect the presence of the alga. Soft corals including *Sinularia flexibilis* and *Sinularia* spp. have been shown to be highly space competitive organisms overgrowing scleractinian corals (Nishihira 1981, La Barre and Coll 1982, Sammarco *et al.* 1985). It is suggested that the requirement for shade in the growth of *P. hamatum*, and the competition for space between the alga and the much larger shade providing organism (in this case *Sinularia* spp.) has selected for an allelopathic mechanism which allows the algal species to successfully persist in the highly competitive coral reef environment.

8. CHEMICAL DEFENCES OF THE TEMPERATE RED ALGA *DELISEA PULCHRA*

8.1. Introduction

Algal secondary metabolites deter feeding by a range of marine herbivores including echinoderms, molluscs and fishes (Hay and Fenical 1988). In the few studies in which the herbivore deterrent properties of purified algal secondary metabolites have been tested under field conditions, the deterrent effects of a compound were a specific function of the structure of the compound and the species of herbivore; "ecological function could not be predicted from structural class alone" (Hay *et al.* 1989a). Structurally similar compounds also differ in their effect on food choice by herbivores (Hay *et al.* 1987a, b, 1988,1989a, b; Paul *et al.* 1987; Hay *et al.* 1989a; Paul *et al.* 1989), and metabolites which deter feeding by one herbivore may act as a stimulant for another (Hay *et al.* 1989, Paul *et al.* 1989). Similarly, secondary metabolites vary in their deterrent effect on feeding by juvenile and adult forms of the same herbivore species (Paul *et al.* 1990).

Delisea pulchra (Greville) Montagne (Rhodophyta) is among the most abundant foliose red algae in sub-littoral habitats at Cape Banks, Sydney, New South Wales (Underwood *et al.* 1982, de Nys pers obs.), and is a low preference algal food source for the generalised herbivorous mollusc *Turbo undulata* (Phylum Mollusca, Class Gastropoda) and the generalist echinoid *Tripneustes gratilla* (Phylum Echinodermata, Class Echinodea) (Steinberg and van Altena, in press). Growth rates of *Turbo undulata* and *Tripneustes gratilla* were also low when fed *D. pulchra* (Steinberg and van

Altena in press). The rate of growth of *Tripneustes gratilla* under these conditions was not significantly different from that of starved urchins (Steinberg and van Altena in press). *Delisea pulchra* has no apparent physical defence against herbivory but produces a number of secondary metabolites which possess strong antifungal and antimicrobial properties (Chapter 2.3). In order to investigate the potential role of these compounds as chemical defences against herbivores, the feeding-deterrent properties of crude extracts and purified metabolites from the alga were investigated in a series of feeding choice experiments using the herbivores *Turbo undulata* and *Tripneustes gratilla.*

8.2. Methods

8.2.1. Study Site and Organisms

Field experiments were conducted at the University of Sydney Marine Scientific Research Area, Cape Banks, Sydney, New South Wales, Australia (34° S,151°11' E). This was also the site of collection of *Delisea pulchra* for chemical analyses. The study area is a subtidal sandstone shelf with *Sargassum* spp., *Ecklonia radiata,* and *Delisea pulchra* as the dominant algal species (Underwood *et al.* 1982, de Nys pers. obs.) (Fig. 24a). The benthos of this area has been well studied and documented (Fletcher 1987). *Tripneustes gratilla, Turbo undulata,* and the echiniods *Centrostephanus rodgersii* and *Heliocidaris* spp. are the dominant herbivore species (Fig. 24b). *Turbo undulata* inhabits the shallow sub-littoral and lower-littoral zones (Worthington and Fairweather 1988) while *Tripneustes gratilla* is restricted to the sub-littoral zone.

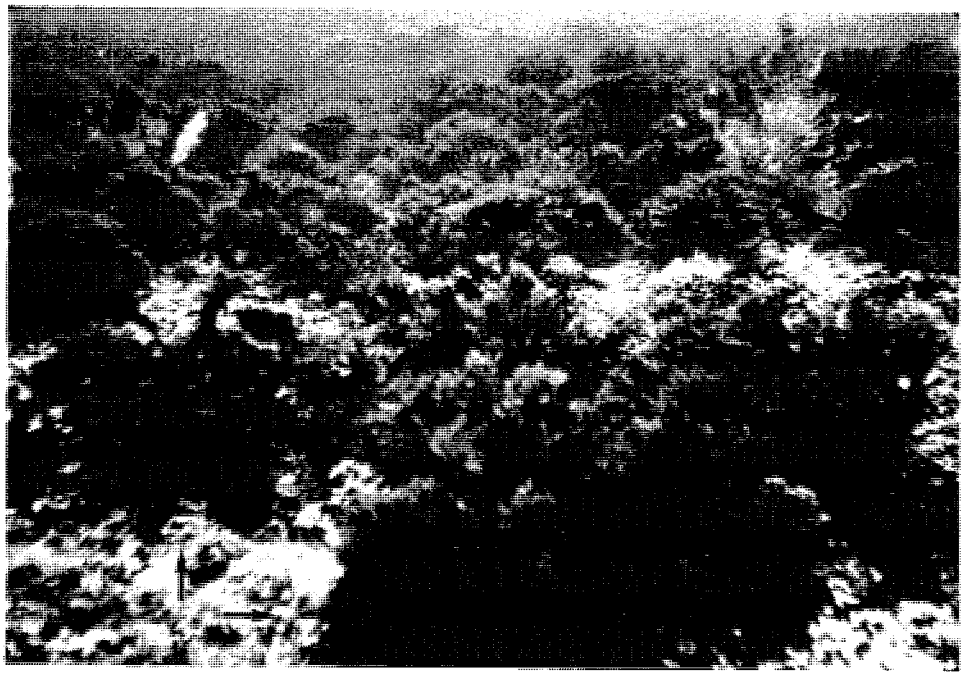
Figure 24. Detail of the experimental area at Cape Banks, N.S.W., and the experimental apparatus used in the feeding deterrence bioassays.

(A) Dense population of *Delisea pulchra* (arrowed) in the vicinity of the experimental area (*D. pulchra* thalli height appprox. 15 cm).

(B) The echinoderm *Centrostephanus rodgersii*, and the alga *D. pulchra* adjacent to a 'barren zone' resulting from grazing.

(C) Experimental apparatus showing *Phyllospora comosa* samples being placed in a prawn crate. Each crate is divided into four individual chambers, with each chamber containing the herbivore used in the bioassay.

(A)



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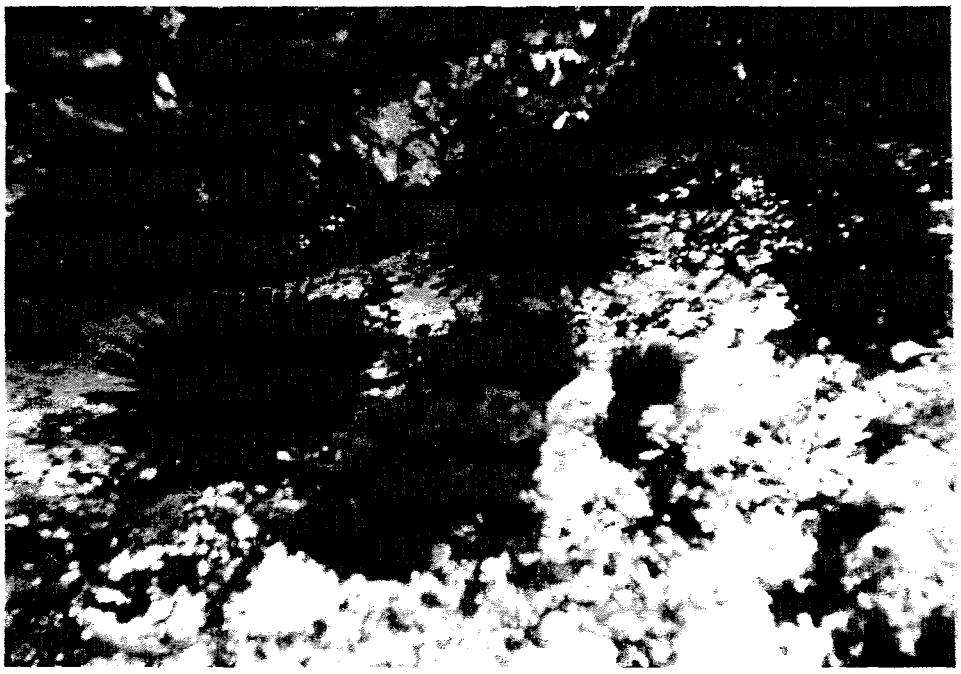

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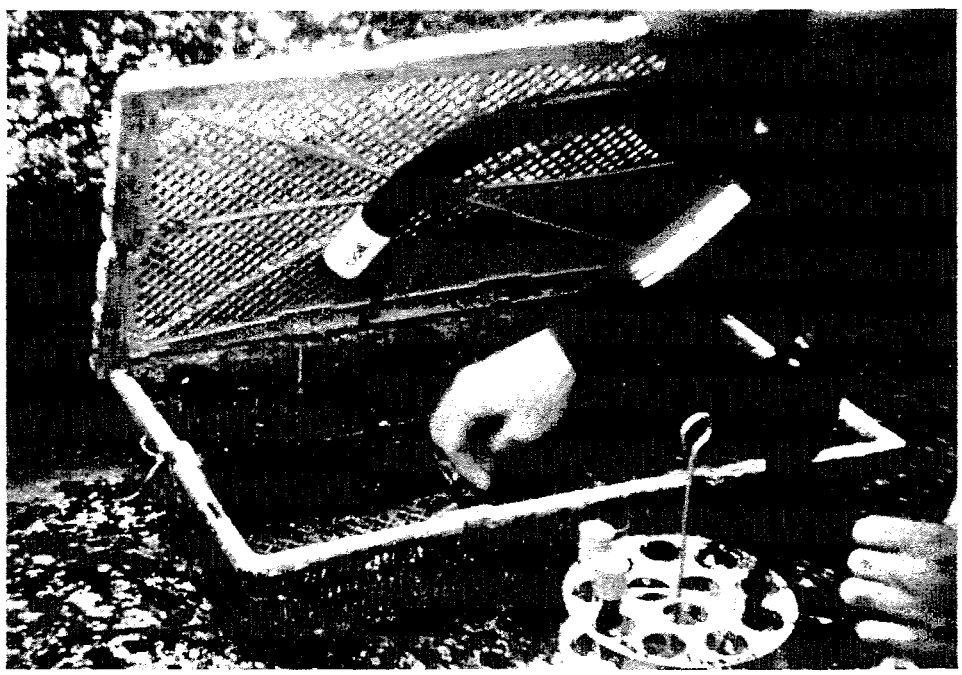


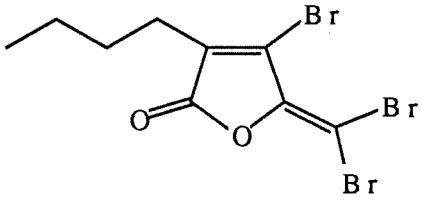
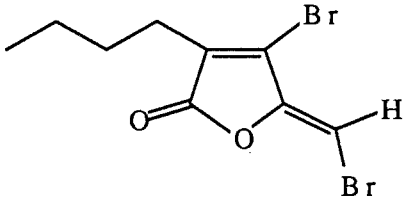
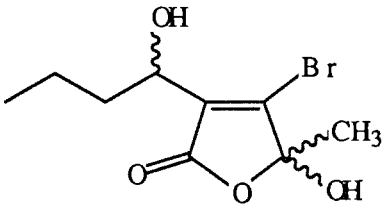
8.2.2. Chemical Procedures

The isolation and structural elucidation of the secondary metabolites from *D. pulchra* is detailed in chapter 2.3. of this thesis. The purity of all compounds used in feeding deterrence bioassays was determined by ^1H n.m.r. prior to use. The crude extract used in bioassays was prepared from a fresh collection of *D. pulchra* taken from the study site directly prior to extraction (03/ix/90).

8.2.3. Feeding Deterrence Bioassays

Whole plants of *Phyllospora comosa* (Phaeophyta), were collected fresh from Cronulla, Sydney for each experiment. *P. comosa* is a preferred food of the herbivores *Turbo undulata* and *Tripneustes gratilla* (Steinberg and van Altena, in press). The laterals from freshly collected *P. comosa* thalli (0.50-0.80 g) were removed from the plants, grouped in samples of three, blotted with paper tissues to remove excess water, and weighed. Laterals were sorted such that groups of three weighed between 1.8-2.5 g. Each sample was then treated (coated) with a solution of either the crude extract of *Delisea pulchra*, or with isolated metabolites at the concentration at which they were extracted from the alga. The crude extract and isolated metabolites were dissolved in diethyl ether such that a 100 µl application of solution on each side of the thallus sections produced a loading equivalent (µg/g fresh weight) to the natural concentration of the extract or metabolite isolated from *D. pulchra*. The diethyl ether evaporates leaving the metabolites adhering to the fronds (see Chapter 7). The metabolites and concentrations used in the experiments are presented in Table 25. Untreated control samples were coated with an equal quantity of diethyl ether only. Experiments were also carried out to determine the effects of

Table 25. Crude extract and secondary metabolites from *Delisea pulchra* investigated as feeding deterrents against *Tripneustes gratilla* and *Turbo undulata*

material	structure	total weight (mg)	as % fresh (% **dry**) weight	as % applied to sample
crude extract	various	1900	0.2 % = 2000ppm (**1.1 %**)	0.2 % = (2000 ppm)
(56) see Chp 2.3.		53.6	0.0054 % = (54 ppm) (**0.032 %**)	0.006 % = (60 ppm)
(57) see Chp 2.3.		44.3	0.0044 % = (44 ppm) (**0.027 %**)	0.0050 % = (50 ppm)
(59) see Chp 2.3.		122.0	0.0120 % = (120 ppm) (**0.073 %**)	0.0120 % = (120 ppm)

diethyl ether as a feeding deterrent; in these assays the control (untreated) samples consisted of fresh *P. comosa*.

After coating the samples of *P. comosa*, they were joined together with plastic coated wire and labelled appropriately. The treated and untreated samples were then transported to the research site in seawater in stoppered numbered glass tubes.

The feeding experiments were carried out in plastic prawn crates (Nally Plastics Pty Ltd, 60 x 35 x10 cm in size) with removable mesh lids. The crates were further subdivided into four compartments by fine meshing. The cages were bolted to the substrate at a depth of three to five metres (Fig 24c). Each compartment contained either two urchins (*Tripneustes gratilla*) or three gastropods (*Turbo undulata*). All herbivores were starved for one to two days before each experiment, and were returned to the field after the experiment.

Treated and untreated samples of *P. comosa* were added to each of twelve cages containing herbivores. In all experiments four additional cages containing treated and untreated samples of algae but lacking the herbivores were included to measure autogenic changes in sample weight (Peterson and Renaud 1989). The experiments were conducted for 15-20 hr after which time the algae were recovered and returned to the laboratory so that the change in weight could be measured. The algal samples were carefully dried using paper tissues to remove excess water and then weighed. The change in weight was determined for both treated and untreated samples. The results were analysed using a paired

student's t-test (one-tailed) in which the amount of *P. comosa* sample consumed was corrected for the mean amount and variance of autogenic changes in control algae (Peterson and Renaud 1989).

8.3. Results

8.3.1. Feeding Deterrence

Tripneustes gratilla

The crude extract of *Delisea pulchra* significantly deterred feeding by the urchin *Tripneustes gratilla*. When applied at natural concentrations the extract reduced grazing to 52% ($n=24$, $p< 0.025$) relative to that of untreated samples (Fig. 25a). Of the four purified metabolites tested as feeding deterrents against *T. gratilla*, (56) and (57) both significantly deterred feeding, reducing grazing to 36% ($n=12$, $p<0.001$) and 41% ($n=6$, $p<0.05$) of that of their untreated samples respectively (Fig. a). The metabolite (59) had no effect on feeding preference ($n=12$, $p>0.15$) (Fig. 25a).

The results of the experiments to determine the effects of diethyl ether on feeding deterrence showed that diethyl ether had no significant effect on the feeding preference of either *Tripneustes gratilla* ($n=12$, $p> 0.15$) or *Turbo undulata* ($n=12$, $p>0.15$).

8.3.2. Feeding Deterrence

Turbo undulata

The crude extract of *Delisea pulchra* strongly deterred feeding by *Turbo undulata* when applied at natural concentrations, reducing grazing to 32% of that of the untreated samples ($n=12$, $p<< 0.001$) (Fig. 25b). However, none of the pure metabolites tested , (56), (57)

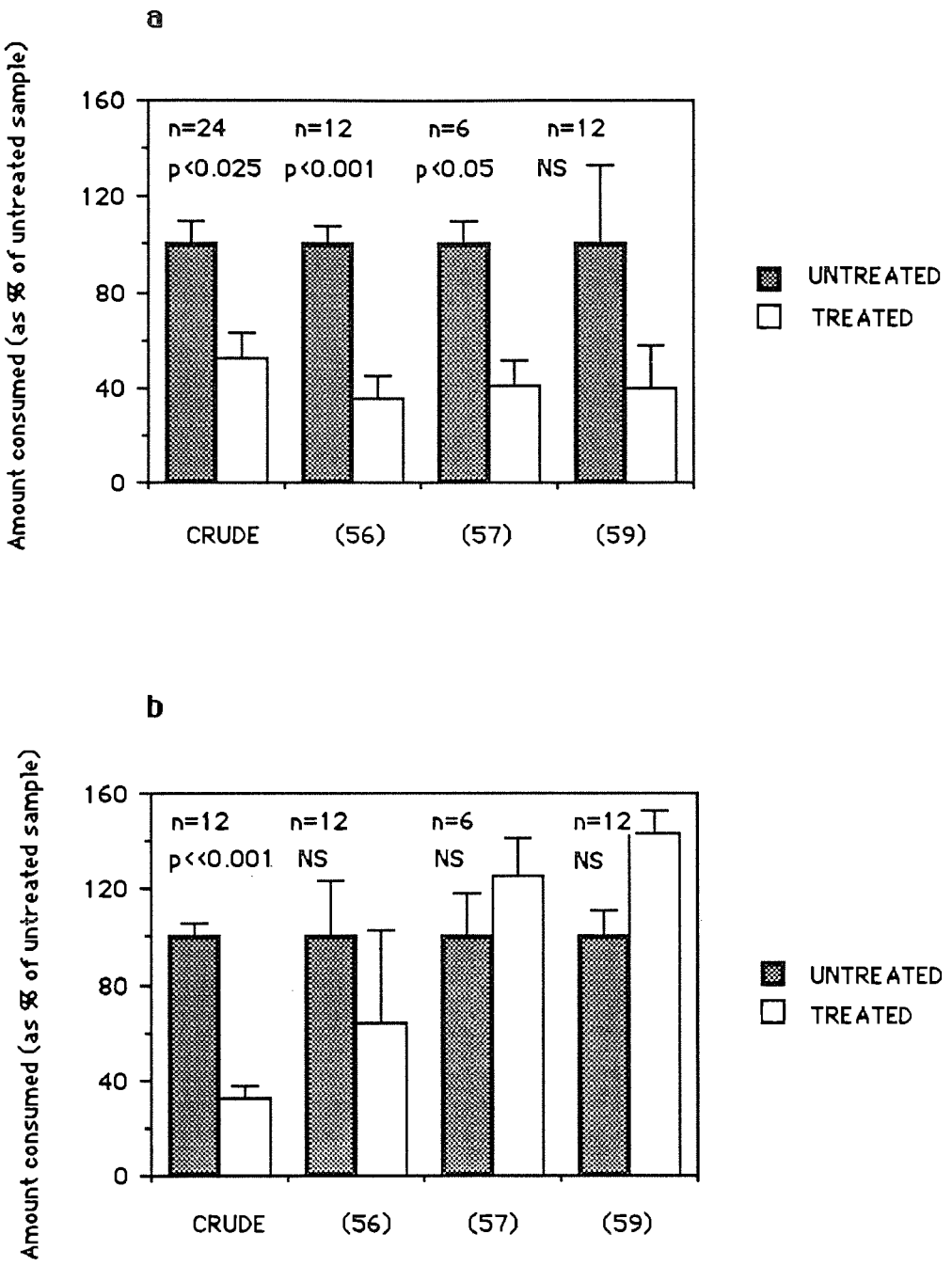


Figure 25 (a and b). The deterrent effect of crude extract and secondary metabolites isolated from *Delisea pulchra* on the consumption of the alga *Phyllospora comosa* by the echinoderm *Tripneustes gratilla* (Figure 25a) and the gastropod *Turbo undulata* (Figure 25b). The amounts consumed are expressed as a percentage of the untreated samples (bars) and standard errors (vertical lines) are shown.

and (59), showed any significant deterrence of feeding by *T. undulata* at natural concentrations (Fig. 25b). The only significant effect of any of the three compounds on *T. undulata* was that of (59), which significantly stimulated feeding. Consumption of treatment samples was 143% ($n=12$, $p< 0.05$) of that of the untreated samples (Fig. 25b).

8.4. Discussion

The majority of studies on the ecological role of secondary metabolites have focused on coral reef systems where herbivory is intense and plays an important role in structuring the algal communities (Hay 1985; Lewis 1985, 1986; Carpenter 1986). Algal secondary metabolites have been demonstrated to act as feeding deterrents in these communities (Hay and Fenical 1988), in particular against herbivorous fishes. Specific secondary metabolites also act as feeding deterrents against some tropical marine invertebrates including gastropods (Paul and Fenical 1986) and urchins (McConnell *et al.* 1982, Hay *et al.* 1987b). Herbivory also plays an important role in structuring temperate intertidal and subtidal algal communities (Dayton 1985), including those in temperate Australasia (Schiel 1990, Underwood and Kennelly 1990). The most important herbivores in temperate marine benthic communities are echinoderms and gastropods (Gaines and Lubchenco 1982, Hawkins and Hartnoll 1983, Andrew 1988). Although herbivory in temperate benthic communities is generally less intense than in tropical reef communities (Gaines and Lubchenco 1982, Steneck 1986), grazing urchins in particular are capable of reducing algal biomass to low levels over extensive areas

(Schiel and Foster 1986, Andrew 1988, Schiel 1990), such as at Cape Banks, where feeding preference experiments have been carried out (Fletcher 1987). The role of secondary metabolites as feeding deterrents in these temperate benthic communities has focused primarily on brown algal phlorotannins (Steinberg 1984, 1988, 1989; Geiselman and McConnell 1981, van Alstyne 1988, Steinberg and van Altena 1991). In the few studies which have considered the deterrent effects of non-polar algal secondary metabolites on herbivores in these communities, specific metabolites have been shown to act as feeding deterrents against fishes (Hay *et al.* 1987a, 1988c) and urchins (Hay *et al.* 1987a).

In the present study, we tested the effects of the crude extract and selected pure metabolites of the red alga *Delisea pulchra* as feeding deterrents against the herbivores *Tripneustes gratilla* and *Turbo undulata*. The two closely related metabolites (56) and (57), differing in structure only in the replacement of a bromine in (56) by a hydrogen in (57) (see Table 25), functioned as significant feeding deterrents against the urchin *T. gratilla* whereas a further related metabolite (59), had no effect on feeding preference (Table 25). The metabolite (59) is structurally similar to (56) and (57), the major difference is that (56) and (57) have planar rings while (59) has an sp^3 centre with substituents which are out of plane. While it is tempting to conclude that since the metabolites (56) and (57) were both active deterrents, there may be some relation between this functionality and the observed activity, this observation is not supported by similar studies where closely related compounds differed significantly in their effect on particular herbivores (Hay *et al.* 1987a, 1988c, 1989; Paul *et al.* 1989). In the case of *D. pulchra*

metabolites, the degree of halogenation does not appear to affect feeding deterrence. There appears to be little difference between the deterrent effects of the tri- and bi-substituted metabolites (58) and (59) on feeding by *T. gratilla*. Similarly, Paul *et al.* (1989) showed that halogenation does not appears to enhance deterrent effects of *Laurencia* metabolites.

The concentration at which the metabolites (58) and (59) are effective as feeding deterrents for *T. gratilla* is much lower than that recorded in most other comparable studies. Both metabolites are effective at a concentration of approximately 0.03% of the dry weight of the alga (0.005% of the fresh weight). These levels are probably less than those present in the alga *in-situ* due to incomplete extraction and losses through purification. The majority of studies of the deterrent effects of purified secondary metabolites (e.g. Hay *et al.* 1989a, Paul *et al.* 1989), have used final concentrations of approximately 1% of the dry weight of the alga as coatings on test specimens (i.e. 30x the concentration used in this study). Although the natural concentrations of secondary metabolites are not well documented, Hay and Fenical (1988) reported maximum published yields as between 0.5 and 3.0% of the dry weight of the alga. The few studies that have examined the effects of secondary metabolites on feeding deterrence at different concentrations have shown that those metabolites active as deterrents become ineffective at concentrations of approximately 0.5%-1.0% (Hay *et al.* 1987a, 1988c, 1989d). The only study which demonstrated feeding deterrence at concentrations of pure compounds comparable to those used in this study is that of Targett *et al.* (1986). The secondary metabolites halimedatetraacetate and halimedin were assayed at natural concentrations (0.07 and 0.06%

of dry weight respectively) in laboratory tests using the parrotfish *Sparisoma radians* (Targett *et al.* 1986). The former metabolite acted as a feeding deterrent at a concentration of 0.4 mg g^{-1} fresh weight (0.004%, 40 ppm), the latter showed no deterrent effect. However, field assays of feeding deterrence using halimedatetraacetate at 1% and 1.5% of the dry weight of the alga have shown variable results in one study (Paul 1987) and active deterrence in another (Paul and van Alstyne 1988) .

Steinberg and van Altena (1991) suggest that non-polar secondary metabolites may play a more important role in feeding deterrence than polyphenolic compounds in temperate Australasia. This is based on Estes and Steinberg's (1988) argument that responses of herbivores to secondary metabolites are based on the relative abundances of different types of algae, and thus herbivores adapt to compounds which are prevalent in common food plants as contact with these metabolites will be high. High levels of polyphenolic compounds are common in Australian algae and generally fail to deter feeding by temperate Australasian herbivores (Steinberg and van Altena 1991). Non-polar secondary metabolites, however while common in a number of temperate Australian algae (e.g. *Dictyota* spp., *Laurencia* spp.), are absent from many of the dominant laminarian (e.g. *Ecklonia radiata*) and fucalean algae (e.g. *Phyllospora comosa*). Steinberg and van Altena (1991) suggest that herbivores in temperate Australasia are faced with a choice of species either rich in non-polar metabolites or lacking these metabolites, and predict that non-specialist herbivores will choose to feed on the dominant algae which lack non-polar metabolites and thus fail to adapt to them. Although the

present study is only the first to demonstrate feeding deterrence by secondary metabolites in this geographic region, the effectiveness of *D. pulchra* secondary metabolites as deterrents at low concentrations supports this hypothesis.

While the crude extract of *D. pulchra* significantly deterred feeding by *T. undulata*, none of the metabolites assayed, including those which were deterrent towards *T. gratilla*, functioned as feeding deterrents towards the gastropod. Other studies have shown that a metabolite may vary considerably in its effect on different herbivores (Hay *et al.* 1987a, b, 1988c, 1989a, b; Paul *et al.* 1987, 1989). Metabolites which deter feeding by one herbivore have been shown to stimulate feeding by another (Hay *et al.* 1989) and can vary in their effect between juvenile and adult forms of the same herbivore (Paul *et al.* 1990).

The ineffectiveness of any of the three compounds assayed as feeding deterrents against *Turbo undulata* may be due to several factors. Kazlaukas *et al.* (1977) characterised 17 metabolites from *D. pulchra* (reported as *D. fimbriata*) which were identified by mass spectrometry, while a reinvestigation of the alga as part of this study identified eight metabolites (see Chapter 2.4.), some of which were in appreciable concentrations, but decomposed before they were able to be assayed for their feeding deterrent properties. The extract of *D. pulchra* also contained a number of secondary metabolites which were neither isolated nor characterised as they were present in very low concentrations. These metabolites may account for the more pronounced deterrent effect of the crude extract. It could also be possible that there are compounds in the

crude extract of *D. pulchra* that function as active feeding deterrents toward *T. undulata*, but which cannot be detected by the usual methods of analysis for non-polar metabolites (e.g. tlc and n.m.r.). The more pronounced activity of crude extracts has been demonstrated in a number of algal species. Wylie and Paul (1988) demonstrated that the crude extracts of *Codium geppi*, *Padina* spp., *Galaxaura oblongata* and *Halymenia durvillaei* acted as feeding deterrents against the herbivorous fish *Zebrasoma flavescens*, but did not contain identifiable secondary metabolites. Renaud (1988) demonstrated that a low polarity fraction of the extract of *Padina gymnospora* significantly deterred grazing by the urchin *Arbacia punctulata*, but no specific feeding deterrent compound was found. Renaud *et al.* (1990) presented further data supporting this evidence, however no specific metabolite has been isolated to explain the deterrent effect of the crude extract. Steinberg and Paul (1990) reported the presence of non-polar metabolites in the crude extract of *Padina* sp. which acted as a feeding deterrent, but failed to isolate any specific metabolites.

It has been proposed by Hay and Fenical (1988) that the models which have been applied to terrestrial plant-herbivore interactions may also be applicable to algal-herbivore interactions. A basic précis of the plant apparency model (Feeney 1976, Rhoades and Cates 1976) and plant resource availability (Coley *et al.* 1985) model as proposed for terrestrial plant-herbivore interactions suggests that "apparent plants", i.e. those bound to be detected by herbivores, invest heavily in generalist defences which are effective against a broad range of herbivores and which are dose dependent; these are termed quantitative defences (e.g. phlorotannins). In

contrast, "unapparent plants", i.e. those likely to escape detection by herbivores, invest in "low cost" defences which are effective at low concentrations; these are termed qualitative defences (e.g. non-polar secondary metabolites). *Delisea pulchra* does not appear to conform to this scheme. It is one of the most abundant algae in the study area, occurring in large stands accessible to a large number of generalist herbivores, yet it produces a wide range of non-polar secondary metabolites which are effective at very low concentrations against specific herbivores. A more detailed discussion of the comparison of terrestrial plant-herbivore theory and marine plant-herbivore interactions is beyond the scope of this discussion, but is presented in Hay and Steinberg (1991).

The data presented here supports the current opinion that the ecological effects of secondary metabolites cannot be based simply on structural characteristics, but are also a function of the type of herbivore, the environmental conditions at the time, and the location of the interaction between the herbivore and alga. The study also underlines the need for further experimental studies and theoretical consideration by both chemists and ecologists, before the role of secondary metabolites in marine systems can be adequately understood.

Chapter 9

Experimental

9. EXPERIMENTAL

9.1. Abbreviations

DCM	dichloromethane
EtOH	ethanol
MeOH	methanol
EtOAc	ethyl acetate
Me	methyl
br	broad
d	doublet
t	triplet
q	quartet
quin	quintet
s	singlet
n.m.r.	nuclear magnetic resonance
i.r.	infrared
u.v.	ultraviolet
n.O.e.	nuclear Overhauser effect
ms	mass spectrometry
EIMS	electron impact mass spectrometry
CI	chemical ionisation
HREIMS	high resolution electron impact mass spectrometry
HPLC	high performance/pressure liquid chromatography
tlc	thin layer chromatography

9.2. General Procedures

9.2.1. Sample Collection and Extraction

Algae were collected intertidally by hand at low tides or using snorkelling or SCUBA when found at greater depths. Subtidal collections of algae were carried out exclusively using SCUBA between 0-20m. Samples were returned to the laboratory wet and a representative sample of each species preserved in 8% formaldehyde solution. These samples were lodged in the James Cook University herbarium. The remainder of the samples was frozen or immediately extracted.

Wet extraction of either fresh or frozen material was carried out using DCM:MeOH, 50:50. The DCM and MeOH extracts were separated and the MeOH extract washed with DCM and again separated. The DCM extracts were combined and the DCM removed *in vacuo* to obtain the concentrated extract.

In some cases frozen material was freeze dried before extraction. The freeze dried material was extracted using DCM or EtOAc. The extracts then had the solvents removed *in vacuo* to obtain the concentrated extract.

9.2.2. Solvents

All solvents (technical grade) were distilled prior to use.

9.2.3. Chromatography

9.2.3.1. Column Chromatography

Column chromatography in which the solvent was drawn through the stationary phase under reduced pressure was used for the fractionation of crude extracts and the purification of individual compounds (Coll and Bowden 1986). The quantity of the solid support depended on the amount of material to be fractioned, but generally was less than five times the weight of applied extract. The stationary phase was silica gel 60 G (Merck), and the elution of the adsorbed mixtures was by step-wise gradient elution (i.e. using mixtures of solvents containing increasing amounts of the more polar solvent). All fractions obtained were examined by thin layer chromatography (tlc) and ^{1}H n.m.r. spectroscopy.

9.2.3.2. High Performance / Pressure Liquid Chromatography (HPLC)

HPLC was carried out using a Waters Associates 6000A or Waters 590 solvent delivery system connected to a Waters Associates Model R401 differential refractometer. HPLC columns used were

1. Techsil 5-20 silica
2. Techoprep 5-20 silica
3. Waters Si-100 7μm
4. (1) and (2), or (2) and (3) in series
5. Whatman Partsil M9 10/50 ODS-2
6. Activon Goldpak Techsphere C18 5μm

9.2.3.3. Thin Layer Chromatography (tlc)

Thin layer chromatography was carried out on plastic sheets precoated with silica gel 60 F (Merck) (0.2 mm) (normal phase) and

glass plates precoated with silica gel RP-18 $F_{254}S$ (Merck) (0.25 mm, reversed phase). After elution, plates were examined under u.v. light (254 nm) to detect u.v. absorbing compounds, and then sprayed with a solution of vanillin in concentrated sulphuric acid before heating to visualise individual compounds.

9.3. Instrumentation

9.3.1. Mass Spectrometry (m.s.)

Low resolution mass spectra were recorded on a Shimadzu GCMS QP 2000 gas chromatograph-mass spectrometer at James Cook University or were performed independently at the Australian National University's Research School of Chemistry. Low resolution EI (70eV) and CI mass spectra were run on a a VG707F mass spectrometer interfaced to an INCOS data system. Ammonia was used as the CI reagent gas. High resolution (~7000) accurate mass measurements were carried out on the same instrument using perfluorokerosene (PFK) as the reference compound.

9.3.2. Infrared Spectroscopy (i.r.)

Infrared spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. Samples were generally run as liquid films on KBr discs.

9.3.3. Ultraviolet Spectroscopy (u.v.)

Ultra-violet spectra were recorded in ethanol on a Varian series 634 spectrophotometer using quartz cells of 10mm path length.

9.3.4. ^{1}H Nuclear Magnetic Resonance Spectroscopy (n.m.r.)

^{1}H n.m.r. spectra were recorded in deuteriochloroform ($CDCl_3$) or perdeuteriobenzene (C_6D_6), internal standards in each case being chloroform ($CHCl_3$, δ7.26) or benzene (C_6H_6, δ7.15) respectively. Coupling constants (*J*) are given in Hertz (Hz) with signal multiplicities designated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) and br (broad). All spectra were recorded using a Bruker AM300 n.m.r. spectrometer (300 MHz, pulse FT mode).

9.3.5. ^{13}C Nuclear Magnetic Resonance Spectroscopy (n.m.r.)

^{13}C n.m.r. spectra were recorded in either deuteriochloroform ($CDCl_3$) or deuteriobenzene (C_6D_6) using a Bruker AM300 instrument (75 MHz). Proton decoupled and DEPT (Pegg *et al.* 1982) ^{13}C n.m.r spectra were routinely obtained to afford carbon signals and their multiplicities. INAPT (Bax 1984, Bax *et al.* 1985) ^{13}C spectra were obtained to afford connectivities for long range coupled proton signals. All proton pulses were obtained by using a proton selective 90° pulse train (DANTE) (Morris and Freeman 1978), established by a series of 30 x 3° pulses separated by a delay of 0.1 msec .

9.3.6. Two Dimensional Nuclear Magnetic Resonance Spectroscopy (2D n.m.r.)

2D n.m.r. spectra were recorded in deuteriochloroform ($CDCl_3$) or perdeuteriobenzene (C_6D_6) using a Bruker AM300 instrument.

Routinely acquired 2D spectra were the ^{1}H-^{1}H shift correlated spectra (COSY and COSYDQF), short range or directly bonded heteronuclear shift correlated (polarisation transfer via J_{XH}) spectra (XHCORRD) and the long range (2 or 3 bond) heteronuclear shift correlated spectra (XHCORRD, COLOC) (Kessler *et al.* 1988).

The short range ^{13}C-^{1}H shift correlated spectra were obtained by employing the standard Bruker microprogram XHCORRD.AU. The long range ^{13}C-^{1}H shift correlated spectra were obtained using the standard BRUKER microprograms XHCORRD.AU or COLOC.AU. Prior to running heteronuclear 2D experiments, sweep widths for both the f1 (^{1}H) and f2 (^{13}C) dimensions were optimised, as were the scan and section numbers. The latter two quantities were optimised on the basis of the number of scans required to obtain a DEPT spectrum with a signal to noise ratio, S/N, greater than three, and the required level of resolution in the 2D n.m.r. spectrum. The short range XHCORRD experiment is performed with delays D3 and D4 for J= 135 Hz. The long range XHCORRD and COLOC experiments are performed with delays D3 and D4 tuned for J= 7-10 Hz.

Inverse short range ^{1}H-^{13}C shift correlated spectra were obtained using the standard program HMQC (heteronuclear multiple quantum coherence, (Bax and Subramanian 1986) (J=135 Hz). Prior to the running of HMQC experiments a series of 1D spectra was acquired using the same pulse sequence to minimise the signals attributed to ^{1}H attached to ^{12}C by optimising the final delay in the standard BIRD pulse (Garbow *et al.* 1982) to $T_{1/2}$. The sweep widths for both the f1 (^{13}C) and f2 (^{1}H) dimensions were optimised, as were the scan and section numbers.

Inverse long range ^{1}H-^{13}C shift correlated spectra were obtained using the standard program HMBC (heteronuclear multiple bond coherence, (Bax and Summers 1986) (J= 7 Hz). D2 =1/(2 x $^{1}J_{CH}$), D4 =1/(2 x $^{3}J_{CH}$). The sweep widths for both the f1 (^{13}C) and f2 (^{1}H) dimensions were optimised, as were the scan and section numbers. The experiments was carried out using the a Bruker AM300 instrument at James Cook University and the ACP 300 n.m.r. spectrometer (300 MHz, pulse FT mode) at the Australian Institute of Marine Science

9.3.7. Nuclear Overhauser Effect (n.O.e.experiments) Spectroscopy

N.O.e's were determined using the standard Bruker microprogram, the n.O.e. difference microprogram: NOEDIFF.AU. A number of two-dimensional phase sensitive n.O.e. experiments were carried out using the standard Bruker program (NOESYPH, Bodenhausen *et al.* 1984). Mixing time for Z-magnetisation exchange (D9) was 1.8 seconds; D9 was randomly varied by 10%.

9.3.8. Optical Rotations ($[\alpha]_D$)

Optical rotations were recorded in chloroform using a Perkin-Elmer 141 Polarimeter. $[\alpha]_D$= α (degrees) / l (dm) x d (gcm^{-3})

9.3.9. Melting Points (mp)

Melting points were determined using a Reichert microscope hot stage apparatus. All melting points are uncorrected.

9.4. RHODOPHYTA

9.4.1. *Laurencia marianensis*

Sample MI871F

The sample was collected from Geoffrey Bay, Magnetic Island (GBR) 11/vi/87 at a depth of 1-3m and was frozen and freeze dried. The freeze dried tissue (346 g) was exhaustively extracted with DCM and the resultant crude extract (10.2 g, 2.9%) was separated by rapid chromatography on silica gel which afforded 16 fractions.

HPLC separation of fractions 4 and 5 combined (Techoprep 5-20 and Si 100 7 μm in tandem) (1:10, EtOAc:light petroleum) yielded the sesquiterpenes (35) and (37).

(35) deoxyprepacifenol. (35 mg, 0.010%); white crystalline solid; m.p. 121-123°C (c.f. 124-125°C, Ireland *et al.* 1976; 122-124°C, Watanabe *et al.* 1989); m.s. characteristics identical to those previously reported.

u.v. λ_{max} (EtOH) 212.5 (ε,4540) nm

i.r. υ_{max} (film) 2981, 2933, 2855, 1711, 1625, 1468, 1437, 1385, 1302, 1232, 1091, 965, 885, 807, 799 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 3.

^1H n.m.r. (C_6D_6, 300 MHz): see Table 3.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 3.

(37) 1-[(1'*S**,3'a*S**,4'*S**,7*R**,7'a*R**)-4'-bromo-1'-isopropyl-3'a-methyl-octahydro-1*H*-inden-7-yl]ethanone. (45 mg, 0.013%); crystalline solid; m.p. 49-53°C; $[\alpha]_D$ -13.0^0 (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 300.1090, $C_{15}H_{25}{}^{79}BrO$ requires 300.1089]

u.v. λ_{max} (EtOH) 203 (ε, 2316) nm

i.r. υ_{max} (film) 2958, 2927, 2887, 2357, 1711, 1466, 1389, 1176, 806 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 6.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 6.

EIMS m/z (%, RA): 300 ($M^{\cdot+}$,<1%), 221 (6), 220 (3), 123 (27), 107 (8), 97 (11), 95 (17), 93 (15), 85 (16), 83 (17), 81 (20), 79 (13), 71 (36), 69 (34), 67 (15), 57 (48), 55 (47), 43 (100).

COSY experiment: The methyl signals δ0.86 and δ0.96 showed crosspeaks to the methine signal δ1.73. Similarly the signal δ1.73 showed crosspeaks to the signals δ0.86 and δ0.96 and to the methine signal δ1.92. The signal δ1.92 showed crosspeaks to the adjacent methylene signals δ1.49 and δ1.71 and to the methine proton signals δ1.73 and δ2.22. The methylene signals δ1.49 and δ1.71 consequently showed crosspeaks to each other, the methine signal δ1.92 and the adjacent methylene signals δ1.28 and δ1.96. Similarly the methylene signals δ1.28 and δ1.96 showed crosspeaks

to each other and to the signals δ1.49 and δ1.71. The methine signal δ2.22 showed crosspeaks to the adjacent methine signals δ1.92 and δ2.44. The signal δ2.44 showed crosspeaks to the δ2.22 and the methylene signals δ1.55 and δ2.08. The signals δ1.55 and δ2.08 showed crosspeaks to each other, the signal δ2.44 and the methylene signals δ2.01 and δ2.15. Similarly the signals δ2.01 and δ2.15 showed crosspeaks to each other and to the methylene signals δ1.55 and δ2.08, and also to the downfield methine signal δ4.22, which in turn showed crosspeaks to the adjacent signals δ2.01 and δ2.15, and the methylene signal δ1.55.

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:10, EtOAc:light petroleum) of fraction 9 yielded the sesquiterpene (36).

(36) pacifenol. (43 mg, 0.012%); white crystalline solid; m.p. 154-156°C (c.f. 149-151°C, Sims *et al.* 1971); m.s. characteristics consistent with those previously reported.

u.v. λ_{max}(EtOH) 204 (ε, 2393) nm

i.r. υ_{max} (film) 3603, 2926, 2855, 1717, 1465, 1391, 1366, 1305, 1217, 1116, 1048, 1024, 1002, 988, 964, 934, 885, 808, 803 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 5.

^{13}C n.m.r. (CDCl , 75 MHz): see Table 5.

9.4.2. *Laurencia majuscula*

Sample MI884E

The sample was collected from Magnetic Island (28/ix/88) and was frozen and freeze dried. The freeze dried tissue (270 g) was exhaustively extracted with DCM and the resultant crude extract (10.2 g, 3.7 %) was separated by rapid chromatography on silica gel which afforded 24 fractions.

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:50, EtOAc:light petroleum) of the combined fractions 7, 8 and 9 yielded the triquinane sesquiterpene (40), and the sesquiterpenes (46), (47) and (48).

(40) (1*S**,2*S**,3*S**,5*S**,8*S**,9*S**)-tricyclo[6.3.0.0^{1,5}]undecan-2-ol (51 mg, 0.019%); mobile oil; ^{1}H and ^{13}C n.m.r. data identical to those previously reported (Coll and Wright 1989a).

(46) (1'*R**,3'*R**)-2-(3'-chloro-1',3'-dimethylcyclohexyl)-5-methyl-phenol. (145.9 mg 0.054 %); mobile yellow oil; $[\alpha]_D$ + 14° (c 0.001); [Found M·$^+$ (EIMS) 252.1279, $C_{15}H_{21}{}^{35}ClO$ requires 252.1281]

u.v. λ_{max} (EtOH) 226 (ε,4081), 278 (ε,2350), 284 (ε,2265) nm

i.r. υ_{max} (film) 3622, 2937, 2887, 2347, 1571, 1426, 1313 cm^{-1}

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 8.

^{1}H n.m.r. (C_6D_6, 300 MHz): see Table 8.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 8.

^{13}C n.m.r. (C_6D_6, 75 MHz): see Table 8.

EIMS m/z (%, RA): 252 ($M^{\cdot+}$,18), 237 (24), 216 (12), 201 (43), 175 (33), 173 (67), 161 (41), 159 (19), 148 (52), 147 (11), 145 (18), 135 (30), 122 (10), 121 (100), 109 (17), 108 (49), 105 (24), 93 (23), 91 (32).

(47) (1'*R**,3'*R**,4'*S**)-2-(4'chloro-1',3'-dimethylcyclohexyl)-5-methyl-phenol. (342.7 mg, 0.128 %); brown mobile oil; $[\alpha]_D$ + 47° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 252.1279, $C_{15}H_{21}{}^{35}ClO$ requires 252.1281]

u.v. λ_{max} (EtOH) 222 (ε,4412), 277 (ε,2177), 282 (ε,2081) nm

i.r. υ_{max} (film) 3602, 2954, 2872, 1617, 1297, 1188 cm^{-1}

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 10.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 10.

EIMS m/z (%, RA): 252 ($M^{\cdot+}$, 15), 237 (21), 201 (29), 173 (15), 161 (26), 159 (10), 148 (42), 145 (11), 135 (30), 133 (10), 122 (10), 121 (100), 109 (13), 108 (54).

Conversion of (47) *to the corresponding acetate* (47a)

(47) (3.2 mg, 0.0127 mmol) was dissolved in DCM (2 ml) to which was added triethylamine (1.1 molar equivalent, 1.5 mg) followed by dimethylamino-pyridine (0.1 molar equivalent, 0.2 mg). Acetic anhydride (1.2 molar equivalent, 1.4 mg) was added to the reaction mixture. The reaction was complete (tlc) after four hours. On completion the reaction mixture was diluted with water (2 ml) and washed successively with 1% HCl (2ml), 5% $NaHCO_3$ (2 ml) and water (2 ml). The organic layer was separated and the solvents removed *in vacuo*. The resultant product was purified by column chromatography (Keiselgel 60H, Merck, ethyl acetate:light petroleum, 1:10) to afford (47a) (3.0 mg, 95%).

(47a) (1'*R**,3'*R**,4'*S**)-2-(4'chloro-1',3'-dimethylcyclohexyl)-5-methyl-phenyl acetate. 3.0 mg (95%); brown mobile oil; $[\alpha]_D$ +22.3° (c, 0.003); [Found $M^{\cdot+}$ (EIMS) 294.1386, $C_{17}H_{23}{}^{35}ClO_2$ requires 294.1387]

u.v. λ_{max} (EtOH) 220 (ε,4846) nm

i.r. υ_{max} (film) 2927, 2854, 2361, 2341, 1745, 1465, 1376, 1236, 803 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300MHz): 1.01 (d, 8'-CH_3), 1.20 (s, 7'-CH_3), 1.50-2.20 (m,7H), 2.30 (s, 7-CH_3), 2.32 (s, CH_3), 4.22 (brs, 4'-CH), 6.79 (s, 6-CH), 6.99 (brd, *J* 7.8 Hz, 4-CH), 7.21 (d, *J* 7.8 Hz, 3-CH).

EIMS m/z (%, RA): 294 ($M^{\cdot+}$,<1%), 237 (14), 201 (14), 175 (12), 161 (15), 148 (18), 135 (12), 121 (37), 108 (24), 105 (13), 97 (18), 93 (11), 91 (16), 85 (28), 84 (11), 83 (28), 81 (14), 57 (100).

Elimination of (47) *to corresponding alkene* (49)

(47) (20 mg) was dissolved in dry MeOH (1 ml) to which was added KOH (1 pellet) dissolved in dry MeOH (1 ml) . The reaction mixture was heated under reflux in a nitrogen atmosphere for four hours (tlc) at which time the reaction was quenched by addition of dilute HCl (0.1 M), and subsequently washed with four aliquots of DCM (5 ml) and the organic layer separated. Solvents were removed *in vacuo* and the reaction mixture separated by column chromatography (Keiselgel 60H, Merck, ethyl acetate:light petroleum 1:10) to yield the alkene (49) (17 mg, 85%).

(49) (1'*R**)-2-(1',3'-dimethyl-3'-cyclohexenyl)-5-methyl-phenol. (17 mg, 85%); yellow mobile oil; $[\alpha]_D$ +47.0° (c, 0.01); [Found $M^{\cdot+}$ (LREIMS) 216, $C_{15}H_{20}O$ requires 216]

u.v. λ_{max} (EtOH) 276 (ε,8162), 219 (s) (ε,6270), 209 (ε,3427) nm

i.r. υ_{max} (film) 3606, 3186, 2924, 1710, 1620, 1503, 1412, 1156 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 12.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 12.

EIMS m/z (%, RA): 216 ($M^{\cdot+}$,29) 215 (26), 201 (17), 200 (15), 173 (60), 148 (78), 133 (25), 109 (21), 97 (38), 81 (49), 69 (83), 55 (100).

Epoxidation of (49) *to* (48)

(49) (24.3 mg) was dissolved in chloroform (2 ml) and cooled to 4°C, to which was added a 1.2 molar equivalent of *m*-chloroperbenzoic acid (25 mg) . The reaction was stirred at room temperature in the presence of Na_2CO_3 for three hours. TLC analysis of the reaction mixture at this time indicated that the reaction was complete. The reaction was quenched by the addition of water and the product was subsequently extracted with three aliquots of chloroform (5 ml). The organic layer was separated and the solvents removed *in vacuo*. The resultant mixture was separated by HPLC (Techsil 5 silica, Si-100 7μm in tandem, ethyl acetate:light petroleum 1:8) to yield (48) (20mg, 82%).

Esterification of (47) *to* (50): *crystalline derivative.*
(46) (38.3 mg) was dissolved in DCM (1 ml) and added dropwise to a stirred solution of benzoyl chloride (55.5 mg) in pyridine (1 ml) and DCM (1.25 ml). The solution was stirred at room temperature for 7hr. The reaction was stopped by the addition of water and was washed with HCl (1 M) and NaOH (1 M). The organic layer was separated and the solvents removed *in vacuo*. The crude product was separated by column chromatography (Keiselgel 60H, Merck, ethyl acetate: light petroleum 1:10) The fraction containing (50) was then further purified by HPLC (Techsil 5 silica, Si-100 7μm in

tandem, ethyl acetate:light petroleum 3:20) and crystallised from light petroleum to give the ester (50) as colourless needles (32mg, 83%).

(50) (1'*R**,3*R**,4'*S**)-2-(4'-chloro-1',3'-dimethyl-cyclohexyl)-5-methyl-phenyl-benzoate. 32mg (83%); colourless crytalline solid; m.p. 77-80°C, $[\alpha]_D$ +19.4° (c, 0.013); [Found $M^{\cdot+}$ (EIMS) 356.1543, $C_{22}H_{25}{}^{35}ClO_2$ requires 356.1543]

u.v. λ_{max} (EtOH) 231 (ε,13496), 208 (ε,11589) nm

i.r. υ_{max} (film) 2947, 2926, 2855, 2360, 2340, 1739, 1679, 1452, 1260, 1232, 1069,1062, 786 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 13.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 13.

EIMS m/z (%, RA): 356 ($M^{\cdot+}$,<1%), 252 (<10), 216 (<10), 201 (<10), 173 (<10), 105 (100).

COSYDQF experiment: The methyl signal δ0.89 showed a crosspeak to the signal δ1.84. The methylene signal δ1.56 showed crosspeaks to the signals δ1.84 and δ2.11. The unresolvable signals for the methine proton δ1.84 and the methylene proton δ1.83 showed crosspeaks to the signals δ0.89 and δ1.56, and δ1.94, δ1.95 and δ2.28. The methylene signals δ1.94 and δ1.95 showed crosspeaks to δ1.84, δ2.28 and δ4.20. The methylene signal δ2.11 showed crosspeaks to the signals δ1.56 and δ2.28 ($^3J_{H-H}$), while the

methylene signal δ2.28 showed crosspeaks to the signals δ1.84, δ1.94, δ1.95 and δ2.11. The aromatic proton signal δ7.05 showed crosspeaks to the signal δ7.27 and visa versa. The aromatic proton signal δ7.57 showed crosspeaks to the signals δ7.67 and δ8.20 while the signal δ7.67 showed a crosspeak to δ7.57. Similarly the proton signal δ8.20 showed a crosspeak to the signal δ7.57.

(48) (1'*R**,2*R**,4*R**)-2,4-(1'-hydroxypropano)-2,4,7-trimethyl chroman. (20.9 mg, 0.007 %); yellow viscous oil; $[\alpha]_D$ 0.00° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 232.1463, $C_{15}H_{20}O_2$ requires 232.1463]

u.v. λ_{max} (EtOH) 220 (ε,4469), 277 (ε,1957), 284 (ε,1913) nm

i.r. υ_{max} (film) 3627, 2928, 2358, 2336, 1504, 1146, 1056 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 11.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 11.

EIMS m/z (%, RA): 232 ($M^{\cdot+}$, 11), 175 (13), 174 (14), 173 (100), 148 (20), 91 (12), 77 (12).

Conversion of (48) *to the corresponding acetate* (48a)

(48) (1.3 mg, 0.0056 mmol) was dissolved in DCM (2 ml) to which was added triethylamine (1.1 molar equivalent, 0.7 mg) followed by dimethylamino-pyridine (0.1 molar equivalent, 0.1 mg). Acetic anhydride (1.2 molar equivalent, 0.6 mg) was added to the reaction

mixture. The reaction was complete (tlc) after four hours. On completion, the reaction mixture was diluted with water (2 ml) and washed successively with 1% HCl (2ml), 5% $NaHCO_3$ (2 ml) and water (2 ml). The organic layer was separated and the solvents removed *in vacuo*. The resultant product was purified by column chromatography (Keiselgel 60H, Merck, ethyl acetate:light petroleum, 1:10) to afford 1.3 mg of (48a) (100%).

(48a) (1'*R**,2*R**,4*R**)-2,4-(1'-acetoxypropano)-2,4,7-trimethyl chroman. 1.3 mg (100%); brown mobile oil; $[\alpha]_D$ +32.1° (c, 0.0013); [Found $M^{\cdot+}$ (EIMS) 274.1569, $C_{17}H_{22}O_3$ requires 274.1569]

u.v. λ_{max} (EtOH) 209 (ε,7826) 219 (ε,6236)

i.r. υ_{max} (film) 2926, 2854, 2361, 2341, 1745, 1465, 1376, 1237, 1226, 1046, 803 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300MHz): 1.34 (m, 3'-CH), 1.36 (s, 10-CH_3), 1.43 (s, 11-CH_3), 1.48 (m, 3-CH), 1.53 (m, 2'-CH), 1.58 (m, 2'-CH), 1.63 (m, CH), 1.85 (d, *J* 13.2 Hz, 3-CH), 2.09 (s, CH_3), 2.27 (t, *J* 1.3 Hz, 7-CH_3), 4.85 (brs, 1'-CH), 6.62 (s, 8-CH), 6.68 (dd, *J* 1.8, 7.8 Hz, 6-CH), 7.05 (d, 7.8 Hz, 5-CH).

EIMS m/z (%, RA): 274 ($M^{\cdot+}$,<1%), 199 (10), 174 (12), 173 (89), 159 (11), 148 (19), 105 (15),97 (16), 95 (11), 85 (22), 83 (26), 81 (15), 57 (100).

HPLC separation (Techoprep 5-20 and Si 100 7 μm in series) (1:10, EtOAc:light petroleum) of fraction 10 further yielded the sesquiterpene (46) and the triquinane (40) as well as the sesquiterpenes (41) and (43).

(41) iso-obtusadiene. (85 mg, 0.031%); clear mobile oil; identical ^{1}H and ^{13}C n.m.r. data identical to those previously published (Gerwick *et al.* 1987, Coll and Wright 1989a)

(43) (1(15)*E*,2*Z*,4*S*,8*R*,9*S*)-8,15-dibromochamagra-1(15),2,11(12)-trien-9-ol. (60 mg, 0.022%); clear mobile oil; ^{1}H and ^{13}C n.m.r. data identical to those previously published (Coll and Wright 1989a)

HPLC separation (Techoprep 5-20 and Si 100 7 μm in series) (1:10, EtOAc:light petroleum) of fraction 11 further yielded and the sesquiterpenes (41) and (43) as well as the sesquiterpenes (42) and (44).

(42) (1*Z*,8*R**,9*R**)-8-bromochamigra-1,11(12)-dien-9-ol.
(40 mg, 0.015%); clear mobile oil; ^{1}H and ^{13}C n.m.r. data identical to those previously published (Wright *et al.* 1990).

(44) (1(15)*Z*,2*Z*,4*S*,8*R*,9*S*)-8,15-dibromochamigra-1(15),2,11(12)-trien-9-ol. (70 mg, 0.026%), clear mobile oil; ^{1}H and ^{13}C n.m.r. data identical to those previously published (Suzuki and Kurosawa 1978, Suzuki *et al.* 1979, Coll and Wright 1989a)

HPLC separation (Techoprep 5-20 and Si 100 7 μm in series) (1:10, EtOAc:light petroeum) of fraction 13 further yielded and the sesquiterpene (44) and the sesquiterpene (45).

(45) *iso*-obtusol (90 mg, 0.033%); colourless crystal; $[\alpha]_D$ and m.p. as previously reported (Gonzalez *et al.* 1979a, b).

u.v. λ_{max} (EtOH) 206 (ε,6006), 264 (ε,3280) nm

i.r. υ_{max} (film) 3556, 2957, 2932, 1636, 1448, 1371, 1244, 1069, 904 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 7.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 7.

9.4.3. ***Dasyphila sp.***

Sample HR891I

The sample was collected from Flinders Reef, Coral Sea (02/viii/89) and was frozen and freeze dried. The freeze dried tissue (232 g) was exhaustively extracted with DCM and MeOH and the resultant crude extract (1.12 g, 0.50%) was separated by rapid chromatography on silica gel using mixtures of light petroleum and ethyl acetate as eluents which afforded 21 fractions.

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:12, EtOAc:light petroleum) of the combined fractions 7 and 8 yielded the vinyl acetylene (51) and the sesquiterpene (53).

(51) (3*E*,6*S*,7*S*,9*S*,10*S*,12*S*,13*R*)-10-chloro-12-bromo-6,9;7,13-bisepoxypentadec-3-en-1-yne. (8.9 mg, 0.004 %); mobile oil; ^{1}H and ^{13}C n.m.r. data identical to that previously reported (Coll and Wright 1989b).

(53) 4(2'-methyl-1'-propenyl)-1,5-dimethyl-octahydro-1*H*-inden-3a-ol. (22 mg, 0.01 %); mobile yellow oil.

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 15.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 15.

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:12, EtOAc:light petroleum) of the combined fractions 10-12 yielded the vinyl acetylene (52).

(52), (3*E*,6*S*,7*S*,9*S*,10*S*,12*S*,13*R*)-10-hydroxy-12-bromo-6,9;7,13-bisepoxypentadec-3-en-1-yne. (5.9 mg, 0.003 %); mobile yellow oil; $[\alpha]_D$ +8.0° (c 0.003); [Found $M^{\cdot+}$ (EIMS) 231.1381, $C_{15}H_{21}O_3{}^{79}Br$ - $H_2O^{79}Br$ requires 231.1385; Found $M^{\cdot+}$ (EIMS) 277.0439, $C_{15}H_{21}O_3{}^{79}Br$ - C_4H_3 requires 277.0440]

u.v. λ_{max} (EtOH) 223 (ε,2951) nm

i.r. υ_{max} (film) 3313, 2927, 2854, 1736, 1461, 1373, 1152, 1100, 1063 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 14.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 14.

CIMS (NH_3) m/z (%, RA): 362 (11), 360 (10), 345 ($M^{\cdot+}$ NH_4^+, 37), 344 (11), 343 (39), 327 (19), 326 (23), 325 (11), 313 (27), 277 (12), 263 (20), 231 (45), 195 (22), 179 (24), 165 (34), 135 (38), 109 (100).

9.4.4. *Delisea pulchra*

Sample SY891A

The sample was collected from Cape Banks, Sydney, N.S.W. (01/ix/89), frozen upon collection and sent frozen to Townsville. The sample was freeze dried (168.2 g) and the tissue exhaustively and sequentially extracted with DCM and EtOAc. The resultant crude extract (1.9 g, 1.1%) was separated by rapid chromatography on silica gel using increasing portions of ethyl acetate in light petroleum which afforded 28 fractions.
HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:50, EtOAc:light petroleum) of fractions 3-8 combined yielded three compounds; two halogenated lactones (fimbrolides): (56) and (57), and a halogenated ketone (60).

(56) Fimbrolide II, 3-*n*-butyl-4-bromo-5-(dibromomethylidene)-2(5*H*)-furanone. (53.6 mg, 0.032%); yellow mobile oil.

u.v. λ_{max} (EtOH) 207 (ε,11687), 303 (ε,14776) nm

i.r. υ_{max} (film) 2956, 2926, 2865, 1789, 1599, 1546, 1455, 1250, 1212, 1106, 1015 cm^{-1}

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 16.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 16.

(57) Fimbrolide IIa, (5Z)-3-*n*-butyl-4-bromo-5-(bromomethylidene)-2(5*H*)-furanone. (44.3 mg, 0.026%); yellow mobile oil.

u.v. λ_{max} (EtOH) 205 (ε,9756), 286 (ε,8605) nm

i.r. υ_{max} (film) 3097, 2960, 2929, 2860, 1792, 1549, 1212, 986 cm^{-1}

^{1}H n.m.r. ($CDCl_3$, 300 MHz): see Table 17.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 17.

(60) 1,1,2-tribromo-oct-1-en-3-one. (11.9 mg, 0.007 %); yellow mobile oil; ^{1}H, ^{13}C n.m.r. and i.r. characteristics identical to literature values (Rose *et al.* 1977).

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:20 EtOAc: light petroleum) of fraction 9 yielded three compounds (63) and (64)

(61) (2*S*)-1,1,3-tribromo-2-hydroxydodec-3-ene. (25.4 mg, 0.014%); yellow mobile oil; $[\alpha]_D$ -40.0° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 417.9139, $C_{12}H_{21}{}^{79}Br_3O$ requires 417.9142]

u.v. λ_{max} (EtOH) 205 (ε,6238) nm

i.r. υ_{max} (film) 3572, 2927, 2856, 1466, 1378, 1150, 1101, 800, 723 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 19.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 19.

EIMS m/z (% RA): 418 ($M^{\cdot+}$,<1%) 249 (20), 247 (21), 149 (52), 137 (17), 136 (19), 107 (18), 97 (23), 95 (23), 93 (26), 85 (26), 83 (32), 82 (13), 81 (43), 79 (17), 71 (34), 70 (12), 69 (50), 67 (37), 57 (64), 55 (62), 53 (25), 43 (99), 42 (15), 41 (100).

OXIDATION OF (61)

To a stirred solution of pyridinium chlorochromate (7.7 mg, 0.0036 mmol) in DCM (2 ml), was rapidly added (61) (10.0 mg, 0.0024 mmol) in DCM (2 ml). The reaction mixture was stirred at room temperature for 4 hrs by which time the reaction appeared by tlc to have gone to completion. The reaction was quenched by addition of diethyl ether (20 ml) and the solution filtered. The solvent was removed *in vacuo* to yield the crude product (4.7 mg).
HPLC separation (Techsil 5 silica HP Si-100 7 μm, ethyl acetate:light petroleum (8:1)) of this material afforded the ketone (61a).

(61a) (1.1 mg, 23%); clear mobile optically inactive oil.

u.v. λ_{max} (EtOH) 204 (ε,4868), 264 (ε,2972) nm.

i.r. υ_{max} (film) 2927, 2882, 2856, 1717, 1484, 1359, 1323, 1217, 848 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 300 MHz): 0.88 (t, *J* 6.8 Hz, CH_3), 1.26 (brs, CH_2), 1.50 (dd, *J* 7.2, 14.2 Hz, CH_2), 2.42 (dd, *J* 7.1, 14.4 Hz, CH_2), 6.68 (s, CH), 7.45 (t, *J* 7.1 Hz, CH).

Horeau determination of (61)

The alcohol (61) (12.5 mg, 0.030 mmol) in pyridine (300 µl) was treated with (+/-)-α-phenylbutyric anhydride (18.6 mg, 0.060 mmol). The mixture was left at room temperature overnight, diluted with water (1 ml) and warmed for 45 mins. The mixture was placed in a separatory funnel with water (5 ml) and benzene (5 ml) and titrated with 0.1M sodium hydroxide solution (phenolphthalein indicator, 0.95 ml, 83% esterification). Benzene (10 ml) was added and the layers separated and the organic layer was extracted with water. The aqueous layers were acidified to pH 1.5 and extracted into benzene (2x10 ml), washed with water, dried over magnesium sulphate and concentrated. The residue was made up to 1 ml with benzene and the polarimeter reading was 99.986, rotation was -0.014. Excess acid was laevorotatory; the absolute configuration was *S* .

(62) (*R*,*S*)(1Z) 6-acetoxy-1,1,2-tribromo-oct-1-en-3-one. (33.2 mg 0.020 %); yellow mobile oil; $[\alpha]_D$ 0.0° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 374.8253, $C_{10}H_{13}{}^{79}Br_3O_3$ - CH_3CO requires 374.8231]

u.v. λ_{max} (EtOH) 218 (ε,7587), 208 (ε,6413), 277 (ε,1587) nm

i.r. υ_{max} (film) 2969, 2927, 2880, 2855, 1739, 1721, 1463, 1373, 1240, 1117, 1019, 807, 741 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 20.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 20.

EIMS m/z (% RA): 375 ($M^{\cdot+}$-CH_3CO,<1%),115 (22), 97 (12), 69 (18), 57 (31), 55 (18), 43 (100)
CIMS (NH_3) m/z (%RA): 442 (20), 440 ($M+NH_4^+$, 60), 438 (58), 436 (22), 365 (42), 354 (13), 363 (100), 362 (17), 361 (98), 360 (10), 359 (46), 281 (12), 280 (14), 279 (23), 278 (11), 203 (16), 201 (18).

Attempted hydrolysis of (62) *to the corresponding alcohol* (62a)

The acetate (64, 10 mg) was treated with K_2CO_3 (5 mg) in 95% MeOH at room temperature for 1 hr. The resultant product appeared to have undergone cyclization, no simple alcohol was detected in the complex mixture.

Enzymatic hydrolysis of (62) *to the corresponding alcohol* (62a)

An excess of the lipase; (Fluka, from *Pseudomonas fluorescens*, SAM-2, EC 3.1.1.3, 50 mg)) was dissolved in tris-phosphate buffer (0.1M, pH 7.2) (2 ml) and added to the acetate (62) (10 mg). The mixture was left for 48 hrs in a shaker bath (30°C). The reaction was stopped by the addition of diethyl ether (5 ml) and the organic layer separated. The aqueous layer was subsequently extracted with four aliquots (5 ml) of diethyl ether and the organic fractions

combined and concentrated. TLC confirmed the presence of reaction product while ^1H n.m.r. confirmed the presence of the secondary alcohol of the acetate (50% yield: integral of δ4.83 to δ3.56; 1:1). The mixture was separated by column chromatography (Keiselgel 60H, Merck, ethyl acetate:light petroleum, 5:95) and the two compounds further purified by HPLC (Techsil 5 silica, Si-100 7μm, ethyl acetate:light petroleum, 5:95). The remaining purified reactant (3 mg) was taken up in 1 ml CCl_4 and polarimeter reading was 99.991; $[\alpha]_D = -3.0^\circ$ (C 0.003)

(62a) (1Z) 6-hydroxy-1,1,2-tribromodec-1-en-3-one (3 mg).

^1H n.m.r ($CDCl_3$, 300 MHz): 0.96 (t, *J* Hz, CH_3), 1.26 (brs, CH_2), 1.56 (m, CH_2), 1.72 (m, CH_2), 1.89 (m, CH_2), 2.97 (dd, *J* Hz, CH_2, two proton integral), 3.56 (m, CH).

HPLC separation (Techoprep 5-20 and Si 100 7 μm in tandem) (1:10 EtOAc: light petroleum) of fraction 10 yielded the acetoxyfimbrolide (58).

(58) (Acetoxyfimbrolide 2B), (1*R*, 5*Z*)-3-(1-acetoxybutyl)-4-bromo-5-(bromomethylidene)-2(5*H*)-furanone. (76 mg, 0.045 %); yellow mobile oil; ^1H, ^{13}C and i.r. characteristics identical to literature values (Kazlaukas *et al.* 1977, Pettus *et al.* 1977).

HPLC separation (Whatman Partsil M9 10/50 ODS-2) (40:60 H_2O:MeOH) of fractions 18-20 combined yielded a single compound (59).

(59) (*R*,*S*) 3-(1'-hydroxybutyl)-4-bromo-5-hydroxy-5-methyl-2(5*H*)-furanone. (122 mg, 0.072%); yellow mobile optically inactive oil; [Found: $M^{\cdot+}$ (CIMS) 266, $C_9H_{11}{}^{81}BrO_4$ requires 266), (EIMS) 222.9444, $C_9H_{13}{}^{79}BrO_4 - C_3H_7$ requires 222.9429]

u.v. λ_{max} (EtOH) 229 (ε,7183) nm.

i.r. υ_{max} (film) 3358, 2962, 2933, 2874, 1753, 1655, 1372, 1294, 1256, 1194, 1138, 1106, 1044, 994, 955, 903, 807, 800 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 18.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 18.

CIMS m/z (% RA): CI (NH_3) 285 (10), 284 (100) ($M+NH_4^+$), 283 (11), 282 (95), 267 (61), 266 (14), 265 (68) (MH^+), 249 (32), 247 (29), 158 (22).
EIMS m/z (%RA): 223 ($M^{\cdot+}$,13%), 221 (14), 205 (23), 203 (22), 55 (15), 53 (16), 43 (100).

9.5. Chlorophyta

9.5.1. *Microdictyon obscurum*

Sample HR892A, HR892J

The sample was collected from Holmes Reef, Coral Sea (02/viii/89) and was frozen and freeze dried. The freeze dried tissue (HR892A 56.6 g, HR892J 72.0 g) was exhaustively extracted with DCM followed by MeOH. The resultant crude extracts (HR892A 507.8 mg, 0.90%, HR892J 768 mg, 1.06%) which were the same by thin layer chromatography were combined. The combined extract was separated by rapid chromatography on silica gel which afforded 25 fractions. ^{1}H n.m.r. and tlc studies of all these fractions indicated no secondary metabolites of interest had been isolated.

9.5.2. *Chlorodesmis fastigata*

Sample JB871A

The sample was collected from John Brewer Reef (26/ii/87) and was frozen and freeze dried. The freeze dried tissue was exhaustively extracted with DCM and the resultant crude (0.81 g) was separated by rapid chromatography on silica gel which afforded 17 fractions.

HPLC separation of fraction 5 Techoprep 5-20 and Si 100 7 μm in tandem) (1:20, EtOAc:light petroleum) yielded the diterpene acetate (63).

(63) 14,15-didehydrotrifarin. (27 mg, 3.3 %); clear mobile oil; ^{1}H and ^{13}C n.m.r. data identical to literature values (Wells and Barrow 1979, Wright 1988).

HPLC separation of fraction Techoprep 5-20 and Si 100 7 μm in tandem) (1:20, EtOAc:light petroleum) yielded the diterpene (66).

(64) (2*E*,6*E*,10*E*)-1-acetoxy-3-acetoxymethyl-7,11,15-trimethylhexadeca-2,6,10,14-tetraene. (17 mg, 2.1% of crude); clear mobile oil; ^{1}H and ^{13}C n.m.r. data identical to literature values (Wright *et al.* 1990).

9.6. Phaeophyta

9.6.1. *Sargassum* sp. cf. *linearifolium*

The sample was collected from Alma Bay, Magnetic Island (08/89) and was frozen and freeze dried. The freeze dried tissue (205 g) was exhaustively extracted with DCM and the resultant crude extract (1.0 g, 0.50 %) was separated by rapid chromatography on silica gel which afforded 20 fractions. ^{1}H n.m.r. and tlc studies of all of these fractions indicated no secondary metabolites of interest had been isolated. The only compound isolated was the ubiquitous brown algal sterol fucosterol.

9.7. Cyanophyta

9.7.1. *Lyngbya* sp.

Sample BR891A

The sample was collected from Bougainville Reef, Coral Sea (04/viii/89) and was frozen and freeze dried. The freeze dried tissue (175 g) was exhaustively extracted with DCM and the resultant crude extract (1.47 g, 0.85%) separated by rapid chromatography on silica gel which afforded 20 fractions. ^{1}H n.m.r. and tlc studies of all of these fractions indicated no secondary metabolites of interest had been isolated.

9.8. Alcyonaria

9.8.1. *Heliopora coerulea*

The sample was collected from Cattle Bay Orpheus Island 14/xi/1991 at a depth of 3-6m. The tissue (718 g fresh weight) was exhaustively extracted with DCM followed by MeOH to yield 2.87 g of crude extract. The crude extract was separated by rapid chromatography on silica gel which afforded 15 fractions.

HPLC separation (Techoprep 5-20 and Si 100 7μm in tandem) (35:100, EtOAc:light petroleum) of fractions 10 and 11 yielded the novel metabolites (66) and (67).

(66) Helioxenicane A, (1*R*,1'*R*,2'*S*,3'*S*,4a*S*,7*S*,8*S*,9*R*,11a*S*)-1-acetoxy-4(1',2'-diacetoxy-3',4'-epoxy-4'-methylpentyl)-8,9-epoxy-7-hydroxy-7-methyl-11-methylene-1,4a,5,6,7,8,9,10,11a-decahydrocyclonona[c]pyran. (106 mg, 3.7%); a yellow oil, $[\alpha]_D$ -17° (c, 0.0013); [Found $M^{\cdot+}$ (EIMS) 406.1979, $C_{26}H_{36}O_{10}$ - $C_4H_6O_3$ requires 406.1991]

u.v. λ_{max} (EtOH) 207 (ε, 10292) nm

i.r. υ_{max} (film) 3565, 2965, 2927, 2853, 1744, 1651, 1455, 1369, 1223, 1169, 1024, 799 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 21.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 21.

EIMS m/z (%, RA): 406 ($M^{\cdot+}$- $C_4H_6O_3$,<1), 288 (<10), 245 (<10), 173 (<10), 164 (<10), 147 (12), 145 (12), 135 (13), 123 (15), 109 (23), 95 (69), 83 (53), 55 (100).

CIMS m/z (%, RA): CI (NH_3) 526 ($M+NH_4^+$,<10), 509 (MH^+, <10), 484 (11), 449 (36), 424 (19), 407 (37), 389 (54), 347 (100), 329 (86), 289 (83), 271 (12), 259 (11), 245 (10).

COSY experiment: The methine proton signal δ2.80 showed a crosspeak to the doublet of doublets δ4.94. Similarly the signal δ4.94 showed crosspeaks to the methine signals δ2.80 and δ5.47, while δ5.47 showed a crosspeak to δ4.94.
The methine signal δ6.10 showed a crosspeak to the methine signal δ2.66, while δ2.66 showed crosspeaks to δ6.10 and the methine signal δ3.20. Similarly the δ3.20 showed a crosspeak to the signal δ2.66. The methine proton signal δ3.00 showed a large crosspeak to the signal δ2.72-2.75 corresponding to the adjacent methine (δ2.74, 62.5 ppm) and methylene (δ2.73, 32.3 ppm) signals, similarly these signals showed a crosspeak to the signal δ3.00. The geminal methylene proton signals δ1.72 and δ2.00 showed crosspeaks to each other.

(67) Helioxenicane C, (1'*S**,2*R**,3b*S**6*S**,7*S**,8*R**,10a*S**,11*S**,12a*R**) -2-(1',2'-epoxy-2'-methylpropyl)-7,8-epoxy-6,11-dihydroxy-6-methyl-10-methylene-2,3b,4,5,6,7,8,9,10,10a,11,12a-dodeca hydrocyclonona[4,5-e]furo[2,3-b]pyran. (24 mg, 0.85%); mobile yellow oil, $[\alpha]_D$ -92° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 346.1779, $C_{20}H_{28}O_6$ - H_2O requires 346.1780]

u.v. λ_{max} (EtOH) 205 (ε, 7754) nm

i.r. υ_{max} (film) 3503, 2926, 2844, 1724, 1716, 1699, 1639, 1583, 1451, 1246, 1109 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 22.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 22.

EIMS m/z (%, RA): 346 ($M^{\cdot+}$- H_2O,<1), 215 (<10), 201 (<10), 147 (11), 145 (13), 131 (15), 121 (17), 119 (17), 107 (24), 95 (54), 83 (65), 71 (45), 55 (100) .

CIMS m/z (%, RA): CI (NH_3) 347 (MH^+ -H_2O, 100), 331 (11), 329 (14), 289 (10), 273 (19), 247 (13), 229 (14), 221 (23), 201 (13), 191 (23), 177 (22), 163 (31), 149 (26), 135 (30), 125 (33), 109 (44)

The second sample of a portion of a female colony of *Heliopora coerulea* was collected from Cattle Bay Orpheus Island 22/xii/1990 at a depth of 2-5m and was frozen and freeze dried. The freeze dried tissue was exhaustively extracted with DCM followed by MeOH yielding 24.5 g of crude extract. The crude extract (9 g) was separated by rapid chromatography on silica gel which afforded 14 fractions.

HPLC separation of fractions 1 and 2 combined (Whatman Partsil M9 10/50 ODS-2) (Acetonitrile) yielded the sesquiterpenes (69) and (68).

(69) (1*R**,3a*R**,7*S**,8a*S**)-7-isopropylene-1-methyl-4-methylene-1,2,3,3a,4,5,6,7,8,8a-decahydroazulene. (65 mg, 0.72%); mobile yellow oil; $[\alpha]_D$ -3.0° (c,0.001); [Found $M^{\cdot+}$ (EIMS) 204.1877, $C_{15}H_{24}$ requires 204.1878]

u.v. λ_{max} (EtOH) 211 (ε,3043) 242 (sh) (ε,1228) nm

i.r. υ_{max} (film) 3077, 2924, 2361, 1727, 1638,1445, 1378, 891 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 23.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 23.

EIMS m/z (%, RA): 204 ($M^{\cdot+}$,<10), 189 (10), 162 (12), 161 (17), 147 (20), 135 (22), 133 (23), 121 (28), 107 (53), 93 (68), 91 (95), 83 (24), 82 (27), 81 (100).

(68) calamenene. mobile oil; $[\alpha]_D$ +9.9° (c, 0.001); ^1H n.m.r. data similar to that previously reported (de Mayo *et al.* 1965).

^1H n.m.r. ($CDCl_3$, 300 MHz): δ0.77 (d, *J*6.7 Hz, CH_3), 1.04 (d, *J*6.7 Hz, CH_3), 1.25 (d, *J*7.0 Hz, CH_3), 2.30 (s, CH_3), 1.60-1.90 (m, 2x CH_2), 2.25 (m, CH), 2.59 (ddd, *J*6.8, 6.8, 7.0 Hz, CH), 2.86 (ddd, *J*6.8, 6.8, 7.0 Hz, CH), 6.96 (dd, *J*1.4, 8.0 Hz, CH), 7.04 (s, CH), 7.05 (d, *J*8.0 Hz, CH).

^{13}C n.m.r. ($CDCl_3$, 75 MHz): 17.5 (q), 19.5 (t), 21.1 (q), 21.4 (q), 23.3 (q), 28.6 (t), 31.0 (d), 32.4 (d), 43.5 (d), 126.2 (d), 128.4 (d), 128.6 (d), 134.4 (s), 139.7 (s), 139.9 (s).

HPLC separation (Techoprep 5-20 and Si 100 7μm in tandem) (light petroleum) of fractions 4 and 5 yielded novel metabolite and (70).

(70) Helioproene A, (1*S**,3*R**,7*S**,9a*R**)-3-(2'-methyl-1'-propenyl)-1,4,7-trimethyl-5,6-methylenedioxy-2,3,7,8,9,9a-hexahydro phenalene. (45 mg, 0.50%); white crystalline solid; m.p. 96-98° C; $[\alpha]_D$ -85° (c, 0.001); [Found $M^{\cdot+}$ (EIMS) 312.2091, $C_{21}H_{28}O_2$ requires 312.2089].

u.v. λ_{max} (EtOH) 219 (ε, 21192) nm

i.r. υ_{max} (film) 3447, 2926, 2856, 1734, 1700, 1539, 1488, 1423, 1375, 1089, 890 cm^{-1}

^1H n.m.r. ($CDCl_3$, 300 MHz): see Table 24.

^{13}C n.m.r. ($CDCl_3$, 75 MHz): see Table 24.

EIMS m/z (%, RA): 312 ($M^{\cdot+}$,70), 297 (45), 255 (30), 213 (25), 203 (10), 199 (19), 165 (20), 152 (18), 141 (21), 128 (29), 115 (30), 91 (28), 77 (33), 69 (47), 55 (100).

COSYDQF experiment: The methyl signal δ1.67 (25.6 ppm) showed crosspeaks to the methyl signal δ1.74 (17.7 ppm) and the methine signal δ5.10 (130.2 ppm). Similarly the methyl signal δ1.74 showed

crosspeaks to the methyl signal δ1.67 and the methine signal δ5.10 while the methine proton δ5.10 showed crosspeaks to both methyl signals δ1.67 and 1.74 and to the methine proton signal δ3.58 (35.2 ppm). The methine proton signal δ3.58 showed crosspeaks to the methine signal δ5.10 and the methylene proton signals δ1.65 (39.7 ppm). The methylene proton signals δ1.65 showed crosspeaks to the methine signals δ3.58 and δ1.59 (29.6 ppm) and to the methyl signal δ1.03 (20.8 ppm), while the methyl signal δ1.03 showed crosspeaks to the signals δ1.65 and δ1.59. The methine proton signal δ1.59 showed crosspeaks to the methyl signal δ1.03, the methine proton signals δ1.65 and the methine proton signal δ2.01 (44.8 ppm) while the methine proton signal δ2.01 showed crosspeaks to δ1.59 and the methylene proton signals δ1.15 and 2.15 (28.3 ppm). The methylene proton signal δ1.15 showed crosspeaks to the methylene proton signal for its geminal partner and the adjacent proton δ2.15-2.17 (28.3 and 32.3 ppm) and to the methylene proton signal δ1.40 (32.3 ppm), while δ2.15-2.17 (28.3 and 32.3 ppm) showed crosspeaks to δ1.15, 1.40 and the methine proton signal δ3.07 (28.9 ppm). The methine proton signal δ3.07 showed crosspeaks to the methyl proton signal δ1.27 (22.3 ppm) and methylene proton signals δ1.40 and 2.17 (32.3 ppm).

NOESYPH experiment: The methyl proton signal δ1.03 showed crosspeaks to the methylene proton signal δ1.65, and the signal for the two methylene protons δ2.15 and 2.17. Similarly the signal for the two methylene protons δ2.15 (28.3 ppm) and 2.17 (32.3 ppm) showed crosspeaks to the methyl signal δ1.67, the methylene signals δ1.15 and δ1.40 and the methine signal δ3.07. The methylene signal δ1.15 showed crosspeaks to the signal for the

methylene protons δ2.15 and 2.17, and the methine proton signal δ2.01 (44.8 ppm). The methylene proton signal δ1.40 showed crosspeaks to the methyl proton signal δ1.27, the methine signal δ2.01 (44.8 ppm) and the signal for the methylene protons δ2.15 and 2.17. The methyl signal δ1.27 showed a crosspeak to the methine signal δ3.07, while the signal δ3.07 showed crosspeaks to the methyl signal δ1.27, and the signal for the methylene protons δ2.15 and δ2.17. Correlations from the methyl proton signal δ2.01 showed crosspeaks to the methyl signal δ1.74 and the methine signals δ3.58 and δ5.10. Similarly the signal δ3.58 showed crosspeaks to the methyl signal δ2.01, the methyl signal δ1.74 and the methylene proton signals δ1.65. The methyl signal δ1.74 showed crosspeaks to the aromatic methyl signal δ2.01 and the methine signal δ3.58. The methine signal δ5.10 showed crosspeaks to the methyl signals δ1.67 and δ2.01 while the signal for the overlapping methylene signals δ1.65 (39.7 ppm) and the methyl signal δ1.67 (25.6 ppm) showed crosspeaks to the methyl signals δ1.03 and δ1.67, and the methine signals δ3.58 and δ5.10.

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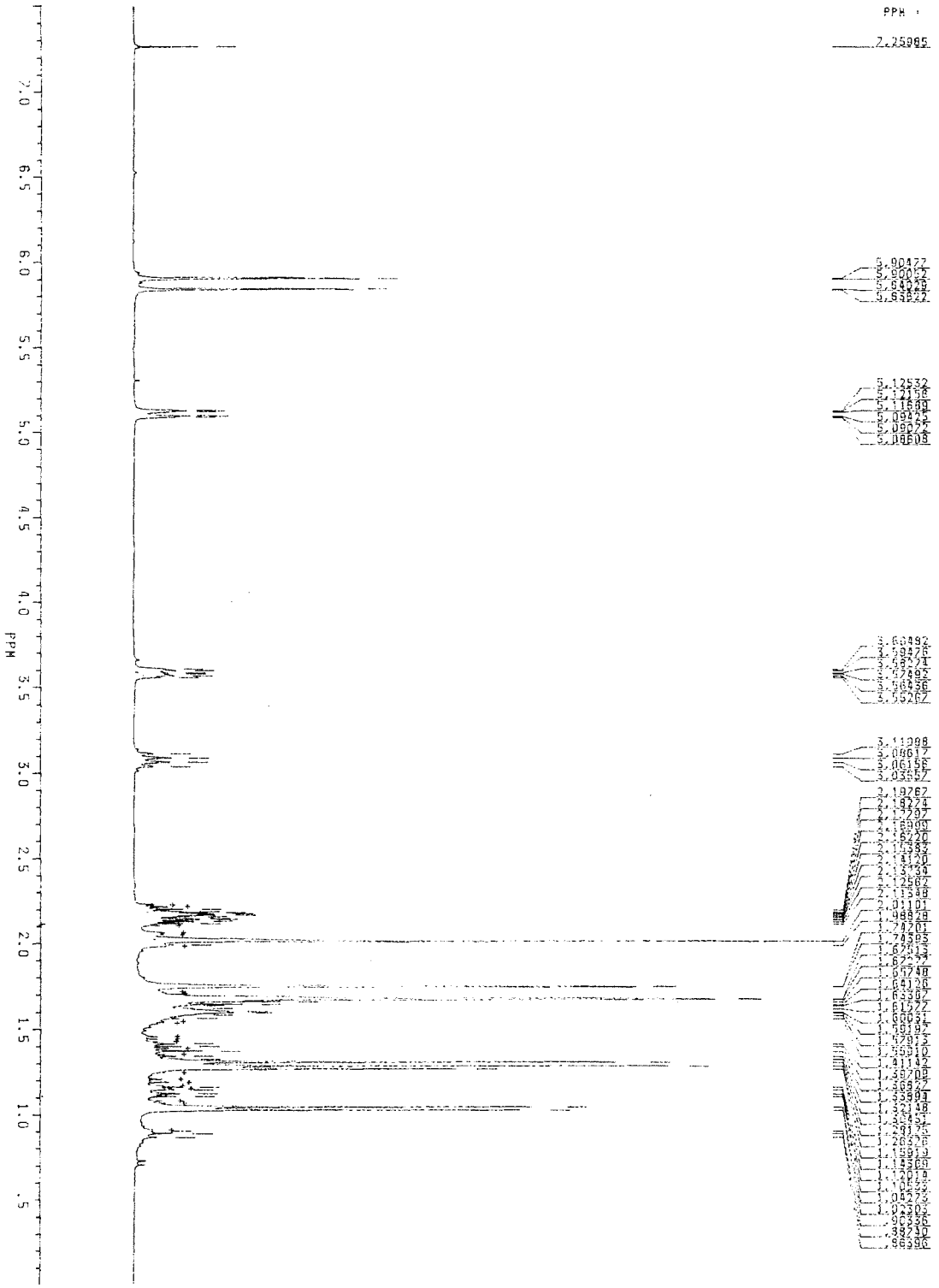
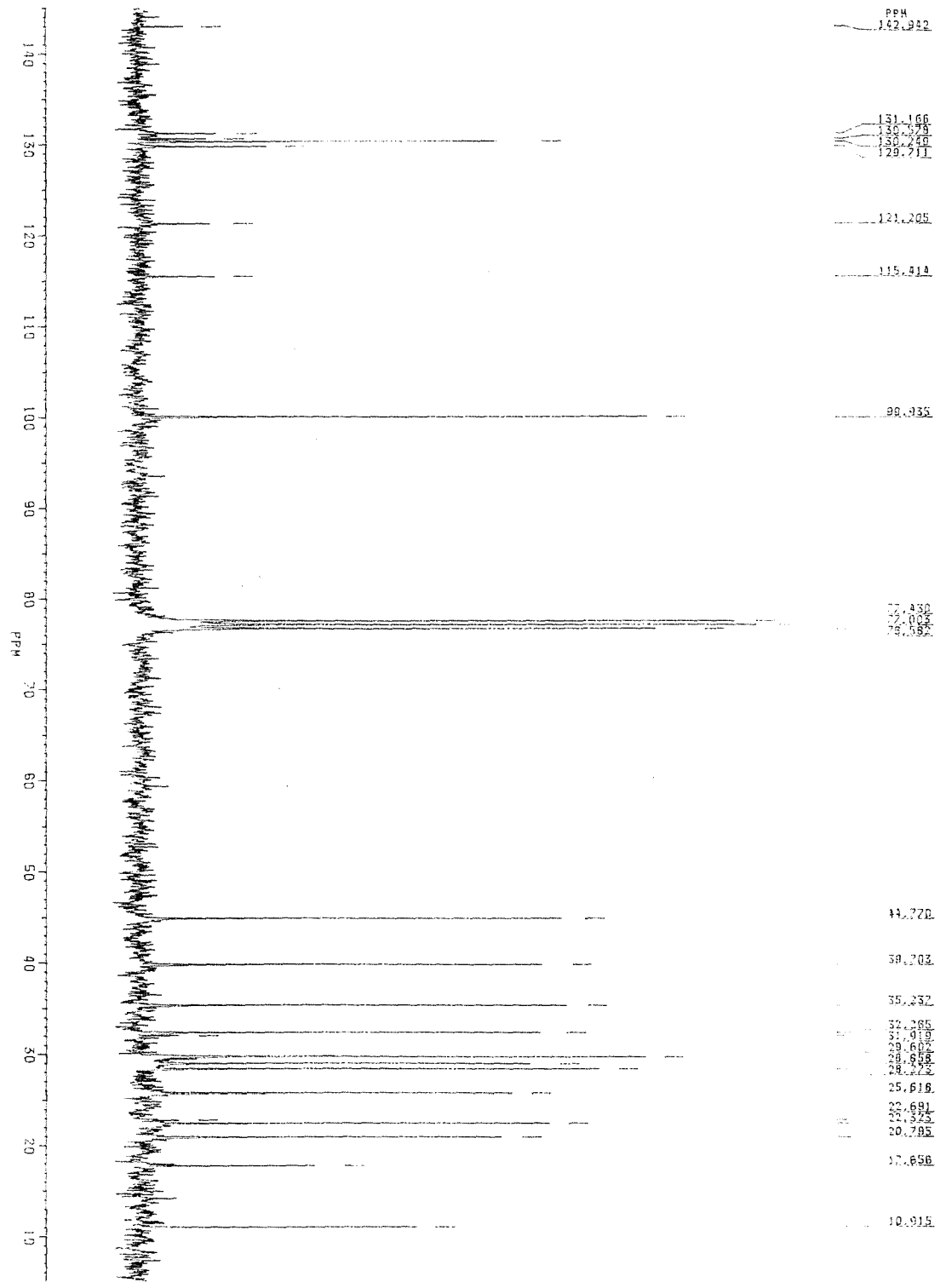
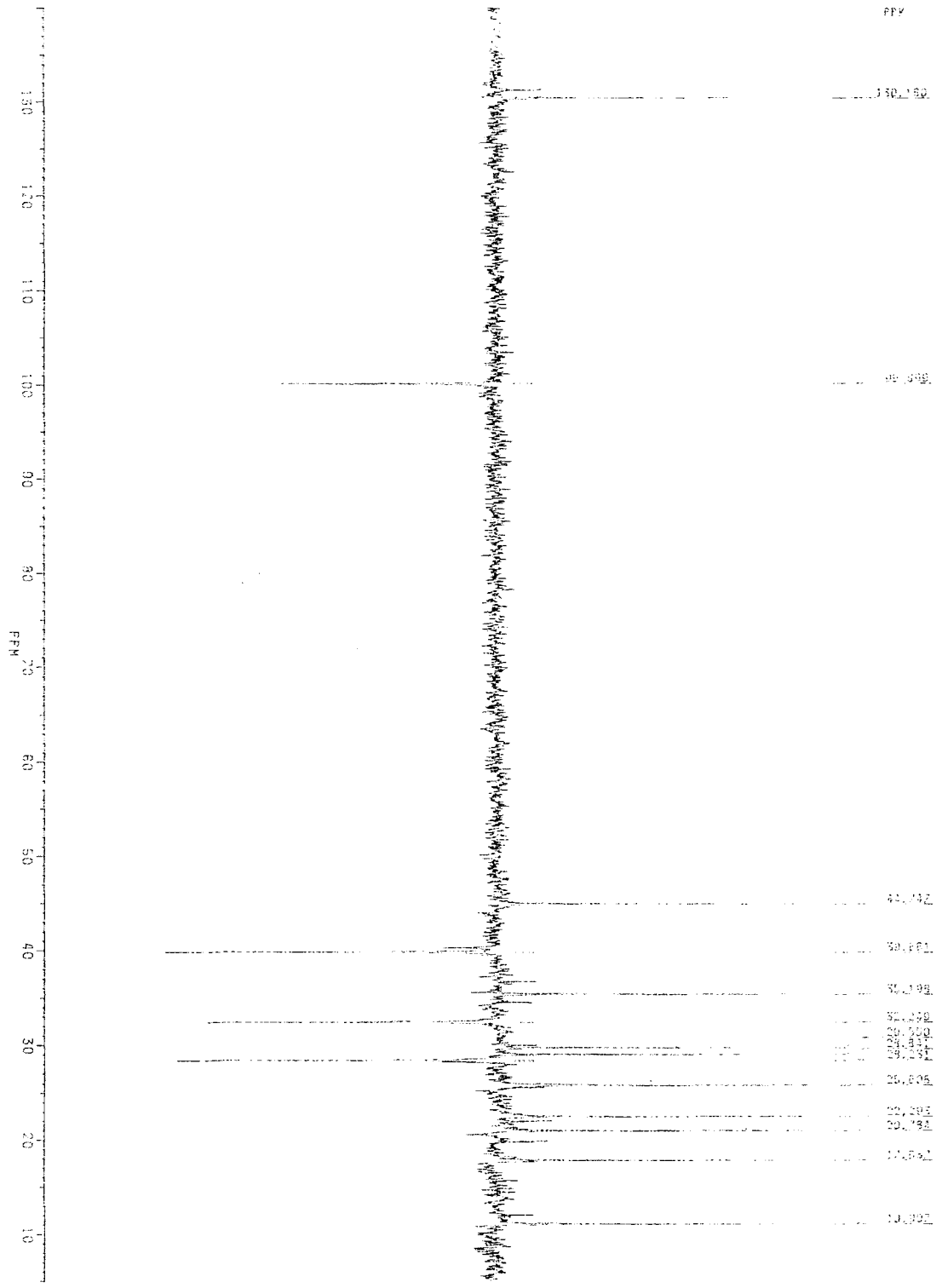
Appendix I



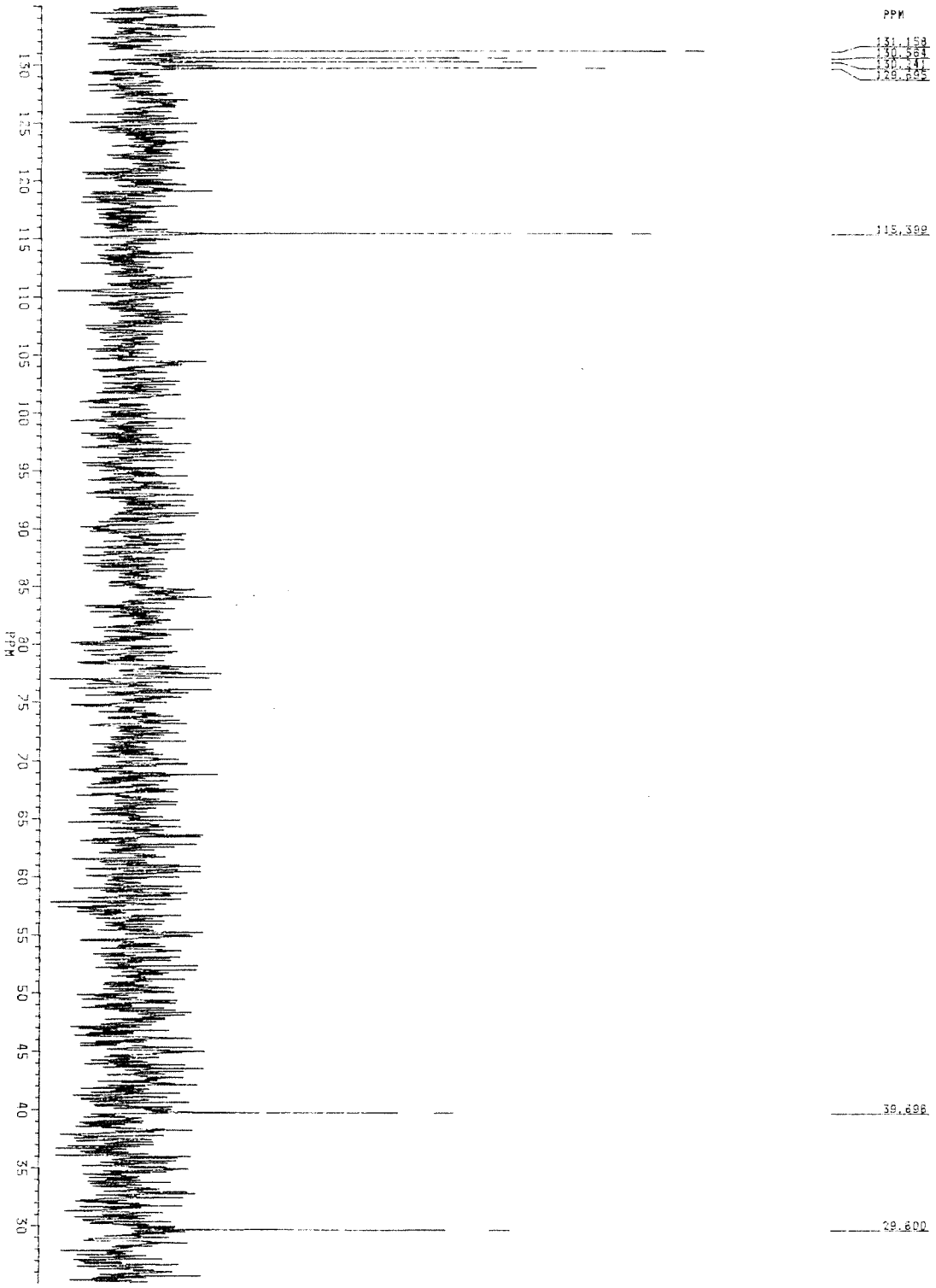
^{1}H n.m.r. spectrum of (70)



^{13}C n.m.r. spectrum of (70)

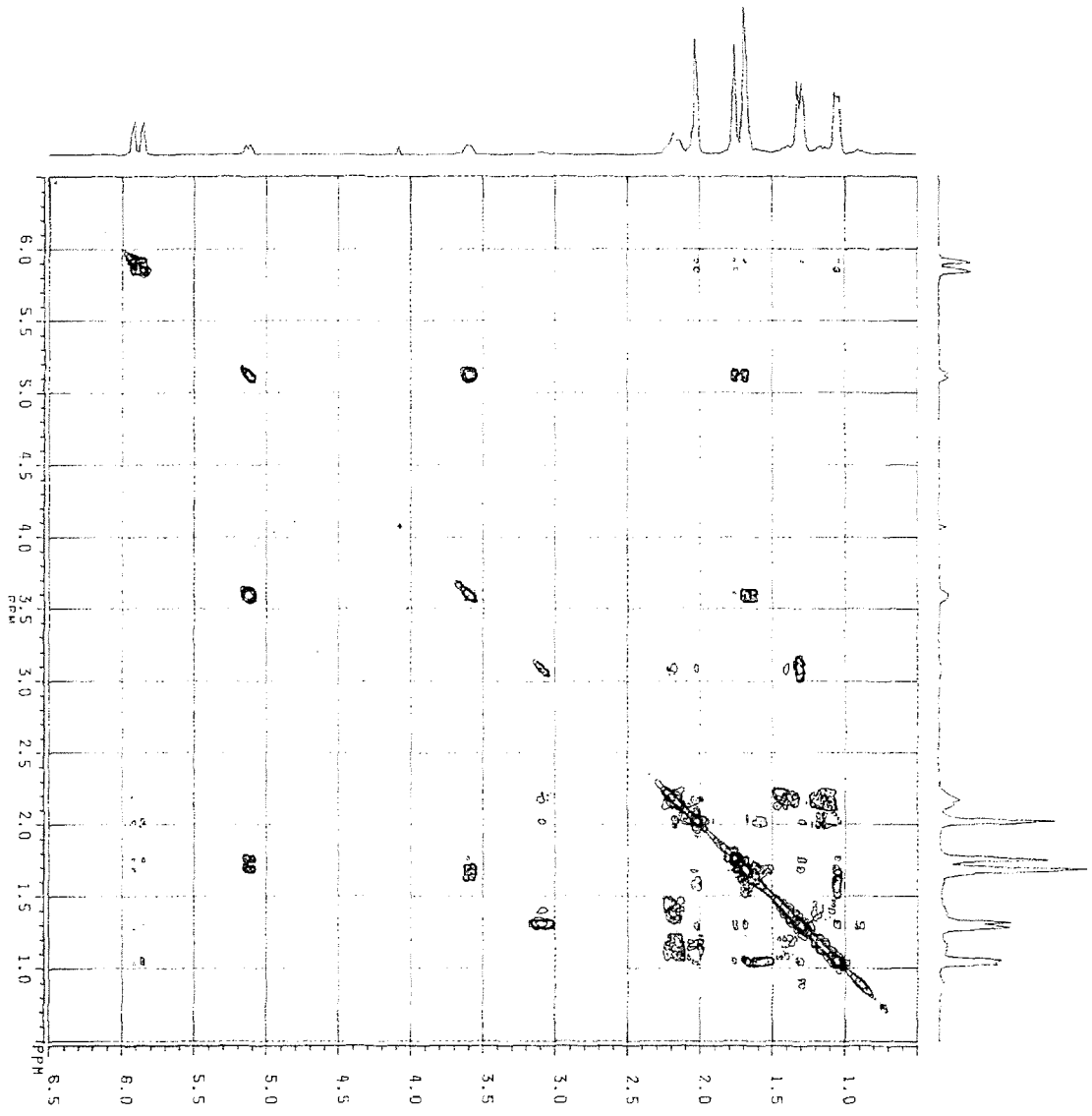


DEPT (135^0) spectrum of (70)

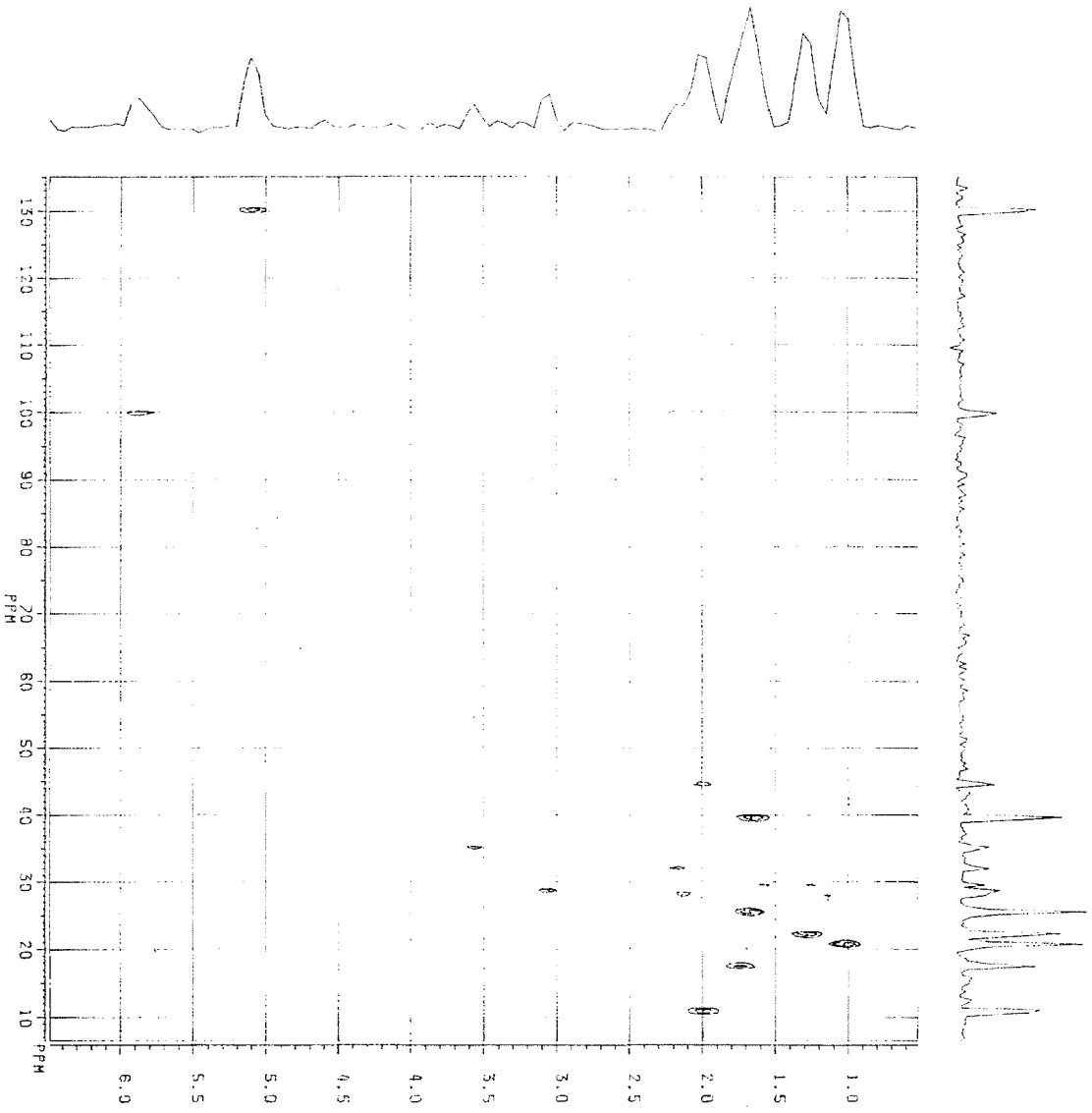


INAPT (J= 7 Hz) spectrum of (70)

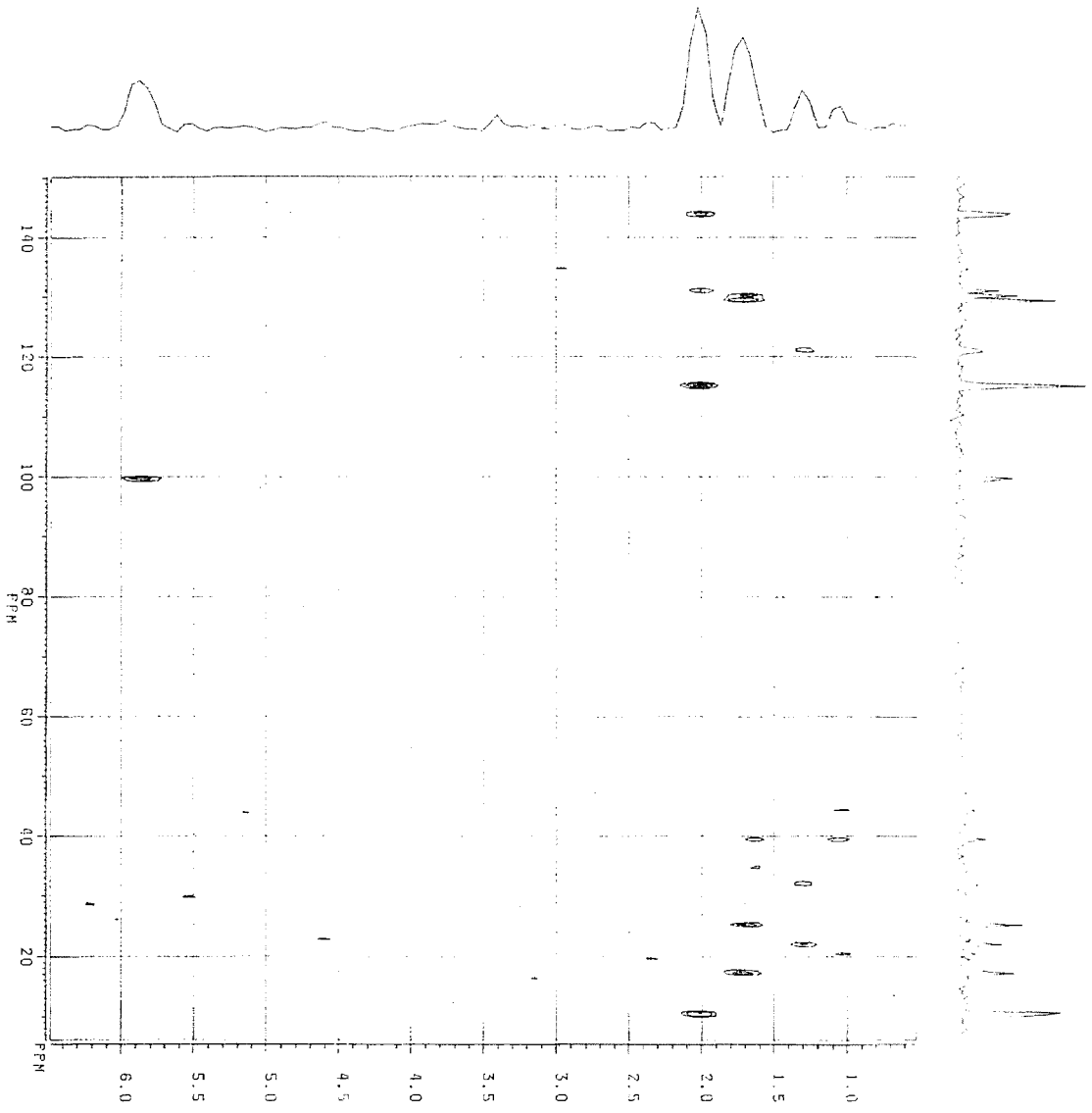
(selective irradiation of the methine proton signal δ3.58)



COSYDQF spectrum of (70)



Short range XHCORRD (J= 135 Hz) spectrum of (70)



Long range COLOC (J= 10 Hz) spectrum of (70)