Secondary Metabolites from the Red Seaweeds *Plocamium* Species

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Summary

The red seaweeds *Plocamium* species are generally distributed around the world and continuously produce potential secondary metabolites. This review intends to show typical secondary metabolites from *Plocamium* from 1984 to present focusing on methods of isolation, structure determination, and biological activity where possible. Monoterpenes are the major isolated secondary metabolites from *Plocamium*.

Keywords: Red seaweeds; *Plocamium*; Secondary metabolites; Monoterpenes; Biological activity

INTRODUCTION

Natural products are compounds that are intrinsically produced by an organism and known as secondary metabolites. However, they are not typically essential for the organism's survival. Whereas the photosynthetic process and the Krebs cycle products, e.g. carbohydrates, proteins, nucleic acid, amino acids, and fats are primary metabolites, which involve in the life processes (Haslam, 1986; Torssell, 1997). Secondary metabolites perform such functions as chemical defenses, toxins, antifouling, and overgrowth inhibition (Pawlik, 1993). Therefore, secondary metabolites are basically important in terms of chemical ecology among organisms.

The field of natural product chemistry has been developed as a part of organic chemistry since around the middle of the 19th century. Nowadays, the feasibility to explore natural products from the ocean world rather than focusing only on terrestrial organisms is increasing as a result of the possibility to access marine organisms by scuba diving or from deeper water by special submersibles (Wright, 1998), which allows chemists to explore an otherwise untouchable water world. In addition, the problems of taxonomy, marine biologys and spectroscopy continue to be diminished because of increasing tools, experts, and the development of instrumentation (Kelly-Borges, 1998; Reynolds & Enríquez, 2002). The cooperation of chemists, biologists, and taxonomists is essential in order to overcome the overall problems and to help one another solving the problems better.

Chemical ecology, pharmacology, microbiology, biotechnology including genetic engineering and total synthesis are related to the field of natural products. Some secondary metabolites from both terrestrial and marine sources are of interest. Terrestrial source, e.g. paclitaxel or taxol from the bark of the Pacific Yew Tree *Taxus brevifolia*, which are used as drugs and michellamine B from the leaves of the liana *Ancistrocladus korupensis* are potential and useful metabolites. Marine sources, e.g. bryostatin-1 from the bryozoan *Bugula neritina*, and dolastatin 10 from the sea hare *Dolabella auricularia* are in the stage of trial drugs and being tested as antimitotic compounds for microtubules (Andersen, 2001; Cragg et al., 1999).

Marine natural products have been annually reviewed by Faulkner since 1984 till thelast for his age (Faulkner, 1984; Faulkner, 2002). Then the New Zealand group (Blunt et al., 2003) has continued. The reviews discuss and classify secondary metabolites from various types of marine organisms. Faulkner also reviewed chemical defenses of marine molluscs such as sea hares of the genus *Aplysia*, ascoglossans, nudibranchs, other opisthobranchs (e.g. cephalaspidean mollusc, notaspidean mollusc), marine pulmonates (e.g. onchiids, siphonariids, trimusculiids), limpets, prosobranch mollusks (Faulkner, 1992).

McClintock and Baker edited a recent review in marine chemical ecology. This book demonstrated background and introduction to chemical ecology of marine natural products including biosynthesis and as an evolutionary narrative in the first section, an organismal approach to understanding the role of secondary metabolites in mediating trophic interrelationships in the second section, review of cellular and physiological aspects of marine chemical ecology in the next section, and applied marine chemical ecology in the last section (McClintock & Baker, 2001).

SECONDARY METABOLITES FROM *PLOCAMIUM*

Seaweeds are very important among the primary producers as they are significant links in the food web. They function as food organisms to

the marine and fresh water environments. Benthic seaweeds constitute one of the major pathways for energy and material flows in shallow coastal ecosystems and are instrumental in the turnover of nutrients in the near-shore benthos. Seaweeds are the basis of a multibillion dollar enterprise which is quite diversified, including food, textiles, pharmaceuticals, cosmetics and biotechnological fields. Their diversity will assure that new secondary metabolites will be discovered all the time and will be beneficial to mankind. Seaweeds have been increasingly used as biodetectors and to analyze autoecological as well as synecological problems, by combining physical, chemical, and biological measurements to collect relevant information for the management of coastal zone (Misheer et al., 2006).

Seaweeds probably produce halogenated organic compounds as a part of the defence system against microorganism infections, herbivore grazing, space competitors, detrimental fouling by different kinds of epiphytes, or excess of self-genereated hypochlorite and hydrogen peroxide. Halogenated secondary metabolites are common in marine organisms because of the abundance of chloride and bromide ions in seawater. Organisms that have the ability to form halogenated compounds have been found among various species of bacteria, seaweeds, sponges, mollusks, coelenterates, and several marine worms. However, an enormous number of organohalogens have been isolated from most genera of Rhodophyta. A significant number of halogenated metabolites have shown biological properties ranging from antimicrobial to insecticidal activities. The discovery of the acyclic monoterpene halomon, which displays selective antitumor activity in the National Cancer Institute's human tumor and disease oriented *in vitro* screen (Kladi et al., 2004).

The red seaweed genus Plocamium is distributed around the world in cool to temperate sea. *Plocamium cartilagineum* (L.) Dixon is an example, which belongs to Phylum Rhodophyta, class lorideophyceae, order Gigartinales, and family Plocamiaceae. Species of *Plocamium* are normally found on coasts of strong to moderate wave zones. They are quite common as drift on the shore or at depths of 2-26 m (Fuhrer et al., 1981; Womersley, 1994).

Previous reviews of halogenated monoterpenes from *Plocamium* have appeared in the literature from time to time. Halogenated compounds from the Rhodophyta were reviewed (Fenical, 1975). Seventeen monoterpenes from red seaweeds were described, six of which were from *Plocamium cartilagineum* and three from *Plocamium* spp. The other eight metabolites, which were acyclic monoterpenes, were from *Desmia hornemanni*. Among the seventeen compounds, the only cyclic monoterpene was from *Plocamium violaceum*.

Types of halogenated monoterpenes from Plocamium as 1, 5, 7-octatriene, 1, 5-octadiene, 2, 7-octadiene, 1-vinyl-1, 5-dimethyl-cyclohexane, 2-vinyl-1, 5-dimethyl-cyclohexane and dihydropyran derivatives were reported (Crews, 1977b).

Biosynthesis of halogenated cyclic monoterpene metabolites were reported from *Plocamium cartilagineum*, which was carried out through four steps of allylic chlorination with displacement of an oxygen substituent, allylic halogenation, allylic chlorination involving the shift of a double bond and cyclisation (Barrow, 1983).

A critical review of marine monoterpenes was reported (Naylor et al., 1983). No simple correlation of secondary metabolites and organism taxonomy could be concluded.

As said before, since 1984 there have been annual reviews for marine natural products. In addition, monoterpenes in naturally occurring organohalogen compounds have also been surveyed (Gribble, 1999), and the diversity of naturally occurring organobromine compounds has been reported (Gribble, 1996).

This review will attempt to discuss all monoterpenes and nonterpenoid metabolites discovered in the red seaweed genus *Plocamium* covering the literature published from 1984 to 2007, focusing on secondary metabolites especially novel compounds and methods of isolation, structure determination and biological activity where possible. Secondary metabolites will be discussed in compound types. The drawing of structures is based on the original publications.

Polyhalogenated acyclic monoterpenes

Plocamenone (1) from *Plocamium* sp., which was collected off the coast of New South Wales, Australia was reported (Stierle & Sims, 1984). The ketone was isolated as the major constituent from hexane extraction and followed by silica gel chromatography. In addition, plocamenone has been shown to be a potent mutagen in the Ames reversion assay.

The same year, Crews et al. (1984) described a new (2) and a revised (3) monoterpenes from *Plocamium violaceum* and *Plocamium cartilagineum*, which were intertidally collected from Patrick's Point, Humboldt County and Davenport Landing, Santa Cruz County, California, respectively. Acyclic compound (2) was purified by HPLC, 97% hexane/ethyl acetate of fractions 7-9 from gradient elution flash chromatography. Moreover, carbon nuclear magnetic resonance (^{13}C NMR) chemical shifts were used to explain halogen regiochemistry or six-membered ring substituent stereochemistry in marine monoterpenes, together with using

new additivity parameters and model compounds in ^{13}C NMR chemical shifts data. However, no incremental constants were available to calculate ^{13}C chemical shifts of tertiary halogenated carbons. It is possible to distinguish between C-Br and C-Cl by ^{13}C NMR since α-substituent effects are large (see Figure 1).

Eight new compounds (4-11) from *Plocamium cartilagineum* were investigated (Blunt et al., 1985). The plants had different appearances and were separated into type I from St. Kilda Rocks and type II from the Old Wharf, Kaikoura, New Zealand. Type I was collected in February, 1979, while type II was collected in February, 1980. The samples were collected from the two areas, which are geographically close but their constituents were found to be quite different. The old Wharf sample (type II) contained primarily the C_8 dichlorodienol (12), while the St. Kilda Rocks sample (type I) contained the linear monoterpenes dienes, epoxides and alcohols (4-11, 13) and only a trace of the dichlorodienol (12). It was concluded that the genetic differences occurring between type I and II were the cause of the different metabolites elaborated by *Plocamium cartilagineum* from different times and from different locations around the Kaikoura Peninsula.

The structure of plocamenone (14) has been revised from previous structures (15-16) (Naylor et al., 1983) by using new ^{13}C substituent effect values on model compounds (Naylor et al., 1985) (see Figure 2 and Figure 3).

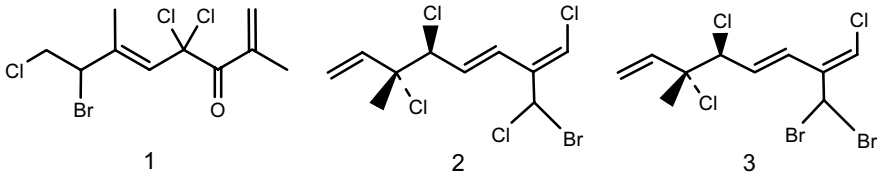
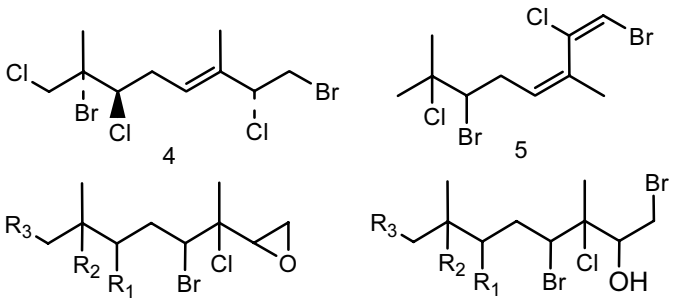


Figure 1. Stuctures of polyhalogenated acyclic monoterpenes (1-3).



6 R_1=Cl, R_2=Br, R_3=H
7 R_1=Br, R_2=Cl, R_3=H
8 R_1=βCl, R_2=αBr, R_3= Cl

9 R_1=Cl, R_2=Br, R_3=H
10 R_1=Br, R_2=Cl, R_3=H
11 R_1=βCl, R_2=αBr, R_3= Cl

Figure 2. Structures of polyhalogenated acyclic monoterpenes (4-11).

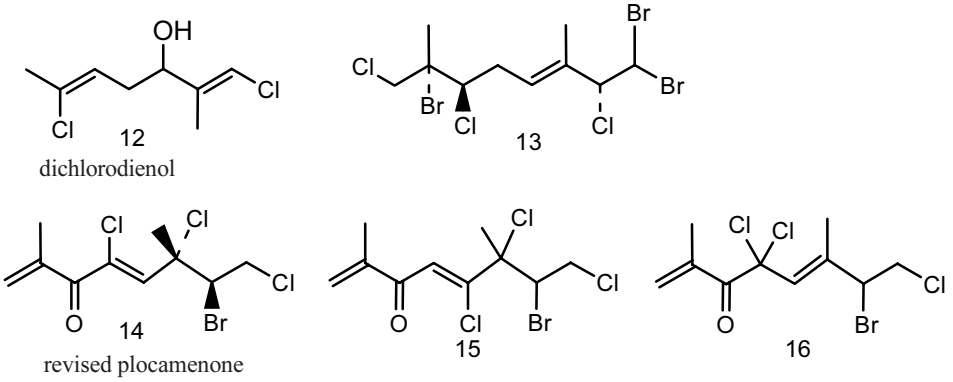


Figure 3. Structures of polyhalogenated acyclic monoterpenes (12-16).

Two new acyclic monoterpenes (17-18) (see Figure 4) were also reported from *Plocamium hamatum*, which was collected from the channel between Orpheus Island and Pelorus Island, Palm Island group in Australia (Coll et al., 1988).

One new acyclic (19) and a known (20) halogenated monoterpenes were isolated from *Plocamium cartilagineum* which was collected at Rada Covadonga, Antarctic Peninsula (Rovirosa et al., 1990). Mass spectrometry of the acyclic monoterpene (19) did not display the expected halogen cluster at the molecular ion but the cluster resulting from the loss of an -OH group from the molecular ion was observed. The absolute configuration of the compound (19) was not determined. All two compounds (19-20) showed antibiotic activity against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*. Compound (19) showed the strongest activity against the microorganism *Staphylococcus aureus*. However, all two compounds were inactive against *Staphylococcus epidermidis*, *Bacillus arthacis* and *Saccharomyces cerevisiae* (see Figure 5).

One new acyclic (21) and a known (22) polyhalogenated monoterpenes were reported from *Plocamium cartilagineum* which was collected from northern Spain (König et al., 1990). High performance liquid chromatography (HPLC) of combined fractions 1 and 2 from vacuum liquid chromatography, using reverse phase C18 with methanol:acetonitrile:water (64:9:27) as an eluent, afforded compound (21) as a clear oil. The alicyclic compounds (21-22) were not toxic towards both *Biomphalaria glabrata* and *Artemia salina* at the concentrations tested. Only relative stereochemistry was assigned for the new metabolite (21).

Polyhalogenated acyclic monoterpene aldehyde (23) was isolated from *Plocamium cartilagineum* from the Portuguese coast (Abreu & Galindro, 1996). A sequence of silica gel, preparative high performance thin layer chromatography (HPTLC), and reversed phase HPLC techniques was used for purification of compound (23). The structure was determined using the basic NMR, double quantum filtered-correlation spectroscopy (DQF-COSY), proton-proton correlation spectroscopy (^{1}H-^{1}H COSY) and mass spectrometry (MS), however only the relative stereochemistry was addressed. The hexane extract containing (23) was lethal for the fish *Lesbites reticulatus* in one hour at concentrations of less than 50 mg/L (Abreu & Galindro, 1996) (see Figure 6).

One new (24) and three known (25-27) acyclic halogenated monoterpenes were also investigated from *Plocamium costatum* which was collected from Deep Glen Bay, Eaglehawk Neck, Tasmania, Australia at a depth of 25-30 m (König et al., 1999). The dichloromethane extract and compound (26) deterred settlement of barnacle larvae, suggesting a potential ecological role. All (24-27) showed little or no effect on antimicrobial and antialgal activities, and none demonstrated inhibition of reverse transcriptase of the human immunodeficiency virus type 1 (HIV-1-RT), tyrosine kinase p56^{lck} (TK). Compound (27) had only weak effects and only toward brine shrimp at a test concentration of 0.5 mg/mL with *Artemia salina* and *Caenorhabditis elegans* (see Figure 7).

Two new (28-29) and two known (30-31) polyhalogenated acyclic monoterpenes were isolated from *Plocamium cartilagineum*, which was collected from the East coast of Tasmania, Australia (Jongaramruong & Blackman, 2000). The fraction containing halogenated monoterpenes showed one hundred percent mortality at concentrations of 92.5 µg/mL or greater after 15 hours with *Artemia salina* (see Figure 8).

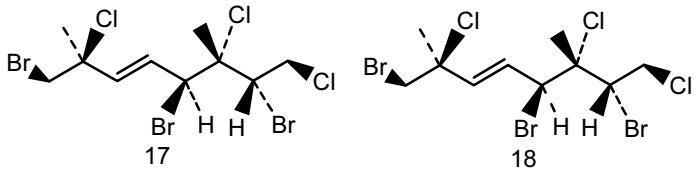


Figure 4. Structures of polyhalogenated acyclic monoterpenes (17-18).

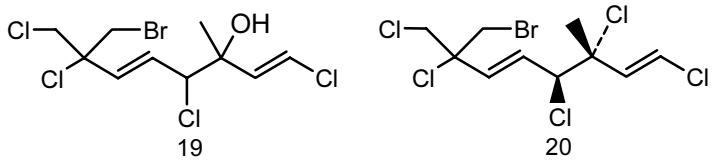


Figure 5. Structures of polyhalogenated acyclic monoterpenes (19-20).

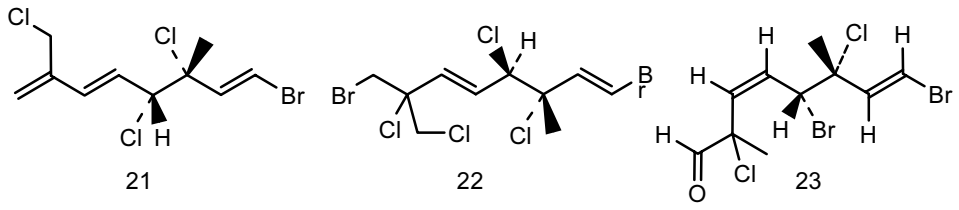


Figure 6. Structures of polyhalogenated acyclic monoterpenes (21-23).

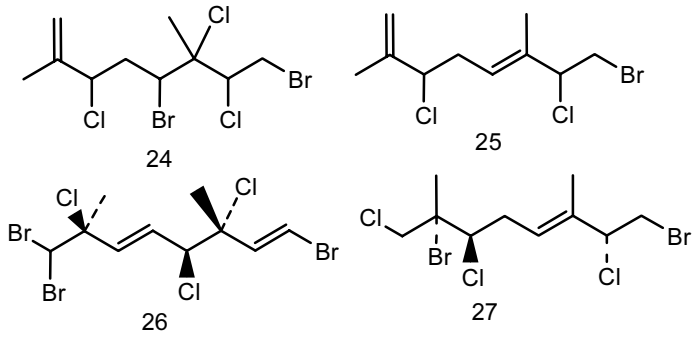


Figure 7. Structures of polyhalogenated acyclic monoterpenes (24-27).

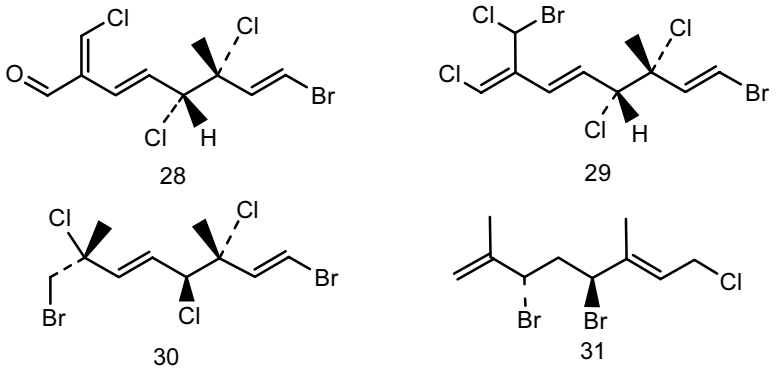


Figure 8. Structures of polyhalogenated acyclic monoterpenes (28-31).

Metabolite (32) was isolated from *Plocamium* (Wessels et al., 2000) and demonstrated significant cytotoxicity toward three cancer cell lines HM02 (gastric carcinoma), HEP G2 (liver carcinoma), and MCF 7 (breast carcinoma) at the 50% growth inhibition concentration (IC_{50}) of 1.0 to 1.5 μg/mL.

The chloroform extract of *Plocamium cartilagineum* (Ankisetty et al., 2004) was subjected to gradient flash chromatography to yield a fraction (90:10 hexane/ethyl acetate) from which anverene (33) was obtained.

A new acyclic polyhalogenated monoterpene (34) has been isolated from *Plocamium cartilagineum*, which was collected by scuba diving at El Quisco, V Region of Chile. It was extracted with a mixture of hexane/ethyl acetate/dichloromethane/methanol (1:4:4:1). The crude extract was chromatographed by flash chromatography on silica gel, followed by gel filtration on LH-20 column and further purified on HPLC (Díaz-Marrero, Rovirosa et al., 2002).

Three new minor linear polyhalohydroxylated marine monoterpenes, plocamenols A-C (35-37), have been isolated from *Plocamium cartilagineum*, which was collected from the Chilean coast (V Region) using scuba diving. It was extracted with ethyl acetate and the crude extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane/ethyl acetate (75:25) was further separated by filtration chromatography to give a fraction which was chromatographed on a silica gel column to obtain a mixture of monoterpenes (35-37). It was further purified by recycling-HPLC, using chloroform as the eluent (Díaz-Marrero, Cueto et al., 2002).

Two minor linear dihalo-vinyl monoterpenes (38-39) have been isolated from *Plocamium cartilagineum*, which was collected off by scuba diving at El Quisco, V Region of Chile. Air-dried *Plocamium cartilagineum* was extracted with ethyl acetate. The fraction eluted by flash

chromatography on silica gel with hexane/ethyl acetate (96:4) was further separated by gel filtration chromatography to afford a fraction which was purified by HPLC (normal phase) with hexane/ethyl acetate (90:10) to give the compound (38). Compound (39) was obtained after gel filtration chromatography of the fraction eluted with hexane/ethyl acetate (75:25) by flash chromatography. In this report mentioned the comparison of the ^{1}H and ^{13}C NMR data of (38) with those of prefuroplocamioid (40) to determine the regiochemistry and geometry of the rare 1,2-bromochloro vinyl system (Díaz-Marrero et al., 2004).

Three new ocimene-type polyhalogenated monoterpenes, plocoralides A-C (41-43) have been isolated from *Plocamium corallorhiza*, which was collected from the intertidal zone at Kalk Bay near Cape Town, South Africa. The frozen sample was steeped in cold methanol and extracted with dichloromethane. The extract was separated with silica gel chromatography using hexane with increasing proportions of ethyl acetate. The first fraction was further purified by repeated normal phase HPLC (hexane/ethyl acetate, 95:5 to 100:0) to give plocoralides A-C (41-43) (Knott et al., 2005).

Two known compounds, 4,6-dibromo-1, 1-dichloro-3,7-dimethyl-2*E*,7-octadiene (41) and 1, 4,8-tribromo-3,7-dichloro-3,7-dimethyl-1*E*, 5*E*-octadiene (44) (see Figure 9).

The four new halogenated monoterpene aldehydes (45-48) have been isolated from the South African marine red alga *Plocamium corallorhiza*. It was collected at low tide near Kenton-On-Sea, South Africa and extracted sequentially with methanol and dichloromethane/ methanol (2:1). The extracts were combined and fractionated by solvent partitioning. Aldehyde proton signals were present in the ^{1}H NMR spectra of the hexane and dichloromethane fractions. These fractions were further fractionated by silica gel column chromatography and final purification was achieved by semipreparative normal phase HPLC to obtain two known and four new, unstable, halogenated monoterpene aldehydes (45-48) (Mann et al., 2007) (see Figure 10).

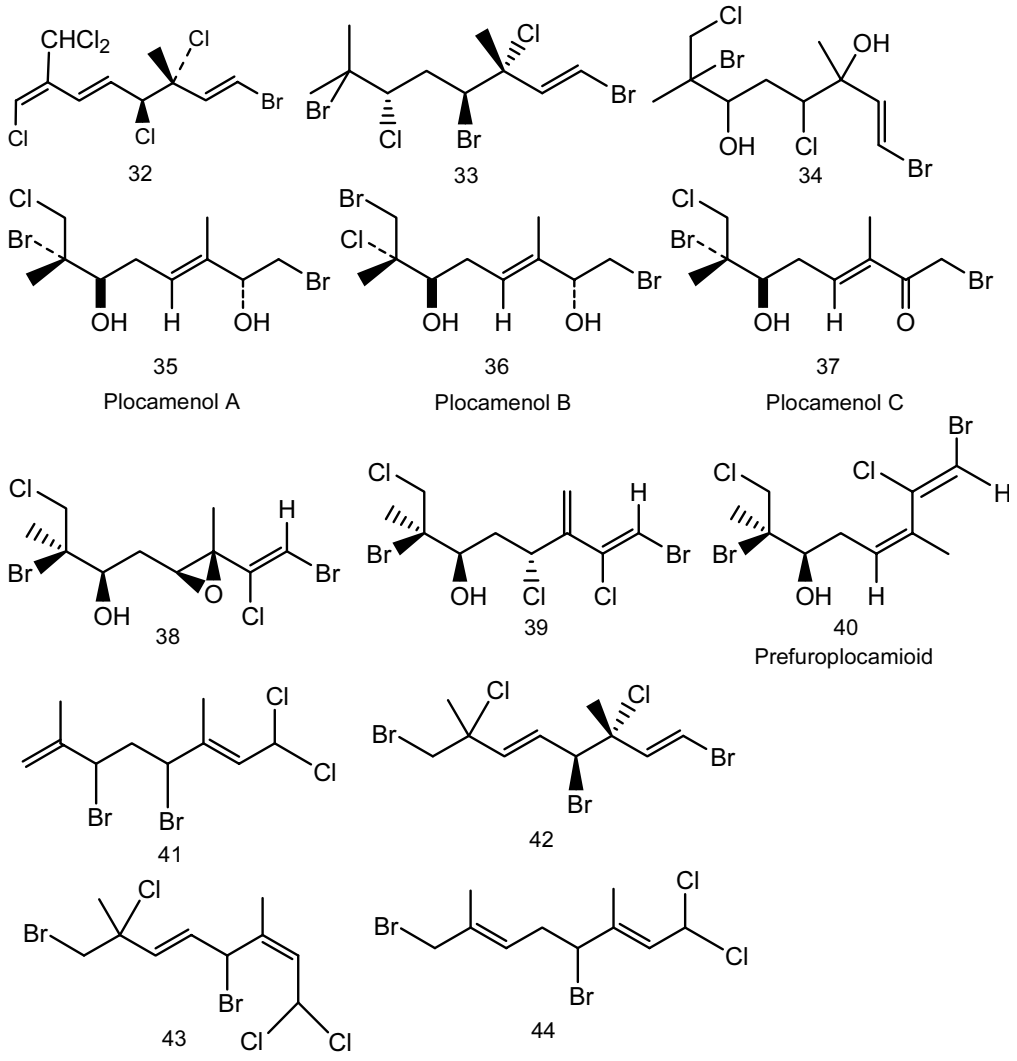


Figure 9. Structures of polyhalogenated acyclic monoterpenes (32-44).

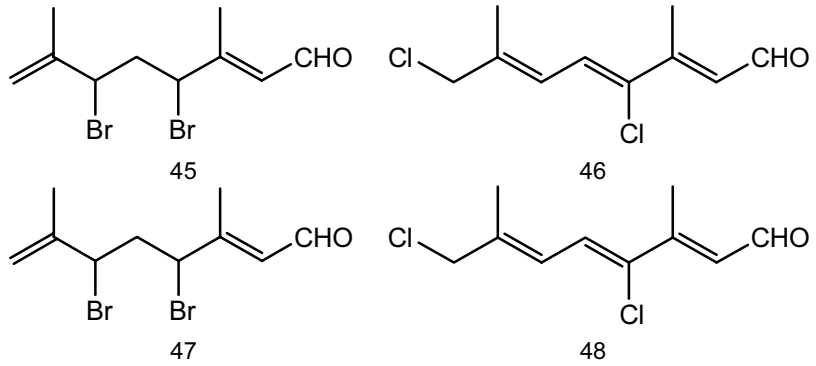


Figure 10. Structures of halogenated acyclic monoterpene aldehydes (45-48).

Polyhalogenated cyclic monoterpenes

Two compounds were isolated from the red seaweeds *Plocamium mertensii* and unclassified *Plocamium* sp., which were collected off Carnac Island and from the drift on Rottnest Island, Western Australia, respectively (Capon et al., 1984). A new compound, (1*R*,2*S*,4*S*,1'*E*)-2-bromo-1-chloro-4-(2'-chloroethenyl)-1-methyl-5-methylenecyclohexane (49), was purified by sublimation from a dichloromethane soluble portion and combined with recrystallization from methanol/water of rapid silica filtration to give an identical compound to the sublimed one, mp. 46.5-47.5 °C. Whereas the unidentified *Plocamium* species yielded (1*R*, 2*S*, 4*R*, 5*R*, 1'*E*)-4-bromo-1,2-dichloro-5-(2'-chloroethenyl)-1,5 dimethylcyclohexane (50), which was purified through Sephadex LH-20 and recrystallized from hexane as white needles, mp. 105.5-106 °C. The close correspondence of the ^{13}C NMR data of (50) with that previously reported for (51) strongly suggested these two compounds were identical. Although no comparison with the authentic mertensene sample in this paper was done, the corrected structure of mertensene was confirmed to be (50), not (51) by X-ray crystallography (see Figure 11).

One new (52) and six known (53-58) compounds were found from *Plocamium coccineum* Lyngb., which was intertidally collected at Bastiagueiro, Aguino, La Lanzada and Patos, and by dredging at Ria de Arosa from the coast of north-west Spain between April and October (Castedo et al., 1984). The different batches were shown by HPLC to contain the same composition of compounds (52-58). Coccinene (52) was separated as a crystalline solid, mp. 65 °C from reverse phase HPLC (see Figure 12).

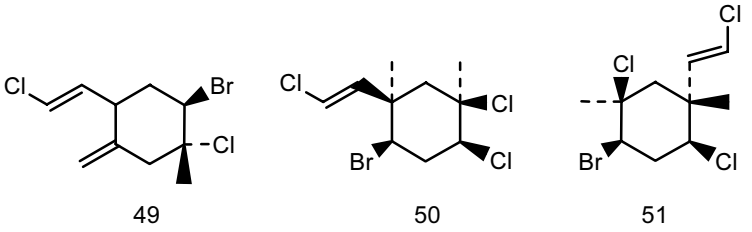


Figure 11. Structures of polyhalogenated cyclic monoterpenes (49-51).

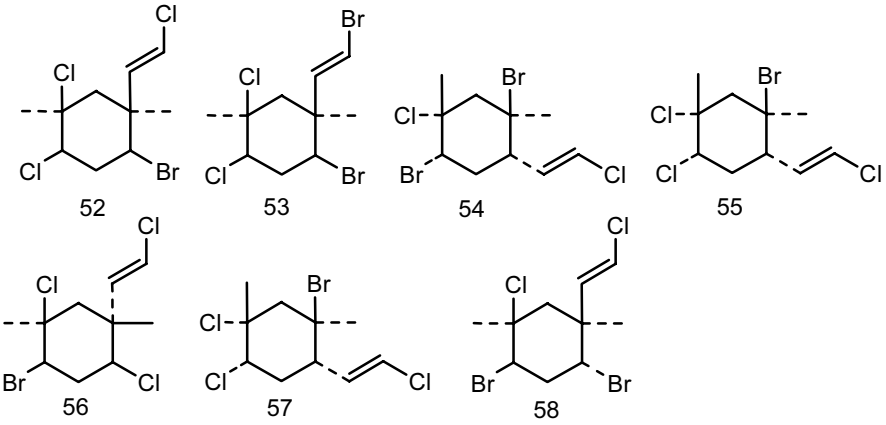


Figure 12. Structures of polyhalogenated cyclic monoterpenes (52-58).

Two new (59-60) and some structurally revised (61-63) cyclic monoterpenes were also reported from *Plocamium cartilagineum* and *Plocamium violaceum* in California (Crews et al., 1984). Two structures (64-65) were revised from *Plocamium coccineum* by using two dimensional nuclear magnetic resonance (2D NMR) and Nuclear Overhauser effect (NOE) difference spectroscopy (Sardina et al., 1985). It was considered that crystals of the previous assigned structures were damaged by X-ray irradiation (Crews, 1977b) (see Figure 13).

^{13}C NMR chemical shifts in the stereochemistry of cyclic monoterpenes (66-70) from *Plocamium coccineum* were discussed (Sardina et al., 1986). The calculated minimum energy conformers of the compounds agreed with the geometries deduced experimentally by using a Karplus equation. Using 2D NMR and NOE, different spectroscopy and molecular mechanics calculations led to a complete structural analysis (see Figure 14).

Variations in composition of a number of known cyclic monoterpenes (71-78) of *Plocamium cartilagineum* from the Chilean Coast by gas chromatography (GC) analysis were reported (San-Martin & Rovirosa, 1986). Neither ^{1}H NMR nor GC on the semipurified oils could detect acyclic compounds (see Figure 15).

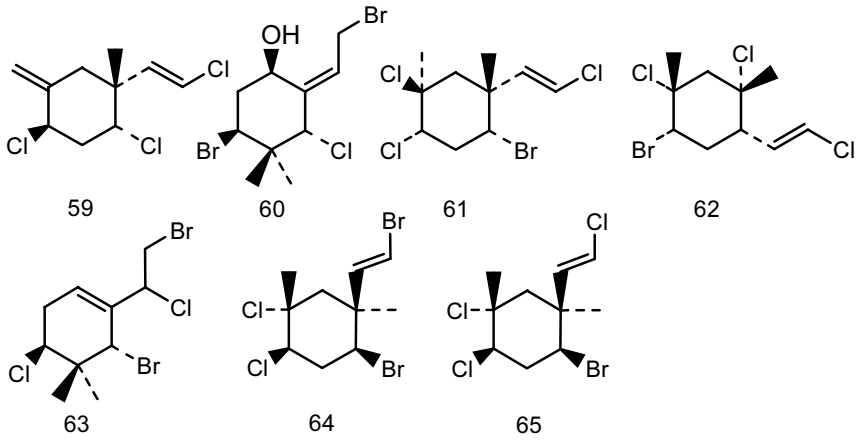


Figure 13. Structures of polyhalogenated cyclic monoterpnes (59-65).

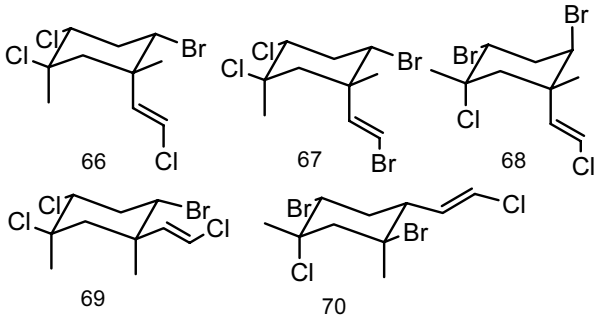


Figure 14. Structures of polyhalogenated cyclic monoterpnes (66-70).

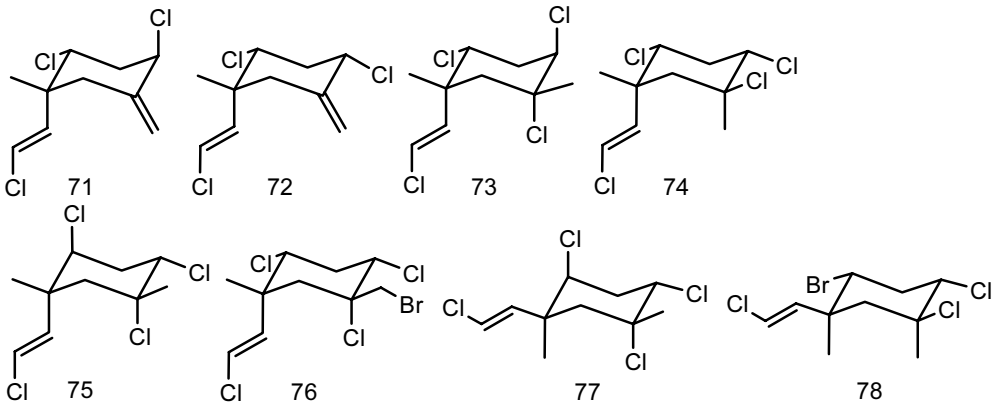


Figure 15. Structures of polyhalogenated cyclic monoterpenes (71-78).

Three new halogenated monoterpenes, one cyclic (79) and two acyclic monoterpenes, (22-23) and a revised mertensene (80) were reported from *Plocamium hamatum*, which was collected from the channel between Orpheus Island and Pelorus Island, Palm Island group in Australia (Coll et al., 1988). The structures were confirmed by X-ray crystallography.

A mixture of cyclic polyhalogenated monoterpenes (66-67, 81-84) from the digestive gland of *Aplysia punctata* was reported but no halogenated compounds were found in *Aplysia depilans*. *Aplysia punctata* were collected at lowtide at Agnino (La Coruna) and Patos (Pontevedra). *Aplysia depilans* were collected by diving at La Coruna and Villagarcia de Arosa (Quinoa et al., 1989). In order to find the origin of these compounds, some red seaweeds were investigated. The red seaweeds *Plocamium coccineum*, *Laurencia pinnatifida*, *Ceramium ciliatum*, *Calliblepharis lanceolata*, *Plumaria elegans*, *Corallina officinalis*, *Gracillaria verucosa*, *Porphyra laciniata* and *Lomentaria articulata* were collected at low tide on the coast of La Coruna and Pontevedra. The HPLC and gas-liquid chromatography (GLC) traces of *Plocamium coccineum* extracts were almost identical with those of the mixture obtained from *Aplysia punctata*, but no halogen-containing compounds were found in the other red seaweeds. In addition, the crude mixture of compounds (66-67, 81-84) was toxic to the larvae of the crustacean *Artemia salina*, with a LC_{50} of 1 μg/0.5 mL. (see Figure 16).

Telfairine (85) was isolated from the red seaweed *Plocamium telfairiae* which was collected at Wakasa Bay, Fukui Prefecture, Japan (Watanabe et al., 1989). Its structure was elucidated by ^{1}H, ^{13}C NMR, MS and NOE difference spectrum analysis. This compound exhibited 100% insecticidal activities against mosquito larvae, *Culex pipiens* pallens at 10 ppm.

Two insecticidal polyhalogenated monoterpenes, telfairine (85) and aplysiaterpenoid A (86) were also isolated from *Plocamium telfairiae*, which was collected at Wakasa Bay, Fukui Prefecture, Japan (Watanabe et al., 1990). Telfairine (85) and aplysiaterpenoid A (86) showed strong insecticidal activities as a 80 and 60 percent mortality against the German cockroach *Blatella germanica*, respectively. LC_{50} values of telfairine (85) against the susceptible strains of mosquito larvae *Anopheles gambiae* was 1.1 ppm and could not be calculated for the dieldrin-resistant strains of mosquito larvae *Anopheles gambiae* since the mortalities at 1.25 and 2.5 ppm were 0% and 100%, respectively. While LC_{50} values of aplysiaterpenoid A (86) against the susceptible and the dieldrin-resistant strains of mosquito larvae *Anopheles gambiae* were 0.1 and 0.24 ppm, respectively.

Two known cyclic monoterpenes (87-88) and one new acyclic (21) monoterpene were reported from *Plocamium cartilagineum* in Spain (König et al., 1990). The cyclic monoterpenes (87-88) exhibited potent toxicity towards both *Biomphalaria glabrata* and *Artemia salina* at the concentrations tested. A known cyclic monoterpene (89) was also reported from *Plocamium cartilagineum* from the Antarctic Peninsula (Rovirosa et al., 1990). Four new alicyclic monoterpenes (90-93) were found from *Plocamium cartilagineum*, collected from the Chilean coast, together with four known cyclic monoterpenes (94-97) (San-Martín et al., 1991).

Aplysiaterpenoid A (88) was isolated from the red seaweed *Plocamium leptophyllum*, which was collected at Toyama Bay, Japan (Sakata et al., 1991). This compound, which was previously isolated from the red alga *Plocamium telfairiae* and the sea hare *Aplysia kurodai*, showed feeding inhibitory activity at a level of 40 μg against the marine herbivores, the abalone *Haliotis discus*, the gastropod *Turbo cornutus*, the top shell *Omphalius pfeifferi*, and the sea urchin *Strongylocentrotus intermedius* (see Figure 17 and 18).

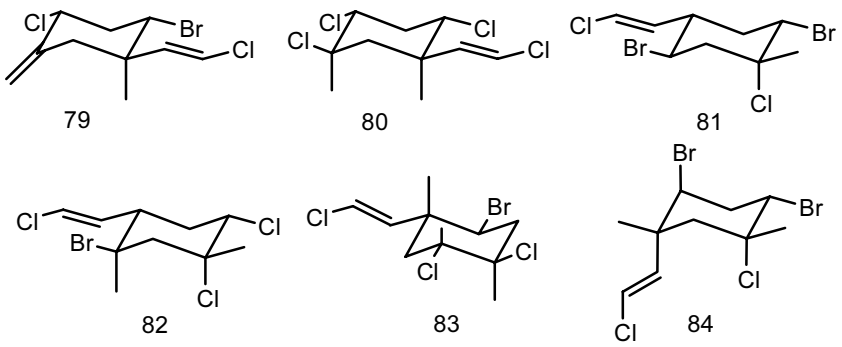


Figure 16. Structures of polyhalogenated cyclic monoterpenes (79-84).

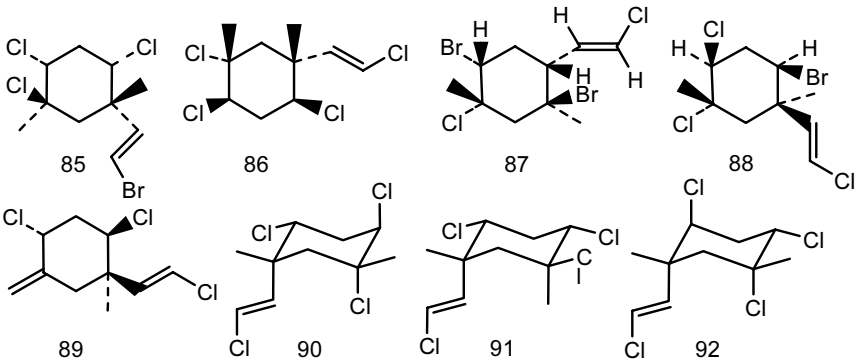


Figure 17. Structures of polyhalogenated cyclic monoterpenes (85-92).

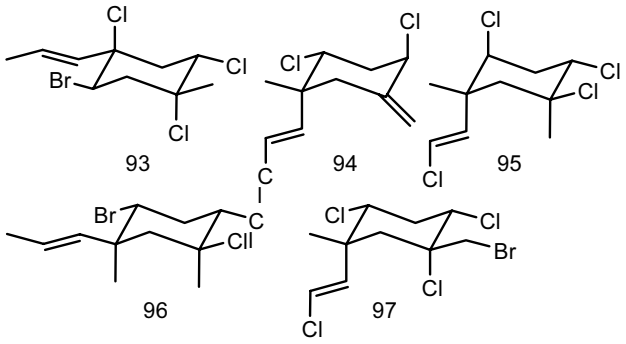


Figure 18. Structures of polyhalogenated cyclic monoterpenes (93-97).

Polyhalogenated epoxymonoterpenes

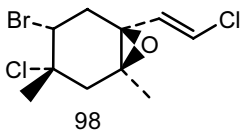
The chloroform extract of Plocamium cartilagineum was investigated (Abreu & Galidro, 1998). It was collected in Sesimbra, Portugal. The extract was fractionated by silica gel flash chromatography using hexane with increasing proportions of ethyl acetate to give 4-bromo-5-chloro-2-(*E*)-chlorovinyl-1, 5-dimethyl-1,2-epoxycyclohexane (98), mp. 49-50 °C. Mass spectrometry, basic NMR, carbon-insensitive nuclei enhanced by polarization transfer (^{13}C-INEPT), NOE, and COSY experiments were used for the structure elucidation, and a halogen regiochemistry was assigned by comparison of its ^{13}C NMR data with those of model compounds (see Figure 19).

Two metabolites (99-100) were investigated from *Plocamium* (Wessels et al., 2000) and exhibited activity in a brine shrimp bioassay in the range of 100% lethality within 24 hours to 40% after 48 hours. The metabolite (100) showed moderate antimicrobial activities (2-25 mm total/growth inhibition of several organisms), with 99 also being strongly algicidal at the minimum inhibitory concentration (MIC) of 7-11 μg/filter disk (see Figure 20).

Epi-plocameme D (101) was purifed by reversed-phase HPLC. It was originally reported from *Plocamium cartilagineum* (Ankisetty et al., 2004) collected from Anvers Island, Antarctica. This metabolite was the most active feeding deterrent toward the amphipod (19% of treated pellets eaten vs 50% of controls; p90.005) at isolated concentration (and only 1% of treated pellets eaten vs 51% of controls at three times isolated concentration; p90.005) but, like the other terpenes studies, had no effect on feeding by the sea star *Odontaster validus* (see Figure 21).

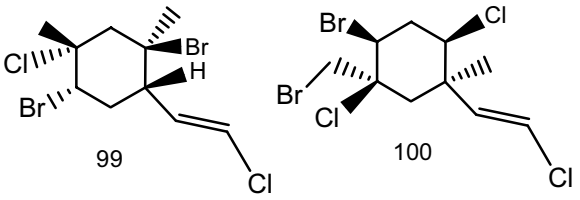
Pyran halogenated monoterpenes

Four new tetrahydropyran monoterpenes, plocamiopyranoid (102) and compound (103) were isolated from *Plocamium cartilagineum* and *Pantoneurines plocamioides*. Pantoneurines A (104) and B (105) from *Pantoneurines plocamioides*, were collected off the Chilean coast (Cueto et al., 1998). Structures were determined by using COSY, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond coherence (HMBC), NOE and a complete molecular modeling package for a personal computer or workstation (PCMODEL). In addition, two dimensional rotational nuclear Overhauser effect spectroscopy (2D ROESY NMR) confirmed the structure of (102). The interesting structural relationship of these compounds from *Plocamium cartilagineum* and *Pantoneurines plocamioides*, which belong to different orders (Gigartinales and Ceramiales, respectively), suggested a requirement of taxonomic revision (see Figure 22).



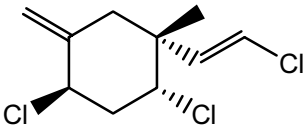
98

Figure 19. Structures of polyhalogenated cyclic epoxymonoterpenes (98).



99 100

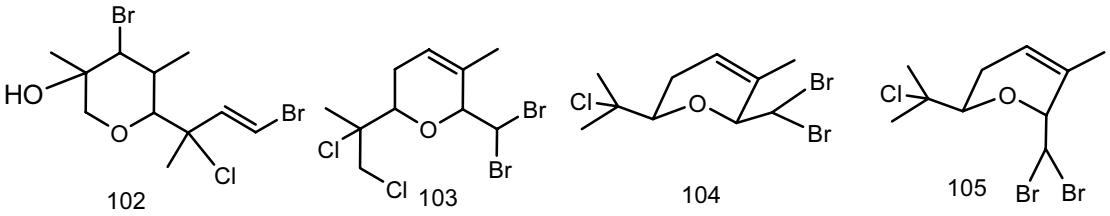
Figure 20. Structures of polyhalogenated cyclic monoterpenes (99-100).



101

epi-plocameme D

Figure 21. Structure of halogenated cyclic monoterpene (101).



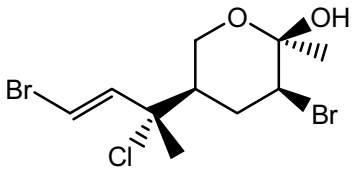
102 103 104 105

Figure 22. Structures of pyran halogenated monoterpenes (102-105).

Pyranoid 106 was isolated from *Plocamium cartilagineum* (Ankisetty et al., 2004) collected off the Chilean coast. It was obtained by repeated reversed-phase HPLC purification. Compound 106 is a similarly selective antifungal metabolite, displaying inhibition of *C. albicans* (8 mm zone; no activity against other Wyeth strains), but was largely insignificant as a feeding deterrent in field assays. (see Figure 23).

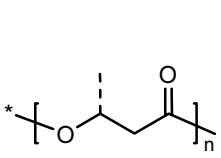
Nonterpenoid compounds

Poly(β-hydroxybutyrate) (PHB) (107) and 2-O-α-D-galactopyranosyl-glycerol (fluridoside) (108) were isolated from the red seaweed *Plocamium cartilagineum*, which was collected from Figueira da Foz, on the coast of Portugal (Abreu et al., 1997). The red seaweed *P. cartilagineum* was extracted with hexane, chloroform and ethanol. Repeated treatment of the chloroform extract with hexane and acetone gave five fractions of a colourless crystalline solid PHB, mp. 175-176 ^{o}C. This was the first report of PHB occurrence in a macroalga. Reversed phase flash chromatography of the ethanol extract using water with increasing proportions of methanol as eluent, afforded the new secondary metabolite (108) as a water-soluble colourless solid, mp. 120-122 ^{o}C. Floridoside is the main low molecular carbohydrate occurring in many red seaweeds and this was the first time that it had been reported in *Plocamium cartilagineum*. Both structures were established on the basis of NMR experiments. In addition, the structure of floridoside was confirmed by gas chromatography mass spectrometry (GCMS) analysis of the permethylated derivatives and the hexaacetate resulting from acid hydrolysis of floridoside followed by $NaBH_4$ reduction and peracetylation. (see Figure 24).



106

Figure 23. Structure of halogenated pyranoid monoterpene (106).



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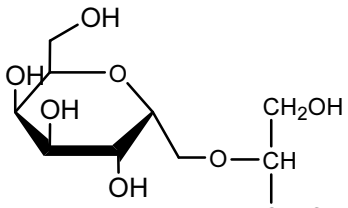


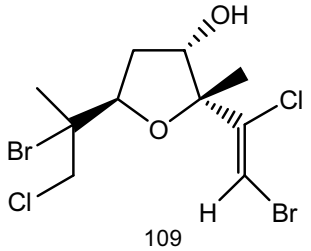
108

Figure 24. Structures of nonterpenoids (107-108).

Polyhalogenated furanoid monoterpenes

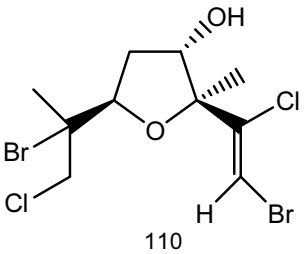
Novel polyhalogenated furanoid monoterpenes, furoplocamioids A-C (109-111), with an unusual chlorobromo vinyl functional group, have been isolated from *Plocamium cartilagineum*, which was collected from the Chilean coast (V Region) by scuba diving. It was extracted with ethyl acetate/dichloromethane/hexane (1:1:1). The crude extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane/ethyl acetate (95:5) was further separated by filtration chromatography to give a fraction which was chromatographed on a silica gel column to obtain a mixture of monoterpenes (109-111), homogeneous by TLC. Acetylation of the monoterpenes (109-111) was prepared and purified using recycling HPLC eluted with chloroform to afford the acetates of each monoterpene. Each of the acetates was saponified using an excess of potassium carbonate in methanol to give the alcohols (109-111) (Darias et al., 2001).

Two new related tetrahydrofuran halogenated monoterpenes (112-113) and a new acyclic polyhalogenated monoterpene (34) were isolated from *Plocamium cartilagineum*, collected at El Quisco, Chile. They were obtained after flash chromatography followed by gel filtration and successive HPLC (Díaz-Marrero, Rovirosa et al., 2002) (see Figure 25).



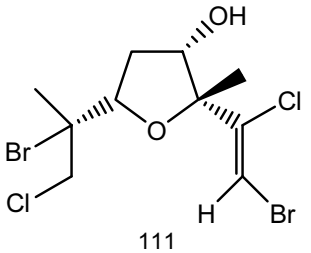
109

Furoplocamioid A



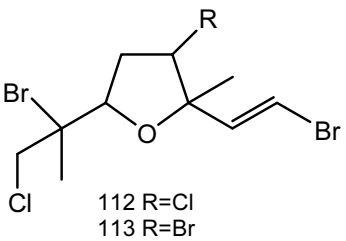
110

Furoplocamioid B



111

Furoplocamioid C



112 R=Cl
113 R=Br

Figure 25. Structures of polyhalogenated furanoid monoterpenes (109-113).

CONCLUSIONS

This review intends to gather secondary metabolites from *Plocamium* species. *Plocamium* gave a variety of secondary metabolites in the past twenty-five years. Most of them were halogenated monoterpenes, which displayed significant biological activities. However, numerous secondary metabolites of *Plocamium* have been declined in terms of novel compounds since 2002. Why do halogenated monoterpenes often exhibit biological activities? The reason is probably their structures containing halogens which might affect the activities somehow. As we all know that halogenated compounds are well known and used for insecticide, drug, cosmetics, etc. In addition, antifeedant effects of the marine halogenated monoterpenes were exhibited against several divergent insect species (Argandona et al., 2002) and antioxidant activities of the lipophilic extracts of *Plocamium telfairiae* and other species of seaweeds were presented (Huang & Wang, 2004). The halogenated secondary metabolites are possibly not delicious to taste. Seaweeds are though quite rare to be marine-derived natural products in clinical and preclinical trials comparing to marine invertebrates such as bryozoans, sponges or ascidians. However, they are valuable to explore in terms of chemical ecology and biotechnology, which will be increasingly important to our world and environment, for example.

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