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BIOLOGICALLY ACTIVE MARINE METABOLITES: SOME RECENT EXAMPLES[1]

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In the last ten years or so the investigation of marine organisms for metabolites with biological activity has become an important aspect of natural product chemistry. This research has been rewarded with the discovery of some unique chemical structures possessing important biological activity. Some recent examples are reviewed.

Au cours des quelques dix dernières années, la recherche, chez les organismes marins, de métabolites bioactifs est devenue un aspect important de la chimie des produits naturels. Cette recherche a permis la découverte de structures chimiques uniques possédant d'importantes activités biologiques. Quelques examples récents sont présentés.

Introduction

Nature has been found to be amazingly versatile in producing compounds with important biological activity. Often quite simple biosynthetic manipulations result in complex molecules with remarkable potency. Thus, natural product chemistry has provided us with many unusual and challenging structures, some of which are the basis of todays pharmaceutical industry. In fact, it is this search for biologically active compounds from natural sources that has, to a large extent, maintained the early momentum of natural product chemistry.

To date terrestrial microorganisms and plants have been the principal sources of biologically active materials. However, in the last 15 years or so, there has been a considerable increase in interest in marine natural products, fostered to a large extent by discoveries of a varied range of unique chemicals—many of which possess some form of biological activity.

Nevertheless, compared with studies of terrestrial organisms, progress in the chemistry of marine natural products has been slow. There is knowledge of biological activity among marine organisms which dates back to Biblical times (Halstead 1965), yet only in the last decade has significant progress been made in identifying the chemical agents. Several factors contributed to this slow start: (i) The success in dealing with natural products from microbes and land plants concentrated the major research thrusts in that direction, (ii) the comparative difficulty in locating and identifying marine species, (iii) our inability to mimic the high yielding laboratory cultivation of microorganisms using marine plants or animals, (iv) difficulties in purifying the lipophilic and often heavily pigmented extracts of marine organisms.

There is no clear-cut relationship between (a particular) biological activity and the type of organism. Consequently, there is no obvious guide that will lead a natural product chemist towards a source of bioactive metabolites. The most common approach is to establish a screen for the biological activity of interest, and examine groups of organisms displaying certain biological and ecological indicators. For example there is a belief that mollusks lacking protective shells, such as sea-hares and nudibranches, are prone to predation and thus likely to have adopted some form of chemical defense. This may also be true for algae which are able to flourish in an area of intense herbivorous pressure. Again, the symbiotic

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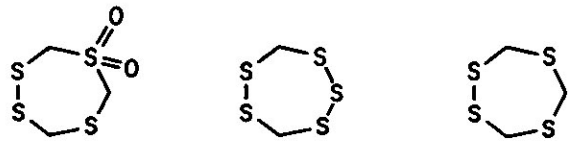
relationship between microorganisms and sponges may account for the isolation from sponges of several biologically active compounds which appear to be of microbial origin.

The variety of chemical structures and range of biological activities that have been reported from marine sources is remarkable and the aim of this review is to illustrate this with some fairly recent examples from the literature. Other pertinent reviews or accounts of the variety, structure, and biological activity of marine metabolites include those by Braslow (1969), der Marderosian (1969), Faulkner (1978), Fenical (1982), Glombitza (1979), Halstead (1965), Moore (1982), Scheuer (1973), Wells (1979), and Youngken (1969), as well as the excellent series of volumes edited by Scheuer (1978). The compounds reviewed here are separated into groups according to their effect upon mammalian cells or on organisms affecting mammalian cells.

Antimicrobial Agents

There are many reports of antimicrobial activity from marine plant and animal sources. Most likely this is because antimicrobial assays are relatively easy to perform and are often used as a preliminary screen for other pharmacological activity.

Since a crude extract of a plant or animal contains an enormous variety of compounds, an assay for bioactivity of any sort will often lead to the discovery of a metabolite that would otherwise have been overlooked: but for the antimicrobial activity of the cyclic polysulphides (1) - (3) from the brown alga *Chondria californica* (Wratten & Faulkner 1976) it is likely these compounds would have been overlooked as solvent impurities in the ^{1}H NMR spectrum of the extract. Curiously other species of *Chondria* which have been examined possess neither antimicrobial activity or cyclic polysulphides, though (2) and (3) have previously been isolated from the mushroom *Lentinus edodes*.



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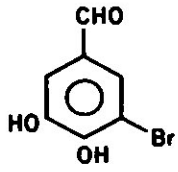
Several red algal genotypes produce antimicrobial metabolites containing halogen. Bromine is the most frequently encountered element despite the fact that the molar ratio of Cl^- to Br^- in seawater is approximately 60:1. The introduction of halogen is catalysed by peroxidase, and it has been proposed that the selectivity in halogen distribution among marine metabolites is based on oxidation-reduction potentials for a particular peroxidase as well as the intracellular halide concentration (Theiler et al. 1978).

Historically the presence of halo-organics in red algae has been known for many years (Augier 1953), and the first definite structure proof was presented for 3-bromo-4,5- dihydroxybenzal dehyde (4) a product of *Polysiphonia morrowii* almost three decades ago (Saito & Ando 1955). Some years later several di-bromophenols were described (Craigie & Greunig 1967; Katsui et al. 1967) and since then a considerable variety of brominated and sulphonated phenolics have been isolated, usually based on the 4- hydroxybenzyl alcohol and 3,4 dihydroxybenzyl alcohol skeleton (5 - 10) (Glombitza 1979).



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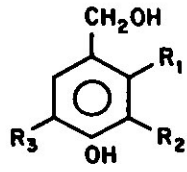
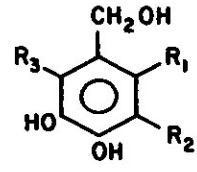


	R_1	R_2	R_3
5	H	Br	H
6	H	Br	Br
7	Br	Br	Br



	R_1	R_2	R_3
8	H	Br	H
9	Br	Br	H
10	Br	Br	Br

Red algae of the family Bonnemaisoniaceae have a prodigious repertoire of non-aromatic halo-organic antiseptics (McConnell & Fenical 1979). Polyhalo-methanes, - acetic acids, - acetones - acrylic acids and - octenones containing various amounts of chlorine, bromine, and iodine, have been isolated (Fig. 1). These compounds

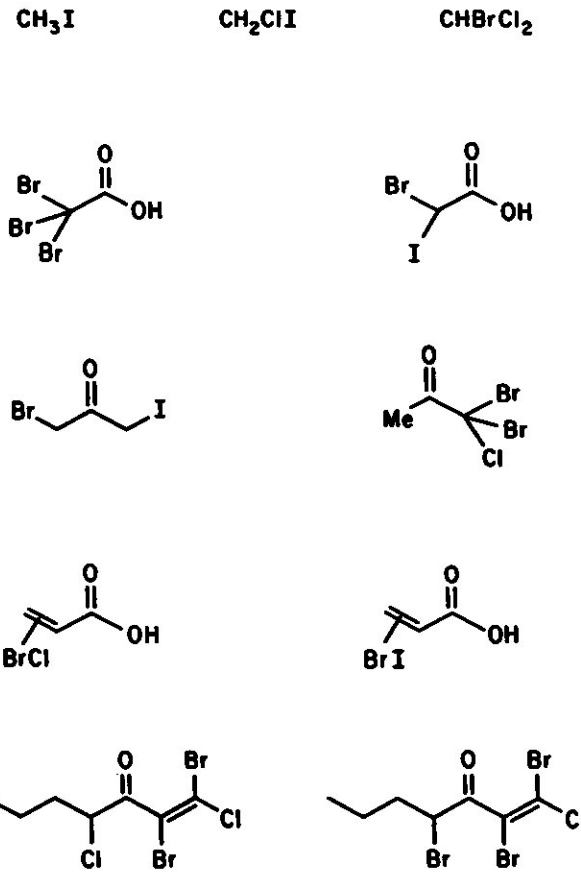
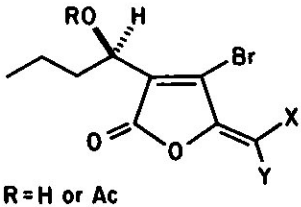


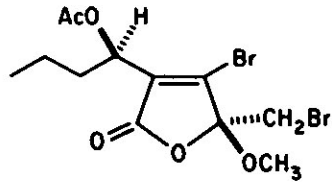
Fig 1. Halogenated polyketides of the family Bonnemaisoniaceae

appear to be polyketide in origin and the polyhalo-1-octen-3-ones may share a common biosynthetic origin with the lactonic fimbrolides (11 - 17) from another red alga *Delisea fimbriata* (Kazlauskas et al. 1977a; Pettus et al. 1977). Over 60% of the dichloromethane extract was composed of these lactonic metabolites, as the acetoxy (major) and hydroxy (minor) derivatives. Conclusive structure proof came from an X-ray crystallographic analysis of the methanol adduct (18).



R=H or Ac

	X	Y
11	Br	H
12	H	Br
13	I	H
14	H	I
15	Cl	H
16	H	Cl
17	Br	Br



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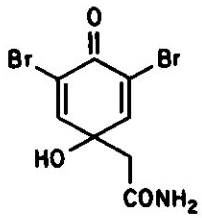
Algae are not the only marine source of halogenated metabolites. Sponges have a consistent record of yielding halogenated antibacterial substances and the genus *Verongia* is well known for its supply of bromo compounds. Sharma and Buckholder (1967) isolated the dienone (19) from *V. cauliformis*. The same authors subsequently reported (Sharma et al. 1970) the structure of another antimicrobial component as the ketal (20) which they assumed was a natural product. Later, however, the mixed ketal (21) was isolated from a species of *Verongia* following ethanol extraction (Anderson & Faulkner 1973). Even more significant was the discovery that (21) was a mixture of diastereoisomers suggesting that the dienone (19) and the ketals are all derived from a single intermediate [perhaps (22) or (23)]



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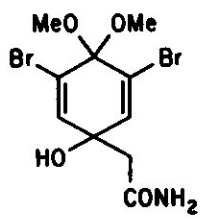

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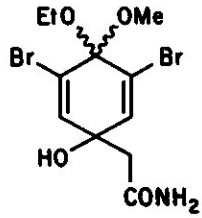

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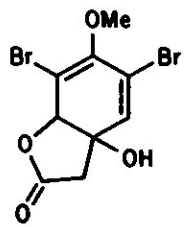

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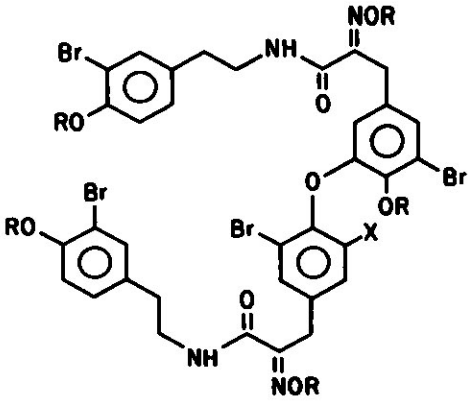
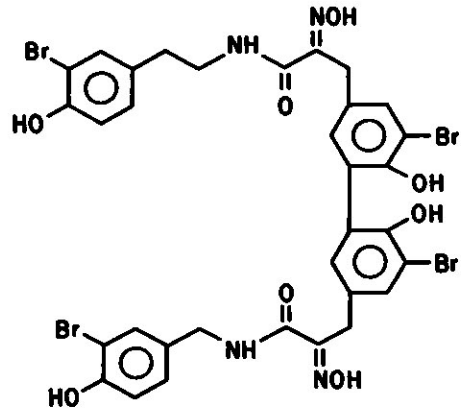
by the addition of water, methanol, or ethanol. Another Verongid sponge *Ianthella basta* has provided to date the most complex and varied source of bromotyrosine based metabolites (Kazlauskas et al. 1981). The methanol extract showed potent *in vitro* and some *in vivo* activity against Gram-positive bacteria and this was attributed to a series of related compounds which could be separated into two fractions. The most polar fraction contained bastadin-1 (24), bastadin-2 (25) and bastadin-3 (26). The less polar fraction contained bastadin-4 (27), bastadin-5 (28) [separated only as the tetramethyl ether (31)] bastadin-6 (29) and bastadin-7 (30). [The structures were assigned mainly on the basis of their spectral data.] It was thought that the biological activity of these cyclic compounds might be related to



	R	X
24	H	H
25	H	Br



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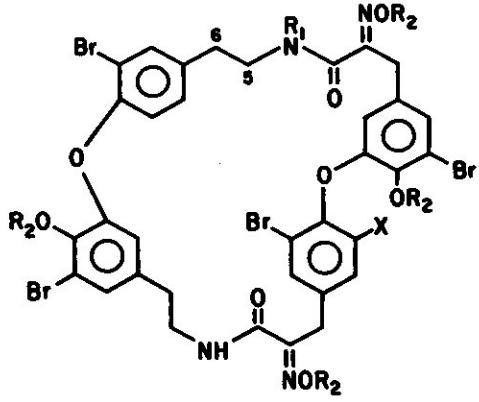
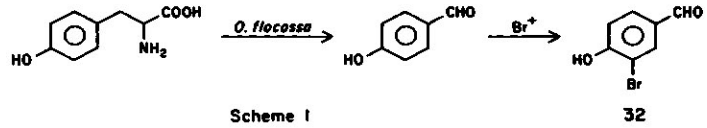
their ability to accommodate small mono-or di-valent metal cations. However, the X-ray crystal structure of bastadin-5 tetramethyl ether (31) revealed the central hole was blocked by one of the two methoxyimino groups and so it is improbable that these macrocyclic compounds can act as ionophores.



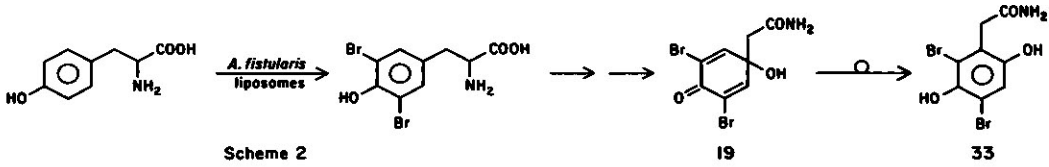
	R_1	R_2	X	Δ(E)
27	H	H	Br	5,6
28	H	H	H	
29	H	H	Br	
30	H	H	H	5,6
31	Me	Me	H	

Clearly most of these halo-aromatics appear to be derived from tyrosine, but few biosynthetic studies have been performed to substantiate this. Manley and Chapman (1978, 1980) have shown that a cell-free homogenate of the red alga *Odonthalia floccosa* converts tyrosine to 3-bromo-4-hydroxybenzaldehyde (32). Bromin-

ation occurs after formation of p-hydroxybenzaldehyde (Scheme 1). Conventional biosynthetic studies with marine organisms are often hampered by very low incorporation of radiolabelled precursors. In a biosynthetic study of brominated phenols and quinones from the sponge *Aplysina fistularis (Verongia aurea)*, Tymiak and Rinehart (1981) used liposome-enclosed precursors to improve incorporation levels. Both phenylalanine and tyrosine were converted to the dienone (19) as well as the rearrangement product dibromohomogentisamide (33) (Scheme 2).



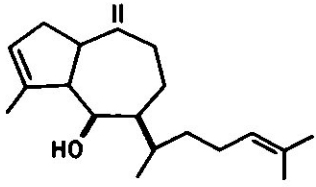
Scheme 1: Biosynthesis of 3-bromo-4-hydroxybenzaldehyde from tyrosine by the red alga *Odonthalia floccosa*



Scheme 2: Biosynthesis of the dienone (19) and dibromohomo-gentisamide (33) from tyrosine by the sponge *Aplysina fistularis*

Terpenoid antimicrobials containing halogen are common metabolites of certain red algae: most common are mono- or sesquiterpenoids all containing bromine and occasionally chlorine. Although the biosynthetic ability to produce such chemicals seems confined to a few genera, the variety and novelty of these terpenes is remarkable (Martin & Darias 1978; Erickson 1983). For example, the genus *Laurencia* provided at least six new terpenoid classes and several of these metabolites display antimicrobial activity (Fig. 2). In many cases their structures were established by X-ray crystallography.

In contrast, certain brown algal genera produce mainly diterpenes, usually containing no halogen. The antimicrobial pachydictyol A (34) from the Pacific seaweed *Pachydictyon coriaceum* was the first of a host of related metabolites to be



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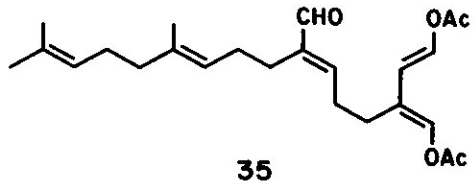

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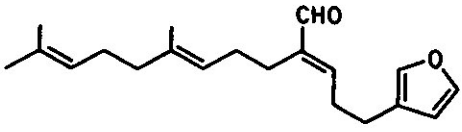

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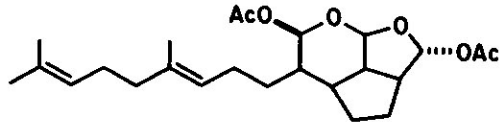

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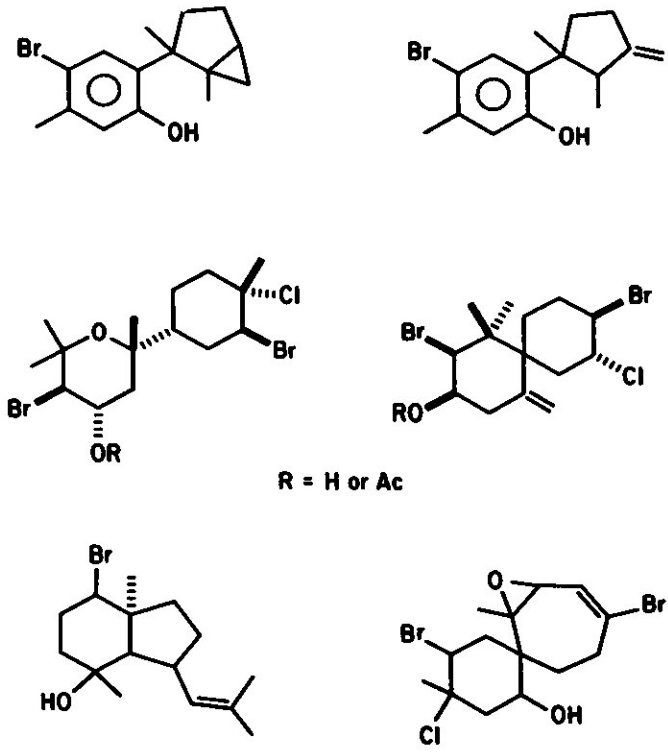
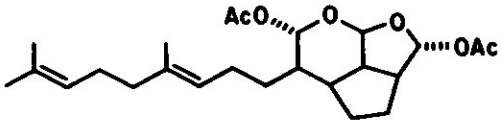


Fig 2. Halogenated terpenoids of *Laurencia* spp.

isolated from the biologically related plants *Pachydictyon, Dictyota, Dilophus* and others. Chemical and spectral studies (Hirschfield et al. 1973) led to the conclusion that (34) possessed a new terpenoid skeleton, confirmed by X-ray crystallography of the p-bromophenylurethane derivative.

Ethanol extracts of the calcareous green alga *Udotea flabellum* display moderate antimicrobial activity. The terpenoid constituent udoteal (35) was isolated from fresh extracts of the plant (Paul et al. 1982), but older ethanolic extracts contained udoteafuran (36) and a series of related cyclic compounds isolated as their acetates (37) - (38) (Nakatsu et al. 1981). All the acetates were optically active and are presumed to be derived from "udoteatrial" (39) which exists as an inseparable mixture of hydrates and ethanolates. Interestingly, a related calcareous alga of the



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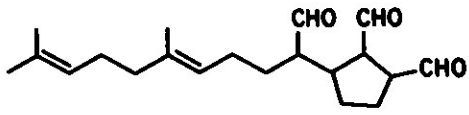

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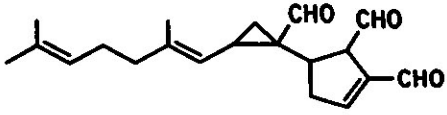

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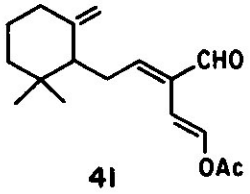
genus *Halimeda* produces a similar terpenoid trialdehyde (40) (Paul & Fenical 1983). In addition to antimicrobial activity this compound displays a range of deleterious biological effects and may offer relief to this macroalga from herbivores which inhabit the same area.

Similar metabolites containing the unsaturated aldehydo and enol acetate functions have been discovered in marine animals, though it is open to debate whether they are synthesized *de novo* or are simply dietary products. When irritated, the opisthobranch mollusc *Onchidella binneyi* secretes a white mucus containing the antimicrobial sesquiterpenoid enol acetate (41) (Ireland and Faulkner 1978). Similar functionality appears in the sesterterpenoid antibiotics (42), (43) and (44) which were isolated from the sponge *Luffariella variabilis* (deSilva & Scheuer 1981).



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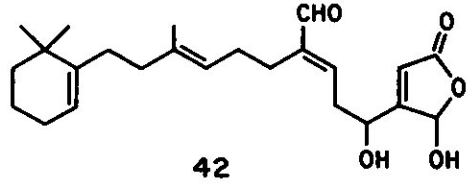

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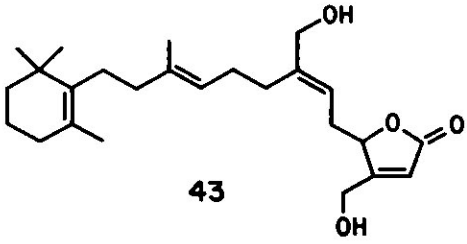

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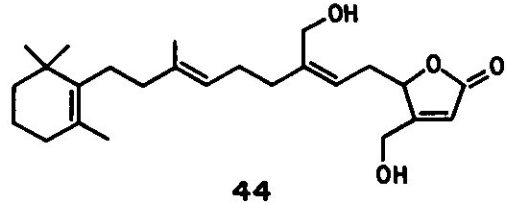

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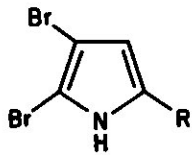
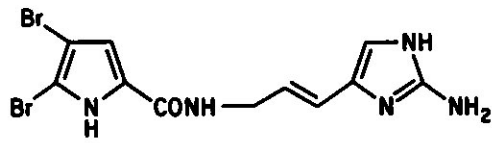
The sponge *Agelas oroides* contains 4,5-dibromopyrrole-2-carboxylic acid (45), the corresponding nitrile (46) and amide (47) as well as a fourth product, oroidin, containing the amide moiety linked to a substituted 2-aminoimidazole grouping (Forenza et al. 1971). This original structure proposed for oroidin was later revised to (48) based on comparison of the reduction product (49) with authenic synthetic material (Garcia et al. 1973). A separate study, by Faulkner's group, of *Agelas* sponges from the Caribbean area resulted in the isolation of sceptrin (50) a strong antimicrobial agent from *Agelas sceptrum* (Walker et al. 1981). The structure was established by X-ray crystallography. Sceptrin (50) is related to oroidin (48) by a



	R
45	COOH
46	CN
47	$CONH_2$



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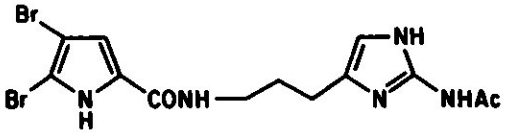

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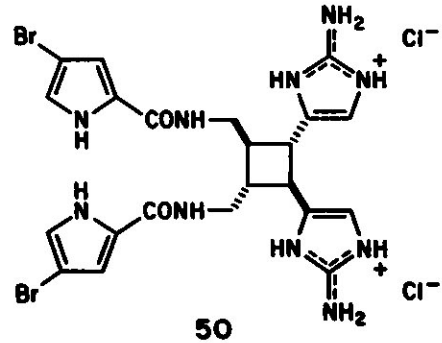
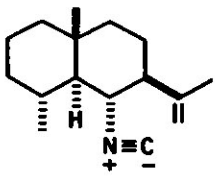


50

head-to-head [$_{\pi}2_s$ + $_{\pi}2_s$] cycloaddition reaction. This is an allowed photochemical process but attempts in the laboratory to achieve the photodimerization of oroidin failed. The authors also point out that the biosynthesis of (50) cannot be regarded as a simple photodimerization of debromooroidin: there is insufficient light at the depth where *Agelas sceptrum* was found (from -20 to -30 m) and more significantly sceptrin is optically active while debromooroidin must be achiral.

The antimicrobial activity of the sponge *Acanthella acuta* is due to the sesquiterpenoid isonitrile (51) (Minale et al. 1974). Although the isonitrile function is relatively rare in nature, several examples have been isolated from sponges. Another two terpenoid isonitriles are implicated in the antimicrobial activity of a different marine sponge *Halichondria* sp. (Burreson et al. 1975). The cyclic sesquiterpenoid derivative (52), based on the amorphane skeleton, is substituted at C-10 by an isocyanide group. The second isonitrile metabolite is the linear diter-



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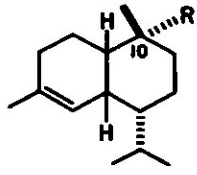
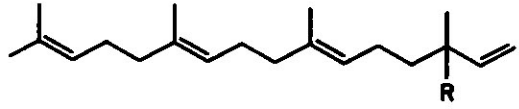
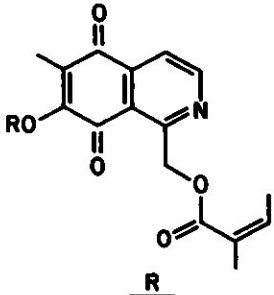


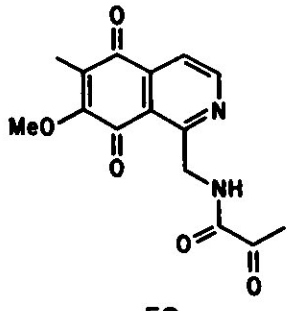
R	
52	N≡C
54	NHCHO
56	N=C=S



R	
53	N≡C
55	NHCHO
57	N=C=S



R	
58	Me
64	H



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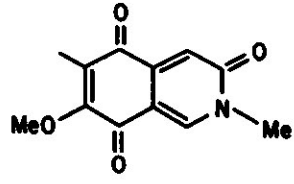
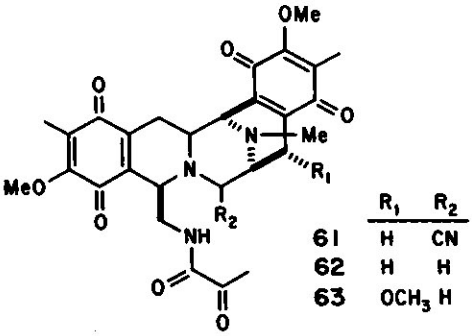


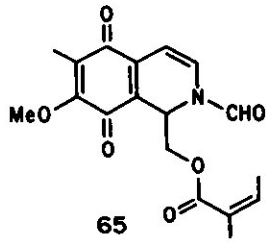
60

pene (53), an isocyanide analogue of the jasmine constituent geranyllinalool. Interestingly both terpenoid isonitriles were accompanied by the corresponding formamides (54) and (55) and isothiocyanates (56) and (57), implying that the formamides are biosynthetic precursors of the isocyanides.

In the study of marine metabolites, there are several examples of products which are a likely consequence of symbiosis. This is most frequently encountered in sponges which are suspected of playing host to marine microorganisms and is beautifully illustrated by the recent isolation of several heterocyclic antibiotics from *Reniera* sp. a bright-blue sponge (McIntyre et al. 1979; Frincke & Faulkner 1982). The major antimicrobial agent was renierone (58), an orange-red pigment which bears a remarkable similarity to mimocin (59) a metabolite of *Streptomyces lavendulae* No. 314 (Kubo et al. 1980). This terrestrial microbe also produces the antibiotics mimosamycin (60) (Fukumi et al. 1978), saframycin A (61) (Arai et al. 1980), saframycin B (62) and saframycin C (63) (Arai et al. 1979). Remarkably, further examination of the metabolites of *Reniera* sp. (Frincke & Faulkner 1982) resulted in the isolation of a series of compounds including mimosamycin (60) and several other isoquinoline metabolites (64) - (66), the isoindole (67) and four "dimeric" metabolites called renieramycins A-D (68) - (71) which clearly are related to the saframycins.



	R_1	R_2
61	H	CN
62	H	H
63	OCH_3	H



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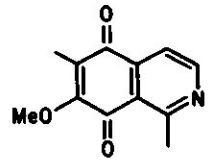

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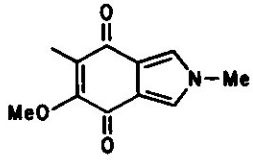

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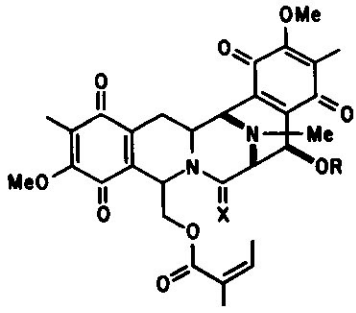


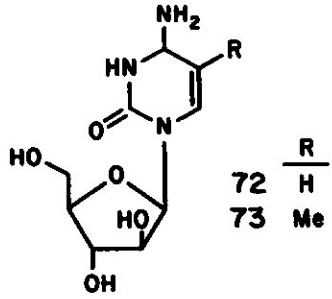
	R	X
68	H	H_2
69	Et	H_2
70	H	O
71	Et	O

Antineoplastic and Antiviral Agents

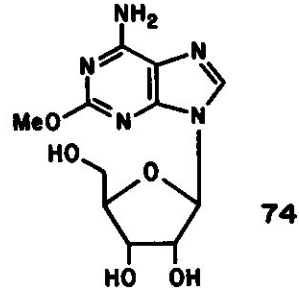
As marine natural product chemistry developed, the discovery of unusual organic compounds, frequently very different from those found in terrestrial organisms, prompted the idea that new and effective anti-cancer drugs may be present among this assortment of structures. In fact preliminary results of the National Cancer Institute screening program have been encouraging (Suffness & Douros 1981) and several marine derived materials will be developed to clinical trials in the next few years.

It is worth adding a cautionary note. Although *in vivo* testing for antineoplastic activity is more meaningful, it is slow and expensive. Consequently, in the interests of speed and convenience, researchers have often adopted an *in vitro* screen using cancer cell cultures. Since these assays only indicate cytotoxicity, and there is no clear correlation between cytotoxicity and antineoplastic activity, such measurements may be misleading unless correlated with animal testing data.

Very early work on marine natural products (Bergmann & Burke, 1955, 1956) led to the isolation of three new nucleosides, spongouridine (72), spongothymidine (73) and spongosine (74), from the sponge *Cryptotethia crypta*. The discovery and biological activity of these metabolites led, almost twenty years later, to the clinical use of the synthetic analogues adenine arabinoside (Ara-A) and cytosine arabinoside (Ara-C) as anti-viral and anti-cancer agents.



	R
72	H
73	Me



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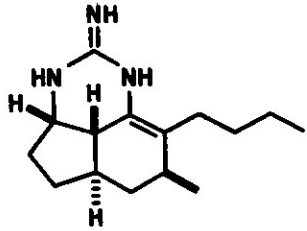

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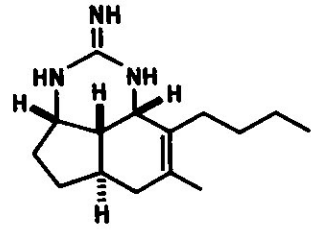
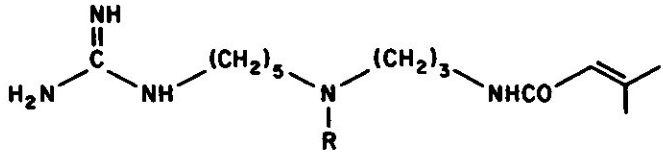


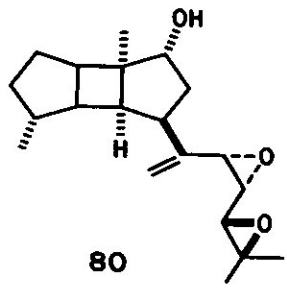
76

The potent cytotoxic and antimicrobial activity in crude extracts of another sponge, *Ptilocaulin* aff. *P. spiculifer*, was found to be associated with two cyclic guanidines, ptilocaulin (75) and isoptilocaulin (76) (Harbour et al. 1981). The guanidine function crops up again in the acarnidines (77) - (79) a novel group of sponge metabolites that display anti-viral and anti-bacterial activity (Carter & Rinehart 1978a). They have in common the unique substituted homospermidine skeleton and differ in the fatty acid substituent which is either isovalerate, laurate or myristate.

A variety of novel anti-viral and anti-tumor terpenoid metabolites have been isolated from marine plants and animals. The unusual diterpene spatol (80) a metabolite of the brown alga *Spatoglossum schmittii* inhibits cell division of sea-urchin



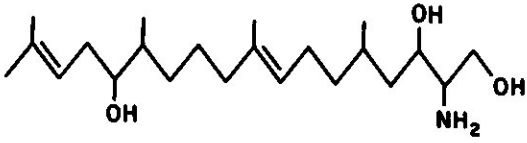
	R
77	$-CO(CH_2)_{10}CH_3$
78	$-CO(CH_2)_3CH \overset{cis}{=} CH(CH_2)_5CH_3$
79	$-CO(CH_2)_3(CH \overset{cis}{=} CHCH_2)_3CH_3$



80

eggs in a manner similar to the antileukemic compounds, colchicine, vincristine and vinblastine (Gerwick et al. 1980). Following extensive chemical and spectroscopic investigations the final stereochemical details were obtained by an X-ray crystallographic study of the p-bromobenzoate derivative. A much simpler terpene, the linear derivative (81) was identified as the responsible agent from an extract of the marine tunicate *Aplidium* sp. which displayed inhibitory activity toward Gram-positive and Gram-negative bacteria, fungi and Herpes virus type I, as well as cytotoxicity towards tumor cells (Carter & Rinehart 1978b).

Soft corals and gorgonians have been a rich source of cembrane diterpenoids, many of which are cytotoxic to cancer cells *in vitro*. Investigations for biologically active metabolites in the Caribbean gorgonian *Plexaura crassa* yielded the first marine cembranolide crassin acetate (82) whose structure was established after extensive chemical studies and X-ray analysis of the corresponding p-iodobenzoate ester (83). (Hossain & van der Helm 1969). Investigations of other gorgonians has



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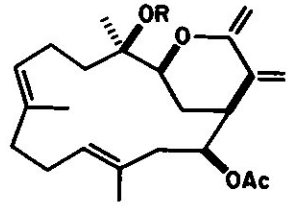
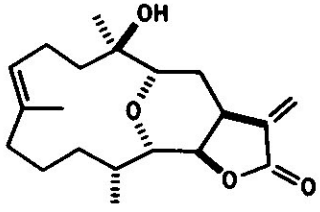
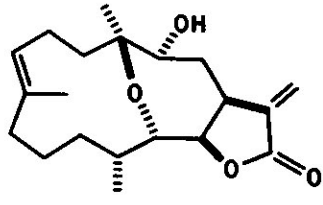


	R
82	H
83	C_6H_4I



84



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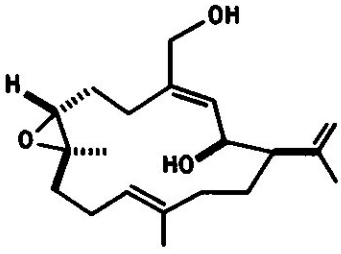

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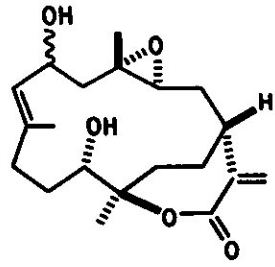

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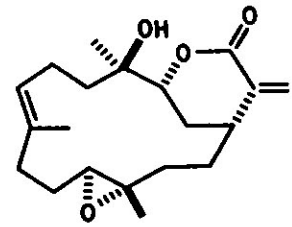
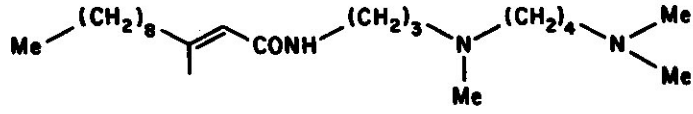


88

led to the discovery of a series of inter-related metabolites. Bahamian strains of *Euricea mammosa* contain the oxygen-bridged lactone eunicin (84) (Weinheimer et al. 1968; Hossain et al. 1968) whereas Caribbean samples of the same species contain the closely related cembranolide jeunicin (85) (van der Helm et al. 1976). Potent biological activity is often associated with the exomethylene lactonic ring but a new nonlactonic cembrane, asperdiol (86), isolated from two gorgonians *Eunicea aspenula* and *E. tourneforti*, displays significant *in vitro* anti-tumor activity in the N.C.I. test systems (Weinheimer et al. 1977).

Soft corals have yielded even more cembrane derivatives than have gorgonians. For example, soft corals of the genus *Sinularia* from Hawaii, Indonesia and Australia have been examined by a number of independent groups (Fenical 1978) and typical structures are represented by sinulariolide (87) and flexibilide (88). Although the bioactivity of soft corals is often associated with these cembranolides,



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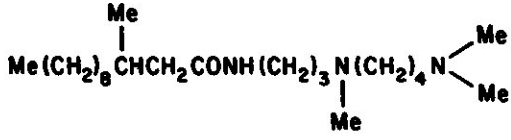

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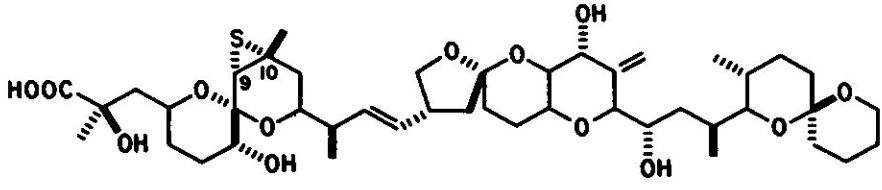


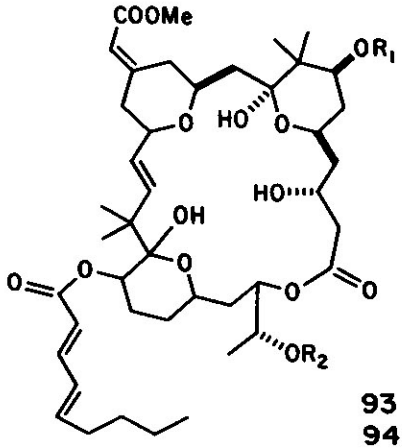
91

92 de-S, $\Delta^{9,10}$

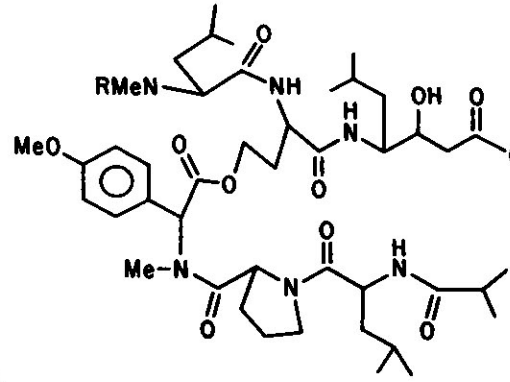
it was found that the cytotoxic activity of *Sinularia brongersmai* was due to the two spermidine derivatives (89) and (90) present as a 9:1 mixture (Schmitz et al. 1979). Catalytic reduction of the mixture yielded a single saturated amide which following acid hydrolysis and esterification gave methyl 3- methyldodecanoate.

Without a bioassay system to pinpoint activity, the novel anti-tumor agent ancanthifolicin (91) from the sponge *Pandaros acanthifolium* may have gone undiscovered. Antineoplastic activity in the lipid fraction is often associated with palmitoleic and oleic acids. However, in this case repeated purification revealed (91) as a very minor component (Schmitz et al. 1981). The structure of this novel episulphide-containing polyether was established by X-ray crystallography, and is clearly related to okadaic acid (92) isolated from two other sponges of the genus *Halichondria* about the same time (Tachibana et al. 1981). In fact, ancanthiofolicin can be smoothly converted to okadaic acid using a Zn-Cu couple in refluxing ethanol. The structural features of these compounds class them as ionophores, which hitherto had not been isolated from a marine source. Very recently the structures of two related cyclic ionophores bryostatin 1 (93) and bryostatin 2 (94) from collections of the ubiquitous bryozoan *Bugula neritina* have been reported (Pettit et al. 1982a, 1983). These metabolites and as many as fifteen related, but very minor components, all display very potent antineoplastic activity. The structure of (93) was confirmed by X-ray analysis, and that of bryostatin 2 (94) by comparison of the high resolution ^{1}H nmr data with that of (93).

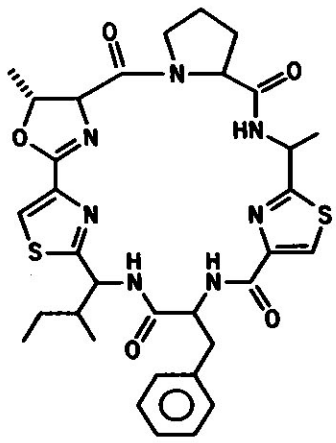
A number of novel peptides possessing considerable biological activity have been isolated from certain marine invertebrates. Initial extracts of the Caribbean tunicate *Didemnum* sp. showed significant cytotoxicity to monkey kidney cells and powerful anti-viral activity towards several DNA and RNA viruses (Rinehart et al. 1981). Purification of the active extract yielded three components, the major one didemnin A (95) and two minor ones, didemnins B (96) and C (97). Both A and C are anti-viral agents, whereas B has anti-cancer activity. The didemnins all contain an hydroxyisovalerylpropionyl (HIP) group, as well as statine, an amino-acid recently discovered in pepstatin a pepsin inhibitor of microbial origin (Morishima et al. 1970). Another marine tunicate *Lissoclinum patella* yielded the bioactive cyclic peptides ulicyclamide (98) and ulithiacyclamide (99) following MeOH extraction and purification (Ireland & Scheuer 1980). Threonine is masked as an oxazoline



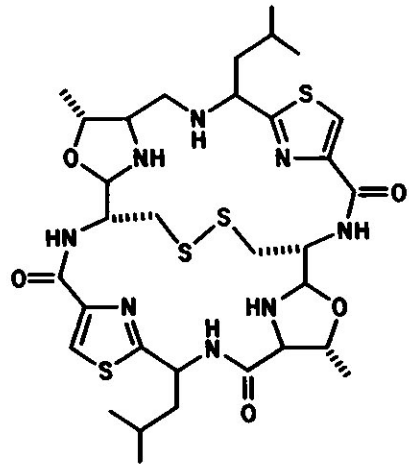
	R_1	R_2
93	COMe	H
94	H	H



	R
95	H
96	MeCH(OH)CO-Pro
97	MeCH(OH)CO

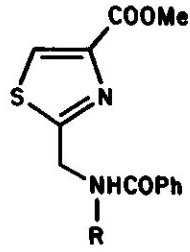


98



99

moiety and hydrolysis of (98) yielded five amino acids Pro, Phe, Thr and two thiazoles (100) and (101). The structural similarity of the second constituent (99) was indicated by the spectroscopic data and hydrolysis of this symmetrical peptide furnished cystine, threonine and thiazole (102) in a 1:2:2 molar ratio. Both ulithiacyclamide (99) and another symmetrical cyclic peptide (103) displaying

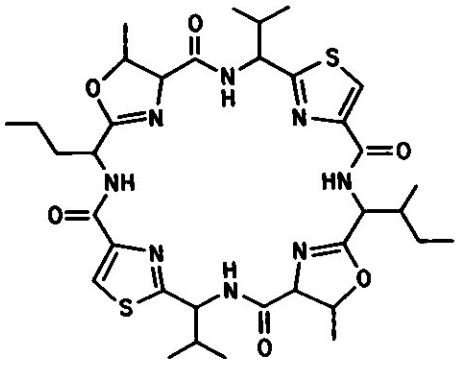


	R
100	Me
101	$MeCH_2CHMe$
102	Me_2CHCH_2

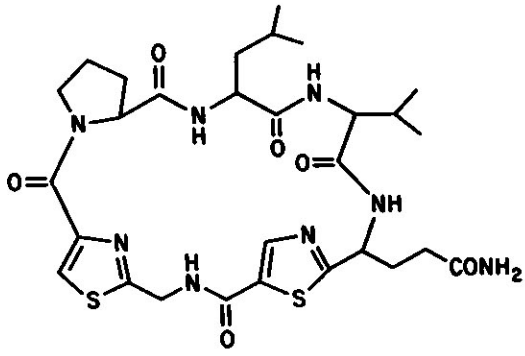
cytoxicity were isolated from an unidentified ascidian (Hamamoto et al. 1983). In this case hydrolysis yielded leucine, threonine and a thiazole moiety containing a valyl side-chain. The cyclic peptide dolastatin 3 (104) is one of nine antineoplastic and/or cytotoxic substances isolated from the sea hare *Dolabella auricularia* (Pettit et al. 1982b). The similarity with the other tunicate-derived cyclic peptides is obvious. The structure of dolastatin 3 was established on the basis of extensive NMR and MS data.

Toxic Agents

The toxicity of certain marine organisms was probably the first and most widely known feature of their biological activity and dates back to Biblical times (Halstead



103

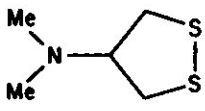


104

1965). However very few of the chemicals were identified until recently. This was due to difficulties in purifying the toxins, the often minute amounts produced by the organism, and the complex structures of the toxins themselves.

One of the first toxins to be characterized was isolated from the marine annelid *Lumbriconeris heteropoda*. Fishermen noted that flies landing on the worm quickly expired. From chemical extracts of the worm the cyclic disulphide nereistoxin (105) was identified as the responsible insecticide (Okaichi and Hashimoto 1962). Based on this lead, simpler synthetic analogues such as (106) (Konishi 1968) have been successfully employed as commercial insecticides.

Saxitoxin (107) one of the so-called paralytic shell fish poisons (PSP) was originally isolated from the Alaskan butter clam *Saxidomus giganticus*. Although often sus-



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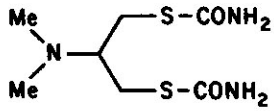

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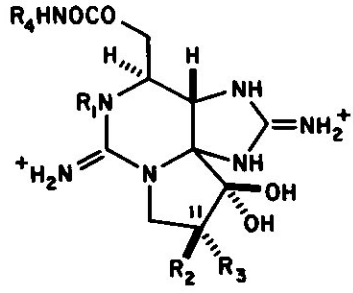
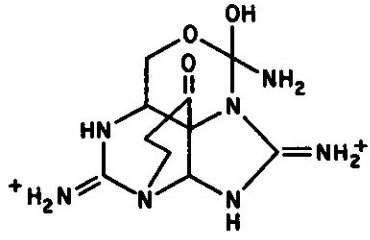


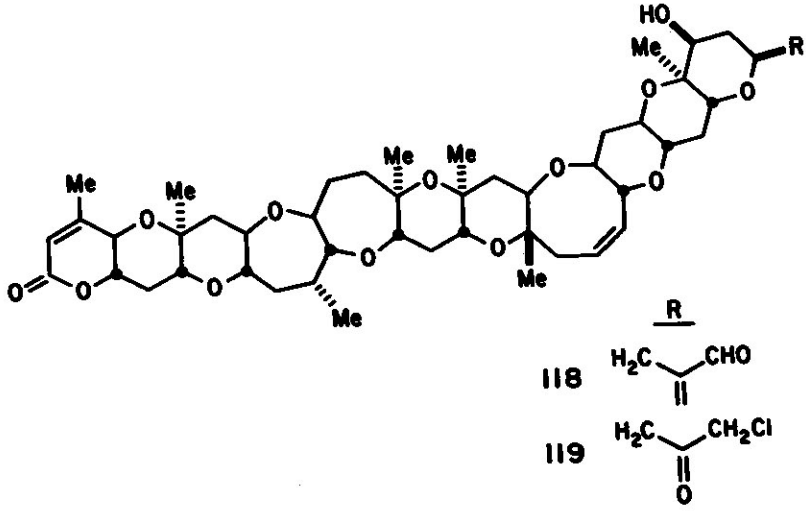


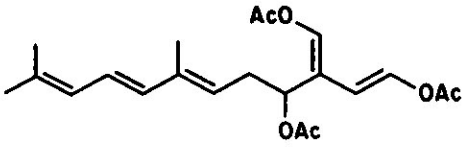
108

	R_1	R_2	R_3	R_4
107	H	H	H	H
109	OH	H	H	H
110	H	H	OSO_3^-	H
111	H	OSO_3^-	H	H
112	H	H	OSO_3^-	SO_3^-
113	H	OSO_3^-	H	SO_3^-
114	OH	H	OSO_3^-	H
115	OH	OSO_3^-	H	H
116	OH	H	OSO_3^-	SO_3^-
117	OH	OSO_3^-	H	SO_3^-

pected, it was some years before the toxin from the clam and the toxin from the dinoflagellate *Gonyaulax tamarensis* (Protogonyaulax)—responsible for the red tide—were shown to be the same chemical (cf. Shimizu 1978). After investigations spanning more than fifteen years, structure (108) was proposed to account for the spectroscopic data and most of the degradation products (Wong et al. 1971). However, this was changed a few years later when another group succeeded in preparing a crystalline di-p-bromobenzene sulphonate of the toxin (Schantz et al. 1975). The final structure is similar to that proposed earlier, the major difference being in the position of the propionyl group and the existence, in the crystalline form at least, of the hydrated ketone. Neosaxitoxin (109), an N-hydroxy derivative of saxitoxin, was first isolated as a minor constituent in the clam *S. gigantens*, but later it was discovered as a major component of the dinoflagellate *G. tamarensis* (Shimizu et al. 1978). Currently eight related toxins (110) - (117) have been isolated from a variety of sources including clams, mussels, and dinoflagellates (Alam et al. 1982). Structure variety is based on the saxitoxin and neosaxitoxin skeleton, and 11-0H epimers which occur as sulphate esters. The observation that brief exposure of a mixture of PSP toxins to acid resulted in enhanced toxicity, led to the discovery of a series of latent toxins containing the hitherto unreported sulphonatocarbamoyl function (Kobayashi & Shimizu 1981, Wichmann et al. 1981). The presence of this functional group considerably reduces the toxicity of the molecule but this returns following mild hydrolytic removal of the sulphate.

Another dinoflagellate *Ptychodiscus brevis (Gymnodinium breve)* has caused devastating red tide blooms in the Gulf of Mexico, resulting in massive fish kills. The toxicity is caused by a new group of compounds called the brevetoxins. Unlike the tetrodoxin/saxitoxin group of paralytic shell-fish poisons which are water soluble and block sodium channels (Shimizu 1978) the brevetoxins are ether soluble neurotoxic shell-fish poisons which activate sodium channels (Risk et al. 1979). The crude ether-soluble toxin mixture from *P. brevis* contains 3 components: brevetoxin A (minor constituent, most potent) brevetoxin B (major component) brevetoxin C (minor component). An X-ray crystallographic study established the structure of brevetoxin B (118) and the structurally related toxins as large polycylic molecules possessing contiguous trans-fused ether rings (Lin et al. 1981).





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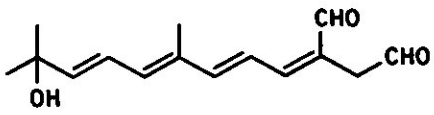

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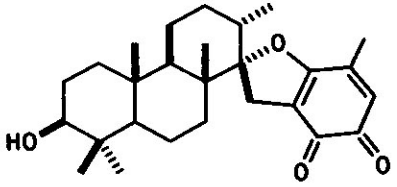

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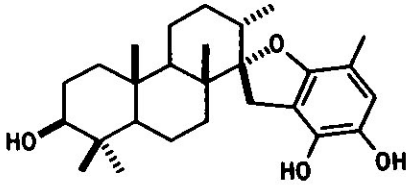
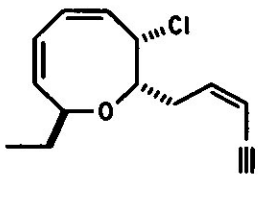


123

Shortly after, the structure of brevetoxin C (119) was elucidated by comparison of its spectral data with these of brevetoxin B (118) (Golik et al. 1982).

Several ichthyotoxins or fish repellants are produced by marine plants. A defensive role for these compounds seems obvious. For example, in an area subjected to predation by herbivorous fish the flourishing green alga *Rhipocephalus phoenix* produces two linear sesquiterpenoids rhipocephalin (120) and rhipocephenal (121) which induce pronounced feeding avoidance behaviour in the herbivorous fish *Eupomacentrus leucostictus* (Sun & Fenical 1979.) Another algal product which is both ichthyotoxic and cytotoxic is the novel terpenoid stypoldione (122) (Gerwick & Fenical 1981) from the brown alga *Stypopodium zonale*, which produces stypoldione and several related ichthyotoxins. The o-quinone is found in the surrounding sea-water of the plant, and is in fact the air oxidation product of the more toxic stypotriol (123) the major intracellular toxin.

Antifeedants have also been found in marine animals, though frequently they are of algal origin. Sea hares are large slow-moving slug-like gastropod molluscs, that seem vulnerable to predation. However, when tested, sections of the mollusc *Aplysia brasiliana* (eg. body wall, digestive gland, etc) were unpalatable to sharks. Chemical investigation of the digestive gland uncovered three halogenated cyclic ethers brasilenyne (124) *cis*-dihydrorhodophytin (125) and *cis*-isodihydrorhodophytin (126), all of which are produced by an alga which grows in an area inhabited by the sea-hare (Kinnel et al. 1979). Another example of sea hares accumulating noxious chemicals through their diet is illustrated by two recent reports from independent laboratories. From the brown alga *Dictyota crenulata*, Fenical's group isolated acetoxycrenulide (127) a major diterpenoid metabolite which is severely debilitating to the herbivorous fish *Eupomacentrus leucostictus* (Sun et al. 1983). The sea-hare *Aplysia vaccaria* is reported to feed on brown algae and from the digestive glands of these molluscs Sims' group isolated acetoxycrenulide (127) and three other crenulides (128) - (130) (Midland et al. 1983). The novel bicyclo [6.1.0] nonane skeleton of the crenulides, proposed following chemical investigation of the algal material, was confirmed by X-ray analysis of dihydroxycrenulide (129).



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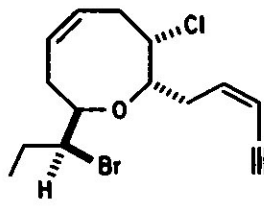

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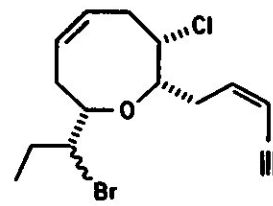

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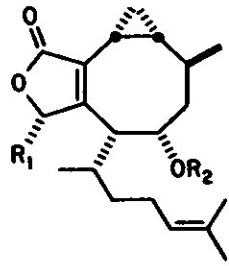
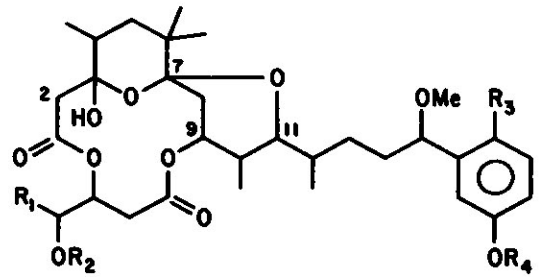


	R_1	R_2
127	H	Ac
128	H	H
129	OH	H
130	OH	Ac

A chemical examination of the toxic principles of another sea-hare *Stylocheilus longicauda* led to the isolation and identification of two toxins aplysiatoxin (131) and debromoaplysiatoxin (132) from an ether extract (Kato & Scheuer 1974). In a separate study Mynderse and co-workers (1977) identified debromoaplysiatoxin as the antineoplastic agent from a deep water variety of the blue-green alga *Lyngbya majuscula* upon which the mollusc *S. longicanda* was observed feeding. Somewhat surprisingly, aplysiatoxin (131) was not found in the alga, though shallow water varieties do generaly contain both toxins. Debromoaplysiatoxin (132) and oscillatoxin A (133) were identified as two of the compounds responsible for antineoplastic activity in two other blue-green algae *Schizothrix calcicola* and *Oscillatoria nigroviridis* (Mynderse and Moore 1978).

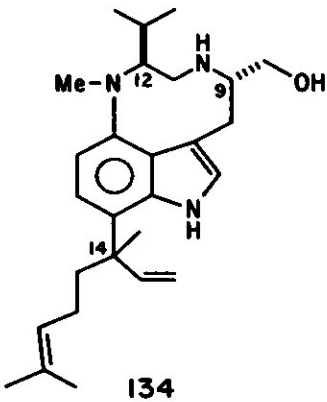
However, not all toxic strains of *L. majuscula* contain debromoaplysiatoxin or its congeners and another investigation of a shallow water variety revealed that the



	R_1	R_2	R_3	R_4
131	Me	H	Br	H
132	Me	H	H	H
133	H	H	H	H

marginal activity of this species was due to a completely different compound, lyngbyatoxin A (134) (Cardellina et al. 1979). The UV spectrum of the toxin was typical of an indole - a relatively rare structure in marine plant products - and other spectroscopic data established the nature and placement of a non-hydrolyseable N-methylvaline unit and a linalyl group. The algal toxin is identical with the C_{14} epimer of teleocidin A, a metabolite of *Streptomyces mediocidicus* (Takashima et al. 1962). This bacterium also produces another toxin teleocidin B (135) which exists as a mixture of four C-14, C-17-diastereoisomers. Interestingly, only one epimer of lyngbyatoxin A has been found in the alga *L. majuscula*. Comparison of the ORD and CD measurements of the algal product and the teleocidin mixture indicated that the chirality at C-9 and C-12 in all the toxins is the same.

Contact with *Lyngbya majuscula* can result in severe dermatitis for swimmers in Hawaii during summer months, and debromoaplysiatoxin (132) aplysiatoxin (131) and lyngbyatoxin A (134) are each responsible for this effect. Not surprisingly, the teleocidin producer *S. mediocidicus* is responsible for a contact dermatitis which affects workers in the antibiotic industry (Moore 1982). A much simpler but no less unusual chemical that causes contact dermatitis—the so-called Dogger Bank itch—is the sulfonium ion (136) produced by the marine bryozoan *Alcyonidium gelatinosum* (Carlé & Christophersen 1980).



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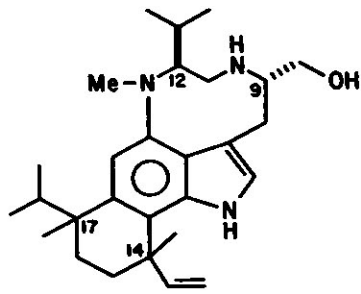

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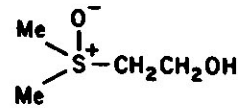
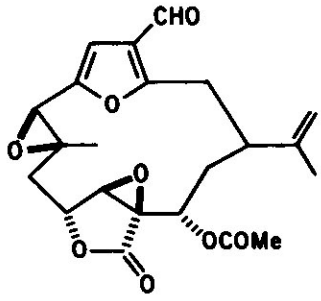


136

Considerable cytotoxic, ichthyotoxic and antibacterial activity was displayed by alcohol extracts of several gorgonians belonging to the genus *Lophogorgia*. From these extracts a powerful neuromuscular toxin, lophotoxin (137) was isolated (Fenical et al. 1981), which is another example of the cembrane class of diterpenes. We have seen that terpenes of this group frequently display biological activity but lophotoxin is unusual in that it contains furanoaldehyde and α,β-epoxy-γ-lactone functional groups, relatively rare features among natural products. Both functional groups seem important for the pharmacological activity of lophotoxin, and chemical modification of either group results in diminished toxicity. Along with lophotoxin, the extract also yielded a small amount of another cembrenolide, pukalide (138), an essentially inactive metabolite previously isolated from the soft coral *Sinularia abrupta* (Missakian et al. 1975).

Cardiovascular/Cardiotonic Agents

Few compounds displaying cardiotonic activity have been reported from marine organisms but this may be due to the fact that assays for such biological activity are less frequently performed.



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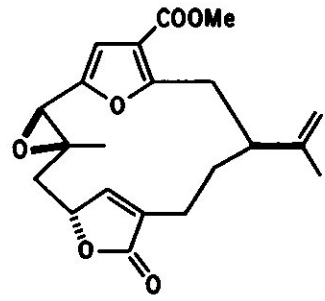
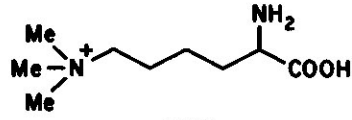


138

Laminine (139) a widely distributed metabolite of brown algae has long been known as a hypotensive agent. More recently another hypotensive agent, autonomium (140), which also possesses a quaternary ammonium head group, was isolated from an aqueous-alcoholic extract of a sponge *Verongia fistularis* (Kaul 1981). The aqueous extracts of several holothurians (eg. *Pentacter arassa, Thelenota ananus* and *Stichopus chloronatus*) were found to possess hypotensive activity and in each case the causative agent was the known compound 5-hydroxytryptamine (Gregson et al. 1981b). An aqueous extract of the soft coral *Nepthea* sp. caused an increase in heart rate and blood pressure of test animals. A variety of aromatic amines including histamine, tyramine and dopamine were detected in the extract but none of these was responsible for the cardiovascular activity. Further



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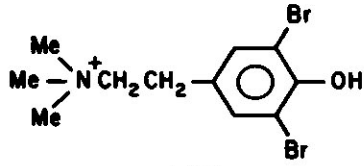

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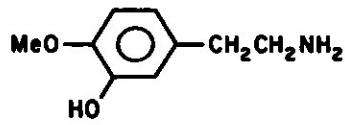
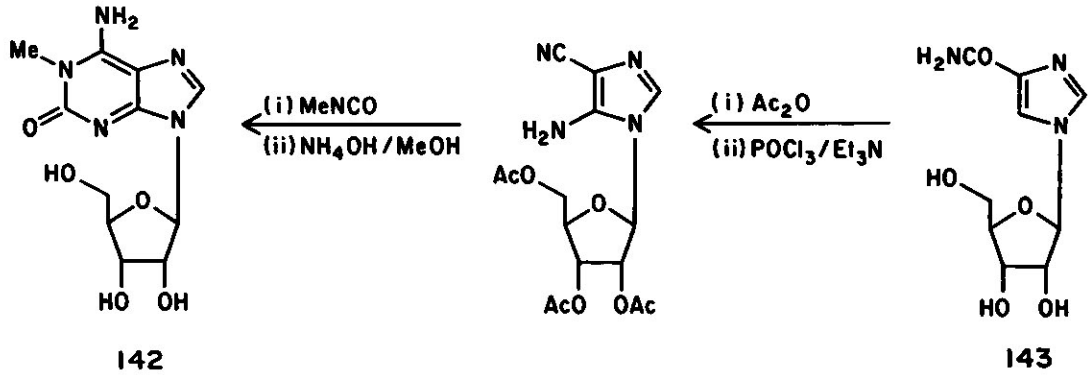


141

purification yielded a pure active fraction identified as 3-hydroxy-4-methoxyphenethylamine (141). Hitherto unreported from a marine source, this amine had already been found in cacti and human urine (Gregson et al. 1981a).

Free nucleosides are rare in nature but most possess some form of biological activity. An aqueous extract of the sponge *Tedania digitata* displayed muscle relaxant, antihypertensive, and anti-inflammatory properties, and all these pharmacological properties were eventually attributed to a single compound, 1-methylisoguanosine (142) (Quinn et al. 1980). Chemical degradation and spectroscopic evidence established the presence of D-ribose and 1-methylisoguanine, and a simple synthesis from the imidazole (143) (Scheme 3) yielded a product identical with the natural product. The same compound (named doridosine) was isolated by another group from extracts of the digestive gland of a nudibranch *Anisodoris nobilis* (Fuhrman et al. 1980).



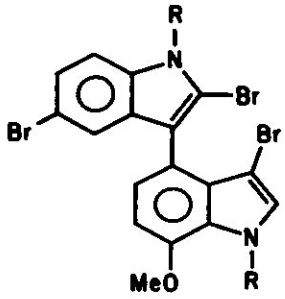
Scheme 3

Scheme 3: Chemical synthesis of 1-methylisoguanosine (142) from the imidazole (143)

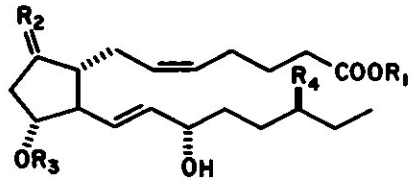
Anti-inflammatory Agents

Marine bi-indoles are extremely rare yet one of the most potent naturally occurring anti-inflammatory compounds ever discovered is the brominated bi-indole (144) isolated, together with several analogues, from a methylene chloride extract of the blue-green alga *Rivularia firma* (Norton & Wells 1982). The compound also displays anti-anaphylaxis properties which are enhanced by formation of the diacetate (145). An interesting feature of these bi-indoles is that despite their lack of a chiral centre most of them are optically active due to restricted rotation within the molecule.

A convincing case for serendipitous research was made by Weinheimers group following their isolation of unusually large concentrations of prostaglandins from the Caribbean gorgonian *Plexaura homomalla* (Weinheimer & Spraggins 1969). The excitement over this discovery stimulated activity in gorgonian fishery and raised the question as to the origin of these compounds since many of these primitive animals enjoy a symbiotic relationship with algae. Despite intensive searching it was ten years before another marine prostanoid was discovered and was in fact the first report of prostaglandins from a plant: the derivatives PGE_2 (146) and PGF_{2a} (147) were isolated from the red alga *Gracilaria lichenoides* (Gregson et al. 1979), and several others (148) - (151) from the soft coral *Lobophyton depressum* (Carmely et al. 1980).



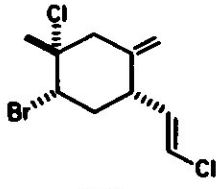
	R
144	H
145	Ac



	R_1	R_2	R_3	R_4
146	H	O	H	H
147	H	αOH,H	H	H
148	Me	αOH,H	Ac	H
149	Me	αOH,H	Ac	OAc
150	H	αOH,H	Ac	H
151	H	αOH,H	Ac	OAc

Central Nervous System (CNS) Agents

Many mono- and diterpenes isolated from marine organisms have non-specific depressant activity on the central nervous system (CNS) (Taylor et al. 1981). In contrast, the halogenated monoterpene plocamadiene A (152) isolated from the red alga *P. cartilagineum* (Higgs et al. 1977) causes an excitatory action on mouse behaviour. The effect is long-lasting and may have promise as a tool with which to study receptors of the CNS. Another drug with significant anti-depressant activity is methylaplysinopsin (153) a yellow crystalline metabolite isolated from the sponge *Aplysinopsis reticulata* together with the less active compound aplysinopsin (154)



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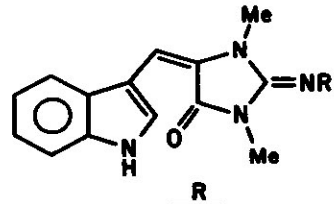
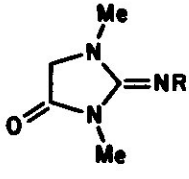
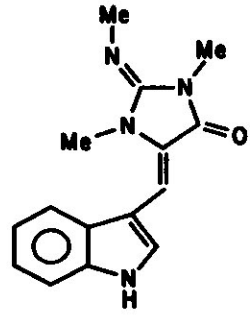


	R
153	Me
154	H



	R
155	Me
156	H



157

(Kazlauskas et al. 1977b). Both natural products, and many analogues, were synthesized by piperidine catalysed condensation of indole-3-aldehyde with the requisite creatinine derivatives [(eg. (155) and (156)]. Both X-ray crystallographic analysis and nuclear magnetic resonance studies established the E configuration of the double bond in the natural products (c.f. Wells 1979.) The Z (157) isomer of methylaplysinopsin can be prepared but the antidepressant properties of this isomer are drastically reduced.

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