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Chapter 18

Marine Metabolites: Oceans of Opportunity

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Chapter Outline

Learning Objectives

- To gain an understanding of the importance of marine natural products chemistry in drug development
- To be able to map the process involved in drug development from marine natural products
- To gain an appreciation of the range of biological activities associated with compounds isolated from micro- and macroorganisms
- To identify the marine-derived drugs which are undergoing clinical evaluation

18.1 INTRODUCTION

Over 75% of the earth's surface is covered by vast expanses of ocean. Its inhabitants are diverse with 15 of the 34 phyla occurring exclusively in the oceans with only one phylum (Onychophora) being reported as present on land only [1]. The marine environment provides an array of structurally unique and diverse constituents produced by an equally diverse consortium of marine organisms living on our coral reefs and in benthic communities. The marine organisms are highly variable in species, color, and morphology and belong to several phyla including Porifera (sponges), Ascidiacea (sea squirts), and Octacorallia (soft corals). The metabolites of marine origin emanate from a variety of parts of the plants and animals and are thought to be produced as a form of chemical communication, defense, or to ward off potential predators [2–12] (Figs. 18.1–18.3).

Of the 34 animal phyla recorded, **15** are ONLY found in the ocean with only 1, Onychophora present on land only.

The pioneers in the field, Paul Scheuer of the University of Hawaii at Manoa and D. John Faulkner of *Scripps* Institution of Oceanography, University of California at *San Diego*, La Jolla, set the foundation with active research programs sourcing marine compounds from a wide range of species including algae, sponges, coelenterates, ascidians, and bryozoans. Bioprospecting efforts over the last 40 years have yielded over 20,000 compounds of marine origin with

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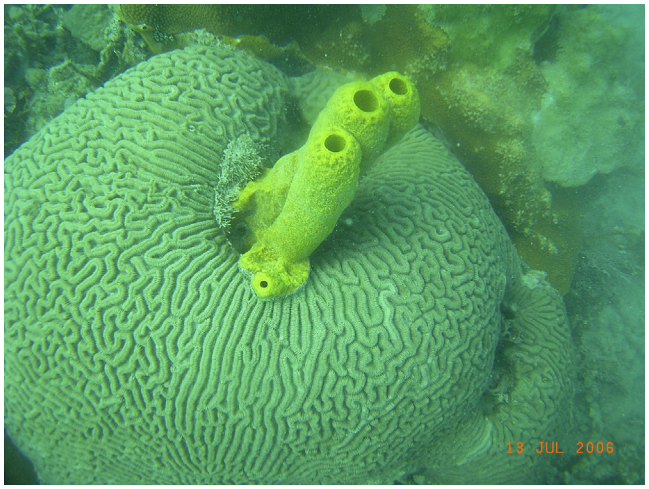


FIGURE 18.1 Tropical sponge growing on a brain coral, Port Royal, Jamaica.

FIGURE 18.2 Purple ascidian growing on soft coral, Drunken Man's Cay, Port Royal, Jamaica.

FIGURE 18.3 Diverse assemblage of marine species growing on a mangrove root.

the potential for a range of applications including anticancer, antibacterial, antiviral, antiinflammatory, antimalarial, antituberculosis activity, as well as pharmacological and industrial applications. The classes of compounds manufactured by marine organisms include alkaloids, terpenoids, shikimates, peptides, and polyketides [2–15].

18.2 COLLECTION, EXTRACTION, AND ISOLATION OF MARINE NATURAL PRODUCTS

The decision regarding which marine organisms to collect may be based on different considerations including the nature of the collection location and the novelty of the organisms being collected. It has been reported that specimens collected in different geographical locations have different secondary metabolite profiles and factors such as salinity, temperature, light intensity, pollution levels, as well as predation pressures, and the nature of bacterial symbionts may play a part in the occurrence and concentrations of specific secondary metabolites [16–18].

Organisms to be collected, depend on:
1. Collection environment (salinity, temperature, light intensity, pollution levels, predation pressures, nature of bacterial symbionts)
2. Novel organisms
3. Nonfouled species

Other specimen collections are made based on what may be observed with the organism. For example, if there is an organism which appears not to be fouled by other species which are competing for space in the marine habitat, it could be surmised that the organism may be producing secondary metabolites to ward off the competition, thereby preventing fouling. Such an organism may therefore be selected for investigation on the premise that ecologically bioactive compounds may also have therapeutic or industrial potential [19]. Organisms which were not previously investigated have a significant likelihood of yielding novel metabolites boasting unique biological activity (Fig. 18.4).

18.2.1 Collection

Marine organisms are collected by *snorkeling* or *wading* in water for specimens in shallow locations while scuba diving is required when procuring specimens in deeper waters (2–30 m). NITROX diving can facilitate the collection of specimens up to 80 m in depth (Figs. 18.5 and 18.6).

The use of the *remotely operated vehicle* (ROV) has revolutionized the study of the ocean and its organisms. The ROV can be deployed to depths unsafe or inaccessible to scuba divers. In fact, the photographing and collection of specimens including uncommon and new species at depths of up to 12,000 m has been facilitated by this technology. Consequently, many previously unexplored sites across the world's oceans are now being opened up to chemists, biologists, archeologists, as well as oil exploration teams. Sensors on the ROV are capable of retrieving data on the water

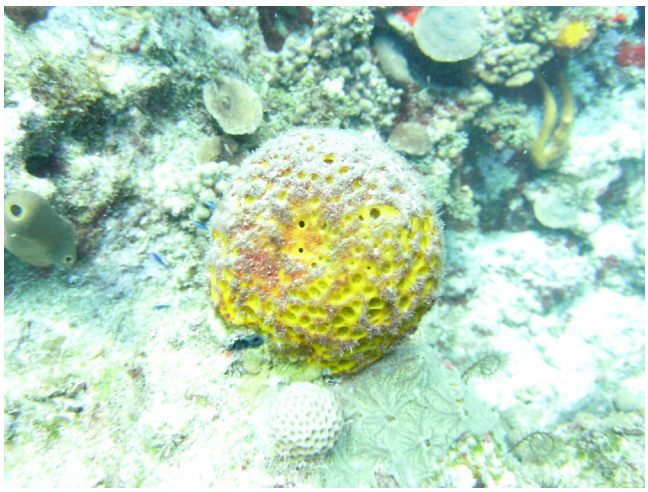


FIGURE 18.4 Orange ball sponge fouled by red algal species.

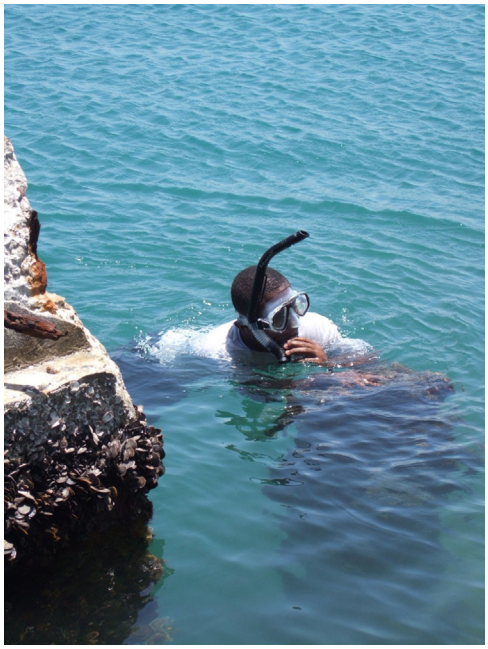


FIGURE 18.5 Student snorkeling to collect marine specimens.

FIGURE 18.6 Divers collecting sponge specimens.

temperature, salinity, pH, and dissolved oxygen concentrations in the water, thereby providing useful information to facilitate environmental studies [20,21].

Caution should always be exercised in the collection of marine species. Gloves should be worn in the collection and subsequent handling of specimens. Scuba divers should be clad in wet suits to protect against the possible deleterious effects of chemicals being exuded into the water by the organisms being collected. The personal unfortunate experience (author's) of hours of severe discomfort and rashes as a result of collecting the sponge *Neofibularia nolitangere* from a reef in Discovery Bay, Jamaica, provides clear evidence regarding the level of respect which should be accorded to

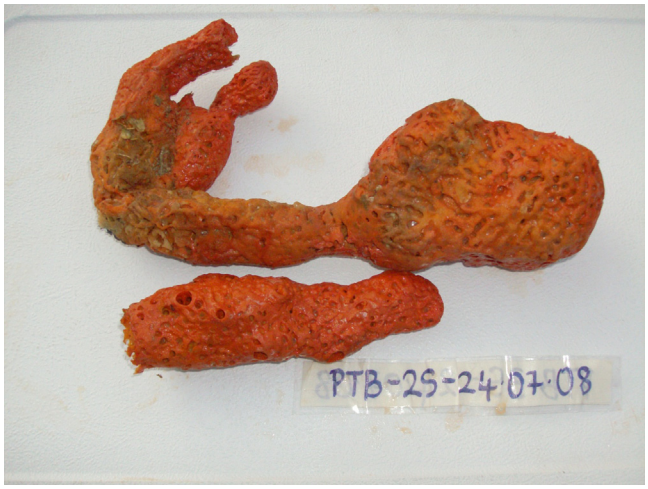


FIGURE 18.7 Coding of collected sponge specimen.

FIGURE 18.8 Boats are often used to access collection locations.

marine organisms whose chemistry is yet to be investigated. Records are made of the depth, habitat, global positioning system coordinates (latitude and longitude), color, morphology, and associated organisms. An appropriate coding system should be employed to distinguish specimens. Where possible, the specimens are photographed in situ as well as by the dockside (Figs. 18.7 and 18.8).

A voucher specimen of each organism is usually preserved in 70% aqueous ethanol for the purpose of taxonomic identification. Ascidians are usually preserved in seawater containing menthol crystals with more long-term storage in 10% formalin solution [22].

It should be noted that the recollection of organisms has proved to be a challenge in some instances. An ascidian species, e.g., found to be thriving on the mangrove in the summer of one year could all but disappear from the ecological landscape 6 or 12 months later, while a healthy bed of algae may be short-lived if there are dynamic factors involved in their growth. For example, the occasional nutrient runoff or groundwater seepage event could provide the ideal environment for the growth of selected algal species. Environmental factors are key in the marine landscape and often provide a source of frustration to the specimen collector.

18.2.2 Extraction

Prior to extraction of the collected organism, the specimens may be frozen, air-dried, freeze-dried, or could be retained in the fresh state. The majority of the marine organisms are extracted fresh or frozen while the remaining specimens are

lyophilized or dried in air before extraction [22]. In some instances, dried algal species are ground to a powder prior to extraction as described by Sansom and coworkers who isolated an antiproliferative bis-prenylated quinone from the alga *Perithalia capillaris* [23]. The extraction of marine organisms may be carried out using a range of organic solvents including hexanes, dichloromethane, acetone, ethyl acetate, as well as more polar solvents such as ethanol and methanol. In many instances, a mixture of polar and medium polarity or nonpolar solvents is utilized in the extraction protocol. For example, the extraction of the Madagascar sponge *Monanchora dianchora* was achieved in CH_3Cl:MeOH (1:1) to yield two polycyclic guanidine alkaloids [24]. Extractions are usually exhaustively performed over several days with at least three aliquots of the solvent being used. The solvent is then removed in vacuo by rotary evaporation. Solvent partitioning is another strategy employed in the extraction of the organisms. This involves single one-step or two-step partitioning systems usually involving an aqueous phase portioned with a solvent immiscible with that phase. The Kupchan and modified Kupchan procedures are often employed in natural products as was described in the isolation of a diterpene from an *Axinella* species [25]. In this procedure, the concentration of the aqueous layer is progressively adjusted to afford three or four different fractions. Complex partitioning procedures are also employed, albeit rarely so. Simple partitioning has been most commonly employed with Kupchan schemes being utilized with less frequency [22].

18.2.3 Isolation and Component Identification

Chromatographic methods of separation include gravity column chromatography, flash column chromatography, and vacuum liquid column chromatography utilizing silica gel as the packing material. With silica gel, the components of the marine extract are separated on the basis of polarity of the compounds. As the polarity of the eluting solvent increases compounds of increasing polarity are eluted from the column with hydrocarbons, e.g., eluting before alcohols. The elution of the components of a column is monitored by using thin layer chromatography (TLC) plates which are spotted to show the sequence of elution of the compounds (Figs. 18.9 and 18.10).

Bonded reverse phase silica is employed in instances where the constituents of the marine extract include polar metabolites. Bonded phases include ODS (C_{18}), C_8, cyano, and diol columns.

Separation of constituents may also be effected using gel permeation chromatography which effects separation of constituents on the basis of the size of the compounds. In this regard, Sephadex LH-20 is commonly utilized in marine natural products isolation work [26]. Resins such as BioBeads, Amberlite, XAD-2, and XAD-4 are also utilized in separating components of relatively high polarity. The use of XAD-2 in the separation of antiviral trisulfated triterpene glycosides from the sea cucumber *Staurocucumis liouvillei* is one such example in marine natural products isolation work [27].

Chromatographic methods
1. Gravity column chromatography
2. Flash column chromatography
3. Vacuum liquid column chromatography
4. Gel permeation chromatography
5. High-performance liquid chromatography (HPLC)
6. Medium pressure liquid chromatography (MPLC)
7. Recycling HPLC

The use of HPLC employing a reversed phase stationary phase system is commonplace in marine natural products isolation work with C_{18} and C_8 semipreparative and preparative columns being used. MPLC and recycling HPLC techniques are related techniques for purification of a range of metabolites including alkaloids, peptides, and terpenoids.

Tandem systems such as liquid chromatography-mass spectrometry systems are also employed to assist with dereplication efforts. Unusual MS peaks in the profile suggest that novel components are present in the fraction or extract being evaluated. Those fractions with unusual constituents may then become the focus of the research efforts. Solid-phase extraction methods are also employed in separating compounds.

The structural identification of compounds isolated from the range of marine sources is facilitated by the use of spectroscopic techniques such as 1D and 2D nuclear magnetic resonance (NMR) spectroscopy and infrared (IR) spectroscopy. X-ray crystallographic techniques are also important in aiding in the determination of the stereochemistry of the compound. The identification of nanogram quantities of a novel compound is becoming increasingly more facile with the use of the cryoprobe, capillary probe, and Mans probe [15].

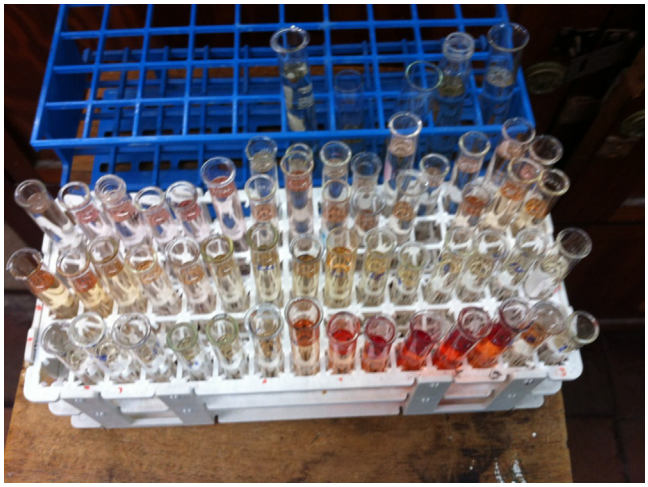


FIGURE 18.9 Test tube fractions from a silica gel chromatography column on a marine extract.

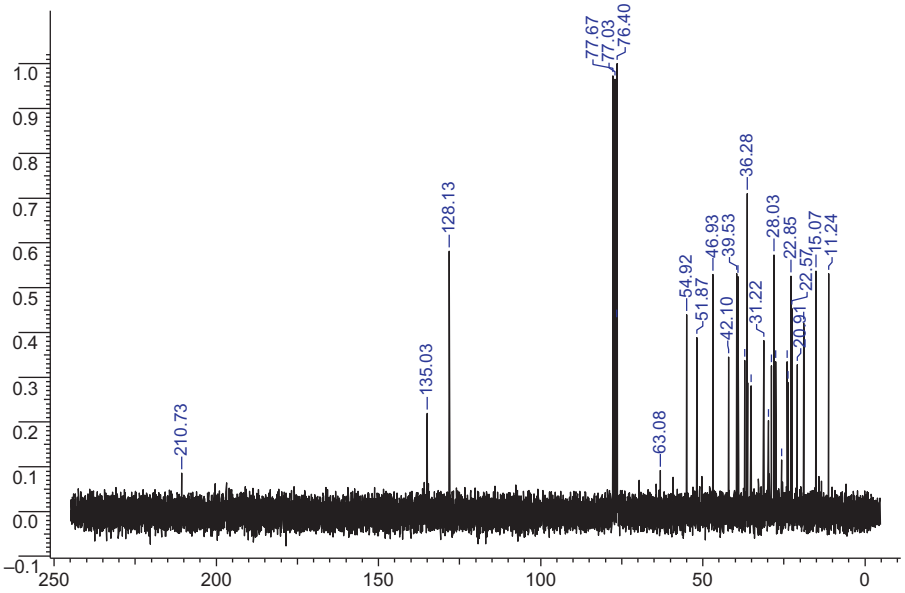


FIGURE 18.10 NMR spectrum of a compound isolated from a sponge.

18.3 IN VIVO AND IN VITRO BIOACTIVITY OF METABOLITES FROM MACROINVERTEBRATES, MACROALGAE, AND MICROORGANISMS

In vitro activities of marine metabolites have been investigated for a diverse range of cell systems including antiinflammatory, antimicrobial, and anticancer activities. Crude extracts, fractions from crude extracts, as well as pure compounds are typically evaluated for biological activity. The in vitro biological evaluation of the isolated compound may be performed using cell lines from human subjects or animals. Brine shrimp, fish, and sea urchin are among the organisms employed in the evaluation of compounds or extracts for ecological and therapeutic importance (Figs. 18.11 and 18.12). A summary of the biological activity of some of the organisms discussed in this section is presented in Table 18.1.

18.4 EVALUATION OF MARINE EXTRACTS

Preclinical trials are an essential component of the process of evaluation of the therapeutic potential of a compound. These trials often include animal models such as rats, dogs and monkeys. The major sources of biologically relevant

FIGURE 18.11 Student with sea urchin specimen.

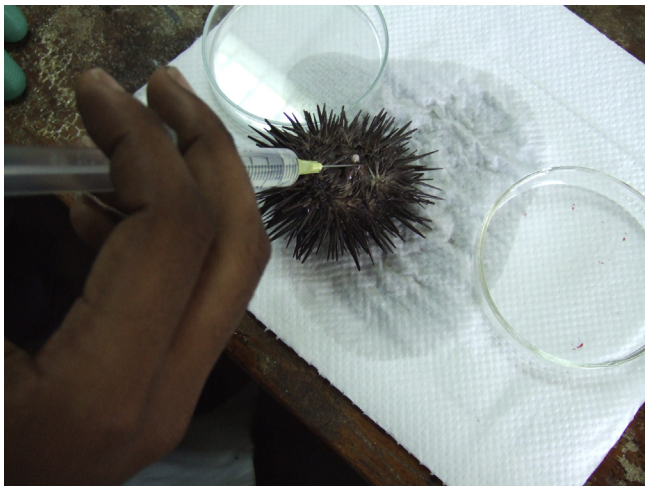


FIGURE 18.12 Injection of sea urchin with salt solution to obtain eggs and sperms for biological evaluation.

compounds have been found to be from sponges, coelenterates, algae, echinoderms, ascidians, molluscs and microorganisms [14].

18.4.1 Metabolites From Macroinvertebrates

Macroinvertebrates include sponges, ascidians, and soft coral. It has been found that the vast majority (75%) of novel compounds obtained from the marine environment have been sourced from the Porifera and Coelenterata (Cnidaria) phyla [15]. Scheme 18.1 shows representative structures of compounds isolated from macroinvertebrates.

Macroinvertebrates include:
1. Sponges
2. Ascidians
3. Soft coral

TABLE 18.1 Representative Macroinvertebrates, Macroalgae, and Microorganisms Investigated to Obtain Biologically Active Metabolites

Source	Species	Biologically Active Compound	Reported Biological Activity
Sponge	*Halichondria okadai*	Halichondrin B	Anticancer
	Agelas mauritianus	Agelaspin	Anticancer
	Agelas nakamurai	Agelasine D	Antimicrobial
	Discodermia dissoluta	Discodermolide	Anticancer
	Mycale sp.	Mycalamide A	*Herpes simplex* virus inhibitor
	Jaspis sp.	Jasplakinolide	Antimicrobial
	Monanchora arbuscula	Batzelladine alkaloids	Antibacterial
Soft coral	*Cespitularia taeniata*	Cespitulactam K	Anticancer, antimicrobial
	Clavularia inflata	Dolabellane diterpenes	Anticancer
Ascidian	*Trididemnum solidum*	Didemnin B	Antiviral
	Didemnum guttatum	Cyclodidemniserinol trisulfate	Antiviral
Macroalgae	*Dictyota* sp.	8α,11-dihydroxypachydictyol A	Antimalarial
	Stypopodium zonale	Zonaquinone acetate	Anticancer
	Laurencia undulatea	Polyphenols	Antiinflammatory
	Halimeda monile	Phenols	Antioxidant
Microorganisms	*Pseudoalteromonas*	3,3′, 5,5′-tetrabromo-2,2′-diphenyl diol	Antibacterial
	Corallospora pulchella	Melinacidins and gencidin	Antibacterial
	Marinospora sp.	Lynamicins A-E	Antibacterial

Sponges (Porifera) are sedentary, filter feeding metazoans which utilize a single layer of flagellated cells (choanocytes) to pump water current through their bodies in a unidirectional manner. There are over 5000 species of sponges accounting for much of the epifaunal biomass. Extracted fresh or freeze-dried, sponge extracts are an important source of biologically active compounds. These isolates exhibit an impressive array of biological activities, some of which are described here.

One sponge which has gained a place in history due to the promising biological activity being displayed is *Halichondria okadai*, the producer of halichondrin B, which underwent evaluation as an anticancer agent. Okadaic acid, also from *H. okadai*, exhibited inhibitory activity against phosphatase-1 and phosphatase-2A [28] (Fig. 18.13).

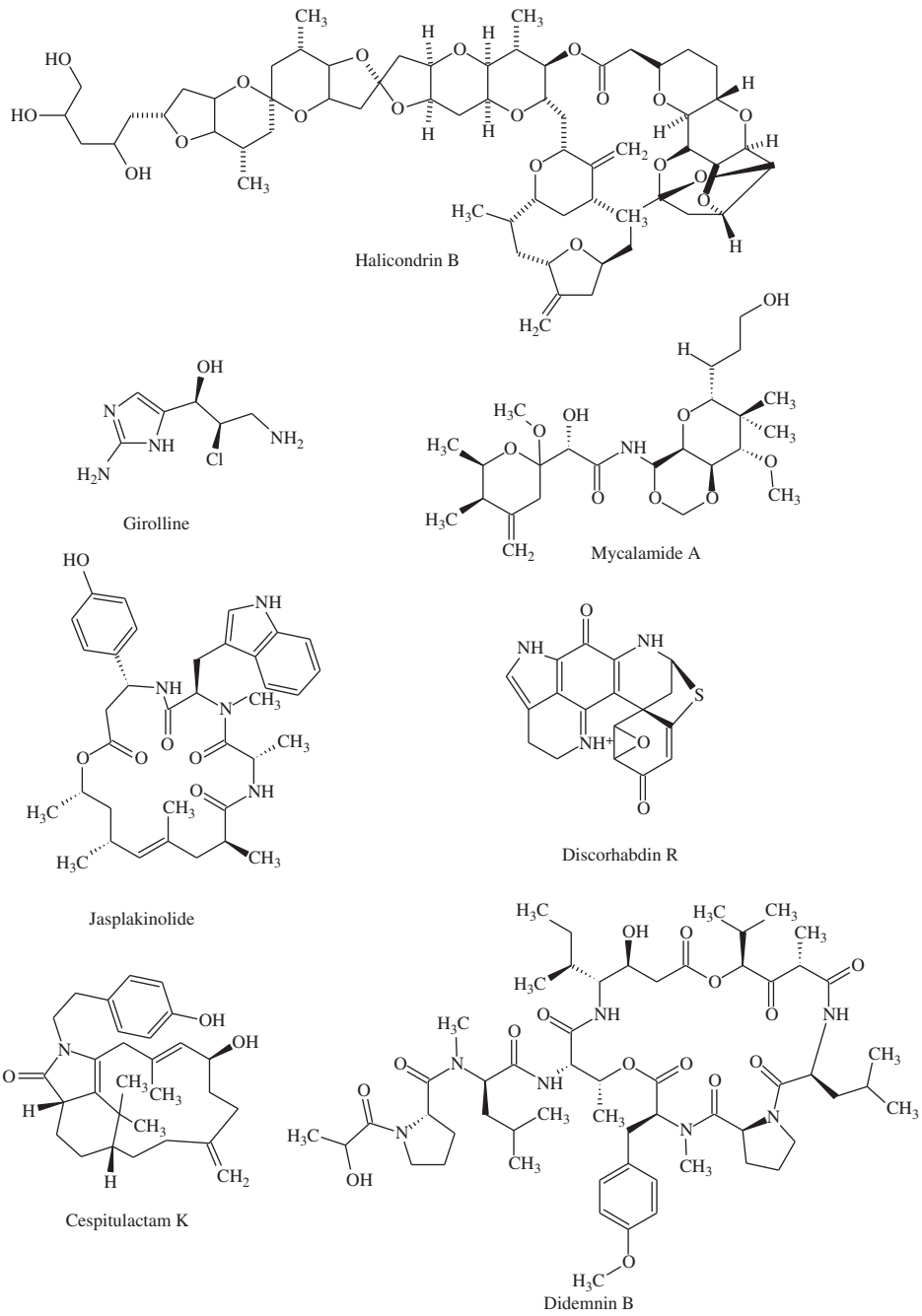
Agelaspin, an antitumor glycosphingolipid obtained from the marine sponge *Agelas mauritianus*, demonstrated antitumor activity in vivo against murine B16 melanoma. This compound was also found to stimulate the immune system. A derivative of agelaspin, KRN-7000, underwent clinical investigations for cancer immunotherapy [28]. More recently, the extracts of another *Agelas* sp., *A. nakamurai*, contained the compound agelasine D which exhibited high antibacterial activity [29].

The deep water sponge *Discodermia dissoluta* produced discodermolide, a polyhydroxylated lactone which exhibited anticancer activity, as well as immunosuppressive activity. It was found to stabilize microtubules in a manner similar to the drug taxol and underwent evaluation for use in tumors resistant to taxol [30,31]. *Dysidea arenaria* was found to contain arenastatin A which showed potent activity against KB cell lines ($IC_{50} = 5$ pg/mL) [32].

Girolline is a substituted imidazole isolated from the sponge *Pseudaxinyssa cantharella* which functions by inhibiting the termination step in eukaryotic protein synthesis. Having entered Phase 1 clinical trials, it was withdrawn due to its adverse hypertensive effects seen in treated patients [28].

Mycalamides A and B are protein synthesis inhibitors isolated from the New Zealand sponge *Mycale* sp. In vivo activity against A59 coronavirus was observed in mice when treated with a 2% mycalamide mixture at a dosage of 0.2 μg/kg daily with 100% survival over a two-week period. Pure mycalamide A inhibited the *Herpes simplex* virus 1 and Polio virus type 1 at a concentration of 0.005 μg/disk. Mycalamide B was found to exhibit more potent antiviral activity and cytotoxicity than mycalamide A [28]. The baculiferins I, J, L, and M from the marine sponge *Iotrochota baculifera* have been found to inhibit human immunodeficiency virus-1 (HIV-1) with IC_{50} values between 0.2 and 7.0 μm [33].

Jasplakinolide, the first example of a cyclodepsipeptide isolated from a sponge, is a 19-membered macrocyclic depsipeptide from the *Jaspis* sp. exhibiting in vitro antimicrobial activity at a minimum inhibitory concentration of



SCHEME 18.1 Representative bioactive compounds isolated from macroinvertebrates.

25 μg/mL against *Candida albicans*. With a topical administration of 2% jasplakinolide solution, an effect similar to that of miconazole nitrate was achieved in vivo [28].

Discorhabdin R is a novel pyrroloiminoquinone isolated from the southern Australian sponge *Negombata* sp. and Antarctic *Latruncula* sp. which was found to display antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*) and Gram-negative bacteria (*Serratia marcescens* and *Escherichia coli*), respectively [34].

Antibacterial activity against a strain of the bacterial parasite *Plasmodium falciparum* was reportedly identified in *Monanchora arbuscula* with the active agents being the batzellidine alkaloids ($IC_{50} = 0.2-0.9$ μm) [35].

An important isolate from a *Spongia* sp. is the polyhydroxylated steroid, agosterol A, which functions by reversing multidrug resistance caused by the overexpression of two kinds of membrane glycoprotein in cancer cells [36].

From the phylum Cnidaria the genera *Sinularia* and *Briareum* have proven to be prolific sources of novel compounds. Cembranoids, 5,8-epidoxysteroids, sinulaflexiolides, and africanenes have been isolated from *Sinularia* species [1] (Fig. 18.14).

Examples of other species of soft corals include the Taiwanese soft coral *Cespitularia taeniata* which was extracted with ethanol to yield a group of verticillene diterpenoids including cespitulactam K. The compounds were evaluated against human epidermal carcinoma and murine L1210 leukemia cell lines. Cespitulactam K exhibited activity against the cancer cell lines (3.7−5.1 μg/mL) and also showed marked antimicrobial activity against *M. luteus* and *Cryptococcus neoformans* [37].

The methanol extract of the octocoral *Muricea austera* showed in vitro activity against chloroquine-resistant *P. falciparum* and was found to contain a range of different classes of compounds including tyramine derivatives, steroidal pregnane glycosides, and sesquiterpenoids [38].

Cytotoxic dolabellane diterpenes were isolated from the Formosan soft coral *Clavularia inflata* var *luzoniana* and bioactivity against P388 cell lines with ED_{50} values between 0.5 and 3.6 μg/mL was observed [39].

Tunicates, sea squirts, or ascidians belong to the subphylum of Tunicata (Urochordata). They are so named because of their cellulose-containing protective tunic surrounding the organism. Tunicates attach to a substratum, usually a marine solid surface such as a mangrove root, rocks, jetties, or even algal species (Fig. 18.15).

FIGURE 18.13 Color morphs of a mangrove sponge.

FIGURE 18.14 Dried specimen of a soft coral (Phylum Cnidaria).

FIGURE 18.15 *Ascidia curvata* growing on a mangrove root, Port Royal, Jamaica.

Much like sponges and soft corals, ascidians have also been found to be a good source of bioactive agents. Didemnin B, isolated from the tunicate *Tridemnum solidum*, is one such bioactive compound, showing remarkable antiviral and cytotoxic activity. Didemnin B demonstrated activity against P388 and L1210 murine leukemia cell lines. It was advanced into preclinical and clinical trials 1 and 2, but had to be withdrawn due to its harsh toxicity [30]. Aplidine, formally known as dehydrodidemnin, an isolate from the Mediterranean tunicate *Aplidium albicans*, is one such bioactive compound. Being structurally related to didemnin B, aplidine was found to be up to 10× more active and less toxic than didemnin B. It entered into Phase 1 clinical trials in 1999 under investigation for the treatment of solid tumors and non-Hodgkin's lymphoma. Broad spectrum activity was displayed in vitro and in vivo against leukemia, melanoma, breast, ovarian, colon, and lung (nonsmall cell) cancer. Having advanced to Phase 2 clinical trials, aplidine affects protein synthesis through GTP-dependent inhibition of elongation factor 1-α [30].

The extract of the Palauan ascidian *Didemnum guttatum* afforded the sulfonated serinolipid cyclodidemniserinol trisulfate which exhibits an antiviral effect by inhibiting HIV-1 integrase, an attractive target for antiretroviral chemotherapy [30].

18.4.2 Metabolites From Macroalgae

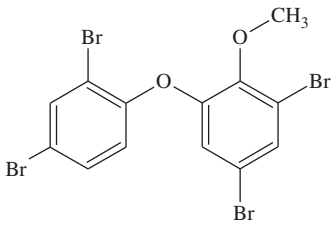
Macroalgae belong to three main phyla: Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae). Biological activities identified in extracts and metabolites of algal origin include anticancer, antiobesity, neuroprotective, and antioxidant activity and Scheme 18.2 shows chemical structures of representative bioactive compounds isolated from the macroalgae. A wide range of algal species are utilized in fresh or dried forms as food particularly in Asian countries where folklore traditions govern their industrial and medicinal usage [40].

Macroalgae are the source of agar, carrageenan, and alginate, which are all of importance in the food industry. The range of compounds isolated from algal sources has been variable. Representative examples of bioactive constituents from macroalgae are mentioned below.

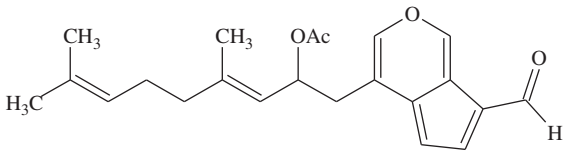
Cytotoxic activity has been identified in 8α,11-dihydroxypachydictyol A, a diterpenoid compound from a *Dictyota* sp. collected on Bangsaen Beach in Thailand. Antimalarial activity was also found in the diterpene isolated from this extract when the compound was tested with malarial parasites [41].

Stypolactone, an isolate from the brown alga *Stypopodium zonale*, was found to exhibit weak cytotoxic activity in vitro when evaluated with A-549 and H-116 cell lines [42]. Zonaquinone acetate, obtained from Jamaican populations of *S. zonale*, displayed in vitro activity against breast and colon cancer cell lines [43]. Specimens of *Taonia atomaria* produced atomarianones A and B which were reportedly found to be cytotoxic against NSCLC-N6 and A-549 cell lines [44] (Fig. 18.16).

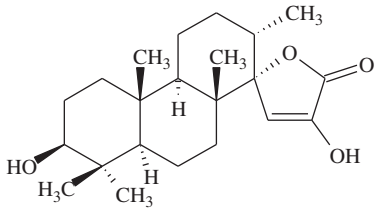
Crude extracts of algal species have been found to exhibit a range of biological activities. For example, aqueous extracts of *Gracilaria corticata* and *Sargassum oligocystum* exhibit bioactivity against cancerous human leukemia cells [45,46] while a methanol extract of *Plocamium telfairiae* was observed to display bioactivity against HT-29 colon cancer cells [47].



2- (2, 4'-dibomophenoxy) -4, 6-dibromoanisol



Halitunal



Stypolactone

SCHEME 18.2 Representative bioactive compounds isolated from macroalgae.

FIGURE 18.16 Specimen of the brown alga *Stypopodium zonale*.

Antiinflammatory activity was found in the green alga from which 2-(2,4′-dibromophenoxy)-4,6-dibromoanisol was isolated. This activity was identified using a snake toxin-induced mouse limb model [48].

Also exhibiting antiinflammatory activity is a mixture of phytosterols obtained from *Dunaliella tertiolecta*. When administered in a sheep model of inflammation-induced cytokine production, an inhibitory effect was observed [49].

Polyphenolic extracts from the red alga *Laurencia undulatea* displayed antiinflammatory activity in vivo. These extracts served to inhibit asthmatic reactions in mice sensitized and challenged with ovalbumin which was used to induce murine allergic reactions in test subjects [50].

Antiinflamatory agents floridoside and D-isofloridoside from the South Korean alga *L. undulatea* were found to inhibit free radical oxidative stress at IC_{50} values between 22 and 43 μm [51].

Biologically active compounds have been isolated from the brown seaweed *Dictyota cervicornis* from which was obtained sulfated polysaccharides with powerful anticoagulant activity [52].

Antioxidant activity, evaluated using the DPPH method, was reported in phenolic isolates of *Halimeda monile* when liver injury was induced in a rat model. The phenolic fraction was administered over a 20-day period and led to protective effects against chemicals harmful to the liver [53].

With IC_{50} values between 0.5 and 2.9 μm, potent antimalarial activity against the human malarial parasite *P. falciparum* was identified in new macrolides bromophycolides J, M, N, O, P, and Q from the red algae *Callophycus serratus* [54] collected in Fiji.

The marine alga *Halimeda tuna* was studied by Koehn and coworkers, leading to the isolation of halitunal, a diterpene displaying in vitro antiviral activity against murine coronavirus A59 [55].

Ecologically important roles are played by some compounds from alga sources. For example, halimedatrial, a diterpene isolated from *Halimeda lamouroux*, exhibited toxicity toward reef fishes and appeared to be a feeding deterrent. Antimicrobial activity was also reported from this compound [56].

18.4.3 Metabolites From Microorganisms

Almost 20% of all bioactive marine compounds currently being studied are obtained from marine microorganisms [15]. These microbes are found in swabs from the surfaces of marine plants and animals, suspended in the water from geothermal vents and deep water environments, or on sediment surfaces. They thrive in a variety of environments including locales characterized by high pressures of up to 600 atmospheres, high temperatures, and high salinities. Efforts at culturing some of the microorganisms have met with varying degrees of success. The ability to propagate these microorganisms in an economically feasible way will be of great significance as potent bioactive metabolites are discovered [57] (Figs. 18.17–18.19).

Marine microorganisms are found
1. On the surface of marine plants and animals
2. Suspended in water
3. On sediment surfaces

Historically, terrestrial microbes have been a potent source of pharmaceutical agents with the seminal discovery of penicillin. The discovery of new antibacterial agents is a serious priority because of the development of potent resistance to current antibiotics on the market.

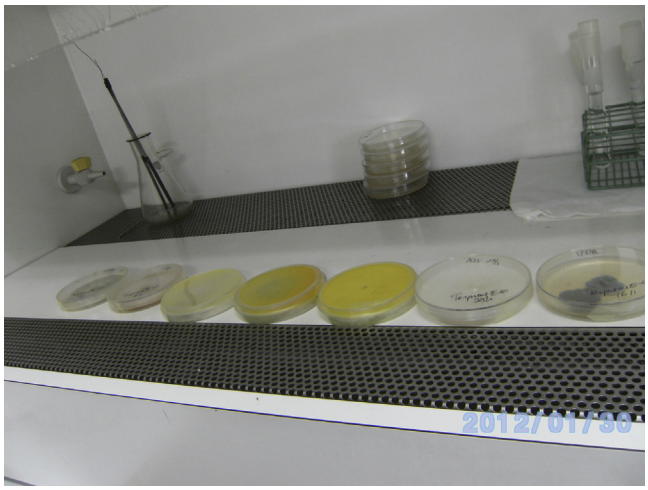


FIGURE 18.17 Plates of fungi isolated from a sponge species.

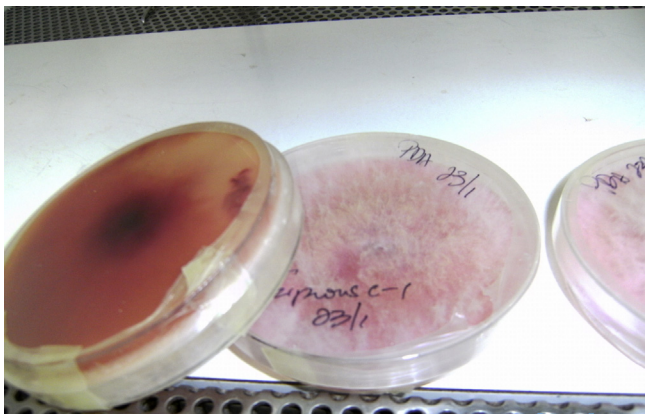


FIGURE 18.18 Pink unidentified fungus isolated from a mangrove sponge.

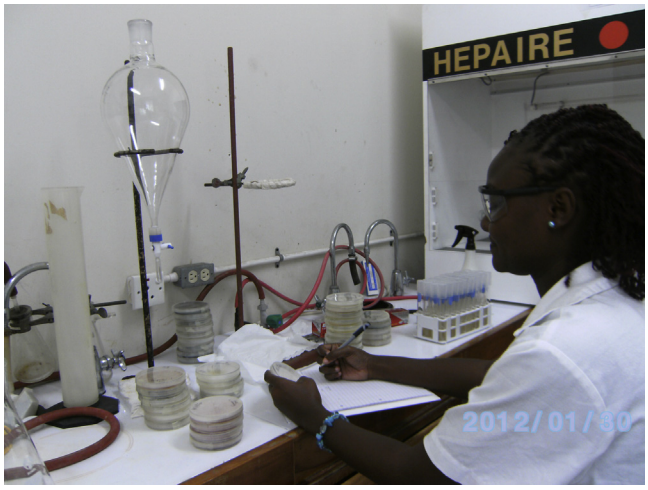
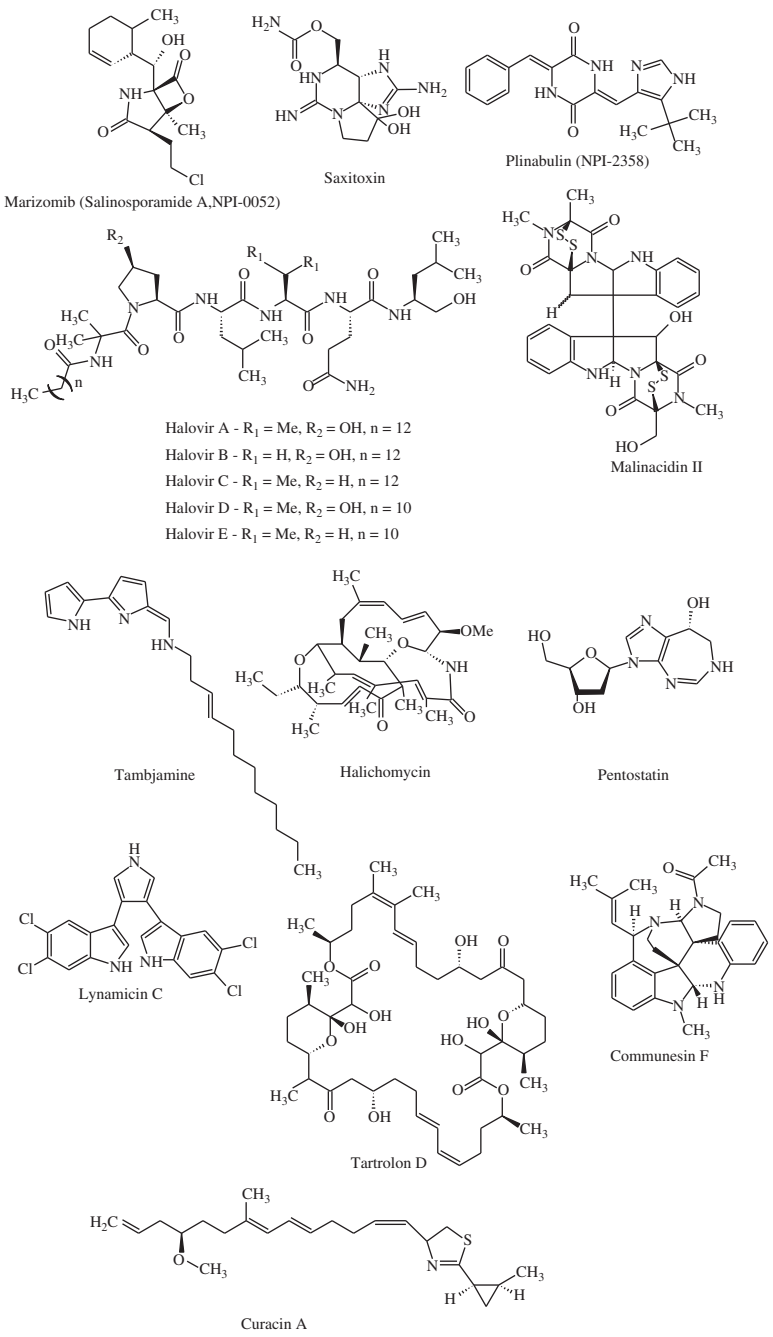


FIGURE 18.19 Student examining fungal isolates from a sponge.

Marine bacteria produce a wide variety of secondary metabolites for the purpose of defending themselves against other microbes. Scheme 18.3 shows structures of representative compounds from microorganisms associated with marine specimens. Marine bacteria which produce compounds of biological significance include *Pseudoalteromonas* species which was found to produce 3,3′, 5,5′-tetrabromo-2,2′-diphenyl diol, an inhibitor of methicillin-resistant *S. aureus*. The class of 4-methoxypyrrole-containing compounds, the tambjamines, isolated from *P. tunicata*, was found to be active antifungal, immunosuppressive, and antimicrobial agents. Biologically active compounds from marine bacteria also include *Streptomyces* species from sediment and fish gut from which anticancer (e.g., halichomycin and δ-indomycinone) and antibacterial agents (e.g., phenazines) have been obtained [58–60]. *Vibrio* species obtained from sponge specimens have produced phenolic and trisindole compounds with antibacterial activity [61,62]. A *Micromonospora* sp. obtained from a soft coral produced thiocoraline, a compound exhibiting anticancer activity [63].

Marine fungi have also been known to produce compounds with a range of bioactivities including antiviral, antifungal, enzyme inhibition, and anticancer and antibacterial activities. The isolation and cultivation of fungi from the marine environment is of critical importance for propagation of the microbes from which biologically relevant compounds may be obtained. Protocols have been established for this work [64]. Fungal species which have produced antibacterial compounds include *Corallospora pulchella* isolated from sand. This species produced melinacidins and gencidin [65].

Anticancer activity has been reported from metabolites of *Aspergillus* sp. (including the aspergillamides and fumiquinazolines) and *Penicillium* sp. sourced from a marine alga which was found to contain pentostatins and communesins among other compounds [66,67]. Antiviral activity, attributable to the presence of halovirs, was identified in a *Scytalidium* sp. collected from a seagrass species. Potent antiviral activity against *H. simplex* virus (type 1) was observed and may be acting by binding directly to the virus [68].



SCHEME 18.3 Representative bioactive compounds isolated from microorganisms.

Actinomycetes have been the source of a wide range of antimicrobial agents, the most common of which include tetracycline and streptomycin. Other bioactive compounds originating from actinomycetes include antitumor and antimicrobial agents. A *Marinospora* sp. produced a group of bisindole pyrroles, lynamicins A−E, which exhibited biological activity against Gram-positive and Gram-negative species. Importantly, activity was also shown against drug-resistant pathogens including methicillin-resistant *S. aureus* [69].

Anticancer activity against lung, colon, and breast cancer cell lines was exhibited by isolates from the fermentation of a *Streptomyces* sp. (MBG-04-17-069). Tartrolon D was found to be the bioactive agent [70].

Microalgae are found in seven phyla. These include Chlorophyta, Phaeophyta, Rhodophyta, Crystophyta, Cryptophyta, Eugelophyta, and Pyrrhophyta. The blue-green algae, Cyanophyta, are cyanobacteria which have been

found to share characteristics with eukaryotic algae. These microalgae produce compounds with a high degree of structural diversity and species, such as *Lyngbya majuscula*, have produced a vast array of biologically active compounds [71]. Curacin A, e.g., isolated by Gerwick and coworkers in 1994 [72], was found to function by disturbing microtubule assembly, thereby functioning as a lead compound in chemotherapy. *Microcystis aeruginosa* is the source of potent protein phosphatase-1 and phosphatase-2A inhibitors identified in microcystins [73].

Other microalgal species under examination include dinoflagellates which produce an array of bioactive toxins including saxitoxin and maitotoxin which function by blocking or activating sodium/calcium channels. Challenges exist with respect to the culturing of these organisms due to relatively low proliferation rates and the large quantities of culture required to obtain small amounts of bioactive compounds. Diatoms, microscopic unicellular colonial algae, grow at a faster rate and are amenable to culturing but few bioactive metabolites have been identified from these microalgae [28].

Some marine compounds sourced from microbes are of clinical significance, undergoing evaluation as potential pharmaceutical agents.

18.5 DRUGS IN CLINICAL TRIALS

The marine-derived drug pipeline, almost nonexistent in decades gone by, now has a range of candidates at various stages of development as shown in Table 18.2. Representative structures of marine-derived compounds in clinical trials are shown in Scheme 18.4.

Drugs in Phase three clinical trials include tetrodotoxin, a guanidinium alkaloid under the trademark name Tectin obtained from the Pufferfish [34]. Affecting the sodium channels, this drug is being investigated for the treatment of chronic pains (Scheme 18.5).

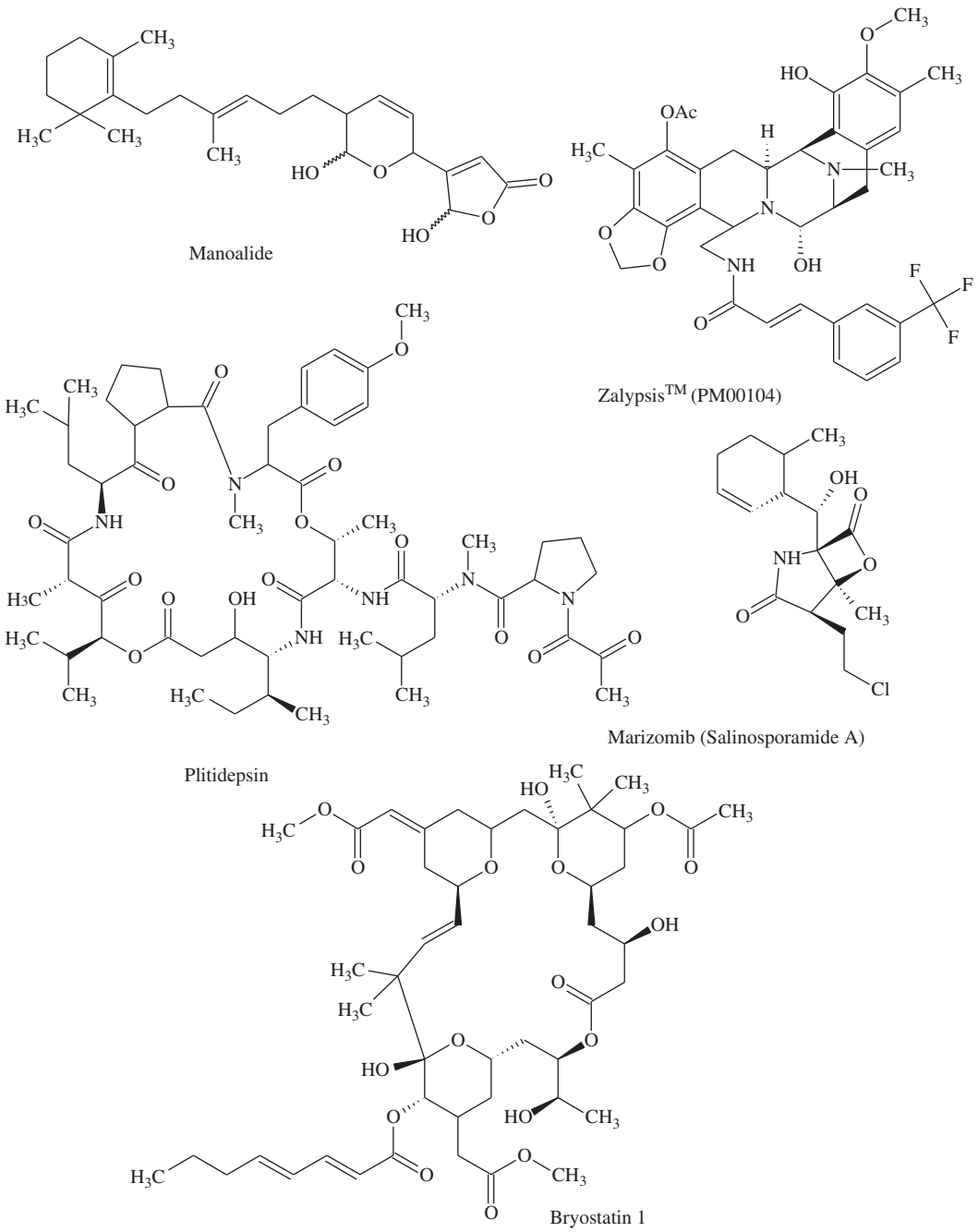
A depsipeptide from a tunicate, plitidepsin, is being tested by Pharmamar in the treatment of a variety of cancers, namely leukemia, multiple myeloma, and lymphoma. Another drug under evaluation by Pharmamar for cytotoxic activity is Zalypsis (PM00104) sourced from a mollusc which targets the DNA-binding capacity of diseased uterine, lymphoma, cervical, and endometrial cancer cells. The alkaloid-derived compound PM01183 is another drug candidate from Pharmamar being evaluated for its efficacy against a range of cancers including ovarian, breast, lung, acute leukemia, and endometrial cancer [74,75].

Bryostatin I, from the bryozoan *Bugula neritina* has been involved in a battery of clinical trials being investigated for its potency against cancer. It is currently under phase I evaluation as a treatment for Alzheimer's [74]. In the early years, the challenge associated with the supply of the drug was underscored by the fact that, in order to obtain 18 g of a cGMP quality bryostatin I, 13 tonnes of *B. neritina* had to be collected in Californian waters [13,76]. The gene cluster of the uncultivated microbial symbiont of *B. neritina, Candidatus endobugula sertula* has been successfully identified, thereby opening the potential for the supply of the compounds [77].

Kahalalide F, a cyclic depsipeptide, was found in the mollusc *Elysia rufescens* as well as the green algae *Bryopsis* sp. on which it feeds. This compound is currently in Phase I/II trials as a treatment against prostate cancer [74] (Fig. 18.20).

TABLE 18.2 Representative Marine-Derived Compounds in Clinical Trials

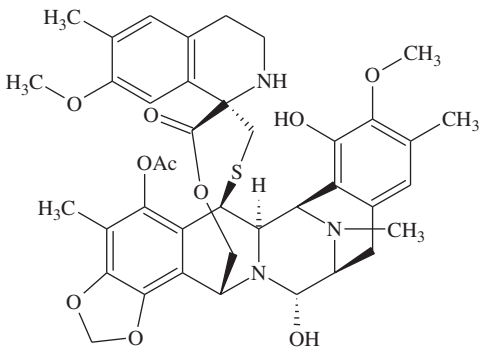
Trial Stage	Marine Source	Compound	Molecular Target	Main Disease Target
Phase III	Ascidian	Plitidepsin	JNK/Rac1 activation	Cancer (lymphoma/leukemia/multiple myeloma)
	Pufferfish	Tetrodotoxin (Tectin)	Sodium channels	Chronic pain
	Mollusc	PM00104 (Zalypsis)	DNA-binding	Cancer (uterine/cervical/endometrial)
	Worm (Annelid?)	DMXBA (GTS-21)	α7 Nicotinic acetylcholine receptor	Alzheimer's disease, schizophrenia, attention deficit hyperactivity disorder (ADHD)
	Ascidian	PM01183	DNA	Cancer (ovarian/breast/lung/endometrial)
Phase I	Bacterium	Marizomib (Salinosporamide A)	20S Proteasome	Cancer (nonsmall cell lung/pancreatic/melanoma/multiple myeloma)
	Bryozoan	Bryostatin	Protein kinase C	Cancer (prostate/pancreatic/kidney/lung/fallopian tube)



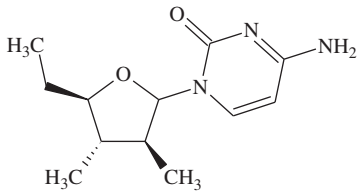
SCHEME 18.4 Representative marine-derived compounds in clinical trials.

DMXBA [(3-(2,4-dimethylxybenzylidene)]-anabaseine is a derivative of anabeseine, an alkaloid found in marine worms. Found to improve cognition in animal models, DMXBA and other related compounds have demonstrated neuroprotective activity in both in vitro and in vivo screens. Thought to have an effect on macrophage 7 receptors, antiinflamatory activity was also observed in animal models. Phase I evaluation of healthy males and schizophrenics have shown that DMXBA has led to marked improvements in cognitive function [74].

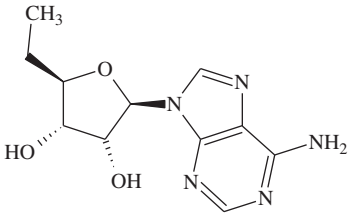
There are several marine compounds sourced from microbes which are of clinical significance. Clinical trials are being conducted on Plinabulin (NPI-2358), a vascular disrupting agent obtained from a marine fungal extract with potential for activity against multidrug resistant tumor cells. Marizomib (Salinosporamide A, NPI-0052), an isolate from a marine bacterium *Salinospora tropica*, is a novel proteasome inhibitor which is currently under investigation for



Trabectidin (ET-743)



Cytarabine (Cytosar-U)



Vidarabine (Vira A)

SCHEME 18.5 Representative marine-derived pharmaceuticals.

FIGURE 18.20 Collection site in Hawaii for the mollusc *Elysia rufescens*.

its efficacy against solid tumor models. The compound exhibits low cytotoxicity to normal cells and has significant potential for oral and intravenous administration [74].

18.6 DRUGS OF MARINE ORIGIN TO TREAT DISEASES

The ultimate goal of many marine natural products and synthetic chemists is that the isolated or synthesized molecule possesses therapeutic applications.

There are several Food and Drug Association (FDA)-approved drugs of marine origin obtained from sponges, a fish, a cone snail, a mollusc, and cyanobacterium species, while Yondelis (Trabectidin) obtained from the ascidian

Ecteinascidia turbinata, has been approved in the European Union. The antitumor effects of aqueous ethanol extracts of *E. turbinata* were observed from 1969. In vitro trials had been carried out on a 60 human cancer cell panel by the company developing the drug, Pharmamar, and the National Cancer Institute. Aquaculture of the ascidian proved to be the initial strategy used to obtain sufficient quantities for evaluation of the efficacy of the compound. Semisynthetic procedures involving the fermentation of *Pseudomonas florescens* are now currently employed in the pharmaceutical preparation of the drug which is sold in over 80 countries, including South Korea and Russia, under the trade name Yondelis. Yondelis is also used in patients with relapsed platinum-sensitive ovarian cancer. This drug is currently under evaluation in phase II for breast, prostate, lung, and pediatric cancers.

The sponge *Tethya crypta (Cryptotethia crypta)* was the original source from which the drug Cytarabine was developed. Cytarabine is a synthetic analogue of the nucleoside which was originally isolated from the sponge. Sold under the trade name Cytosar-U, this cytotoxic agent inhibits deoxyribonucleic acid (DNA) polymerase and DNA synthesis. Acute lymphocytic leukemia, non-Hodgkin's lymphoma, and acute myelocytic leukemia are among the conditions being treated by this drug approved by the FDA in 1969 [74].

Produced by fermentation of *Streptomyces griseus*, cytarabine has limited bioavailability but improvements in the delivery system have been made [78]. A slow-release liposomal form of cytarabine (Depo Cyle) has been approved in the United States and Europe for the prolonged administration/exposure in cerebrospinal fluid.

A related drug, Vidarabine (Vira-A), was developed from spongouridine and found use as an antiviral treatment for epithelial and superficial keratitis caused by the *H. simplex* virus types 1 and 2. Viral DNA polymerase and DNA synthesis of herpes are inhibited by this drug which was discontinued over 10 years ago. This drug is still in use in Europe for ophthalmological challenges.

Prialt (Ziconotide) was obtained from a peptide ω-conotoxin MVIIA isolated from the cone snail *Conus magus.* With a unique mode of action, this drug acts by reversibly blocking N-type calcium channels in some specific nerves in superficial layers of the spinal cord. This drug is used for the management of severe and chronic pains in patients suffering from cancer and Acquired immunodeficiency syndrome who are unable to use or are unresponsive to other drugs such as morphine.

Ziconotide had to be synthesized using solid-phase peptide synthesis due to the insufficient quantities supplied by the cone snail, *C. magus* [79]. The blockage of the spinal cord induced by this drug prevents the release of neurotransmitters responsible for pain from specific neurons. Related *Conus* peptides are undergoing evaluation in human clinical trials [80].

Brentuximab vedotin (SGN-35) is being marketed under the trade name Adcetris by Seattle Genetics and has gained repute for the treatment of Hodgkin and systemic anaplastic large cell lymphoma [81]. This drug is an analogue of dolastatin 10, a compound isolated from the sea hare *Dolobella auricularia*, which was later found to be produced by diet-associated cyanobacteria *Symploca hydnoides* and *L. majuscula.*

Preliminary phase I and II clinical trials of dolastatin 10 and a related analogue were largely unsuccessful. Antibody-drug conjugates function by selectively delivering the drug to the cancer cell by linking the dolastatin 10, e.g., to an antibody that targets a cell membrane protein on the surface of Hodgkin's lymphoma cells. This technology has proven to be a seminal development.

Omega-3 fatty acids from fish oils are being marketed under the trade name Lovaza by GlaxoSmithKline. Used in the treatment of hypotriglyceridemia, the drug controls ethyl esters of eicosapentaenoic acid and docosahexaenoic acid and functions by lowering triglyceride levels. [81].

Eribulin mesylate (E 7389), with the trade name Halaven was formulated from the macrolide halichondrin B sourced from the sponge *H. okadai.* Studies related to the anticancer activity of simpler analogues of halichondrin B showed that the efficiency is retained leading to the development of eribulin mesylate which is more water soluble than the parent macrolide. Now approved for use, potent and irreversible inhibition in cancer cells medicated by this drug resulted in the death of the cells by apoptosis. In the absence of tubulin, cell growth grinds to a halt. Related compounds are currently being evaluated in Phase II trials [81].

One of the more recent formulations on the market is Carragelose, an antiviral nasal spray which functions by creating a physical antiviral barrier in the nasal cavity. The company Marinomed Biotechnologie GmbH, utilized iota-carrageenan, sulfated polysaccharides found in the Rhodophyceae seaweed as well as other seaweeds. The product is effective against the early symptoms of the common cold [81].

It should be noted that, in addition to the pharmaceutical applications of marine-sourced therapies, a range of cosmetic applications also exist and are thriving industries. The foray into cosmetic applications was led by Estee Lauder with the antiaging skin care remedy Resilience which contains an extract from the Caribbean Sea whip *Pseudopterogorgia elisabethae*. The active antiinflammatory and analgesic agents are the pseudopterosins, tricyclic

diterpene glycosides, which have been found to inhibit PLA2 and 5-lipoxygenase. Derivatives of the pseudopterosins underwent phase I and II trials to examine wound healing efficiency but the lipophilic and insoluble nature of the compounds have served to limit its potential as an effective drug. Compounds from this group of tricyclic diterpene glycosides also underwent preclinical evaluation as antiinflammatory drugs [81].

Abyssine is marketed as a product used to soothe and reduce irritation in skin sensitive to ultraviolet B light as well as chemical and mechanical attack. It consists of an extract from an *Alteromonas* species and contains a high molecular weight polymer with two different oligosaccharides (exopolysaccharide), while Seacode represents another exopolysaccharide which occurs as a mixture of extracellular glycoproteins and other glucidic exopolymers produced by fermentation of a *Pseudoalteromonas* sp. This product has been found to improve skin roughness after up to four weeks of administration.

RefirMAR, a recent product to be introduced, was obtained from an intracellular extract from a fermentation of a new *Pseudoalteromonas* sp. Isolated from a deep (2300 m) hydrothermal vent in Portugal's Exclusive Economic Zone, extraction of the cultured biomass afforded a mixture of macromolecules which inhibit muscle contraction. The hydrating and antiaging potential of the product has been evaluated in vivo and in topically applied formulations [81].

18.7 DISCUSSION

The area of marine natural products chemistry has clearly developed leaps and bounds as evidenced by the relatively large number of marine-derived drugs undergoing evaluation as potential therapeutic agents. Buoyed by the potential for the development of natural products from the sea, research work continues to advance with the discovery of new bioactive compounds and new applications for previously isolated molecules [2–15].

The supply issue, however, remains one of critical importance as it relates to the development of drugs from a marine organism. For example, (+) spongistatin 1 has been reported to be highly cytotoxic. It has been deemed to be the most active of all natural and synthetic compounds investigated by the National Institute of Cancer (USA). Three tonnes of the sponge yielded 0.8 mg of the compound. Another collection and processing of 400 kg of the sponge afforded 10 mg of the compound. This isolation work facilitated structure elucidation work. The IC_{50} value for this compound was evaluated at 10^{-6} M in colon cancer cells and 10^{-12} M for breast cancer cell lines [82]. Synthetic approaches to the compound have been presented by research groups including Petit and coworkers [83,84].

Total synthesis of biologically active marine compounds is often fraught with its attendant challenges due to the length of multistep synthetic procedures and the general complexity of the structural motifs which must take into account stereochemical considerations. Propagation through mariculture and aquaculture are also being studied to determine the viability of using these approaches to deal with the challenges associated with procuring sufficient quantities for clinical trials and subsequent formulation into drugs [85].

The timeline from discovering the drug, leading to the entry into the market typically spans a 20- to 30-year period during which time the capital injection is considerable, often necessitating support from the large pharmaceutical entities which are sometimes hesitant about making investments which may not yield significant financial rewards [86]. The Caribbean region, being an important source of marine species with which much research work has been carried out, is not likely to become the recipient of the potential benefits to be derived from the development unless more research work in this area is undertaken in the region with support from the appropriate collaborators.

In the future, it is expected that new strategies will be employed to ensure the supply of large quantities of the target compounds. These include optimization or fermentation techniques for propagation of microbes, including mixed fermentation methods. Biotechnological approaches are likely to include whole genome sequencing, genome mining, genetic engineering, chemoenzymatic synthesis, and in vitro enzymatic synthesis in the hope that new therapeutic drugs will come from our seas [87].

18.8 QUESTIONS

1. If you were required to evaluate an extract for its potential as a drug, what approach would you adopt?
2. Silica gel chromatography is essential for the purification of organic compounds. Identify three methods of chromatography.
3. Design a form which could be used to document information when collecting a specimen.

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