BIOACTIVE NATURAL PRODUCTS FROM MARINE SOURCES

M.J. ABAD* AND P. BERMEJO

Department of Pharmacology, Faculty of Pharmacy, University Complutense, 28040 Madrid, Spain Tel: +34-1-3941871; Fax: +34-1-3941764; E-mail: mjabad@eucmos.sim.ucm.es

ABSTRACT: Natural products from plants and microorganisms have traditionally provided the pharmaceutical industry with one of its most important sources of "lead" compounds in the search for new drugs and medicines. Although twenty thousand plant species are used in traditional medicines, most species have not been thoroughly examined chemically or pharmacologically. Natural product research is increasingly turning to marine animals, plants and microbes as source organisms. The oceans with their millions of species are a rich source of marine plants and animals. In recent years, a number of potential therapeutic agents have been isolated from marine flora and fauna. Several marine natural products are currently in preclinical and clinical evaluation, others show promising biological activities in vitro and in vivo assays, and others are making significant contributions to our understanding of cellular processes at the biochemical level. Although only initiated in the late 1970s, natural drug discovery from the world’s oceans has been accelerated by the chemical uniqueness of marine organisms, and by the need to develop drugs for contemporary, difficult to cure, diseases. The isolation, structure, biological activities, chemical properties and synthesis of compounds from marine sources, have attracted the attention of chemists, biologists and pharmacists. Current research activities have generated convincing evidence that marine drug discovery has an exceedingly bright future. This article deals principally with bioactive constituents characterized in the past decade from marine sources in order to obtain a better understanding of the biological significance of marine flora and fauna. The structural diversity of the medicinal constituents is discussed.

INTRODUCTION

It is well known that plants are an exceptional source of biologically active products which may serve as commercially significant entities in themselves, and which may provide lead structures for development of modified derivatives possessing enhanced activity and reduced toxicity. It is likely that many compounds still await discovery. However, in the last decade the source of natural drugs has expanded to include lower plants, microorganisms and animals as well as marine organisms.
The oceans cover more than 70% of the earth’s surface, which represents over 95% of the biosphere. The oceans are therefore an unexplored area of opportunity for the discovery of pharmacologically active compounds. Although it has been one of man’s principal sources of food for thousands of years, the sea was not considered as a supply of biologically active substances until forty years ago. In the last two decades, the search for marine-derived natural products has been extended to all oceans of the world. The enormous potential of the sea as a source of energy, food and chemicals has led to its being the subject of intense research. The results of this search had been reported in numerous reviews [1-8].

Marine organisms have been shown to be a very rich source of unique and biologically active secondary metabolites that have attracted the interest of both chemists and pharmacologist. Plant and animal marine life forms have been studied with a view to obtaining such products. In particular, in pharmacognosy, the centre of interest is biologically active substances with therapeutical possibilities. Marine natural products represent a vast potential source of new drugs with diverse and often unique structures, many associated with interesting biological properties. Among the properties which have been reported for different marine natural products are very diverse: toxicity, antiviral, antibacterial, antimalarial, antifungal activity, antitumor, anti-inflammatory, analgesic, hypcholesterolemic and hypolipidemic activity. Success in these areas is demonstrated by the agents now in pre- or clinical evaluation.

Biologically active natural products, or secondary metabolites, have become fine tools for pharmacologists and biochemists. Several marine natural products have entered pharmaceutical development, and others are making significant contributions to our understanding of cellular processes at the biochemical level [9,10]. As ligands for cellular receptors, they are used to explore fundamental processes that elicit behavioral responses in living systems, both in homeostasis and in disease states. Biological investigations of marine secondary metabolites have already yielded promising candidates for future drugs, e.g., didemnin B and bryostatin in the area of cancer chemotherapy [11], or have proven useful as biological probes in studying cellular events, e.g., saxitoxin as a sodium channel blocker [12].

The marine environment, comprising approximately half of the total global biodiversity, offers a rich diversity of species, which is in many ways
comparable to that of tropical rain forests. This environment also contains a great number of organisms for which there are no terrestrial counterparts, and which offer an enormous source of novel and biologically active compounds. Biological and chemical investigations of marine ecosystems have provided insights into a wonderful and complex underwater world. As a direct result of these investigations, the structural classes which can be obtained from certain taxonomic groups is to some extent becoming predictable, and some emphasis is now being placed on the biological properties of extracts, fractions and isolated pure metabolites. Ecological pressures on marine organisms, which include competition for space, maintenance of an unfouled surface, deterrence of predation, and the ability to reproduce successfully, may have led to the evolution of unique secondary metabolites, which are responsible for the chemical components of these actions and interactions.

The oceans support a stable and thriving community of sponges, corals, echinoderms and many other invertebrates, that have adapted to the freezing temperatures, low nutrient levels and periodic low light levels. It has been suggested that marine habitats lack sufficient predation pressure to drive sessile invertebrates to produce defensive metabolites. Sessile marine organisms possess various defense systems against predators, larvae of other sessile organisms and pathogenic microorganisms. Since marine invertebrates do not produce antibodies, their defense mechanisms are based primarily on phagocytosis by leukocytes, aided by producing and exuding secondary metabolites. The presence of endogenous secondary metabolites is believed to endow marine organisms with a chemical means of defense.

Both chemists and biologists have been intrigued for many years as to the role of secondary metabolites of terrestrial and marine origins. Dudley Williams (1989) [13] proposed that “secondary metabolites are a measure of the fitness of the organism to survive by repelling or entrapping other organisms”. The accumulation of biologically active substances in marine invertebrates has been observed as a general phenomenon which reflects the defensive strategy of these often sedentary filter-feeding organisms.

Although only initiated in the late 1970s, natural drug discovery from the world’s oceans has been accelerated by the chemical uniqueness of marine organisms, which have the highest probability of yielding natural products with unprecedented carbon skeletons and interesting biological activity. Marine organisms, specially sponges, have attracted the attention
of natural product chemists because of their versatility in using different metabolic pathways, that have no counterpart in the terrestrial world. Because life began in the ocean, it is hardly surprising that marine organisms have not only adapted to the high salt concentrations in the ocean, but have incorporated halogens into their chemical constitutions [14]. While ocean water is universally known for its chloride ion content, it is also an abundant source of bromide, and to a lesser extent iodide. An important consequence of halogen ion availability has been the extensive utilization of halogenation reactions by various marine organisms in their evolutionary biosyntheses of defensive and other necessary constituents.

Additionally, during the past decade interest in the secondary metabolites of marine microorganisms and fungi has been increasing at a slow but definitive pace. Marine microorganisms have become recognized as an important and untapped resource for novel bioactive compounds [15-17]. The vast area of research into marine microorganisms, comprising marine bacteria, ranging from archaebacteria to glinding bacteria, fungi, and a whole range of microalgae, e.g., dinoflagellates, diatoms and protozoa, is just emerging and already showing immense potential. Microbiological investigations of marine environments have yielded a number of new biologically active microorganisms [18-22].

Moreover, symbiotic marine organisms, e.g., sponges and algae and/or microorganisms are common in all marine environments, and are believed to be of great importance in the biosynthesis of biologically active natural products within these organisms. Cases where secondary metabolites have been intimated as products of the microorganisms-containing symbionts of a sponge or algae have attracted much attention. However, it is difficult to rigorously sort out which compounds are metabolites elaborated by the marine organisms or by the symbiont, and most of the suggestions on this point are based on circumstantial rather than hard experimental evidence [23].

In this review, data have been presented to illustrate the diversity of organisms living in the sea and the plethora of chemical compounds that have been discovered from them. Since the late 1970s, there has been a veritable explosion of activity and many new marine metabolites have been isolated and identified. Therefore, the present review will cover only recent development in this area. The information was obtained from a review of the scientific literature, and the sources are referenced at the end of the chapter. Bioactive compounds found in marine environments include
terpenoids and steroids, alkaloids, peptides and proteins, phospholipids, poliketides, carbohydrates, macrolides and toxins.

TERPENOIDS

The diverse, widespread and exceedingly numerous class of natural products that are derived from a common biosynthetic pathway based on mevalonate as parent, are synonymously named terpenoids, terpenes or isoprenoids, with the important subgroup of steroids, sometimes singled out as a class in its own right. Monoterpenes, sesquiterpenes, diterpenes and triterpenes are ubiquitous in terrestrial organisms and play an essential role in life, as we know it. Although the study of terrestrial terpenes dates back to the last century, marine terpenes were not discovered until 1955.

Sponges remain the primary target in the search for "drugs from the sea". It is known that sponges produce the greatest variety of secondary metabolites of any animal group. Diterpenes are one of the most abundant non-steroidal secondary metabolites isolated from marine sponges, with a wide range of biological properties. Structurally, the diterpenes from sponges possess polycarbocyclic skeletons, which are sometimes very degraded with loss of one or two carbon atoms to give nor- or bis-nor-derivatives.

Fig. (1). Structure of agelasimines
From the Thai marine sponge belonging to the genus *Mycale*, two labdanes diterpenes, mycaperoxides A and B were isolated. Both compounds exhibited antibacterial and antiviral activity, and showed significant cytotoxicity against tumor cell lines [24]. The marine orange sponge *Agelas mauritiana* and others of the same genus, yielded two novel derivatives of a bicyclic diterpene, agelasimine A and B, Fig. (1), both exhibiting a wide range of biological activity [25]. Cytotoxicity was reported from both compounds, and both caused relaxation in smooth muscle of rabbit gut and bovine coronary artery. Recently, these marine diterpenes have been reproduced by chemical synthesis using sigmatropic rearrangement and Ritter reaction [26]. Novel diterpenoids, including nakamurol A with unique thelepogane skeleton, were isolated from another *Agelas* sponge species, *Agelas nakamurai* [27]. A Philippine marine sponge of the genus *Strongylophora* yielded new meroditerpenoids with antimicrobial and antifungal activity [28], while sponges of the genus *Diacarnus* yielded epidioxy-substituted nor-diterpenes with antimalarial properties [29].

Additionally, marine organisms have provided a large number of compounds of mixed biogenesis, originating partly from mevalonate and partly from a benzenoid precursor. A number of linear or cyclic prenylhydroquinones have been described with a terpenoid portion from one to eight isoprene units. Many sponges belonging to the family Spongiidae are chemically characterized by a series of terpenoids containing 21 carbons and displaying two β-substituted furan moities at the end of the molecule [30,31]. These unusual compounds are probably biogenetically derived from higher terpenoids. These marine sponges are a well known source of novel furanoterpenes. *Hippospongia* sp., from the southern Australian sea, produced hippospongins A-F, with antibiotic activity [32]. The sponges *Spongia officinalis* and *Fasciospongia cavernosa* yielded furanoditerpenes, including the novel ambliofuran 2 [33]. *Ircinia* sp. yielded bioactive furanoterpene sulfates, which specifically inhibited the neuropeptide Y receptor in vitro, and also showed cytotoxicity against KB cells [34]. The furanoterpene ircinin has been shown to inhibit phospholipase A$_2$ (PLA$_2$) activity and to affect human neutrophil functions like superoxide generation and degranulation [35]. Recently, this marine natural product has been synthesized [36].

Sponges of the genus *Acanthella* have previously been shown to be rich sources of terpenes having various nitrogen-containing groupings, with
antimicrobial and antifungal activities [37,38]. From the Okinawan sponge *Acanthella cavernosa*, novel kalihinane diterpenoids, Fig. (2) were isolated, with potential antimalarial activity [39,40]. Recently, 15 diterpenes which contain isonitrile, isothiocyanate and isocyanate groupings were also reported from the tropical marine sponge *Cymbastela hooperi*. The majority of them demonstrate significant and selective *in vitro* antimalarial activity [41,42].

![Fig. (2). Structure of kalihinol A](image)

Besides sponges, other marine organisms such as corals and algae are beginning to receive attention from natural product chemists. Soft coral are symbiotic associations of coral animals with their algal partners. They are a rich source of terpenoids, notably cembranoid diterpenes with cytotoxic and antifungal activity [43,44]. Their abundant production and accumulation of diterpenoids is intriguing, as it seems unlikely that these compounds act solely as repellents against predators. Recently, new bioactive cembrane-type diterpenoids have been isolated from octocorallia [45,46].

![Fig. (3). Structure of pseudopterosin E](image)

Pseudopterosins are a series of tricyclic diterpene glycosides from the
Caribbean coral *Pseudopterogorgia elisabethae* discovered by Fenical *et al.* [47]. Pseudopterosin E, Fig. (3) is the one with the best pharmacological profile, combining low toxicity and potent anti-inflammatory activity. In human neutrophils, pseudopterosin E inhibits degranulation and formation of leukotrienes [2]. In 1991, phase I clinical trials were initiated with pseudopterosin E as a topical anti-inflammatory agent. Recently, novel anti-inflammatory natural products have been isolated from this Caribbean soft coral [48,49]. Japanese researchers reported the isolation of a group of compounds designated helioporins A-E, which are related to the pseudopterosins [1].

Several other soft corals have been investigated in recent years. *Eleutherobia aurea* yielded two novel diterpenoid glycosides, elenthosides A and B [50]. Seo *et al.* [51] isolated three pigments of the guaiazulene class from the gorgonian *Calicogorgia granulosa*. From the Japanese soft coral *Simulud nanolobata*, new amphilectane-type diterpenoids with cytotoxicity activity were isolated [52], while a sample species of *Simulud* genus from the Indian Ocean yielded aromadendrane diterpenoids with larvicidal activity [53]. Another *Simulud* species also showed interesting biological properties, such as antispasmodic activity from *Simulud flexibilis* [54]. New cytotoxic and antitumor diterpenes were isolated from the Caribbean gorgonian *Eunicea tournefortii* [55], the Formosan gorgonian coral *Briareum excavatum* [56,57], the Okinawan soft coral of the genus *Xenia* [58], and the European *Eunicella cavolinii* [59].

Brown algae of the family Dictyotaceae yielded diterpenes of the dolabellane, xenicane, crenulide as well as extended germacrane and hydroazulenoid types. Some of these compounds were identified as capable of demonstrating appreciable selectivity as antimalarial agents [60], and are being synthetised in the laboratory [61]. The brown alga *Dilophus ligulatus* yielded diterpenoids with cytotoxic activity [62]. The novel xenicane diterpenoid dilopholide, was also obtained in this study. New secospatane diterpenes were recently isolated from another *Dilophus* alga, *Dilophus okamurai* [63].

From the marine alga *Stypopodium flabelliforme*, several diterpenoids with interesting biological properties were isolated. The diterpenoid epitaondiol exhibited a potent anti-inflammatory activity related to inhibition of human PLA₂ activity and leukocyte accumulation [64]. Additionally, epitaondiol has been shown as a potent calcium antagonist in
a study of the cardiovascular system [65]. The diterpenoid 14-keto-stypodiol diacetate, isolated from this alga, inhibited the proliferation of human prostate cells [66]. This compound and several derivatives are being synthetised in the laboratory as the racemate in a stereoselective manner [67]. More recently, new terpenoid compounds have been reported from another Stypopodium species, Stypopodium zonale, as tyrosine kinase inhibitors [68]. From the blue-green alga Tolypothrix nodosa, an anti-inflammatory diterpenoid, tolypodiol, was isolated [69]. Tolypodiol showed strong anti-inflammatory activity in the mouse ear edema assay. Another anti-inflammatory diterpene, pheophytin, was isolated from the edible green-alga Enteromorpha prolifera [70]. Bioactive diterpenoids were also isolated from marine microorganisms, such as phomactin derivatives, Fig. (4) reported from the marine fungus Phoma sp. as platelet activating factor antagonists [71].

Marine organisms have also been intensively examined for their sesquiterpene content. Dysidea herbacea is a sponge species which has yielded new metabolites for more than 20 years, and no doubt further collections from different locations will continue to reveal new chemistry.
The terpenoid metabolites reported from *Dysidea* sp. are predominantly sesquiterpenes [72]. They possess a spiro moiety as in herbadysidolide, Fig. (5), herbasolide, Fig. (6) and spirodysin, Fig. (7), or they are furanosesquiterpenes such as furodysinin, Fig. (8). However, *Dysidea herbacea* from two collection sites on the Great Barrier Reef less than 120 km apart also yielded enantiomeric furanosesquiterpenes [73].

![Fig. (6). Structure of herbasolide](image)

These results suggest that samples of this sponge differ in their enzymatic capabilities concerning the cyclization of geranyl-geranyl-pyrophosphate.

![Fig. (7). Structure of spirodysin](image)

More recently, two new isonakafuran-type sesquiterpenes were isolated from this sponge species [74]. These types of compounds possess interesting antitumor and antifungal activity, and attempts to synthesize them are being conducted [75]. Other bioactive metabolites, such as antifouling sesquiterpenes, have also been recently isolated from *Dysidea herbacea* [76].
From another *Dysidea* sponge species, *Dysidea avara*, the sesquiterpenes avarol, Fig. (9) and avarone, Fig. (10), which show a wide variety of biological activities, were first isolated. Both compounds are potent antileukemic agents *in vitro* and *in vivo*. They were determined to be neither direct mutagens nor premutagens, and they displayed antimutagenic activity.

Both avarol and avarone inhibit replication of the etiological agent of acquired immuno-deficiency syndrome (AIDS) [77]. Additionally, avarol and avarone effectively control acute inflammation in experimental models after either oral or topical administration. Their anti-inflammatory activity may result from inhibition of eicosanoid release and depression of superoxide generation in leukocytes [78]. Several studies reviewed the structures and bioactivity of compounds related to avarone as an anti-human immuno-deficiency virus (HIV), antitopoisomerase II activity and as protein kinase C (PKC) inhibitors [3, 79].
From two sponge samples, *Luffariella* sp. and *Acanthella klethra*, and from red algae of the genus *Laurencia*, several sesquiterpenes with an isonitrile or isothiocyanate functionality were obtained [80]. Some of them exhibited detectable cytotoxic activity against cultured tumor cells [81,82], as well as antibacterial [83] and antimalarial activity [84]. More recently, these types of compounds with antimalarial and antifouling activities were also isolated from marine sponges of the genus *Axinyssa* [85,86].

Additionally, sesquiterpene-substituted quinones and related compounds constitute an important class of cytotoxic natural products of marine origin. Natural products of mixed sesquiterpene and quinol biosynthesis are common to marine algae and sponges. For example, several sesquiterpenoid/quinols have been isolated from a deep water collection of the marine sponge *Siphonodictyon coralliphagum* [87]; cyclorenierins from the sponge *Haliclona* sp. [88] and a Philippine sponge of the genus *Xestospongia* [89]; dactytronic acids from the sponge *Dactylospongia elegans* [90]; two sesquiterpene hydroquinones from *Polyfibrospongia australis* [91]; vinylfurans from *Euryspongia deliculata* [92]; and several sesquiterpene quinones and hydroquinones from *Thorecta choanoides*, a marine sponge from the southern Australian sea [93], and from *Perithalia caudata*, an Australian marine brown alga [94].

In many cases, these compounds showed other interesting biological properties, such as antibiotic [95], anti-inflammatory [96,97], antiviral activity, e.g., peyssonols A, Fig. (11) and B, two anti-HIV sesquiterpenes hydroquinones isolated from the Red Sea alga *Peyssonelia* sp. [98], and cardioactive properties, e.g., halenaquinol, recently isolated from the sponge *Petrosia seriata* [99].
From the marine sponge *Haliclona* sp. (also known as *Adocia* sp.), a family of hexaprenoid hydroquinones called adociasulfates, have been recently reported as inhibitors of kinesin motors [100,101]. These types of compounds were also found in several soft corals, such as *Lemnalia africana* [102], Okinawan soft coral of *Nepthsea* sp. [103], and the gorgonian *Alertogorgia* sp., which yielded the cytotoxic tricyclic sesquiterpene, suberosenone [104].

Marine organisms, specially sponges, have also provided a large number of biologically active sesterterpenoids. The sesterterpenes are the smallest class of terpenoid compounds and consist of alcohol, aldehyde and ketone derivatives of terpene hydrocarbons. The occurrence of sesterterpenes in nature is somewhat uncommon, but for the last two decades an increasing number of examples have been reported. Interestingly, many of the recent additions have been isolated from marine sponges of the order Dictyoceratida. These metabolites may be listed in two main groups: linear sesterterpene molecules terminated by a furan ring at one end and by a tetraonic acid or lactone ring at the other end, and tetra-or pentacyclic-sesterterpenes which are analogues of the scalarane skeleton.

The scalaradial group of marine metabolites exhibit potent biological activity, mainly anti-inflammatory properties [2,105]. Scalaradial, Fig. (12) and other scalaranes were found to completely inactivate the enzyme PLA$_2$ from bee venom directly and irreversibly. Marine sponges are a well-known source of bioactive scalaradial sesterterpenes. *Phyllospongia* sp., collected in the South China Sea, yielded two new scalarane-type sesterterpenes, phyllactone H and I [106]. Scalarolide and scalarin were reported from *Cacospongia* and *Ircinia* sponges, besides other scalarane...
sesterterpenes [107,108].

These types of compounds also showed other interesting properties, such as antineoplastic and cytotoxic activity reported from scalarane-type sesterterpenes of the Indian Ocean sponge *Hyrtios erecta* [109,110], and antituberculosis properties [111].

In recent years, many marine sesterterpenes which are promising candidates for new drugs have been discovered. The sesterterpenoid manoalide, Fig. (13), obtained from the sponge *Luffariella variabilis*, was detected in a program searching for new anti-inflammatory compounds. Manoalide proved to be a potent inhibitor of PLA₂ and has become a useful biochemical tool. Inhibition of phospholipase C and the ability of manoalide to function as a calcium channel blocking agent allows this compound to be used in the study of the role of calcium mobilisation in inflammatory processes, and in a more general sense, in signal transduction pathways [105,112]. A significant number of manoalide derivatives has been isolated and evaluated for their biological activity [113,114]. A total synthesis of manoalide employs an organometallic coupling strategy [115]. Clinical trials are currently underway with some of these and synthesised derivatives, and it is probable that a manoalide-inspired derivative will reach the market [116].
New and interesting anti-inflammatory sesterterpenes have been reported in recent years from marine sponges. Petrosaspongiolides, Fig. (14), isolated from the Caledonian marine sponge *Petrosaspongia nigra* [117], were found to potently inhibit PLA$_2$ on acute and chronic inflammation [118]. In a similar manner, cacospongiolide B, Fig. (15), a sesterterpene isolated from *Fasciospongia cavernosa* [119,120], was shown to be a potent inhibitor of human synovial PLA$_2$ [121].

Fig. (14). Structure of petrosaspongiolides

Fig.(15). Structure of cacospongiolide B

Several other marine sponges have been investigated in the last decade, in the search for novel bioactive sesterterpene molecules. A sample of the sponge *Dysidea herbacea* from the Red Sea is unique in that it contains cytotoxic sesterterpenes with a scalarin skeleton, e.g., scalardysin, Fig. (16) and the C$_{21}$-furanoterpene furospongolide, Fig. (17).
This compound also showed antispasmodic activity [122]. A variety of cytotoxic compounds was isolated, including a bishomosesterterpene and dysidiolide from another *Dysidea* sp., a sulfated sesterterpene hydroquinone from a *Hippospongia* sp., and two new sesterterpenes, lntenolides F and G from the Caribbean sponge *Cacospongia linteiformis* [123,124].

From the Maldives’ Black marine sponge *Hyrtios erecta*, several cytotoxic sesterterpenes were isolated, such as the pentacyclic sesterterpenes designated sesterstatins [125-127] and ppupehenone, Fig. (18) with a quinone-methide system [128]. Three novel cytotoxic nor-esterterpenes, rhopaloic acid A, Fig. (19), B and C, were recently isolated from the sponge *Rhopaloeides* sp.

These compounds also inhibited the gastrulation of the starfish (*Asterina pectinifera*) embryo [129,130]. Both racemic and enantiomeric forms of rhopaloic acid A have been synthesised by very different strategies [131,132].
Additionally, during the search for biologically active sponge metabolites belonging to the sesterterpenoid class, a sulfated sesterterpene hydroquinone, halisulphate, Fig. (20), was isolated from the dark brown sponge *Halichondriidae* sp.

It demonstrated *in vitro* antimicrobial, antifungal and anti-HIV activities [24,133]. Recently, a halisulphate derivative with antithrombin and antitrypsin activity was isolated from the marine sponge *Coscinoderma matthewsi* [134]. The absolute configuration of halisulphate has been determined by application of the chiral amide method coupled with chemical degradation procedures [135].
Triterpenoids are a minor group of sponge metabolites; of these malabaricane/isomalabaricane triterpenoids, Fig. (21) are known from *Jaspis* and *Stelletta* sponge species. All of this group shows cytotoxicity and anti-HIV activity [136-139].

Other cytotoxic triterpenes, designated as sodwanones A-M, Fig. (22), were recently reported in *Axinella weltneri*, a marine sponge from the Indian Ocean [140]. The investigations of small samples of the Mediterranean sponge *Raspaciona aculeata* revealed the presence of raspacionins, Fig. (23), triterpenoids containing two perhydrobenzoxepine systems [141]. Besides sponges, other marine organisms have been reported to produce bioactive triterpenes, including algae from *Laurencia* genus [142], and the holothurian *Psolus fabricii* [143].
As we indicated in the introduction section, halometabolites frequently occur in marine organisms and are known to have basic functions related to the survival of the living creatures producing them. Bromine is by far the halogen most frequently found in these metabolites. Halogenated marine terpenes were first isolated only in 1963. Since most marine organisms have been found to contain halogenated compounds, there are certainly thousands of different, new organohalogenated terpenes in marine organisms awaiting discovery. Among marine organisms, red algae, particularly species of *Laurencia* and *Plocamium*, have provided a rich and diverse collection of halogenated terpenes over the past 25 years [14].

Red algae of the genus *Plocamium* have been shown to be a rich source of acyclic and cyclic halogenated monoterpenes that vary for a given species depending on collection location and season. These algae can be found in many locations ranging from Antarctica to tropical waters. Numerous chemical studies of these species show the presence of bioactive halogenated monoterpenes, whose structure and yield vary greatly [4]. Although the red algae *Plocamium* have been investigated for its chemical content for many years, several new bioactive compounds have been identified recently from these species. For example, plocamadiene A is a polyhalogenated monoterpenes which causes histamine release from mast cells of the guinea-pig and rat *in vitro* [144]. The species *Plocamium cartilagineum* found on the Portuguese coast, produced acyclic polyhalogenated monoterpenes [145]. More recently, new halogenated monoterpenes were isolated from *Plocamium costatum*. These compounds have been shown to deter settlement of barnacle larvae, suggesting a potential ecological role [146].

An array of new and unusual halogenated terpenes have been isolated and characterized from *Laurencia* red algae [14]. This genus is well known as a source of halogenated sesquiterpenes. New chamigrane-type derivatives were isolated from *Laurencia* species, some from *Laurencia*
and some from *Laurencia nipponica* growing in Japan [148]. Examples of iodinated terpenes, which are quite rare and interesting, were found in *Laurencia majuscula* collected in the South China Sea [149]. More recently, Rovirosa *et al.* [150], reported the isolation of new halogenated sesquiterpenes from *Laurencia claviformis*, a species endemic to Easter Island.

Several other red algae have been investigated in recent years. Among them, it is interesting to point out the halogenated monoterpene halomon, Fig. (24) and related compounds, isolated from the red alga *Portieria hornemannii*, which exerts potent antitumor activity *in vitro* and *in vivo* [151-153]. Species of sea hares, a marine mollusk, have also provided a rich source of halogenated terpenes [14]. In many cases, these compounds are derived from the sea hare’s algal diet. New chamigrene-type halogenated sesquiterpenes were isolated from *Aplysia dactylomela* [154], and from the Spanish sea hare *Aplysia punctata*, which exert potent cytotoxic activity [155].

STEROIDS

Since the start of the twentieth century, steroids have continued to be the focus of the research activities of natural product chemists, synthetic chemists, biochemists and clinicians. The reasons are several-fold and related to the fascination of the chemical complexity of sterols and their biochemical functions in living organisms. Sterols and steroids are excellent compounds for the organic chemists to practise their skills upon in the development of new reactions and synthetic procedures. The biological functions of sterols, for example as an essential constituent of membranes, have proved thought-provoking to lipid biochemists.

Marine organisms have been found to be storehouses of sterols,
particularly in terms of unique side-chain structures and unusual functionalization. For example, marine sponges are a rich source of steroids with highly functionalized nuclei and modified side chains. Numerous new sterols have been isolated from marine sponges. Although most have side chains that are polyoxygenated or alkylated, other occurring sterols are known to contain a methylether.

Extensive studies on sterols from marine sponges during the past decades have resulted in the identification of a plethora of unusual forms with interesting biological activities [156]. For example, topsentinols A-J, Fig. (25), new sterols with unusual polyalkylated side chains, were isolated from the Okinawan marine sponge Topsentia sp. [157], while sponges of the genus Ircinia yielded new epoxy sterols [158]. Sterol composition has also been reported from the sponge Faciospongia cavernosa growing in the Adriatic, Aregean and Tyrrhenian Seas [159]. Recently, a new sterol containing an unprecedented seven-membered cyclic enol-ether has been isolated from the Australian Euryspongia arenaria [160]. Most of these compounds showed interesting biological properties, such as antiplasmodial and cytotoxic activity of the steroids from Agelas oroides, a Maltese marine sponge [161]; novel cytotoxic steroids from sponges of the genus Xestospongia sp. [162,163], Biemna sp. [164] and Scleritoderma sp. [165]; an antifouling epidioxy sterol from Lendenfeldia chondrodes, a Palauan marine sponge [166], and antiviral sterols from the marine sponge Petrosia weinbergi [167]. It is interesting to point out the cytotoxic activity of camptothecin and related compounds, with which clinical developments have recently been initiated [168-170].

Additionally, sulfated sterols have been described from a wide variety of marine organisms, particularly sponges and echinoderms, and several of these steroidal sulfates have exhibited a broad range of activities. Halistanol sulfates are a group of sulfated polyhydroxysteroids from
sponges, which are very attractive because of their biological activity. Halistanol disulfate B, isolated from the marine sponge *Pachastrella* sp., was shown as a potent inhibitor of endothelium converting enzyme [171], while halistanol trisulfate, a sulfated steroid derivative isolated from the marine sponges of the genus *Topsentia*, inhibits protein tyrosine kinase activity [172,173]. New trisulfated trihydroxysteroids were also isolated from two different collections of the sponges *Trachyopsis halichondroides* and *Cymbastela coralliophila* [174], while tamosterone sulfates, new polyhydroxylated steroid sulfates, have been reported from a new oceanapiid sponge genus [175]. Acanthosterol sulfates A-J, isolated from the Western Japan sponge *Acanthodendrilla* sp., exhibited antifungal activity [176]. More recently, some cytotoxic bis-steroid sulfates called crellastatin, were isolated from the Vanatua marine sponge *Crella* sp. [177,178]. From the marine sponge *Jaspis* sp., several steroidal sulfates have been reported as inducers of larval metamorphosis and inhibitors hatching enzyme activity in the ascidian *Halocynthia roretzi* [179].

Additional unusual steroid derivatives have been isolated from marine sponges, e.g., polymastiamides, steroid/aminoacid conjugates isolated from *Polymastia boletiformis*, a Norwegian marine sponge [180], and an aminoimidazolium salt of steroid trisulfate from *Topsentia* sp. [181]. Some of these marine sterols are being reproduced by chemical synthesis in the laboratory [182].

Besides sponges, other marine organisms have been investigated in recent years for their steroid content, such as octocorallia [183]. Sarcoaldesters A and B, two new polyhydroxylated sterols together with novel epoxy steroids were isolated from the soft coral *Sarcophytum* sp. [184,185]. Gorgonian of the genus *Muricella* sp. from Jaejn Island of Korea, yielded calicoferols, Fig. (26), new secosteroids with significant cytotoxicity and inhibitory activity against PLA₂ [186,187]. Recently, chemical examination of the soft corals *Clavularia viridis*, *Nephthea chabroli* and *Simularia dissea* yielded novel polyhydroxy steroids [188-190]. Red algae are known to be important sources of cholesterol and desmosterol in the marine environment. Some of these compounds, e.g., oxygenated desmosterols and clerosterols isolated from the red algae *Codium arabicum* and *Galaxaura marginata*, showed interesting cytotoxic properties [191,192]. More recently, a new sterol amide with antimicrobial activity, boophilnine, was isolated from the cattle tick *Boophilus microplus* [193].
ALKALOIDS AND RELATED COMPOUNDS

Alkaloids are extremely difficult to define because they do not represent a homogeneous group of compounds from either the chemical, biochemical or physiological viewpoint. All do occur in plants, but some are found in animals, and practically all have been reproduced in the laboratory by chemical synthesis. Most possess basic properties due to the presence of an amino nitrogen, and many, specially thoses pertinent to pharmacy and medicine, possess marked physiological activity.

Marine organisms are known to be a rich source of alkaloids with unique chemical features and pronounced chemical activities, which suggest potential value as lead structures for the development of new pharmaceuticals [194]. Extensive studies on alkaloids from marine organisms during the past decades have resulted in the identification of a plethora of compounds, sometimes with interesting biological activities. For example, indole alkaloids isolated from marine sponges such as Raphisia pallida [195], Ircinia sp., a Okinawan marine sponge [196], and Hamacantha sp., with antifungal activity [197]. Imidazole alkaloids such as leucettamine A and related compounds isolated from the marine sponge Leucetta micororaphis, have been shown as potent antagonists of leukotriene B4 receptor [198], and antitumor agents [199]. Recently, new imidazole alkaloids were reported from an Australian marine sponge Axinella sp., with interesting bactericidal activity [200,201].

Stellettamide A and B, Fig. (27), indolizidine alkaloids isolated from sponges of the genus Stelletta have been reported as inhibitors of
The absolute configuration of these molecules have been established by synthesis of their enantiomers [204]. From this sponge species, new alkaloids named stellettazoles B and C, which exhibit antibacterial activity have recently been reported [205]. From the Caribbean marine sponge *Agelas dispar*, novel betaines alkaloids which also exert antibacterial activity have recently been isolated [206]. Cytotoxic guanidine-alkaloids have been reported from different samples of marine sponges [207-210].

![Fig. (27). Structure of stellettamides](image)

![Fig. (28). Structure of lamellarins](image)

Another cytotoxic compound, a sulfur-containing alkaloid, was isolated from the ascidian *Polycarpa aurata* [211]. Recently, another series of ascidian alkaloids, the lamellarins, Fig. (28), have been shown as selective inhibitors of HIV virus replication in cell culture [212], together with new indolocarbazole and ergoline alkaloids isolated from the ascidians *Eudistoma toealensis* and *Botryllus leachi*, which showed moderate cytotoxic activity [213-215]. From a Philippine marine sponge, *Oceanapia* sp., an unusual sesquiterpene alkaloid, oceapanamine, was isolated [216], while marine sponges of the genus *Corticium* sp. yielded unusual steroidal
alkaloids [217]. Additional cytotoxic alkaloids were reported from other marine sponges, e.g., peptide alkaloids from _Lissoclinum_ sp. [218], and tryptophan-derived alkaloids from the Okinawan _Aplysina_ sp. [219]. From the Southern Australian sponge _Spongisorites_ sp., a new class of marine alkaloids, dragmacidins, have been reported as potent inhibitors of protein phosphatases [220].

Some of these cytotoxic marine alkaloids are promising candidates for new drugs. For example, ecteinascidins, Fig. (29) are a family of tetrahydroisoquinolone alkaloids isolated from the Caribbean tunicate _Ecteinascidia turbinata_, which have been selected for clinical development. These compounds are presently in pre-clinical and clinical trials for human cancers [221-225]. A series of totally synthetic molecules that are structurally related to the ecteinascidins is currently being prepared and evaluated as antitumor agents [226].

![Figure 29](image-url) Structure of ecteinascidins

However, pyrroloquinolines and pyridoacridines are the alkaloids of major interest as metabolites in sponges and ascidians [227]. Many of these compounds have generated interest both as challenging problems for structure elucidation and synthesis as well as for their cytotoxicities [228-230].

A family of alkaloids characterized by a pyrroloquinone skeleton has been isolated in recent years from several sponges. Included in this family are the batzellines, isobatzellines, damirrones, makaluvamines, discorhabdins, prianosins and wayakin. These alkaloids have shown a
variety of biological activities including cytotoxicity against human tumor cell lines, \textit{in vivo} tumor inhibition and inhibition of topoisomerase I and II. Among these, the makaluvamines, Fig. (30) are the most potent inhibitors of topoisomerase II, suggesting their efficacy as anticancer agents. The principal structural feature of these alkaloids is the core of a planar iminoquinone moiety which can intercalate into DNA and cleave the DNA double helix, or inhibit the action of topoisomerase II [231]. This family of makaluvamines alkaloids was mainly isolated from the Philippine marine sponge \textit{Zyzzya fuliginosa} [232-234]. Recently, these alkaloids are being reproduced in the laboratory by chemical synthesis employing a strategy based upon intramolecular nucleophilic substitution reactions [235-237].

![Fig. (30). Structure of makaluvamines](image)

Discorhabdin alkaloids, Fig. (31), in contrast, are of high cytotoxicity, but they exhibit no inhibition of topoisomerase II. They were isolated from the Anthartic sponge \textit{Latrunculia apicalis} [238], and more recently from a deep-water marine sponge of the genus \textit{Batzella} sp. [239]. The new discorhabdin derivative isolated from this sponge showed \textit{in vitro} cytotoxicity against tumor cell lines.

![Fig. (31). Structure of discorhabdin](image)

Marine sponges of these genus \textit{Batzella} sp. also yielded novel
pyrroloquinolines alkaloids, batzelladines, Fig. (32), with interesting biological properties [240,241]. Many of these types of alkaloids were also isolated from other marine sponges, e.g., Agelas sp. [242], and tsitsikammanine A and B reported from a South African marine sponge, which exhibited antimicrobial activity [243].

As representative of the derivatives of pyridoacridine, eilatin, a marine alkaloid inhibits in vitro cell proliferation in chronic myeloid leukemia patients [244]. Other members of the pyridoacridines, such as alkaloids isolated from a Cystodytes sp. ascidian, inhibit topoisomerase II [245]. Additionally, analogues derivatives of these type of alkaloids showed interesting anti-HIV activity [246].

![Structure of batzelladine A](image)

Fig. (32). Structure of batzelladine A

Fused tetracyclic and pentacyclic alkaloids constitute a relatively new class of natural products isolated mostly from ascidians and sponges. Cytotoxic, antimicrobial and antiviral activities have been reported for many of these compounds. The manzamine alkaloids, Fig. (33) are characterized by a complex pentacyclic diamine linked to C-1 of β-carboline moiety. Manzamine have been isolated mainly from six different genera of marine sponges: Haliclona, Pellina, Xestospongia, Ircinia, Pachypellin and Amphimedon.
Halicionacyclamines, manzamine alkaloids with pronounced cytotoxic activity, were isolated from *Haliclona* sp., a tropical marine sponge [247-249]. Other manzamine-type alkaloids with cytotoxic and antibacterial activity were isolated from the Philippine marine sponge *Xestospongia ashmorica* [250]. Some of these compounds are very attractive because of their biological activity, e.g., manzamine alkaloids isolated from another *Xestospongia* sp., also reported in the marine sponge *Agelas novaecaledoniae*, which are potent somatostatin and vasoactive intestinal peptide inhibitors [251]. These compounds could be promising agents in the research on compounds for therapeutical interventions in cystic fibrosis, Alzheimer’s disease and some tumors.

Manzamine-type alkaloids were also reported in another samples of marine sponges, e.g., the Okinawan *Amphimedon* sp. [252-254], *Pachypellina* sp. [255], and a novel alkaloid called hyrtiomanzamine from *Hyrtios erecta*, with interesting immunosuppressive activity [256]. From
the colonial zoanthid *Zoanthus* sp., a zoanthamine-type alkaloid, Fig. (34) has been reported as a good candidate for an osteoporotic drug [257], while the marine bacteria *Agrobacterium* sp. yielded agrochelin, a new cytotoxic thiazole alkaloid [258]. Recently, investigations have been conducted to reproduce these type of compounds by chemical synthesis in the laboratory [259-261].

![Structure of tauroacidins](image1)

**Fig. (35). Structure of tauroacidins**

Although very few terrestrial plant alkaloids contain halogen, brominated alkaloids have been reported from the marine environment. From the Okinawan marine sponge *Hymeniacidon* sp., several bromopyrrole alkaloids have been described, e.g., tauroacidins A and B, Fig. (35) [262], konbuacidin A, Fig. (36) [263] and spongicidins A-D [264]. Several species of sponges contain hymenialdisine, Fig. (37), which has been shown as a potent inhibitor of nuclear factor kappa B and interleukin-8 production *in vitro* [265,266].

![Structure of konbuacidin A](image2)

**Fig. (36). Structure of konbuacidin A**
New bromopyrrole alkaloids were also isolated from different species of Agelas sp., such as the Caribbean Agelas dispar [267], Agelas nakamura, a Papua New Guinean marine sponge [268,269] and Agelas wiedenmayeri [270]. Two samples of the marine sponge Stylissa carteri collected in Indonesia, yielded two new bromopyrrole alkaloids [271]. Brominated indole alkaloids have been reported from the Caledonian marine sponge Orina sp. [272], while bromotyrosine alkaloids with cytotoxic and antitumor activity have been isolated from several marine sponges, such as Aplysina aerophoba [273], the Okinawan Psammaplysilla purea [274] and Pseudoceratina verrucosa [275].

![Fig. (37). Structure of hymenialdisine](image)

Additionally, marine organisms have proven to be a rich source for a wide variety of modified nucleosides considered worthy for clinical application. For example, arabinoside-nucleosides, constituents of the Caribbean sponge Cryptotethya crypta, have led researchers to synthesise analogues with improved antiviral and anticancer activity [4].

![Fig. (38). Structure of tubercidin](image)

Other bioactive nucleosides have been reported from the marine
environment, e.g., pyrrolo-pyrimidine nucleosides such as tubercidin, Fig. (38) and analogues derivatives from the ascidian *Didemnum voeltzkowi* [276]; caissarone, Fig. (39), a sea anemone iminopurine with adenosine receptor antagonist activity [277]; phenethylguanidine analogues from *Petrosia contignata*, a Indo-Pacific marine sponge [278]; and bioactive bisguanidines from *Stylotella aurantium*, with potent cytotoxic, antibiotic and immunosuppressive activity [279]. More recently, nucleosides have also been reported in the Australian marine sponge *Carteriospongia* sp. [280].

![Fig. (39). Structure of caissarone](image)

**PEPTIDES AND PROTEINS**

The "term" peptide includes a wide range of compounds varying from low to very high molecular weights, and showing marked differences in physical, chemical and pharmacological properties. The lowest members are derived from only two molecules of aminoacids, but higher members have many aminoacid units and form either peptides, simple proteins or more complex proteins, conjugated proteins, for example, lipoproteins in which proteins are combined with lipids.

Marine organisms are a well-established source of unique and biologically active peptides. Complex cyclic peptides and depsipeptides have emerged as an important new class of metabolites present in extracts of marine organisms. Many of these peptides have been found to be extremely potent cytotoxic and/or enzyme inhibitors.
Didemmins are cytotoxic agents belonging to a depsipeptide family isolated from marine tunicates. Didemnin B, Fig. (40), one member of this family obtained from the tunicate Trididemnum solidum, has antiviral, immunosuppressive and potent cytotoxic properties [281-283]. The compound is too toxic to be useful as an antiviral or immunosuppressive agent, but has been in phase I clinical trials as an anticancer agent, and phase II clinical trials are currently underway [284]. Arenastatin A, Fig. (41) is another potent cytotoxic depsipeptide isolated from the marine sponge Dysidea arenaria, which shows selective toxicity against tumor cells [285]. This compound have been reproduced by chemical synthesis in the laboratory [286]. In a similar manner, hapalosin and aplidine, marine cyclic depsipeptides with inhibitory activity against human tumor cell lines, have been obtained by chemical synthesis by a route involving a macrolactamization as an important ring-forming step [287-289].

Other cytotoxic and antiproliferative depsipeptides were recently isolated from the Vanatua marine sponges Axinella carteri [290], Jaspis splendans [291], Geodia sp. [292], and the Papua New Guinea sponge Cymbastela sp. [293]. Marine depsipeptides also showed other interesting biological properties, such as antiviral [294], antifungal [295,296] and hemolytic activity [297]. It is interesting to point out the biological activity of papuamides A-D, new cyclic depsipeptides isolated from the Papua New Guinea sponges Theonella sp., which showed interesting anti-HIV and cytotoxic activity [298].
Marine organisms, specially sponges, have provided a large number of other biologically active peptides in the last decades. Cyclotheonamides, Fig. (42), a family of cyclic pentapeptides isolated from marine sponges of the above mentioned genus *Theonella* sp., have been shown as potent thrombin, trypsin and other serine proteases inhibitors [299-302]. From the marine sponge *Theonella swinhoei* a highly cytotoxic peptide, polytheonamide B, was recently isolated [303].

Phakellistatin, Fig. (43) is a series of cyclic hepta and octopeptides isolated from the Indian Ocean marine sponge *Plakellia* sp., with interesting antineoplastic activity [304,305]. These compounds have recently been reproduced in the laboratory by chemical synthesis using a combination of stepwise coupling and segment condensation [306]. New cytostatic heptapeptides, isolated from marine sponges, were also chemically synthetized using a new synthetic method to elaborate peptide bond [307-309]. Additional biologically active peptides of marine sponge origin include dipuupehedione, a cytotoxic compound from the New Caledonian
Hyrtios sp. [310,311], and the antifungal peptides halicylindramides from Halicondria sp. [312].

Besides sponges, other marine organisms have been reported to produce bioactive peptides, which are promising candidates for new drugs. For dolastatins, e.g. dolastatin 10, Fig. (44), potent antineoplastic peptides isolated from the Indian Ocean molusk Dolabella auricularia, clinical trials are pending [313]. Recently, a structural derivative of dolastatin called auristatin, has been evaluated in human tumor cell lines and has undergone clinical trials [314].

Microcolins, Fig. (45) are lipopeptides isolated from a strain of the blue-green alga Lyngbya majuscula, which revealed interesting cytotoxic and immunosuppressive activity [315]. Several synthetic derivatives are also being evaluated [316]. These compounds resemble majusculamides, which were isolated from another chemovariant of the same species and from marine sponges [317]. Dendroamides, new cyclic hexapeptides, were isolated from another blue-green alga [318].
Marine microorganisms have also been reported to produce bioactive peptides, such as marinostatin from the marine bacterium Alteromonas sp. [319], pentapeptides from the cyanobacterium Anabaena cylindrica [320], and new anti-inflammatory cyclic peptides from the marine Streptomyces sp. [321]. From the marine fungus Hypoxylon oceanicum, several lipodepsipeptides with antifungal activity have recently been reported [322,323].

Besides peptides, marine organisms have been reported to produce biologically active proteins, which are probably involved in the protection of organisms against physiological and stress conditions. Recently, these molecules have been cloned from sponges [324] and marine microorganisms [325].

The marine protein variabilin, Fig. (46) has been shown as a potent dual inhibitor of human secretory and cytosolic PLA2 with anti-inflammatory activity [326]. An interleukin-6 cytokine family antagonist protein was reported from the marine sponge Callyspongia sp. [327]. From the marine sponge Pachymatysma johnstonii, a cytotoxic glycoprotein, pachymatismin was isolated [328,329]. Another active glycoprotein, niphatevirin, isolated from the marine sponge Niphates erecta was reported as an HIV-inhibitory agent [330], together with cambrescidins, proteins isolated from marine invertebrates which also exert antiviral activity [331].

The activities of the purple fluid of the sea hare Aplysia dactylomela, such as toxic, antimicrobial and hemagglutinating properties, have been attributed to a substance of protein nature [332]. Proteoglycans and adhesive glycoproteins present in the extracellular matrix of vertebrates, have also been reported in sponges. These molecules are probably involved in the cell adhesion systems of sponges [333]. Recently, novel marine proteins have been reported, such as silicatein from sponge biosilica [334], and a metallothionein protein from the marine alga Fucus vesiculosus [335]. Metallothioneins have also been isolated from Arctic
bivalves as possible indicators of the availability of trace metals in the
Arctic [336]. Additionally, proteins were also isolated from other species
in the marine environment, e.g., from the Kuruma prawn Penaeus
japonicus [337,338], and the shore crab Carcinus maenas [339].

![Structure of variabilin](image)

Fig. (46). Structure of variabilin

Enzymes are also colloidal in nature and consist of protein or contain
proteins as an essential part. Several enzymes have been reported from
marine organisms, specially sponges and algae, e.g., exopolyphosphatases
from the marine sponge Tethya lyncurium [340], tauropine
dehydrogenases from the Demosponge Halichondria japonica [341], and
more recently phenylalanine hydrolases from the sponge Geodia cydonium
[342]. From this marine sponge Geodia cydonium, oligoadenylate
synthetases were also isolated which may be useful as biomarkers for
environmental monitoring [343,344]. Additionally, these sponge species
contain high levels of telomerase activity, suggesting that they possess a
high proliferation capacity [345].

A protease hydrolyzing casein with proteolytic activity has been
reported from the Papua New Guinea sponge Callyspongia schulzi [346],
while the glutathione-S-transferase activity of the sponge Suberites
domuncula has been used as marker of thermal stress [347]. This enzyme
was also reported in a marine fish, Pleuronectes platessa [348].
Additionally, isomerases were reported from the marine alga Ptilota
filicina [349], while the cyanobacterium Synechocystis sp. yielded β-
carotene hydroxylases [350]. Cyanobacteria also yielded oligomeric forms
of dehydrogenases [351].

Besides sponges and algae, enzymes were also isolated from marine
organisms and microorganisms. For example, polymerases and proteases
from marine Vibrio sp. [352], marine bacterium such as Alcaligenes
faecalis [353], and from archaeaons, such as the psychrophilic
Cenarchaeum symbiosum [354], and the hyperthermophile archaeaons
Pyrococcus furiosus [355], Sulfolobus solfataricus [356], and Aeropyrum
pernix [357]; transferases from marine bacterium such as Vibrio vulnificus
[358], and Photobacterium damsela [359,360]; dehydrogenases from different strains of Nocardiooides sp. [361]; novel alginate lyases from marine bacterium Alteromonas sp. [362], and phenoloxidases from the colonial ascidian Botryllus schlosseri [363], and the marine bacterium Marinomonas mediterranea [364]. More recently, enzymes of the lysozyme family were purified from marine bivalves and conchs [365,366].

Additionally, the marine sponge Spirastrella sp., in symbiotic associations with marine fungi and bacteria, produces enzymatic activities, e.g., serine-type acetylcholinesterase with the marine bacterium Arthrobacter ilicis [367]; urethanase activity with Micrococcus species [368]; and asparaginase and amylase activity produced by the fungus Mucor sp. associated with this sponge [369,370].

Aminoacids occur in plants and animals, both in the free state and as the basic units of proteins and other metabolites. Aminoacid derivatives have been reported in marine environment, such as from marine sponges of the genus Jaspis sp. [371,372], from Suberea creba, a Coral Sea marine sponge [373], and the marine ascidian Leptoclinides dubius [374]. Some of these compounds have been shown to possess interesting biological properties, e.g., cytostatic activity exhibited by axinastatin-4, an aminoacid derivative isolated from a marine sponge [375].

However, tyrosine-derived halometabolites frequently occur in marine organisms. Marine sponges of the order Verongida are of much current biological and chemical interest. An unusual secondary metabolites containing up to four bromotyrosine residues has been isolated from sponges belonging to this order which includes, among others, the genera Aplysinia, Ianthella, Psammaplysilla, Pseudoceratina and Verongula.

Bastadins, Fig. (47) are a family of bromotyrosine-derived metabolites isolated from different samples of the marine sponge Ianthella basta, which exhibit a wide range of biological activity, such as antineoplastic [376], antimicrobial [377], and inhibitory activity of the endothelin A receptor [378]. These types of compounds have recently been reported from another marine sponge, Psammaplysilla purpurea, together with two new dibromotyrosine-derived metabolites [379]. This sponge also afforded brominated benzenoacetonitriles, unusual dibromo-tyrosine derivatives [380]. From the marine sponge Verongula gigantea, a bromotyrosine-derived metabolite, verongamine, has been reported as a potent histamine receptor antagonist. This compound and new acetylenic derivatives are being developed by chemical synthesis. [381].
Other biologically active bromotyrosine-derived metabolites of marine origin include aeroplysinin, Fig. (48) as cytotoxic and tyrosine kinase inhibitor [382,383], fistularin isolated from Aplysina archeri which exhibited antiviral activity [384], and ceratinamides A and B, antifouling metabolites from Pseudoceratina purpurea [385].

**PHOSPHOLIPIDS**

Lipids are esters of long-chain fatty acids and alcohols or of closely related derivatives. The chief difference between these substances is the type of alcohol; in fixed oils and fats, glycerol combines with the fatty acids; in waxes, the alcohol has a higher molecular weight, e.g., cetyl alcohols.

Several monounsaturated phospholipid fatty acids exist in nature, but few cases are known of very long-chain monounsaturated acids longer than 22 carbons. However, marine sponges are unusual in that they have very long-chain fatty acids in their phospholipids. Sponges have provided the most interesting examples of long-chain phospholipid fatty acids since
acids with chain-lengths between 24 and 30 carbons have been reported. This unusual ability of these marine invertebrates to biosynthesize very long-chain fatty acids has been responsible for the many interesting structures which have been reported without counterpart in the terrestrial world. Sponges have long been recognized as a rich source of structurally novel lipids including unique fatty acids, phospholipids and triglycerides. The fatty acids of sponges have attracted considerable interest because of their unique characteristics, such as increased chain length, branching and unusual unsaturation patterns, and because of the implications the structural variations may have, when present in phospholipids, for membrane function.

Common phospholipid fatty acids from marine sponges include 5,9 hexacosadienoic, which occurs in most known sponges, 5,9 heptacosadienoic and 5,9 octacosadienoic. For example, marine sponges of the class Demospongiae contain high levels of characteristic C₂₄-C₃₀ fatty acids and are unique in that they seem to be able to biosynthesize these compounds with amazing ease. Studies have shown that many of these "demospongic acids" possess unusual unsaturation and/or methyl branching not found in the fatty acids of other more common organisms. Many of these compounds have been shown to possess interesting biological properties, e.g., antifungal [386], amidolytic [387], inhibitory activity of PKC and anti-inflammatory activity [388,389] and topoisomerase I [390], and inhibitory activity of HIV reverse transcriptase reported from taurospongin A, Fig. (49), a fatty acid derivative isolated from the Okinawan marine sponge *Hippospongia* sp. [77,391].

![Structure of taurospongin A](image)

Fig. (49). Structure of taurospongin A

Branched fatty acids of longer than unusual chain-length have also recently countered in several other sponges. Sphingosine derivatives, such as plakoside A and B, Fig. (50), two unique prenylated glycosphingolipids isolated from *Plakortis simplex*, have been reported as potent immunosuppressive agents [392]. The Okinawan marine sponge *Agelas*
*mauritianus* yielded a family of new glycosphingolipids named agelasphins, which have been shown as strong antitumor compounds [393]. These lipids have been recently reproduced by chemical synthesis in an efficient manner [394]. Glycosyl ceramides are a family of agelasphin derivatives also with antitumor and immunomodulating activity, which have been reported from different *Agelas* sp. such as the above *Agelas mauritianus* [395] and *Agelas dispar* [396]. These compounds were also isolated from other marine sponges, e.g., *Haliclona koremella*, as an antifouling substance against macroalgae [397], and *Spirastrella abata* as inhibitors of cholesterol biosynthesis [398]. Recently, these compounds have also been reproduced by chemical synthesis [399]. From marine sponges of the genus *Petrosia* sp., several glicerol derivatives have recently been isolated, showing interesting biological properties, such as cytotoxicity against human tumor cell lines [400], inhibitory DNA replication [401], and inhibitory activity of HIV reverse transcriptase [77]. New glicerol derivatives were also isolated from the sponge-associated bacterium *Micrococcus luteus* [402].

![Structure of plakosides](image-url)

*Fig. (50). Structure of plakosides*

Besides sponges, other marine organisms and microorganisms have been reported to produce bioactive fatty acids and phospholipids [403]. These compounds, *Fig. (51)* have been isolated from green algae, such as the Southern Australian *Dictyosphaeria sericea* [404], red algae such as *Pachymeniopsis lanceolata* [405], the cyanobacterium *Lynghya majuscula* [406], and more recently from the brown algae *Laminaria* sp. [407,408], and *Dictyota ciliolata*, which yielded a sulfonoglycolipid with nitric oxide synthetase activity [409]. From a symbiotic marine alga, *Symbiodinum* sp., a long-chain product, zooanthellatoxin B, was also isolated and caused
rabbit platelet aggregation [410] and possess potent vasoconstrictory activity [411]. Exophilin A, a new antibiotic compound, was isolated from the marine microorganisms *Exophiala pisciphila* [412]. Unusual fatty acids were also reported from the Atlantic salmon, *Salmo salar* [413], and more recently from the turbot, *Scophthalmus maximus* [414], the Antartic lamellibranch *Laternula elliptica* [415], and marine bivalves [416].

![Fig. (51).](image)

In the marine environment, several polyacetylenic compounds biogenetically related with the fatty acids have been reported in the last decade. These types of molecules, with interesting cytotoxic properties, have been isolated mainly from marine sponges of the genus *Petrosia* sp. [417-419]. However, other samples of marine sponges also yielded bioactive acetylenic compounds, such as the Okinawan *Adocia* sp. [420,421], and the marine sponges *Pellina* sp. [422], *Reinera* sp. [423] and *Callyspongia truncata*, which yielded polyacetylene derivatives that inhibit fertilization of starfish gametes and showed potent antifouling activity against larvae [424,425]. From the marine sponge *Siliquariaspongia japonica*, new polyacetylenic derivatives with antitumor and antifungal activity have been recently isolated [426,427]. These types of compounds have also been reported in corals [428], and more recently in marine ascidians, such as the Australian *Syngonium prunum* [429].

**POLYKETIDES**

Many of the unusual compounds that indicate the exciting chemistry to be discovered in marine natural products are polyketides. Polyketides are a family of structurally complex natural products that include a number of important pharmaceuticals. They are produced primarily by microorganisms through a specialized metabolism that is a variation of fatty acid biosynthesis [430]. Polyketides fall into two structural classes: aromatic and complex. Polyketides are formed by enzyme complexes
consisting of 4 to 7 monofunctional proteins in which the \(\beta\)-carbonyl groups of the intermediates resulting from the condensation of acetate residues are largely not reduced, and cyclization of the intermediates typically produces aromatic compounds. Complex polyketides are composed of acetates, propionates or butyrates, and the extent of \(\beta\)-carbonyl reduction varies from one cycle to the next. However, a strong sequence and mechanistic similarity among many of the fatty acid and polyketide synthase enzymes has led to paradigms for explaining polyketide biochemistry.

In the marine environment, several polyketides with important medicinal value have been reported in recent years. They have been isolated mainly from marine sponges of the genus *Callyspongia* and *Plakortis*, which yielded potent cytotoxic polyketides, e.g., callystatin A, Fig. (52) isolated from *Callyspongia truncata* [285,431], and several cyclic polyketides named plakortides, Fig. (53), isolated from *Plakortis lita* [432] and *Plakortis simplex* [433].

These polyketides, plakortides, have also been shown as potent activators of cardiac calcium-pumping ATPase [434]. New cyclic polyketides were recently isolated from the Red Sea marine sponge *Acarnus bergquistae* [435], while cytotoxic polyketides have also been reported from sea hares of the genus *Aplysia* and *Dolabella* [436], and the marine sponge *Theonella swinhei* [437].

Marine microorganisms also produced a variety of polyketides wich
sometimes incorporate aminoacids, such as marine myxobacteria and cyanobacteria [438-440]. The saltwater culture of marine fungi isolated from sponges yielded new polyketides, e.g., the cultured fungus *Aspergillus* sp. and *Paecilomyces* sp. separated from the Indo-Pacific sponge *Jaspis coriacea* [441,442], and *Trichoderma longibrachiatum* from *Haliclona* sp. [443]. Some of these marine fungi producing bioactive polyketides were also isolated from marine tunicates, such as the fungus *Phitomyces* sp. separated from the Indo-Pacific tunicate *Oxycorynia fascicularis* [444].

Motivated by the value of these natural products, there has been much research focused on developing guidelines for engineering polyketide synthases to generate natural and novel polyketides [445,446]. Additionally, manipulation of the biosynthetic pathways of microbial polyketides through engineering permits the biosynthesis of bioactive polyketides not generated naturally [447,448].

**CARBOHYDRATES**

Carbohydrates are aldehyde or ketone alcohols containing carbon, hydrogen and oxygen in which the hydrogen and oxygen are generally in the same ratio as in water. In the marine environment, it is known that algae produce the greatest variety of carbohydrates, specially polysaccharides, which exhibit a wide range of biological activity. For example, polysaccharides have been reported in the Clorophyta *Ulva* sp. [449], in the marine alga *Fucus vesiculosus*, which yielded anti-HIV polysaccharides [450], and in the green marine alga *Codium dwarkense* with anticoagulant activity [451].

However, it is interesting to point out the biological activity of calcium spirulan, a sulfated polysaccharide isolated from the blue-green alga *Spirulina platensis* [452]. Extensive studies on calcium spirulan during the past decade have resulted in the identification of a wide variety of biological activity, suggesting it as a useful candidate for a new drug. Calcium spirulan exhibit anti-herpes and anti-HIV activities, related to the inhibition of enveloped virus replication [453]. Additionally, this compound has been shown as a potent antithrombin agent, and more recently as an inducer of plasminogen activator in fibroblast and as inhibitor of metastasis and tumor invasion [454,455].

Recently, polysaccharides have also been reported from marine
prokaryotes, both bacteria and archaea, which offer a number of novel material properties and commercial opportunities, ranging from emulsifiers to adhesives [456].

MACROLIDES

Macrolides are a group of compounds containing a macrocyclic lactone ring and up to 9 conjugated trans double bands. In recent years, new macrolides have been isolated from marine organisms, some of them reported as promising candidates for future drugs.

![Fig. (54). Structure of bryostatin 1](image)

Bryostatins are a unique family of emerging cancer chemotherapeutic candidates isolated from marine bryozoan [457]. They were first discovered in the bryozoan *Bugula neritina*, but problems with supply of sufficient quantities of this natural product hampered the study of this interesting group of marine metabolites for many years. Although the biochemical basis for their therapeutic activity is not known, these macrolactones exhibit high affinities for PKC isoenzymes, compete for the phorbol ester binding site on PKC and stimulate kinase activity *in vivo* and *in vitro*. Bryostatin 1, Fig. (54), one member of this family, is a PKC modulator in a variety of tumor systems [458,459]. Bryostatin 1 is currently in phase II
clinical trial in Europe and USA sponsored by the National Cancer Institute as an anticancer chemotherapeutic agent [460]. A significant number of bryostatin derivatives have been reproduced by chemical synthesis in a computer model, and it is probable that a bryostatin-inspired derivative will eventually reach clinical phase trials [461-463].

Additionally, other macrolides constitute an important class of cytotoxic natural products of marine origin, specially from marine sponges. For example, mycalolides, a family of cytotoxic trisoxazole-containing macrolides isolated from marine sponges of the genus *Mycale* sp. [464,465]; superstolide B isolated from the New Caledonian sponge *Neosiphonia superstes* [466]; jaspisamides, Fig. (55) from the Okinawan marine sponge *Jaspis* sp. [467]; and altohyrtin A, a macrolide isolated from *Hyrtios altum* [285].

![Fig. (55). Structure of jaspisamide A](image)

![Fig. (56). Structure of halichondrin B](image)
Marine sponges of the genus *Haliclona* contain a diverse array of active secondary metabolites, including highly potent cytotoxic macrolides, e.g., halichondrin and related compounds, Fig. (56) [468], and salicylihalamides A and B, Fig. (57) [469]. New macrolides chemically related to salicylihalamides, apicularens A and B, were recently isolated from the myxobacteria *Chondromyces* sp. [470]. From marine bacteria, other cytotoxic macrolides have been isolated, such as octalactin A, Fig. (58) and B, which have been shown as a cell cycle-specific anticancer drug [471], and swinholide, Fig. (59), isolated from symbiotic cyanobacteria with the marine sponge *Theonella swinhoei* [472].

Many macrolides of marine origin have been reported to show other interesting biological properties, such as immunosuppressive [473,474], antifungal [323,475,476], anti-actin [477], and anti-inflammatory activity...
reported from lobophorins A and B, which are two new macrolides recently obtained from a marine bacterium isolated from the Caribbean brown alga Lobophora variegata [478]. Recently, efforts have been conducted in order to design the chemical synthesis of these marine natural products based on a macrolactamisation strategy [479-484].

![Structure of swinholide](image)

**Fig. (59). Structure of swinholide**

**TOXINS**

Toxins are bacterial waste products which are considered poisonous for the animal body. These compounds are released by marine organisms in both fresh-water and marine environments and, when ingested by man and other animals, can cause detrimental or even lethal effects [485,486]. Recent reports have shown that marine toxins are the causative agents of seafood poisoning [487], e.g., pectenotoxin 2 isolated from the European alga Dinophysis fortii [488], manauelides, Fig. (60) from the red alga Gracilaria coronopifolia in Hawai [489], and domoic acid, neurotoxin isolated from the diatom Pseudo-nitzschia multiseries [490,491]. Several chromatographic and fluorimetric methods are being developed for the detection of these paralytic poisoning toxins [492-495].
Biological investigation of marine toxins, which has had ramifications in many areas of biomedical sciences, has reported a wide range of pharmacological properties, and other research has yielded useful candidates as biological probes in studying cellular events [12]. For example, saxitoxins, brevetoxins and more recently, ciguatoxins and maitotoxins involved with ciguatera poisoning, which have been employed in the study of sodium channel action. Saxitoxins are produced by various dinoflagellates and are linked with paralytic shellfish poisonings [496]. This toxin blocks neuronal transmission by binding to the voltage-gated sodium channel [497]. The brevetoxins are "red-tide" toxins of the temperate zones that also cause so-called shellfish poisoning [498,499]. In contrast to these well-understood poisoning, ciguatoxins, Fig. (61) was a problem that was apparently associated with tropical coral reefs [500]. These compounds, originally produced by dinoflagellates, are being sequestered by herbivorous fish and reach high concentrations in carnivorous, predatory species. The related neurotoxin maitotoxin has been shown to induce necrotic cell death in cerebrocortical and breast cancer cell cultures, and calcium-dependent excitatory effects on excitatory membranes such as skeletal, smooth or cardiac muscle [501,502]. This toxin, obtained from the dinoflagellate Gambierdiscus toxicus as a putative calcium-channel activator, has been recently reported as a potent hemolytic and ichthyotoxic agent [503], and to cause shape change followed by aggregation in platelets [504].
Additionally, a number of marine toxins with medical and toxicological importance have been isolated from marine flora and fauna. Okadaic acid, Fig. (62) is the main toxin produced by dinoflagellates, which can accumulate in the hepatopancreas of mussels and caused diarrhetic shellfish poisoning in consumers [505,506]. However, this toxin is also a tumor promoter and a specific potent inhibitor of protein phosphatases which may provokes mitotic arrest and apoptosis of leukemia cells [507-509]. These types of compounds have been reported in shellfish and phytoplankton, and more recently, in Spanish mussels [510], Portuguese bivalves [511], and the diatom *Thalassiosira weissflogii* [512].

Following the first report of tumor promotion by okadaic acid, additional tumor promoters of the okadaic acid activity class have been identified, e.g., microcystin [513,514], and calyculin derivatives, Fig. (63) reported in marine sponges such as *Discodermia calyx* [515] and *Theonella swinhoei* [516] as potent inhibitors of tumor cell proliferation. A two-sponge association, *Poecillastra* sp. and *Jaspis* sp., yielded cytotoxic toxins which exhibited selective activity against several tumoral cell lines [517].

![Fig. (61). Structure of ciguatoxin](image1)

![Fig. (62). Structure of okadaic acid](image2)
Other biological marine toxins have been recently isolated from sea anemones [518,519], the venomous gastropod Conus [520], and marine sponges, e.g., Haliclona exigua and Niphates sp. which yielded xestospongins, neurotoxins that produce depolarizing effects in nerve fibers and inhibit nitric oxide synthase activity [521,522], and a Red Sea sponge which affords latrunculin A, Fig. (64), a potent inhibitor of immunological phagocytosis by macrophages [523]. A related compound, latrunculin B, was recently isolated from the East African nudibranch Chromodoris hamiltoni [524]. Paralytic shellfish toxins were also isolated from blue-green algae, such as Cylindrospermopsis raciborskii from Brazil [525], and the tropical cyanobacterium Lyngbya majuscula [526]. From this alga, curacin A, Fig. (65), was isolated a potent brine shrimp toxin, which has shown promise as an antiproliferative agent due to its inhibition of tubulin polymerization, a mechanisms of proven value in the treatment of neoplastic disorders.
Additional biological properties have been reported from toxins of marine origin, such as actin-inhibitory activity \[527,528\], and inactivation of a serotonin-gated ion channel \[529\]. More recently, marine toxins have also been identified from sea cucumbers \[530\], and coral reef animals \[531\]. These toxins, which have been detected in zoanthid species of the genus \textit{Palythoa}, also occur in various marine organisms living in close association with zoanthid colonies, e.g., sponges, soft corals, mussels and crustaceans.

Some of these marine toxins have been shown as promising candidates for new drugs. For example, mycalamides, potent antitumor and antiviral compounds isolated from a New Zealand marine sponge, which are undergoing to preclinical evaluation. A significant number of mycalamide derivatives have been synthetised and evaluated for their biological activity \[532,533\]. Efforts are currently being conducted to design the chemical synthesis of this group of marine natural products \[534-536\].

To finalize this review, we have to consider that the accumulation of toxic and persistent substances in the marine environment continuously
increases owing to antropogenic activities [537-539]. Particular attention is being paid to the presence of heavy metals, because of their irreversible effects on man. In fact such elements tend to concentrate in all marine environment matrices, and for this reason they are present in the aquatic food chain, becoming dangerous for humans too, as a consequence of the consumption of marine products [540-542]. In recent years, a number of reports have suggested analytical procedures for the determination of heavy metals in marine organisms, showing that a correct purging procedure considerably reduces the metal content in these matrices [543-545].

**ABBREVIATIONS**

PLA$_2$ = Phospholipase A$_2$
AIDS = Acquired immuno-deficiency syndrome
HIV = Immuno-deficiency virus
PKC = Proteinkinase C

**ACKNOWLEDGEMENTS**

The technical assistance of Ms. Brooke-Turner is gratefully acknowledged.

**REFERENCES**

[41] Wright, A.D.; König, G.M.; Angerhofer, C.K.; Greenidge, P.; Linden, A;


1997, 60, 431-438.


[229] Lindsay, B.S.; Christiansen, H.C.; Copp, B.R.; *Tetrahedron,* 2000, 56, 497-505.


Chem, 1994, 37, 3181-3186.


753

Prod. 1994, 57, 1595-1597.