
Taxonomy and Marine Natural Products Research

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Abstract

A taxonomic overview of the marine environment is presented along with specifics on the key phyla. This is accompanied by a brief history of marine natural products research, the aspirations in this field of research, and an analysis, on a taxonomic basis, of the results of the past 60 years of marine natural products research. In the final sections of the chapter, conclusions are drawn from the work as to the phyla that might be of interest for future research.

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1.1 Introduction

“Is it from pride, humour or irony that man has given the name “earth” to his own planet?” [1]

It was undoubtedly pride, not humor or irony, as Earth is the only planet of the Solar System not named from Greek or Roman mythology. Earth probably originates from the Middle English word, *erthe*, which can be traced back through Old English, Old High German, Greek to the Hebrew word *erez*, meaning ground [2]. It is however ironic and indeed humorous when Earth is viewed from space. The land masses our forbears took pride in are simply islands floating in enormous oceans. The 71% of the Earth’s surface that is the oceans is strikingly illustrated by the “Blue Marble” photo of earth taken from Apollo 17 [3] (Fig. 1.1). The oceans are a critical component of the Earth’s biosphere, unique in the Solar System and most probably among the several hundred other planetary systems throughout the Universe that have been identified in recent years.

The essential nonliving components of the biosphere are the atmosphere with its oxygen richness, the hydrosphere, predominantly saline, to an average depth of 3,800 m, and the land mass with its average height of 840 m [4]. In combination, these features support an amazing diversity of life forms.

1.2 How Many Species on Earth?

Systematics attempts to categorize this biological diversity on Earth and sort the various life forms into congruent groups, but there is no one single definition of what constitutes a species. Systematics has been influenced by the influx of new data and new methodology, particularly at the molecular level. As a consequence, taxonomy and recognition of groupings at the species level are constantly evolving. Taxonomists have attempted to impose a classification scheme on the whole of creation during an era of rapidly evolving knowledge. In the opinion of some, genomics will soon be capable of imposing an informatically robust relationship tree on all organisms. Most taxonomists beg to differ, claiming that changes and similarities are expressed through the phenotype not the genotype, and the genomic view disregards the possibility of convergent evolutionary pathways. What is true, however, is that in recent years there have been some major realignments of taxonomic groups based on genomic studies, even in such intensively studied and well-known groups as the higher plants.

The nineteenth-century high-level classification of organisms into plant and animal kingdoms has been abandoned since about the 1960s with the development of cladistic analysis. Since then it has become increasingly clear that certain groups of organisms, some of them previously little studied, such as the cyanobacteria or cyanophytes (so-called blue-green algae; more closely related to bacteria), the chromista (including the brown algae), and the archaeobacteria or archaea, show greater differences in both fundamental biochemistry and genetics from each other and from the so-called higher organisms than higher plants and animals show from each other. It was Whittaker



Fig. 1.1 A view of the earth taken from Apollo 17, December 7, 1972

in 1959 who proposed that the most fundamental division should be between the prokaryotes and the eukaryotes [5], a classification that has now been generally accepted but subsequently modified to include the discovery of the archaea in the 1970s [6].

Major projects are in place worldwide to collate information from available datasets and document the Earth's biodiversity. These include the *Global Biodiversity Information Facility* [7] and the *Species 2000/Integrated Taxonomic Information System* [8] responsible for the *Catalogue of Life*. In the 9th edition (2009), their Annual Checklist, with 1,160,711 distinct species being listed, was estimated to contain just more than one half of the known species [9]. The goal ultimately is to list every distinct species in each group of organisms. At present, some groups are globally complete, some are nearing completion, and others are partial only. At the top level of the taxonomic hierarchy *The Catalogue of Life* lists organisms, based on contemporary perspectives, as the prokaryotic Archaea and Bacteria and the eukaryotes organized as Animalia, Chromista, Fungi, Plantae, and Protozoa. This whole topic has been thoughtfully discussed by Gordon [10] as a management hierarchy or classification system for the *Catalogue of Life*. The partially rooted relationships between these groupings can be depicted diagrammatically in a phylogenetic tree of life [11] (Fig. 1.2).

Based on the known organisms there appears to be consensus [12–15] that the number of known species on Earth is 1.75–1.9 million. These numbers have modest certainty when dealing with the larger organisms or distinctive taxonomic groups, but there is considerable uncertainty when dealing with groups that are harder to classify,

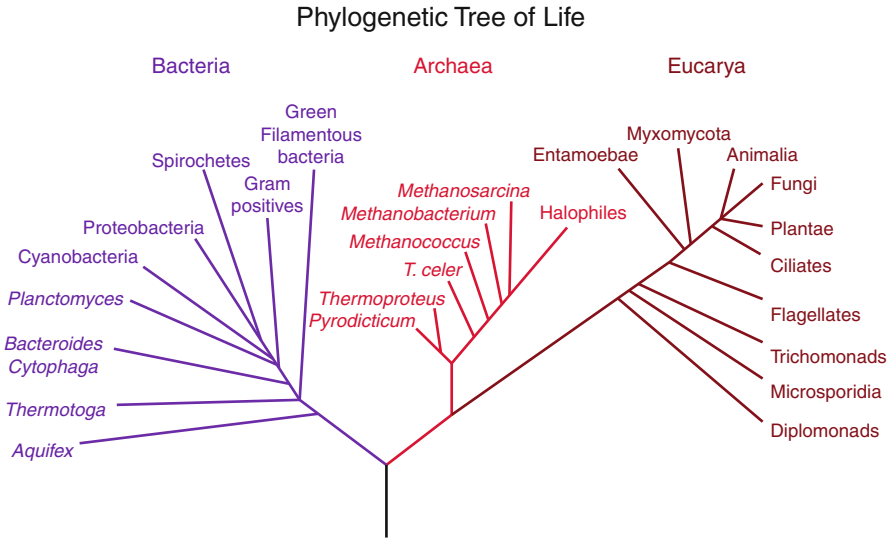


Fig. 1.2 A partially rooted phylogenetic tree of life

Table 1.1 Compilation of species numbers based on higher-order taxonomic groupings

	Total species			Marine species		
	Catalogue of Life [9]	Chapman [13] (<i>World accepted</i>)	Chapman [13] (<i>World estimate</i>)	WoRMS (2009) [16]	Bouchet (2006) [17]	Groombridge and Jenkins (2002) [4]
Archaea	281	–	–	0	0	?
Bacteria	9,773	10,307	~1,000,000	629	5,800	?
Fungi	44,669	98,998	1,500,000	1054	500	500
Protozoa ^a	6,866	28,871	>1,000,000	6,852	>14,800	12,500
Chromista ^a	7,901	~25,044	~200,500	4,098	7,600	1,500
Plantae	211,249	~310,129	~390,800	8,329	8,750	11,000
Animalia	953,661	1,424,153	~6,836,000	139,359	192,667	~205,000
	1,234,500	1,897,502	>10,900,000	160,319	>230,117	~250,000

^aFor comparative purposes the unicellular eukaryotic species have been partitioned between Chromista and Protozoa based on the systematics used by the *Catalogue of Life*. This should be considered speculative as not all databases or authors use these groupings, preferring instead Protocista. The *Catalogue of Life* places the Bacillariophyceae at a phylum level in the Plantae, but acknowledges this as a problem [10]

are microscopic, or are not economically important. It is likely to be many years before definitive answers emerge, but the total number of species will increase. Again estimates vary from 3–5 million [12] to 10–14 million [13, 15], or to 50 [14] to perhaps as many as 100 million species [15]. The breakdown of defined species in the *Catalogue of Life*, as of November 2009, is shown in Table 1.1. These numbers can

be contrasted with the 2009 compilation of world species by Chapman [13] who drew on information from systematists, the taxonomic literature, and previous compilations to arrive at an accepted number of species (~1.9 million) as well as providing an estimate of the total number of species on Earth (~11 million).

The contribution from the marine environment to the total species on Earth is relatively modest. At present the *World Register of Marine Species* (WoRMS) [16] lists 160,829 valid species (Table 1.1), 243,356 species names including synonyms, and 302,955 species to phyla. This data bank aims to capture all of the estimated 230,000 marine species by 2010. Two other sources [4, 17] have data on the estimated number of marine species (Table 1.1). Of the two sources, the data from Bouchet [17], who lists some 230,000 species, is the more definitive and thoughtful. Just as Chapman [13] extrapolated from the accepted number of world species to estimated world species and concluded that there were millions of Arthropod, Bacteria, Chromista, and Protozoa species yet to be characterized, Bouchet carried out a similar exercise for the marine world and speculated on the potential for greater numbers among the microbes and symbionts and the potential for the deep sea to produce a wide array of new species. There is clear evidence based on close field observations and culture-independent molecular techniques that the true number of marine species is considerably greater than that listed in Table 1.1.

The apparent lower species diversity between the terrestrial and hydrospheres (1.67 vs. 0.23 million) is a consequence of the overwhelming number of terrestrial arthropods (~1 million) for which there is no marine equivalent. In the marine world, species from Animalia are totally dominant accounting for 84% of the defined species (see Table 1.2 and Fig. 1.3).

All but one of the Animalia phyla have marine representation; the phylum Onychophora is the sole exception. The other notable feature is that the bulk of the Animalia phyla are exclusively, or nearly exclusively, marine, for example, Porifera, Echinodermata, and Bryozoa. Using data derived by May [14] on the relative numbers and distribution of the Animalia phyla this marine preference can be effectively depicted (Fig. 1.4). The aquatic and marine habitats are provided in the chart.

1.3 Marine Natural Products Chemistry

1.3.1 Historical

Rather surprisingly the natural products chemistry associated with marine species – marine natural products (MNP) – has only emerged over the past 75 years. This is in distinct contrast to the history of terrestrial natural products chemistry, which can readily be traced back to the earliest exponents of natural products chemistry at the start of the nineteenth century. For example, morphine was extracted and subsequently purified from the opium poppy plant about 1804 in Germany [18, 19]. However, the use of extracts from both terrestrial and marine sources as medicines, narcotics, or poisons stretches back to the earliest civilizations and is well documented in der Marderosian's 1969 review on marine pharmaceuticals [20].

Table 1.2 Suggested numbers for the marine phyla

Distribution of the marine phyla (Bouchet [17])			
Archaea	>0	Arthropoda (Chelicerata)	2,267
Archaea	–	Arthropoda (Crustacea)	44,950
		Brachiopoda	550
Bacteria	5,800	Cephalorhyncha (Priapulida; Loricifera; Kinorhyncha)	156
Cyanophyta	1,000	Chaetognatha	121
Bacteria	4,800	Chordata (Ascidiaceae)	4,900
		Chordata (Cephalochordata)	32
Fungi	500	Chordata (Mammalia)	110
Fungi	500	Chordata (Actinopterygi)	16,475
		Cnidaria	9,795
Protozoa	>14,800	Ctenophora	166
Ciliophora	?	Cycliophora	1
Sporozoa	?	Echinodermata	7,000
Euglenophyta	250	Echiura	176
Dinophyta (Dinomastigota)	4,000	Ectoprocta/Bryozoa	5,700
Radiolaria	550	Entoprocta/Kamptozoa	170
Foraminifera	10,000	Gastrotricha	395
		Gnathostomulida	97
Chromista	7,600	Hemichordata	106
Ochrophyta (Phaeophyceae)	1,600	Mesozoa (Rhombozoa; Orthonectida)	106
Ochrophyta (Chrysophyceae)	500	Mollusca	52,525
Haptophyta ^a	500	Nematoda	12,000
“Bacillariophyta”	5,000	Nematomorpha	5
		Nemertea	1200
Plantae	8,750	Onychophora	0
Chlorophyta	2,500	Phoronida	10
Magnoliophyta ^b	50	Placozoa	–
Rhodophyta	6,200	Platyhelminthes	15,000
		Porifera	5,500
Animalia	192,667	Rotifera	50
Acanthocephala	600	Sipuncula	144
Annelida	12,000	Tardigrada	212
Annelida (Pogonophora)	148		

^aFigure extracted from the *Catalogue of Life* [9]

^bBouchet had not included any members of the Magnoliophyta (mangroves, saltmarsh plants) in his estimates. The estimated figure for the Magnoliophyta from Groombridge and Jenkins [4] has been included

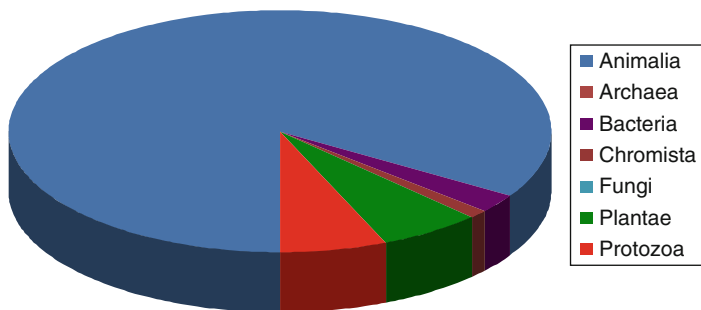


Fig. 1.3 Distribution of marine species by higher-order taxonomic grouping

This review is interesting as it surveyed this new and developing field from a taxonomic perspective. Of course at that stage relatively few compounds had been characterized and the emphasis was very much on toxins, pharmacology, and the biomedical potential for marine natural products. This emphasis was natural following on from Bergman's 1950s discovery of the non-ribose nucleosides spongthymidine and spongouridine from the West Indian sponge *Cryptotethia crypta* [21–25]. The subsequent evaluation of the biomedical potential of these arabinose-based nucleosides undoubtedly stimulated tremendous interest in the potential for pharmaceutical discoveries from the oceans. Bergman's first publication on marine organisms was in 1933 [26] while his 50th and last marine paper *Marine Products L, Phospholipides of Sponges* was in 1961 [27]. Over that 28-year period, Bergman studied sponges, sea anemones (cnidarians), and algae with a structural, not a biomedical, focus. In the 1950s, Nigrelli, Burkholder, and Ciereszko worked and commented specifically on biomedical aspects of marine natural products, for example, the neurotoxic effects of saponins from sea cucumbers, the antibiotic properties of sponge constituents and gorgonians, and antitumor activities associated with molluscs, echinoderms, and annelids [28–33]. In the meantime, work on toxins from marine vertebrates and invertebrates was initiated by Hirata, Scheuer, and Moore [34–38]. The first systematic study of a range of marine animals, both invertebrates as well as vertebrates, was reported in 1970 by Pettit who had collected from broad geographical areas [39]. This early systematic study was expanded by Rinehart with his bioassay-directed collections and onboard screening in the areas of Baja California (1974) and the Caribbean (1978) [40]. By the 1970s, species from across the marine phyla had been collected and were being studied. It was Faulkner, another early leader in the field, who commented that by 1975 there were already three parallel fields of study in marine natural products: marine toxins, MNP chemistry, and marine chemical ecology [41]. The distinct tracks, recognized over 30 years ago, continue today with systematic studies directed toward a particular phylum, toward a particular class of compounds, or following a particular bioactivity in the field of natural products chemistry. The study of marine toxins is still a dynamic aspect of the field and the role of MNPs in ecological studies has flourished. The major development of the past 20 years has

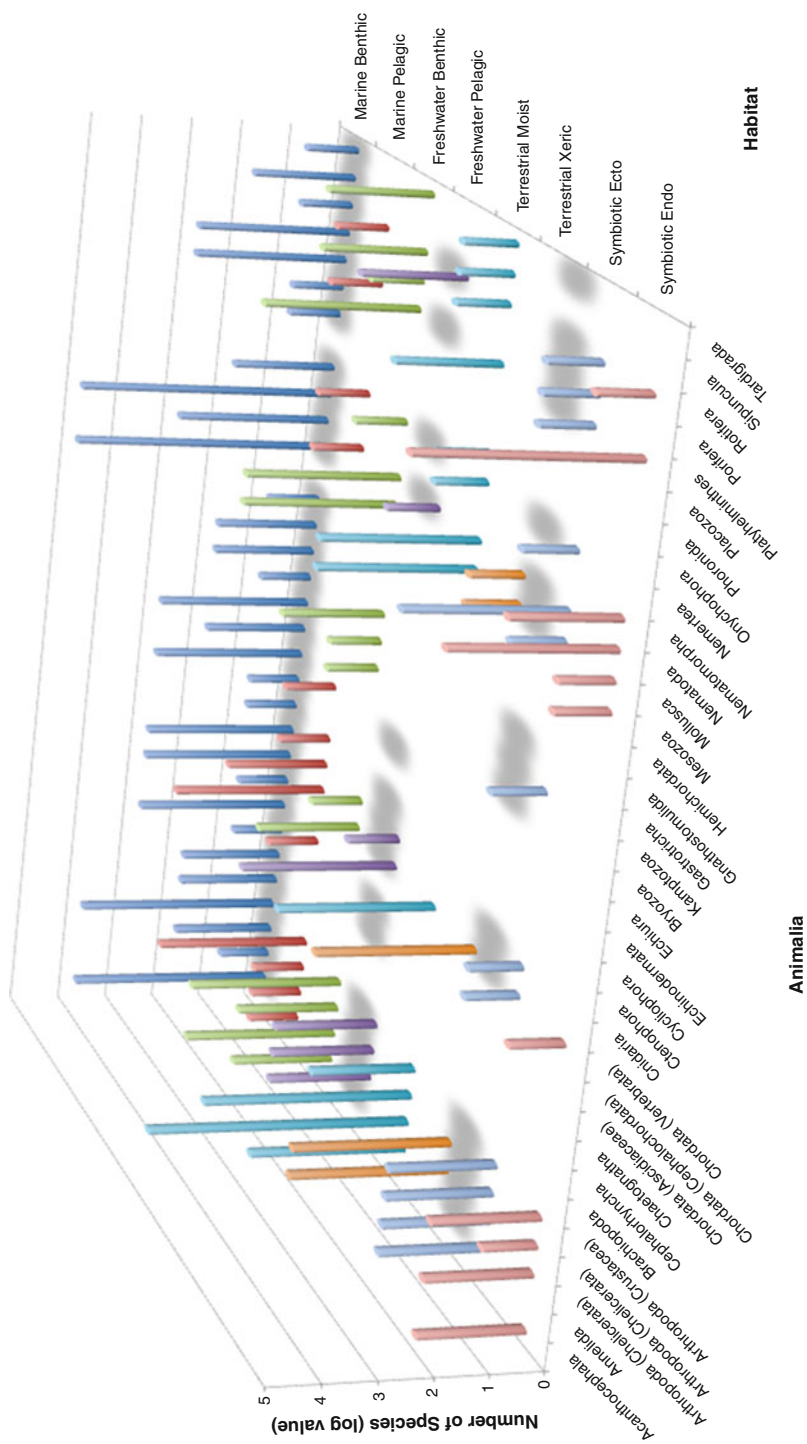


Fig. 1.4 Distribution (log scale) of Animalia species by phyla and habitat

been the discovery and realization of the importance of marine-associated and marine-derived microorganisms. Led by Fenical, the study of marine microorganisms has rapidly become a major focus for a rapidly rising number of researchers [42]. Any survey would also have to note the rapid rise, since 2000, of studies associated with mangroves, whose habitats are right at the marine–terrestrial interface [43].

That study of just a few phyla by Bergman in the 1930s and 1940s was followed by a systematic exploration of the marine environment that has established the marine environment as a rich source of novel, bioactive compounds.

1.3.2 The Marine Resource

The marine environment is an extremely complex one, showing immense biodiversity. It is now clear that, while many marine natural products are located in the tissues of the larger marine animals and plants, and are thus genuine natural products of those organisms, many others are produced by associated endo- or epibiotic microorganisms. Most marine microorganisms have not yet been successfully cultured, and definitive proof of origin is in most cases currently lacking making it extremely difficult to distinguish between commensalism, saprophytism, symbiosis, or parasitism. These products produced by symbiotic, commensal, or epiphytic microorganisms appear in many cases to play a role in chemical defense mechanisms.

To better understand the depth of the marine resource, each of the major divisions/phyla is briefly described. The following descriptions of the characteristic features of each marine phylum have been condensed from the very full descriptions contained in the *Dictionary of Marine Natural Products* (DMNP) [44] which should be referred to for more detail. The DMNP descriptions also include more numerous examples of representative natural products from each phylum, and further surveys of the chemistry of some of the phyla are contained in subsequent chapters of this Handbook. Another particularly useful reference source is *Substances Naturelles d'Origine Marine* by Kornprobst [45].

Alternative taxonomic views are possible. In some groups of organism (e.g., molluscs) there is reasonable agreement among taxonomists about the major subdivisions of the phylum, but in others (e.g., Protozoa and Chromista) there are huge differences between alternative taxonomic schemes. For example, the Annual Checklist of the *Catalogue of Life* has an inconsistent treatment of diatoms and needs correcting. The diatoms appear in three places and it appears that two classification systems are in simultaneous use such that two diatom classes – Coscinodiscophyceae and Fragilariophyceae – are listed under phylum Ochrophyta in kingdom Chromista, whereas class Bacillariophyceae is listed as a phylum-rank entry under kingdom Plantae [10]. In this review of taxonomy and chemistry, the *Catalogue of Life's* higher taxa are used and in general the *Catalogue's* system of assigning phyla adhered to.

1.3.2.1 Archaea

The archaea are prokaryotic organisms inhabiting extreme environments, both marine and terrestrial, such as hydrothermal vents, and also highly saline regions.

There are three generally recognized groups: thermophiles (heat-tolerant), halophiles (tolerant of highly saline media such as in the Dead Sea; some species are also extremely alkali tolerant, growing in media up to pH 12), and methanogens (some species of which are also highly thermotolerant). It is also convenient to recognize a group of “psychrophiles” tolerant of cold Arctic and Antarctic conditions. Although only discovered in the 1970s [6], it now appears that the archaea are in fact the most abundant bacteria in the marine environment [46]. They show major differences from other prokaryotes in their genome, and these are carried through into fundamental differences in membrane structure and biochemistry. Their cell walls do not contain the glycopeptides found in the Bacteria. Stabilization of the membrane structure is effected by esters of glycerol with characteristic branched-chain terpenoid fatty acids, a role that in prokaryotes is performed by carotenoids and/or hopanoids and in eukaryotes by sterols.

1.3.2.2 Bacteria

The Bacteria are characterized by a cell wall structure based on a glycoprotein formed by (1 → 4) linked *N*-acetylglucosamine and *N*-acetylmuramic acid cross-linked by peptide side chains containing unusual amino acids which render different bacterial strains biochemically and immunologically distinct. In Gram-positive bacteria, the glycoprotein coat forms the outermost layer; in Gram-negative bacteria there is an outer membrane coat that prevents this layer being stained by the reagent. Bacteria may be photosynthetic or non-photosynthetic, and the photosynthetic bacteria may be anaerobic (sulfur bacteria) or aerobic (which includes the cyanobacteria). The former group utilizes the bacteriochlorophylls as photosynthetic pigments. Further major subdivisions such as alpha-, beta-, gamma-, and delta-proteobacteria have been delineated according to various schemes, but the overall picture is complex [47–49]. The majority of secondary metabolites are produced by the cyanobacteria and by the actinobacteria, which are described separately in the following sections.

Cyanobacteria

The older term blue-green algae is now considered a misnomer, and the cyanobacteria are considered a subdivision of the photosynthetic Bacteria. They are unicellular organisms that are both marine and terrestrial; some marine species also inhabit fresh water. Some are truly monocellular, but when found unassociated with other organisms, many species adhere via their mucilaginous coats into filaments or tufts visible to the naked eye. Cyanobacteria are among the earliest known organisms. Schemes for the subclassification of the cyanobacteria are based on their mode and degree of such association, or alternatively by the type of spores formed. Attempts have also been made to classify them chemotaxonomically. About 1,000 species have currently been described. Cyanobacteria are responsible for frequent algal blooms, the toxicity of which is associated with their high level of secondary metabolites [50, 51]. Cyanobacteria are present in the tissues of many sponges, often as a major component of the biomass. The most characteristic secondary metabolites are peptidic and are also characterized by a high degree of halogenation.

Actinobacteria

The Actinobacteria are a particular class of the Gram-positive Bacteria showing filamentous growth, and some similarities to fungi. (In the past they have often been classified as filamentous fungi and are sometimes called “higher bacteria.”) They also merit special treatment biochemically speaking because of their vast production of different types of natural products, many of them with strong antibiotic or other pharmacological activity. The most important genera, in terms of natural products, are *Streptomyces* and *Actinomyces*. Actinobacteria occur in marine sediments, and probably as endophytes in many marine organisms. The recent discovery of three marine genera is notable and already significant, and novel chemistry has come from these discoveries (*vide infra*) [52–56]. As a group they tolerate a wide range of salinities.

1.3.2.3 Protozoa

The term “protozoa” is difficult to define taxonomically and is subject to ongoing modification in the light of biochemical studies, which are leading to the reclassification of many groups. It was formerly used as a blanket term to describe almost any kind of unicellular organism, but it is now known that the dinoflagellates are more closely related to the brown algae (Chromista: Ochrophyta; Phaeophyceae) than to other unicellular organisms. The ciliate organisms, for example, *Paramecium*, can be placed here, although it now appears that they are biochemically closest to the dinoflagellates. It is convenient to recognize four subdivisions: flagellates, amoebae, sporozoans (Apicomplexa), and ciliates based on their modes of locomotion, but the reservations expressed above concerning their fundamental dissimilarities must be borne in mind, and a proper classification remains premature.

Dinophyta (Dinoflagellates)

These monocellular organisms are economically important as the causative agents of toxic “red tides.” Biochemical and other studies have shown clearly that they are more closely related to the ciliates and to certain other groups than they are to other flagellate organisms. About 4,000 species are known (Table 1.2). Only just over half are photosynthetic; some are carnivorous. Their main anatomical characteristic is the possession of two flagellae, one equatorial and one longitudinal. Most are unicellular but some are filamentous. They participate in a range of symbiotic associations, especially with corals and with molluscs. Those organisms which are photosynthetic contain chlorophylls *a* and *c*. They contain a range of xanthophylls. The characteristic steroids are a range of 4 α -methyl derivatives representing an intermediate stage between the tetracyclic triterpenoids and the cholestane/ergostane type predominant in the phaeophyta and relatives.

Many shellfish toxins are now known to be dinoflagellate metabolites but may not currently have been specifically described as such in the literature. The known toxins of dinoflagellates fall into two main groups, though the exact type of toxin produced is genus specific. The first main group is polyketide-derived, either long-chain with some cyclic ether formation (e.g., amphidinols [57, 58]) or with multiple ether rings (“polyether ladders”) (e.g., *brevetoxins* (see Fig. 1.5) [59, 60]).

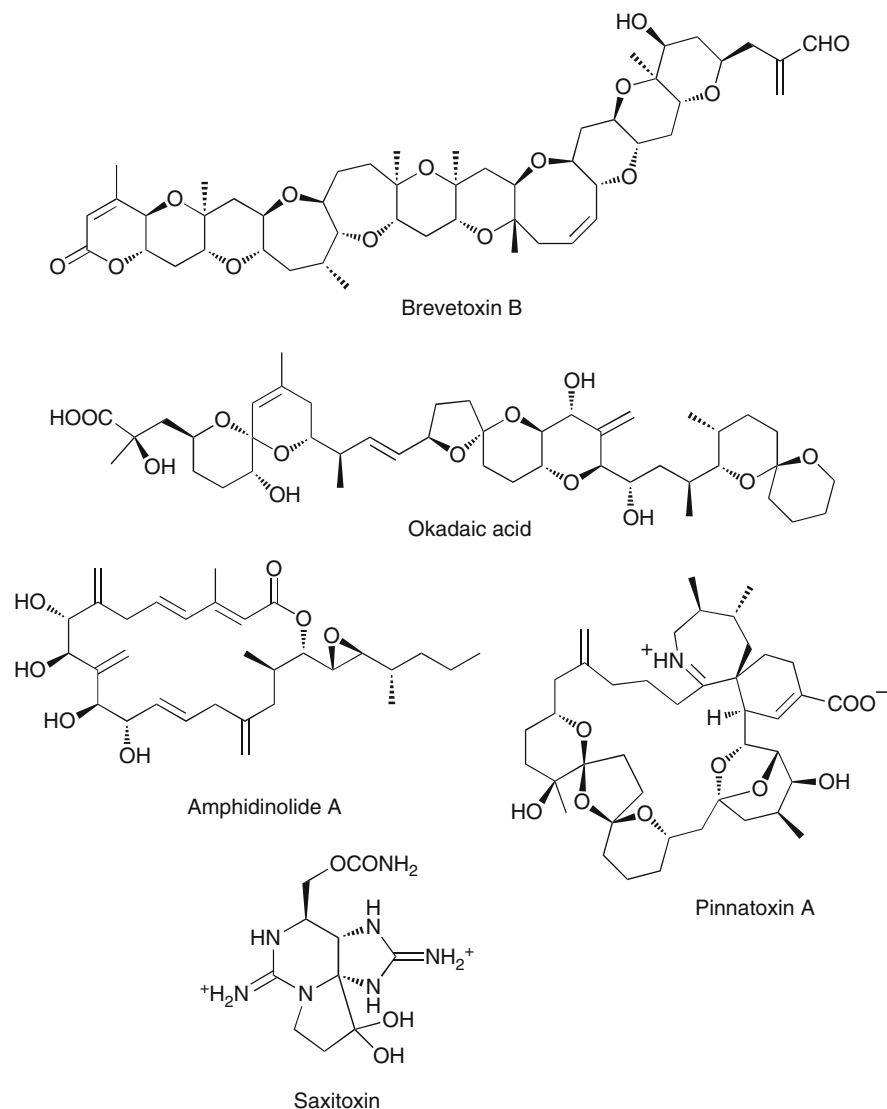


Fig. 1.5 Metabolites from protozoa

Another structural subtype is shown by *okadaic acid* [61], while other polyketides are macrolides such as the *amphidinolides* [62, 63]. Alicyclic nitrogenous polyketides, such as the *pinnatoxins* [64, 65] which have been isolated from shellfish are known to be dinoflagellate produced. It is notable that in known cases the biosynthesis of polyketides in dinoflagellates is by a totally different pathway from that in other organisms. The other main class is composed of nitrogenous guanidino toxins of which *saxitoxin* is the prototype [66].

Ciliophora (Ciliates)

Genera studied chemically include *Euplotes*, *Tetrahymena*, *Litonotus*, and *Pseudokeronopsis*. The ciliates are non-photosynthetic organisms but can often harbor photosynthetic algae as symbionts. Chemical studies have been fairly limited, but a range of sesquiterpenoids, and some highly unusual triterpenes, have been isolated.

1.3.2.4 Chromista

The term Chromista was first introduced in 1981 as a higher-order taxon and includes all algae whose chloroplasts contain chlorophylls *a* and *c* [67]. Previously, the photosynthetic chromists had been classified as plants, while non-photosynthetic chromists were classified with the fungi or animals with the close relationship among chromists not appreciated until the advent of ultrastructural and molecular studies. Chlorophyll *c* and a number of other pigments found in the Chromista are not found in any plant group, and it is these pigments which give them their characteristic brown or golden color. Within the Chromista, the phylum that has been of greatest interest to marine natural product chemists is the Ochrophyta which encompasses the class Phaeophyceae, or the “brown algae.”

Ochrophyta: Phaeophyceae (Brown Algae)

About 1,600 species of brown alga are known, almost exclusively marine. The term “phaeophyta” is to be preferred, even if it is depicted as being at a phylum level. Modern studies have shown that the phaeophyta are only very distantly related to the other algae and the term “brown alga” is therefore a misnomer, although it remains in widespread use. Together with the diatoms and the chrysophytes, they constitute what has been described as the Stramenopiles. Whereas the other two subgroups are entirely monocellular, the vast majority of brown algae are multicellular and macroscopic, sometimes attaining very large size. Most species inhabit cold and temperate, often rough, seas and are sessile, demonstrating a well-defined differentiation into a foot (holdfast), stem (stipe), and frond and growing in surface or relatively shallow waters. The exception is the brown algae of the Sargasso Sea, which are two free-floating *Sargassum* species inhabiting tropical waters. The photosynthetic pigments of all species within the Stramenopiles are chlorophylls *a*, *c*₁, and *c*₂ (characteristic absence of chlorophyll *b*). The carotenoid content is limited to fucoxanthin and lesser amounts of violaxanthin. Both the structural and the storage carbohydrates of the phaeophytes differ from those present in other classes of algae.

The range of sterols found is limited and mostly based on minor modifications of the fucosterol structure, which is the major steroid. There is a wide range of unusual oxylipins, for example, bridged epoxy compounds and prostanoid-like cyclopentanoids.

Brown algae contain a wide range of terpenoids, phenolics, and meroterpenoids, but a striking and somewhat unexpected feature is the paucity of halogenated compounds, and those that are found in small amounts are bromo- and iodo- rather than chloro-substituted. They are unique in their ability to concentrate iodine (and also arsenic) to concentrations of up to 1% dry weight, and although 99% of

the iodine in the tissues is inorganic, the other 1% finds its way into thyroxine and other iodinated tyrosines and a small number of miscellaneous phenolics. The major secondary metabolite content of brown algae is represented by phenols and phenolic meroterpenoids, many of them sulfated and/or halogenated. The content of these in the tissues may reach 20% by weight and they may play a role in the prevention of larval fixation by marine animals and for protection against bacteria. A major series is represented by the phlorotannins, which are radical-induced oligomers of phlorotannin containing C–C or C–O–C linkages. The genus *Cytoseira* has been much investigated and has yielded a large range of structurally diverse meroterpenoids.

Haptophyta and Ochrophyta: Bacillariophyceae (“Bacillariophyta”; Diatoms), Chrysophyceae (Golden Algae)

It was this grouping of unicellular organisms together with the phaeophyta that constituted the Stramenopiles. The term “algae” formerly applied to some of these groups is now considered in biochemical and sub-microstructural studies to be a misnomer (cf. phaeophyta). Although these organism types are linked together in the classification scheme used here, this is a tentative assignment. These organisms have been relatively little studied chemically by comparison with the phaeophyta. The diatoms and haptophytes secrete hard exoskeletons of aluminosilicates and calcium salts respectively, while the golden algae do not, though they may contain silica microspicules. There are ~500 known species of marine golden algae and ~500 haptophytes. The number of marine diatom species, recognized to be 5,000, may be as high as 50,000, constituting the bulk of the phytoplankton at certain times of the year, and therefore of crucial importance to marine ecosystems.

The storage polysaccharide of diatoms and golden algae is chrysolaminarin. Photosynthetic pigments resemble those of the brown algae, with no phycobilins but with chlorophylls *c* and the more recently discovered chlorophyll *c*₃. There have been extensive studies on their lipids on account of their biotechnological importance (cf. algae). The content of sulfur glycerides, especially 1,2-diacylglycerol 6-sulfoquinovosides [68] (also found in other classes of organism), is relatively high. The range of known steroids resembles that of the phaeophyta in being based on limited side-chain modification of the cholestane skeleton, with some sulfation. The prymnesins are toxic polyethers produced by a haptophyte [69]. The range of terpenoids isolated is very narrow and limited so far to simple phytanes. It is noteworthy that in two studied diatom species, the biosynthesis of these (in the chloroplasts) is by a non-mevalonate pathway while the steroids, produced in the cytoplasm, are mevalonate derived. Nitrogenous compounds are similarly few in number, but the toxic pyrrolidine *domoic acid* (see Fig. 1.6), also found in red algae, was isolated as a shellfish toxin resulting from *Nitzschia* infection [70].

1.3.2.5 Plantae

The algae, considered in their totality, can be described as lower, mostly multicellular plants of a simple body plan, lacking well-defined differentiation

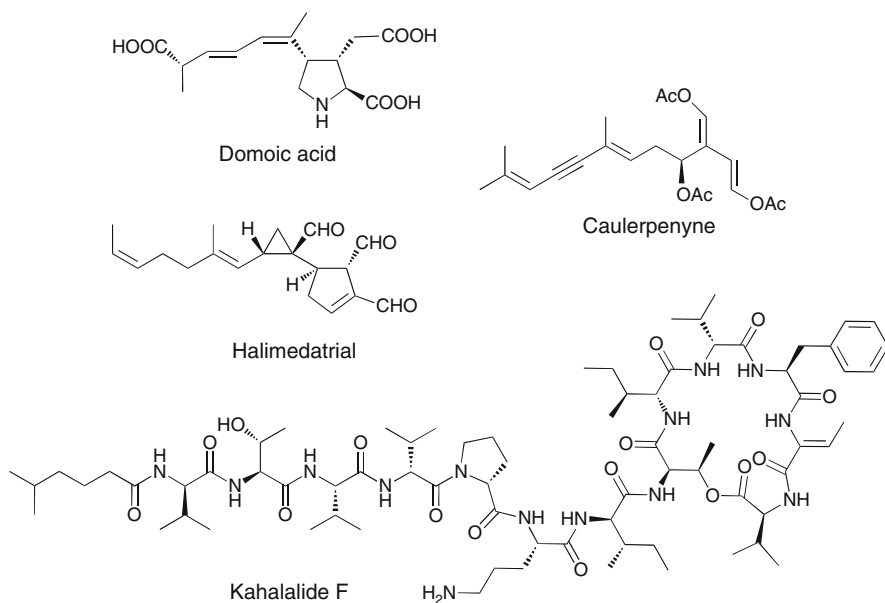


Fig. 1.6 Metabolites from “Bacillariophyta” and Chlorophyta

into roots, stems, and leaves. The higher plants, which show such differentiation, are virtually absent from the oceans, although some species (mangroves; several different spp. of higher plant) are important components of the estuarine saltmarsh environment and have currently become of considerable interest. The classification of algae has undergone a number of changes in recent decades and there is no definitive overall plan that takes care of every subgroup. The most fundamental division is between the brown algal branch, now better described as the phaeophyta as a grouping within the Chromista, and the green algal branch: two groupings which show large biochemical differences one from the other. The “green” branch comprises not only the green algae proper (Chlorophyta), but also the red algae (Rhodophyta), which are now considered more closely related to the green algae than either are to the phaeophyta and relatives.

Chlorophyta (Green Algae)

About 2,500 species are recognized inhabiting mostly surface waters of the calmer seas. Of these, about 20% have so far been investigated chemically, principally in the orders Bryopsidophyceae and Ulvophyceae. In their fundamental biochemistry (photosynthetic pigments, storage polysaccharides, etc.) the Chlorophyta resemble the higher plants. Some members are unicellular, sometimes as endophytes in other species of green algae. Green algae photosynthesize using the common carotenoids α - and β -carotenes, and contain a range of relatively common xanthophylls such as lutein. The most common storage polysaccharides are amylose and amylopectin, and the most common structural polysaccharide is cellulose, although

some groups also secrete β -1,3-xylan and β -1,4-mannan. The most widespread sterols are cholesterol, brassicasterol, sitosterol, and close relatives, although some rarer sterols have been characterized. Studies have not always distinguished between sterols involved in the algal membrane structure and those present in the cytoplasm.

The known secondary metabolites of the green algae are rather limited in structural range and are mostly confined to terpenoids of relatively common skeleton, and a range of aromatics including meroterpenoids. Halogenation is uncommon, and the terpenoids are so far limited to sesqui- and diterpenes and a few triterpenes. Many of the terpenoids contain enoloid functionality, for example, *caulerpenyne*, [71] and/or furan rings formed biogenetically by the cyclization of the related unsaturated aldehydes. Some of these metabolites have also been isolated from species that feed on green algae, such as molluscs. *Halimedatrial* is the only terpenoid so far isolated from green algae containing a carbon skeleton that has not been found elsewhere [72]. Nitrogenous compounds found in green algae tend to be low-molecular-weight amines related to aminoacids, or peptides and modified peptides such as the *kahalalides* [73].

Rhodophyta (Red Algae)

The red algae are characterized by a unique and complex reproductive cycle involving three alternating generations. The great majority of the 6,200 species known are marine, sometimes inhabiting deep water. They may be mono- or multicellular with a complete absence of flagellae. The chloroplasts have a double membrane similar to those of cyanobacteria and presumably arose by endosymbiosis with these organisms. There is no general agreement about the subclassification of red algae. An important biochemical similarity between the red algae and the cyanobacteria is the presence of the phycobilins, phycocyanobilin (blue-green), and phycoerythrobilin (red). It is the latter that is responsible for the red color of the tissues, but the color may be modified or masked by the presence of phycocyanobilin and/or chlorophylls. The red algae contain chlorophyll *a* and the characteristic pigment chlorophyll *d*.

The secondary metabolites of the red algae are characterized by a high proportion of halogenated terpenoids and aromatics, particularly in the intensively studied genus *Laurencia*. The terpene skeletons are strongly weighted toward the lower MW members of the series (especially sesquiterpenes), and there are many representatives of unique terpene skeletons not found in higher plants or elsewhere in the algae. The sesquiterpenes of *Laurencia* are based on more than 20 different carbon skeletons, some of them “traditional” and found also in terrestrial organisms, others novel. Many of these have also been isolated from molluscs and other animals that feed on red algae. There is also a wide range of halogenated (mostly brominated) diterpenes, many derived from the common (marine and terrestrial) skeleton labdane and other skeletons closely related to it. The parguerane skeleton, as found in *parguerene* (see Fig. 1.7) and related compounds, is however unique to marine organisms [74]. Certain *Laurencia* and *Chondria* species have also yielded a series of triterpenoid polyethers derived from squalene, for example, *thyrseferol* [75]. The most characteristic class of natural product isolated from these genera,

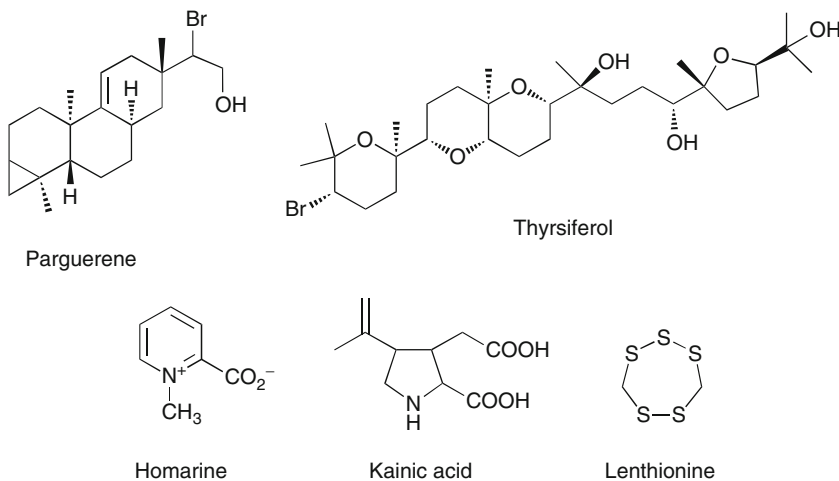


Fig. 1.7 Metabolites from Rhodophyta

however, is the extensive series of mostly halogenated compounds based on a linear C_{15} skeleton, the first of which to be discovered was laurencin in 1968 [76, 77]. A wide variety of structures based on ether formations are found on this basic skeleton (e.g., obtusenyne [78]), which probably arise by loss of a C_1 fragment from a C_{16} precursor. The Ceramiales also contain a range of halogenated phenolics. Nitrogenous natural products are relatively scarce in the majority of red algae, and mostly limited to widely distributed small molecules such as *homarine* [79, 80]. A range of simple halogenated indoles were isolated from *Rhodophyllis membranacea*. Once again, it is the Ceramiales that show a much greater range. A characteristic aminoacid is *kainic acid* [81] and other analogs. The chemotaxonomic unpredictability of this group of organisms is shown by studies of *Chondria californica*, which yielded a range of polysulfur compounds such as *lenthionine* [82]. These were unaccompanied by terpenes, and were not found in apparently closely related species.

1.3.2.6 Fungi

Fungi, part of the Eukaryota, are characterized by the lack of a photosynthetic mechanism and a mode of life which is saprophytic, parasitic, or symbiotic [83]. Another major biochemical difference from algae lies in their cell wall structure usually based on chitin rather than cellulose. Fungi are found throughout a wide range of terrestrial and marine environments and it is not possible to produce a meaningful definition of “marine fungi,” only to refer to a range of halotolerance among the various fungal species that are widely distributed. Thus marine sediments and marine invertebrate tissues yield fungal species from genera also found terrestrially, but which have developed a preference for growing in saline environments. Of the approximately 100,000 fungal species so far described, about

500 have been found in marine environments, but this figure is certain to increase in the light of further research. The majority of fungi fall into the category of higher fungi or Eumycetes having typical fungal biochemistry, and which can be subdivided into the four main classes of Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes. These groups are distinguished by their method of spore formation (zygospores, asci, and basidia, respectively, for the first three groups). The Deuteromycetes are an ill-defined group roughly corresponding with the term fungi imperfecti (the terms Mycelia sterilia and Hyphomycetes are also found according to various schemes). These are fungi in which no reproduction is observable and which are therefore extremely difficult to identify. The Ascomycetes and the Deuteromycetes are the most represented in the marine environment and have been the most investigated chemically [84, 85].

The great majority of fungal secondary metabolites have been isolated from fungi associated with other organisms. Marine algae (green, red, and phaeophyta), like higher plants, harbor a wide variety of endophytic fungal species; for example, 116 different fungal strains were cultivated from a single specimen of *Fucus serratus* [86]. It is not in general known whether any particular relationship should be considered as symbiotic, benign, or pathogenic. There are usually strong structural similarities between the natural products from these epiphytic marine fungi and their terrestrial equivalents.

Fungal mycelia are also found in marine animal tissues. Evidence for their presence is based entirely on culturing experiments and as yet there is no evidence from direct microscopic examination or other techniques. Their role is unknown. In general, compounds produced by fungi associated with marine animals are structurally related to other fungal metabolites and are distinct from natural products produced by the organisms themselves. It does not appear that fungi are the biogenetic source of natural products isolated from marine invertebrates, unlike the situation found with bacteria. The most characteristic sterols of all fungi are ergosterol and related ergostanes. Reports of 5,7-dienic steroids from other marine species are suggestive of fungal contamination. There are very few reports of the incorporation of halogens. Most fungal secondary metabolites are based on a polyketide biogenesis, but some terpenoids are found, for example, the unusual nitrobenzoyl esters of 6,14-dihydroxy-7-drimen-12,11-olide (see Fig. 1.8) [87].

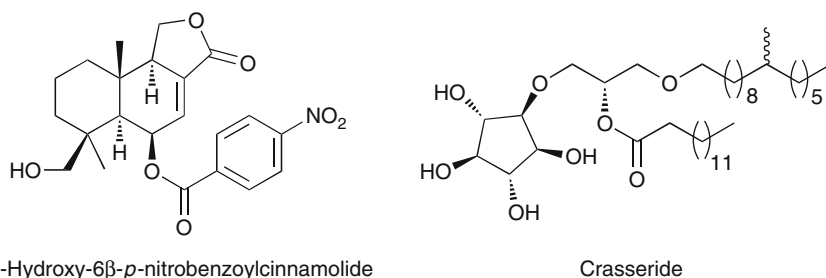


Fig. 1.8 Metabolites from fungi and Porifera

The alkaloids obtained from marine fungi are dominated by diketopiperazines/indoles, as is the case with terrestrial fungi. Very few biosynthetic studies have been reported for natural products specifically from marine fungi.

1.3.2.7 Animalia

All animals are members of the Animalia which range in size from no more than a few cells to organisms weighing many tons. Based on their body plans they are grouped into 30–33 different phyla of which the Arthropoda contains the greatest number of species (~1 million). All members of the Animalia are multicellular eukaryotes, a feature that separates them from bacteria and most protists, and they can be distinguished from plants, algae, and fungi by a lack of rigid cell walls. All members of the Animalia are heterotrophs and so rely directly or indirectly on other organisms for their nourishment. Most ingest food and digest it in an internal cavity with one or two entrances. The bodies of most animals, except the sponges (Phylum Porifera), are made up of cells organized into specialized tissues. Other distinguishing characteristics include a sexual mode of reproduction, at least at some point in the life cycle, and employ differentiated eggs and sperm.

Porifera

The sponges are considered as the most primitive of the multicellular organisms, providing an evolutionary bridge between the monocellular eukaryotes and the rest of the animal kingdom. They are multicellular organisms lacking all organ differentiation (including gonads) and some can uniquely reconstitute themselves after passing through a sieve. They are almost exclusively marine. Sponges are found at all marine depths but the proportion of calcareous sponges diminishes with depth owing to the physicochemical effect of pressure on the ability of the organisms to secrete calcium. The taxonomy of sponges is particularly difficult owing to the paucity of well-marked morphological feature by which they can be distinguished [88]. Many species have been synonymized and genera renamed (e.g., *Aplysina* = *Verongia*), and there are numerous views on their classification at higher levels; a recent multivolume treatise [89] proposes many changes. Three main subdivisions have been generally recognized, depending on the nature of the skeletons that they secrete; calcareous, siliceous, or askeletal. The largest group is the demosponges, about 95% of known species, in which the skeleton is of spongine, a proteinaceous polymer similar to keratin. The Hexactinellida sponges, characterized by silica spicules of sixfold symmetry, are found only at great depth and have been little studied chemically.

Sponges participate in a wide range of symbiotic/commensal relationships, and a large number of the isolations of natural products earlier reported from them are in fact owing to the presence of cyanophytes in particular. Given the extent of these associations, it is not surprising that the diversity of natural products reported from sponges and sponge aggregates covers the whole range of known types. Other natural products reported may be true metabolites of the symbionts [90]. A wide variety of cell membrane components have been isolated, not only extensive series

of both straight-chain, branched, and methylenic (cyclopropanoid) fatty acids but alkylglycerols and hopanoids as well as a vast range of steroids. There are also numerous brominated and α -hydroxyacids. Associated with the membrane structure is a wide variety of glycolipids, many of them of unique structural type [91]. The simpler *N*-containing parents are the sphingosines, the *N*-acyl derivatives collectively known as ceramides, and the glycosides, known as glycosphingolipids or cerebro-sides. The nitrogen-free glycolipid content also includes some structural types not found elsewhere, such as the *crasserides* [92], which as a class have been suggested to be uniquely diagnostic of the Porifera and found in all species examined.

Among calcareous sponges the most investigated genera are *Clathrina* and *Leucetta*. These genera have yielded in particular long-chain unsaturated aminoalcohols, a range of imidazole and other alkaloids, and cyclic peptides. By far the most studied have been the demosponges, reflecting their numerical preponderance and shallow-water accessibility. Demosponges contain a very wide range of steroids, which encompasses not only the conservatively modified structures biogenetically not far removed from cholesterol found in the algae, but also a large number showing more profound modification. These include 19-norsteroids and a range of A-ring abeosteroids (3-hydroxymethyl-A-norsteroids). The most common type of modification however is further side-chain methylation which leads to an extensive series of steroids having various branching patterns up to C₃₂. Side-chain cyclopropasterols occur in the range C₂₇–C₃₁, and there are also a large number of secosterols with fission at 5,6, 8,9, 8,14, and 9,11. There are also many polyhydroxylated and sulfated sterols of the type found in many other marine organisms, and many steroidal glycosides. Halogenated steroids and steroidal alkaloids are rare.

Demosponges of the genera *Plakortis* and *Plakinastrella* (order Homosclerophorida) contain a wide range of oxylipins, including many cyclic peroxides. Another group of unusual natural products found in sponges are the terpenic isocyanides together with their related isothiocyanates, isocyanates, and formamides. A wide range of alkaloids and terpenoids are found in demosponges. Indole alkaloids range from simple halogenated indoles such as the plakohypaphorines to polycyclics such as the pentacyclic pyridoacridines. Demosponges are the most prolific of all marine organisms in terms of the secondary metabolites that have been isolated from (but not necessarily produced by) them.

Cnidaria (Jellyfish, Sea Anemones, Gorgonians, and Corals)

This class of organisms represents the first major development in body plan over the undifferentiated sponges, showing cellular differentiation into cells with different functions, but in general no well-defined organs. The term Cnidarian replaces the older "Coelenterate." This is a class of organisms typified by a carnivorous lifestyle, the presence of specialized stinging cells (cnidocysts) used in the capture of prey and for defense, and a digestive system consisting of a sac with only one opening. They have a basically radial body plan, which may be modified either in the direction of a fixed polyp with a central gastric cavity (hydras) or a free-swimming medusa form (jellyfish) in which the gastric cavity is underneath. Reproduction is sexual, producing a free-swimming larval planula which develops into a free-swimming followed by a

polyp form, although in some species only one of these is formed. About 10,000 species are documented. The sea anemones, gorgonians, and corals have no free-floating phase and have a skeletal structure consisting either of secreted calcareous minerals or of proteinaceous material (gorgonine, analogous to the spongine found in the sponges). They are divided into two groups depending on their symmetry – eightfold in the octocorals (alcyonians or soft corals and gorgonians) and sixfold or a multiple of sixfold (hexacorals, including the sea-anemones and hard corals) [93] – and have been the most studied group of the cnidarians chemically.

In general, relatively few nitrogenous secondary metabolites have been isolated. The proportion of halogenated metabolites is also relatively low, except from *Briareum* spp. Octacorals are rich in prostanoids, steroids, terpenoids (but only sesqui- and diterpenes), and aromatics. The prostanoids include a number identical with those found in higher organisms of the prostaglandin series (A, B, E, and F), but also halogenated prostanoids containing Cl, Br, and I, especially from *Clavularia*. Further oxylipins are now being found in other cnidarians and it appears that their presence may be ubiquitous. The hexacorals also contain a range of polyunsaturated long-chain acids.

Like the sponges, cnidarians contain a wide range of sterols, both typical cholesterol related and those with modified side-chains. An important class are the side-chain cyclopropanoid steroids although it has been shown that these are produced by symbiotic dinoflagellates. There are many polyhydroxylated steroids, often showing side-chain epoxidation and a considerable number of secosteroids. Also encountered are pregnane glycosides, but in contrast to the sponges, *O*-sulfation is absent. The hexacorals produce polyhydroxylated ecdysteroids which are thought to protect the organism against crustacean larvae. The sesqui- and diterpenes found in the octacorals are diverse and include some skeletons unique to them that in most cases are unique to certain families. In contrast, terpenoids are almost absent from the hexacorals. The sesquiterpene hydrocarbon content of octacoral tissues may be exceptionally high and they are thought to play an ecological role as predator and larval implantation repellents. An important feature is the frequent occurrence of common terpenoids enantiomeric to those familiar from terrestrial plants. The hexacorals have yielded only a few sesquiterpenes; there is a complete absence of the isocyanides characteristic of the sponges and only a limited range of terpenoid alkaloids. The octacorals are very rich in diterpenoids, with over 1,900 belonging to 50 isolated skeletal types. As with the sesquiterpenes, some skeletons are widespread throughout the phylum, while others are restricted to a single family and can be considered as chemotaxonomic markers. Particularly widespread skeletons include cembranes, xenicanes, lobanes, briaranes, cladiellanes, dolabellanes, and amphilectanes. As with the sponges, many skeletons are prenylogues of widespread sesquiterpenoid skeletons. Some diterpene alkaloids have been found such as the *sarcodictyins* (see Fig. 1.9) [94]. The hexacorals produce a range of ceramides, often containing unusual sphingosines, and other acyclic amides. Their range of cyclic alkaloids is restricted, but includes the unique class of fluorescent pigments based on the cycloheptadiimidazole skeletons of parazoanthoxanthin A and *pseudozoanthoxanthin* A [95].

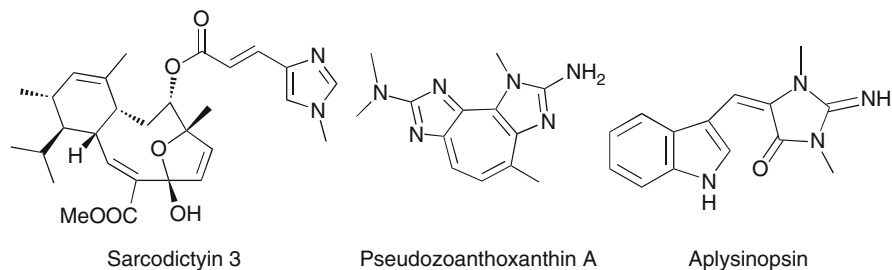


Fig. 1.9 Metabolites from Cnidaria

Hexacorals of the order Zoantharia (genus *Zoanthus*) have yielded a range of polyketide alkaloids. Chemically and pharmacologically, the most significant natural product isolated from cnidarians is probably palytoxin [35, 96]. Hexacorals inhabiting surface waters also contain a range of mycosporins which are closely related to analogs found in fungi and appear to perform a photoprotective function. They also contain a range of small nitrogenous betaines and other amines, some purines, and some simple indole-imidazole alkaloids centered on *aplysinopsin* [97].

Other classes of the cnidarians include the Cubozoa (box jellies), Hydrozoa (hydras), and Scyphozoa (true jellyfish). Chemical studies have been mostly confined to their venoms, which are peptides. One difficulty associated with studying their secondary metabolites is the large amount of water in the tissues, which can reach 98%. Some steroids have been identified, plus a small range of polyketides and simple alkaloids. There are also the nitrogenous compounds associated with the bioluminescence of some species such as *Cypridina*.

Platyhelminthes (Flukes, Tapeworms, and Free-Living Flatworms)

These are the flatworms, characterized by a bilateral body plan and the complete absence of a digestive cavity with about 15,000 species known. They may be terrestrial, freshwater, or marine and many belong to orders which are exclusively parasitic (e.g., flukes). The majority of marine species belong to the class of planarians (turbellarians). These are mobile, carnivorous animals having no physical means of defense and relying entirely on substances absorbed or modified from the diet, or produced by symbiotic organisms, as chemical antifeedants. The most studied genus is *Amphiscolops*. These worms are protected by amphidinolides produced by symbiotic dinoflagellates and other dinoflagellate metabolites [62, 63]. Other planarians feeding on ascidians have yielded alkaloids.

Annelida (True Worms)

These are the segmented worms, having an alimentary canal. They include the polychaetes, oligochaetes (earthworms; mostly terrestrial), hirudineans (leeches; mostly freshwater), echiurians, and Vestimentifera. They locomote by means of bristles which can be irritant or venomous. The best-known genus among the echiurians is the spoonworm *Bonellia viridis*, most studied on account

of its tetrapyrrole pigment bonellin which also plays a role in inducing sexual differentiation in the larva [98]. The Vestimentifera include the recently discovered giant hydrothermal vent dwellers *Riftia* which coexist with sulfur bacteria and store elemental sulfur in the tissues. So far there has been little study of lipid or steroid content. The carotenoid pigments appear to be mainstream components and are derived dietetically. Other annelid pigments are anthracene and anthraquinone based. Annelids also contain brominated phenols and derived aromatics which protect them against bacteria. Certain annelids exhibit bioluminescence and in *Odontosyllis* spp. this is based on pteridines such as 6-propionyllumazine. Nereistoxin is a simple aminodithiolane with powerful cytotoxic properties produced by *Lumbriconereis* sp. [99].

Nemertea and Phoronida

The ribbon-worms or nemerteans have yielded the powerful nicotinic receptor agonist anabaseine, used as a venom by the worm, together with several related oligopyridines. Toxins of the tetrodotxin series are also found in the tissues, and also some peptide toxins. The unsegmented phoronidian worms *Phoronopsis* have yielded antibacterial bromophenols like those obtained from the annelids.

Bryozoa

These colonial organisms are entirely aquatic and mostly marine. They are distinguished by their unique form of gastric cavity, which is surrounded by tentacles forming an organ called the lophophore. The colonies are produced by budding and therefore consist of genetically identical individuals, each of which is surrounded by a bilayered exoskeleton, the inner layer calcareous (not always continuous) and the outer layer chitinous. They are suspension feeders, feeding on plankton and bacteria, and are found at all depths. Bryozoans have so far been less studied than the number of known species (5,700) would justify given the range of interesting natural products already isolated from them. This is probably a consequence of the difficulty of harvesting them.

Terpenoids and steroids have been little studied. The single diterpenoid murrayanolide isolated has a unique skeleton which implies that many more unusual terpenoids may exist in other bryozoans. The similarly limited studies of steroid content have yielded only relatively common types based on cholestane methylated in the side chain and/or hydroxylated. One or two anthraquinone pigments have been characterized, of which the most unusual is bryoanthrathiophene. The most numerous metabolites from bryozoans are defense chemicals, which comprise numerous alkaloids, and the *bryostatins* (see Fig. 1.10) [100], an important series of polyether polyketide toxins with anticancer properties, some of which have also been found in other marine organisms. The ultimate source of these may be a *Candidatus* bacterium [101]. The range of alkaloids is extensive taking into account the limited amount of work that has so far been done. There are simple halogenated phenethylamines, pyrroles, and pyrrolidines. The indoles found are mostly brominated simple indoles, but *Flustra foliacea* yielded a series of alkaloids of the physostigmine type unique in the marine environment [102]. The securamines, chartellines, and chartellamides

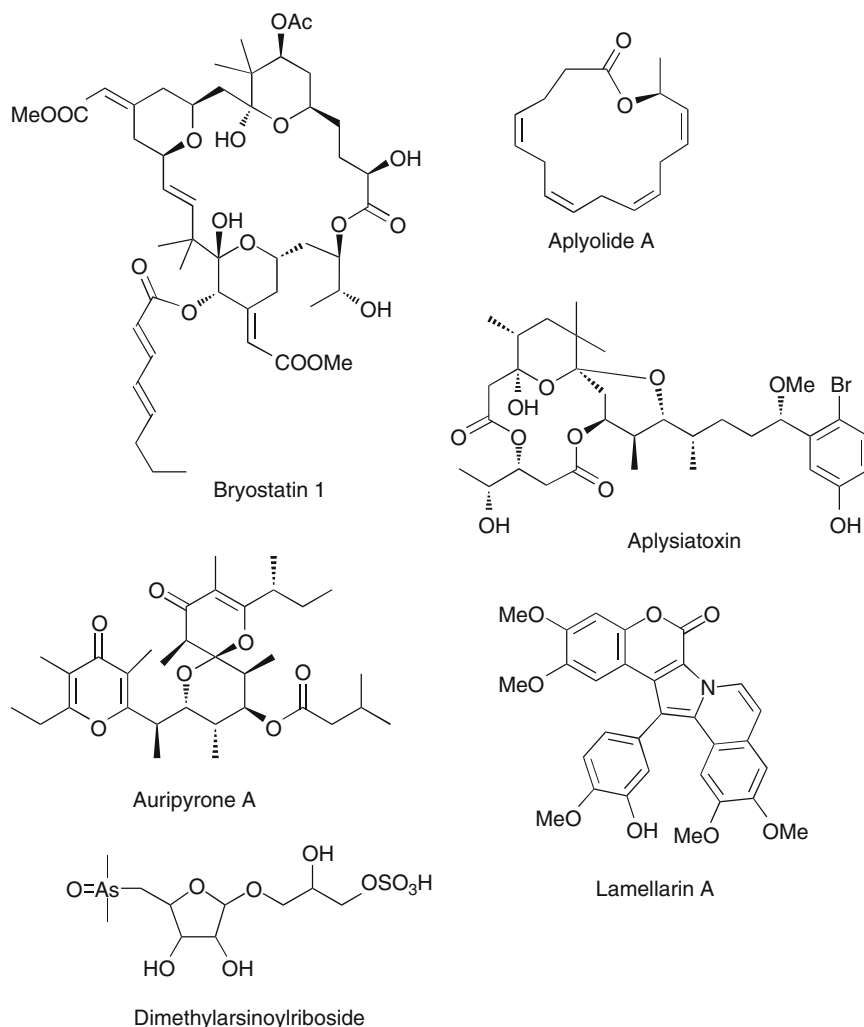


Fig. 1.10 Metabolites from Bryozoa and Mollusca

represent a further elaboration of this structural type up to a maximum of seven rings [103–105]. There are also a number of quinolinequinones, halogenated or bearing thioether substituents. The presence of nitro groups in a few alkaloids is notable.

Mollusca

This is a diverse and widely distributed phylum of organisms [106]. The body plan is basically non-segmented and bilateral, although in some molluscs (the gastropods) it is often modified by torsion into a spiral surrounded by a shell. There are well-developed organs inside a more or less thickened outer layer, the mantle,

which secretes a shell formed of calcareous matter and protein. This shell may be external, as in the gastropods, internal as in squids, or may be totally lacking (octopuses and nudibranchs). There is generally a muscular foot and a cephalic region which may be highly developed into tentacles and other organs, as in the cephalopods. The alimentary canal is well developed and furnished with a rasping radula used in feeding. Different classes of molluscs show variation in this general body plan, for example, the bivalves have a hinged shell, no cephalic region and no radula, and some of them also lack the foot. Many species of mollusc are known, present in marine, freshwater, and terrestrial environments, ranging in size from microscopic to very large. They show a wide range of dietary behavior (carnivores, herbivores, filter feeders, and detritus feeders) and undergo a wide range of symbiotic relationships. In particular, in some molluscs the mantle incorporates symbiotic algae providing toxic antipredator substances. The phylum is usually divided taxonomically into seven unequal classes, but of these four (including Chitons) are numerically limited and have been studied chemically little or not at all [107]. The most important classes both in terms of number of species, economic importance, and chemical studies are the gastropods, the bivalves, and the cephalopods. However, the bivalves, with their well-developed physical protective mechanism of the double shell, appear to have less need for chemical defense mechanisms and their secondary metabolites are less profuse. They have mostly been studied in terms of their economically important shellfish toxins, which are in fact microbial/dinoflagellate products. The cephalopods too have been rather little studied; their most characteristic metabolites are adenochromines [108]. The most studied organisms chemically have been various types of gastropod which have little or no physical defense and rely almost entirely on chemical defense against predators. The numerous gastropods are sometimes further divided into three subclasses: the Prosobranchia, the Opisthobranchia, and the Pulmonata. This division is not recognized by the *Catalogue of Life*, but since it is a convenient subdivision of a large group of natural product-producing organisms, it is followed here.

Terpenoids are numerous. The genus *Planaxis* (Gastropoda, Prosobranchia) has provided a series of cembranoids. Among the opisthobranchs, the sea hares or aplysians, which are herbivorous, feed on cyanobacteria and algae, and their digestive systems and mantles contain a wide variety of unchanged and metabolized secondary metabolites which perform an antifeedant function. These alimentary chains are complex and have been much studied. The two most studied genera are *Aplysia* and *Dolabella*; the former feed mostly on red algae and contain many halogenated and nonhalogenated terpenoids, cyclic halogenated ethers, lactones, and both peptide and nonpeptidal alkaloids. *Dolabella* spp. feed on brown algae, and in accordance with the terpenoid profile shown by these, contain mostly nonhalogenated diterpenoids as well as lactones, peptides (notably the extensive range of highly cytotoxic dolastatins, from cyanobacterial symbiosis), and alkaloids. A few *Aplysia* spp. feed on brown algae and also contain nonhalogenated diterpenes. Other products isolated from this type of mollusc appear to derive from symbiotic/commensal green algae, *aplyolidides* [109], and even fungi, *aplysiatoxin* [110, 111].

The shell-less nudibranchs can incorporate cnidocysts obtained from cnidarians into their mantle, and also rely heavily on compounds, especially terpenes, ingested in the diet as a means of defense. A wide range of skeletal types have been isolated, and include sponge-derived terpenoid isocyanides and compounds derived metabolically from them. However, nudibranchs also synthesize terpenoids *de novo* via the mevalonate pathway. Many terpenoids are present as glyceryl esters. In general it is possible to predict with a fair degree of accuracy what types of compounds (though not necessarily the exact compounds) will be isolable from nudibranch tissues by studying the prey of the different species. The sacoglossan gastropods also contain a range of terpenoids, but these animals are herbivorous and the terpenoids derive from commensal green algae entering into the tissues. Nudibranchs contain a narrow range of carotenoids and steroids. Other defense allomones include quinonoid and related meroterpenoids, and macrocyclic lactones. Also noteworthy is the isolation of prostanoid lactones from *Tethys fimbria*.

The distribution of polyketides in molluscs is patchy. They are found only in some classes of the gastropods, for example, the *auripyrones* (strictly, polypropionates) from *Dolabella* [112]. Pulmonarians have also afforded a number of polypropionates. Long chain aromatic and heteroaromatic metabolites, which do not appear to be derived from the diet, are found in *Navanax* spp., *Haminoea* spp., and other genera. Peptides, especially cyclic oligopeptides often containing unusual aminoacid residues, are probably widely distributed and show structural resemblances to similar compounds found further down the food chain, for example, the kahalalides [73]. Among other nitrogenous metabolites, the best-known from gastropods is the dye-stuff 6,6'-dibromoindigotin, known since ancient times. The genus *Lamellaria* (Gastropoda; Prosobranchia) yielded a wide range of the pyrrole alkaloids, the *lamellarins* [113].

Carnivorous gastropods of the genus *Conus* produce a vast series (there appear to be tens of thousands of chemically distinct compounds) of highly toxic peptides the conotoxins [114], which are delivered to the prey by means of a highly specialized injecting organ. The bivalve toxins (e.g., pinnatoxins [64, 65]) responsible for various kinds of shellfish poisoning are mostly produced by commensal dinoflagellates and have been mentioned above. There are differences in the distribution of different members of the saxitoxin series between the tissues of the bivalves and the originating dinoflagellates [115].

The kidneys of the giant clam *Tridacna maxima* concentrate up to 0.1% of arsenic, the function of which is unknown. It is stored as various *dimethylarsinoyl-ribosides* [116].

Echinodermata (Crinoids, Starfish, Sea Urchins, Sea Cucumbers, Brittle-Star, etc.)

These organisms are characterized by a radially symmetrical body plan (which is acquired in the adult stage, the larvae being bilaterally symmetrical) and a unique system of respiration through water-filled tube feet which also provide locomotion. There is a calcareous endoskeleton. This is the largest phylum of exclusively marine animals, with about 7,000 species known. They may be herbivores,

suspensivores, detritivores, carnivores, or necrophages, and the carnivorous species may prey, for example, on corals or other echinoderms. There are many examples of commensalism and parasitism between echinoderms and other organisms. Although various classification schemes for echinoderms differ in detail, five main groups are generally recognized. The most primitive, widely represented in the fossil record, are the Crinoids in which the mouth and anus are on the same surface. They have a planktonic larval form followed by an adult form that may be sessile (sea lilies) or mobile (feather stars). The other four groups, in which the mouth and anus are on opposing faces, are the starfish (Asterozoa), sea urchins (Echinozoa), sea cucumbers (Holourozoa) [117], and Ophiurozoa.

The presence of steroidal saponins of different types in the starfish and in the sea urchins is unique in the animal kingdom, and serves to delineate them from the other echinoderms and from each other. Other characteristic markers are the dominance of 3α -hydroxylated steroids in the ophiurians, and of quinonoid pigments of different types in the crinoids and in the sea urchins. The various types of echinoderm produce a variety of specialized polysaccharides, the study of which is still in its infancy. One that has been characterized is frondecside [118]. The lipid content of sea urchins and ophiurians is high in polyunsaturates. The starfish have been relatively little investigated but appear to follow the same pattern, with some prostanoid precursors. A high proportion of branched-chain acids have been isolated from sea cucumbers, but these are probably of bacterial origin. Phospholipids and glycosphingolipids appear to be universally present in echinoderms and a wide range of structural types of ceramides and cerebroside have been isolated from starfish and holothuroids. Throughout the echinoderms, halogenation is rare and found only in a few anthraquinone pigments such as the *gymnochromes* (see Fig. 1.11) (from crinoids) [119]. There are also few alkaloids of greater complexity than a range of aliphatic amines. The few exceptions to this generalization are most probably derived from organisms such as dinoflagellates present in the food chain.

The type and extent of steroid content is a major distinguishing feature of different types of echinoderm. The crinoids and echinoids have been little studied, but appear to contain exclusively “classical” steroid types closely related

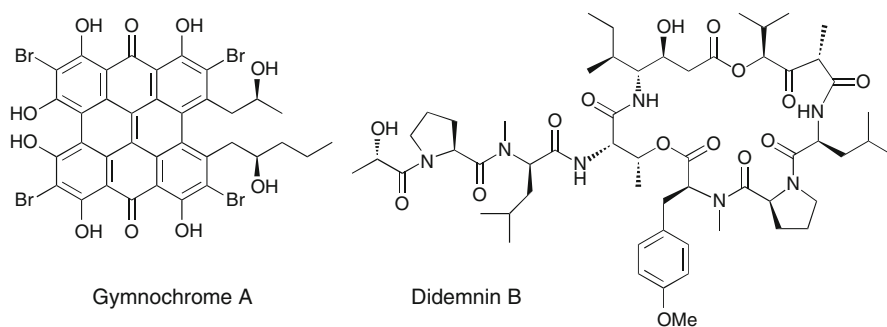


Fig. 1.11 Metabolites from Echinodermata and Ascidiaceae

structurally to cholesterol. The ophiurians, which in some respects are intermediate between the primitive crinoids and the more highly evolved echinoderms, contain many 3α -hydroxysteroids with only one or two glycosides. In the starfish, the range of glycosides and steroid sulfates is extensive, derived from a wide range of side-chain-modified parent steroids, which, however, are mostly 3β -hydroxylated. The side chain may be degraded or cyclopropanated. Polyhydroxylation/*O*-sulfation of the steroid nucleus, which is widespread in marine organisms, is at its most extensive here. The glycosides, such as the asterosaponins play a role in chemical defense through their surface-active properties. Both pentose and hexose residues are found. The holothurians contain many steroids, some biosynthesized de novo from acetate via lanostane triterpenes, others apparently derived from the diet. In *Holothuria*, it has been shown that two biosynthetic routes operate, one via lanosterol and the other via parkeol. The chief distinguishing feature of the holothurians, however, is the exclusive occurrence of specialized triterpenoid glycosides of the holostane type. Examples of the anthraquinonoid pigments are readily extracted from crinoids. Some of these are *O*-sulfates. The sea urchin pigments however are exclusively naphthoquinonoid. The number of alkaloid-like compounds isolated from echinoderms is extremely limited.

Arthropoda (Crustaceans)

The crustaceans, including the decapods, are the only type of animals in the vast arthropod phylum that occur to any extent in the sea. In the past the crustaceans have been considered a separate phylum, but it should be noted that the majority of taxonomists, including the *Catalogue of Life*, now assign the crustacea as a major subphylum of the arthropoda rather than as a separate phylum as they have in common the hard chitinous exoskeleton and the body divided into head, thorax, and abdomen. About 45,000 species are known, some of them (ostracods, e.g., *Cypridina*, and copepods, e.g., *Calanus*) very small planktonic organisms. The best known large species are the decapods (crabs and lobsters). Chitin, derived industrially from crab shells, is an important industrial material.

Chemical studies on crustaceans have been fragmentary and are mostly confined to their carotenoid pigments, some of which have also been obtained from other marine organisms but which were originally characterized as crustacean products. The molting hormones of crustaceans are terpenoid (methyl farnesate) and steroidal. Steroid studies have been fragmentary and have indicated a preponderance of cholesterol and closely related compounds.

Hemichordata

This is a numerically limited class of animal (about 100 species recorded), consisting of two surviving types of organisms; the acorn worms (Enteropneusts) inhabiting temperate and tropical waters, and the pterobranchs, colonial animals inhabiting chitinous tube galleries and found in polar waters. There is differentiation of the body into three well-defined zones, and they have some, but not all, of the morphological characters that define the chordates. Two types of natural products have been identified from them. The first is a range of toxic cyclohexanes,

halogenated phenols, and halogenated indoles isolated from *Ptychodera*, *Balanoglossus*, and *Glossobalanus* spp. These are used by the worms as defense chemicals and are of environmental significance. Of greater biochemical interest are the highly cytotoxic disteroidal metabolites the cephalostatins from *Cephalodiscus gilchristi* [120]. Owing to the difficulty of culturing hemichordate species, there is no information currently available concerning their possible distribution elsewhere in the phylum, or on their biosynthesis.

Chordata; Ascidiaceae (Ascidians, Tunicates)

These simplest chordate animals, also known as the protochordata, are generally divided into two unequal groups the urochordates (which include the ascidians or tunicates) and the cephalochordates which are free-swimming bilaterally symmetrical animals (*Branchiostoma*). Protochordates are the most developed of the invertebrates and have a notochord that is the evolutionary precursor of the spinal column characteristic of the vertebrates. They are exclusively marine. The larger group of urochordates is divided into three classes. Of these, two, including the free-floating salps, have been little investigated. Most chemical studies have been on the third group, the sessile ascidians [121]. These are filter feeders, often harboring commensal cyanobacteria and other organisms that may be the true source of some of the reported natural products. Their chemistry is dominated by the presence of an extraordinary range of mostly biologically potent nitrogen compounds. The ascidians also famously accumulate vanadium to a very high concentration in specialized cells, and some have highly acidic tissues (down to pH 1). The epidermis of ascidians contains a range of sulfated glycans, including some unusual residues such as L-iduronic acid, and the unusual polysulfated polymannose kakelokelose [122]. The membrane lipids have been little studied, and presumably are close to those of other higher organisms in structure. A few oxylipins have been isolated, along with ceramides and cerebrosides. These appear to be widespread as in the echinoderms, with some unusual types such as didemniserinolipids isolated [123]. Ascidians contain a range of carotenoids, both common ones and some rarer examples probably derived from metabolic alteration of more common carotenoids present in the filtered plankton. The steroids so far identified lack the wide structural range shown by the echinoderms and are mostly straightforward cholestanes and ergostanes, with some 5,8-epidioxysteroids and secosteroids. Nonnitrogenous secondary metabolites are few in number and terpenoids are rare. Several series of meroterpenoids have been isolated, especially from the Polyclinidae. The ritterazines from *Ritterella* have disteroid structures linked by a central pyrazine ring [124], showing close structural similarity to the cephalostatins from hemichordates [125].

Among the nitrogenous metabolites, many of which doubtless also spring from commensal organisms, there is a very wide range of structure including some with, and some without, analogies in other phyla. Firstly, there is an extensive range of modified peptides and depsipeptides. Several series of these are macrocyclic thiazoles and oxazoles, for example, the patellamides [125, 126]. Other cyclic depsipeptides are more strictly peptide related, although containing a range of unusual aminoacids.

The most studied have been the *didemnins* and their relatives [127, 128]. Heterocyclic alkaloids are numerous and include the extensive range of pyrroloisoquinolines, the lamellarins which were first isolated from molluscs (*vide supra*), but which are the products of ascidians on which they prey, or possibly of a symbiotic association involving sponges [113, 129]. Quinoline alkaloids are represented and the basic quinoline ring system is further elaborated into pyridoacridines. These too are also found in other marine organism classes. In the ascidians they fulfill the role of pigmentation and are also mostly cytotoxic. Indole alkaloids are also numerous, again known mostly as polycyclic condensed systems. Ascidians also contain a wide range of sulfur compounds, both sulfur-heterocycles and polysulfides.

Chordata; Actinopterygi (Fish)

The fish represent the most numerous of marine vertebrates. They are well studied taxonomically and extensively documented in the online database *Fishbase* (a contributor database to the *Catalogue of Life*). They can be divided into cartilaginous fishes (e.g., sharks), and the larger category of bony fishes, considered more highly evolved.

The cartilaginous fishes contain a wide range of polyhydroxylated nitrogenous and nonnitrogenous sterols based on 5 β -cholestane and include the important drug lead *squalamine* (see Fig. 1.12) [130]. The difference in the color of fish is due to the presence of various carotenoids and xanthophylls.

The fish products that have received the most chemical attention, apart from the lipids, are the toxins produced by various species. These may be steroidal, peptide, or alkaloid like, such as the much studied *tetrodotoxin* and its relatives from

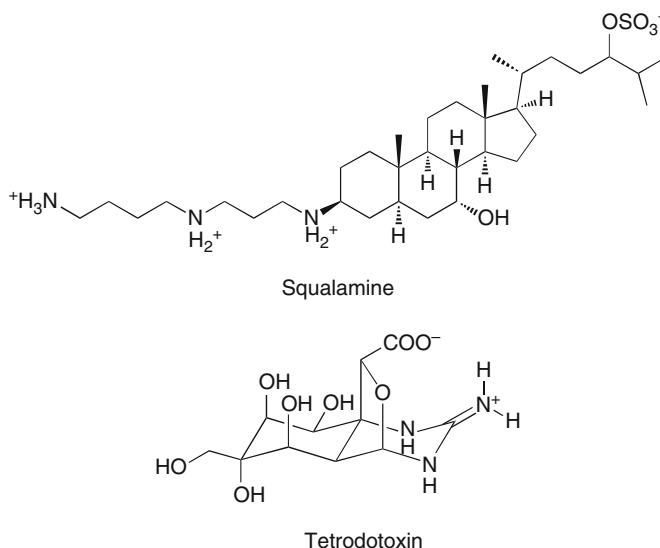


Fig. 1.12 Metabolites from Actinopterygi (fish)

fugu fish (various species of the Tetraodontidae) [131]. The latter, however, are metabolites of *Pseudomonas* bacteria or dinoflagellates in the fish, and are also found in other marine organisms and even terrestrial ones. Some fish secrete peptide venoms in specialized spines to deter prey.

1.4 Marine Taxonomy and Natural Products Chemistry

Biodiversity at the higher taxonomic levels (phyla and classes) is greater in the marine phyla. Of the 76+ phyla recognized by the *Catalogue of Life* across the Eukaryota, about 60 have marine representation compared with around 40 for terrestrial and freshwater environments. This comparison is even more extreme for the Animalia with all but one phylum out of the 30+ recognized having marine representatives (see Fig. 1.4). In contrast, species diversity is lower in the sea with just 250,000 species recognized against the >1.5 million terrestrial and freshwater species.

In looking back over the modern history of marine natural products, definite trends and preferences in the selection of phyla are discernible. To analyze these trends the database MarinLit [132] was used. This is a database of the marine natural products literature. In addition to the usual bibliographic data, the database contains an extensive collection of keywords, trivial names, compound information including structures, formulae, molecular mass, numbers of various functional groups, and UV data. All of these items can be searched for either individually or in various logical combinations. Taxonomic data are also included, permitting the exploration of relationships at various levels from genus up to phylum. The taxonomic descriptors used in MarinLit are aligned with the *Catalogue of Life*, thus avoiding the circumspection associated with alternative taxonomic schemes (cladification vs classification) and the use of pseudonyms. The data extracted from MarinLit were compared in the first instance against Bouchet's modified list of marine species [17] at the higher taxa level. The comparisons are shown in Tables 1.3 and 1.4. The criteria used were the total number of species studied in each taxon, the percentage this represented of that taxon, and the number of papers

Table 1.3 Comparative data for species studied from the higher-order taxonomic levels

Higher taxa	Number of marine species (Bouchet 17))	% of marine higher taxa	Species studied	% coverage of marine higher taxa	Number of papers by taxa	% of papers published
Archaea	>0	>0%	5	-	4	0.02%
Bacteria	5,800	2.5%	504	8.7%	1,622	8.0%
Fungi	500	0.2%	289	57.8%	612	3.0%
Protozoa	>14,800	~6.4%	192	~1.3%	929	4.6%
Chromista	7,600	3.3%	639	8.4%	1,3489	6.6%
Plantae	8,700	3.8%	1,062	12.2%	2,202	10.9%
Animalia	192,667	83.9%	4,071	2.1%	13,596	66.9%
	>230,117		6,762	~2.9%	20,314	

Table 1.4 Comparative data for marine natural products from the higher-order taxonomic levels

Higher taxa	Number of marine species (Bouchet [17])	% of marine taxa	Species producing new compounds (number, %)	Number (%) of compounds isolated	Number (%) of papers on new compounds
Archaea	>0	>0%	2 (?%) ^a	24 (0.1%) ^b	2 (0.03%) ^c
Bacteria	5,800	2.5%	201 (3.5%)	1,178 (5.9%)	545 (7.0%)
Fungi	500	0.2%	196 (39.2%)	969 (4.8%)	369 (4.7%)
Protozoa	>14,800	6.4%	60 (0.4%)	308 (1.5%)	193 (2.5%)
Chromista	7,600	3.3%	182 (2.4%)	1,231 (6.1%)	467 (6.0%)
Plantae	8,700	3.8%	282 (2.1%)	1,975 (9.9%)	798 (10.3%)
Animalia	192,667	83.9%	1,941 (1.0%)	14,372 (71.7%)	5421 (69.6%)
	>230,117		2,864 (~1.25%)	20,057	7,795

published along with the relative percentage contribution to the overall output of papers. Some 84% of all marine species belong to the Animalia. The observation that 4,071 of the 6,762 marine species studied belong to the Animalia is in keeping with this dominance of the Animalia among the marine species. The actual number of species studied is probably higher than this as many of the samples studied were identified to the genus level only. The 129 citations of *Laurencia* sp. or the 132 citations of *Streptomyces* sp. have each been counted as only one in the list of species studied, even though they might represent many different species. However, this will be offset as many will be identical to samples fully identified to the species level. The number of papers published by taxon is generally in step with the relative numbers of species/taxon with 13,596 of the 20,314 papers published up to early 2009 having an Animalia focus. Despite the focus on the phyla of the Animalia, the relative percentage coverage of this taxon (2.1%) is noticeably lower than for most of the other taxa, except the Archaea.

Many aspects of MNPs are covered in MarinLit. These include first syntheses of MNPs, assignment or correction of stereochemistry, environmental studies, as well as the isolation of new natural products, and these are the data covered in Table 1.3. The isolation of new natural products is the focus of Table 1.4. Studies on 2,864 species resulted in the isolation of 20,057 compounds reported in 7,795 papers. These are the data to early 2009. Again the actual number of species is not totally reliable as many samples were reported to the genus level only. Not surprisingly the major focus has once again been on the Animalia as the prime source of new compounds (14,372). There is a good correlation between the papers published and the numbers of compounds isolated. Despite the focus on Animalia the overall percentage of species in this taxon yielding new compounds is only 1%.

The data outlined in the Tables shows the output and general emphasis over the past 75 years in MNP chemistry, but to get to the detail it is necessary to examine the data at a phylum level. These data are portrayed in Tables 1.5 and 1.6. For some taxa, Bouchet's breakdown [17] was not detailed enough and the added phyla are shown in italics as, for example, in Table 1.5, Actinobacteria, etc.

Table 1.5 Comparative isolation and publication data for the Archaea, Bacteria, Fungi, Protozoa, Chromista, and Plantae taxa

Taxa	Marine species	Species with new compounds	Species studied	Compounds isolated	Papers with new compounds	Total papers
<i>Archaea</i>						
Euryarchaeota	–	2	3	24	2	4
	>0	2	3	24	2	4
<i>Bacteria</i>						
Bacteria	4,800	128	295	571	283	699
<i>Actinobacteria</i>		59	116	348	167	357
<i>Bacteroidetes</i>		8	15	16	8	25
<i>Firmicutes</i>		8	13	51	25	60
<i>Proteobacteria</i>		53	151	156	83	257
Cyanophyta	1,000	73	209	607	262	923
	5,800	201	504	1,178	545	1622
<i>Fungi</i>						
Fungi	500					
<i>Ascomycota</i>		195	278	968	368	605
<i>Basidiomycota</i>		1	5	1		4
<i>Zygomycota</i>			6		1	3
	500	196	289	969	369	612
<i>Protozoa</i>						
Ciliophora	?	7	11	27	10	18
Sporozoa						
Phylum not assigned	?					
Class Sporozoa						
Dinophyta (Dinomastigota)	4,000	52	177	278	182	905
Euglenozoa	250	1	3	2	1	5
Radiolaria		–	–	–	–	–
Phylum not assigned	550					
Class Sarcomastigophora						
Foraminifera						
Phylum not assigned	10,000					
Class Granuloreticulosea						
	>14,800	60	191	307	193	928
<i>Chromista</i>						
Ochrophyta	1,600	161	456	1166	437	1120
Class Phaeophyceae						

(continued)

Table 1.5 (continued)

Taxa	Marine species	Species with new compounds	Species studied	Compounds isolated	Papers with new compounds	Total papers
Ochrophyta	500	18	174	57	27	222
Class						
Chrysophyceae						
<i>Labyrinthista</i>		1	3	3	1	2
<i>Oomycota</i>		–	2	–	–	2
Haptophyta	500	1	1	3	1	1
“Bacillariophyta”	5,000	1	3	2	1	2
	7,600	182	639	1,231	467	1,349
<i>Plantae</i>						
Chlorophyta	2,500	60	305	275	113	541
Magnoliophyta	50	26	73	125	43	98
Rhodophyta	6,200	195	682	1,574	641	1,562
	8,750	281	1,060	1,974	798	2,201
Totals	37,450	922	2,683	5,683	2,371	6,712

For the taxa from Archaea through to Plantae, shown in Table 1.5, there are 37,450 species of which 2,683 have been studied at one level or another, some 5,683 compounds have been isolated, 2,371 compound structural papers published, and a total of 6,712 papers refer to these species in one way or another. These seven taxa represent just over 16% of the known/recognized marine species, but just over 7% of these recognized species have been examined with the bulk of this work having been accomplished over the past two decades.

The taxon Animalia, covered in Table 1.6, portrays a somewhat different picture.

This one higher taxon, the Animalia, represents 84% of the established marine species and comprises 30 phyla (Table 1.6), although the actual number of phyla is variable from 30 to 33 depending on the taxonomic authority consulted. Of the 29 phyla with recognized marine representatives, only 13 have been sampled over the years by natural product chemists (Table 1.6). In all 4,071 species out of a total of 192,667 (2.1%) of the Animalia have been examined. Of these 1,554, or 38%, have come from the Porifera alone, another 806 (20%) from Cnidaria, 587 (14%) from Mollusca, and 407 (10%) from Echinodermata. So, 82% of the Animalia species examined have come from just four phyla. Furthermore, about half of the compounds isolated from the Animalia have been of Porifera origin (7,024 out of 14,372) and the phylum accounts for one third of all publications across all branches of marine natural products chemistry. As only 5,683 compounds came from all the other higher taxa combined, this one phylum, the Porifera with an estimated 5,500 species, has been the origin of about 30% of all of the MNP chemistry. In keeping with the isolation and publication rate, the percentage

Table 1.6 Comparative isolation and publication data for the Animalia

<i>Taxa</i>	Marine species	Species with new compounds	Species studied	Compounds isolated	Papers on new compounds	Total papers
Animalia						
Acanthocephala	600	–	–	–	–	
Annelida	12,148	14	52	48	25	90
Arthropoda	47,217	9	77	11	10	88
Brachiopoda	550	–	2	–	–	2
Bryozoa	5,700	28	77	172	75	334
Cephalorhyncha	156	–	–	–	–	
Chaetognatha	121	–	–	–	–	
Chordata	21,517	184	475	957	396	1,513
Cnidaria	9,795	415	806	3,909	1,307	2,545
Ctenophora	166	–	–	–	–	
Cycliophora	1	–	–	–	–	
Echinodermata	7,000	205	407	1,172	446	852
Echiura	176	–	–	–	–	
Gastrotricha	395	–	–	–	–	
Gnathostomulida	97	–	–	–	–	
Hemichordata	106	3	18	29	12	68
Kamptozoa	170	–	–	–	–	
Mesozoa	106	–	–	–	–	
Mollusca	52,525	201	587	1,046	459	1,729
Nematoda	12,000	2	7	2	3	9
Nematomorpha	5	–	–	–	–	
Nemertea	1200	–	1	–	–	7
Onychophora	0	–	–	–	–	
Phoronida	10	–	–	–	–	
Placozoa	–	–	–	–	–	
Platyhelminthes	15,000	2	8	2	2	63
Porifera	5,500	878	1,554	7,024	2,686	6,296
Rotifera	50	–	–	–	–	
Sipuncula	144	–	–	–	–	
Tardigrada	212	–	–	–	–	
Totals	192,667	1,941	4,071	14,372	5,421	13,596

coverage of that phylum for chemistry has been very high with 878 out of the estimated 5,500 species having been examined for chemistry and a further 676 species implicated in environmental studies, reviews, and the like. This equates to a 28% overall coverage of that phylum.

When the outputs and the foci of MNP chemistry are examined, it is obvious that right from Bergman's first papers [21–27] MNP chemistry has been *Porifera-centric*. Is there an anthropomorphic bias to these results? Undoubtedly! There was no doubting the enormous academic and medicinal chemistry interest in

Bergman's discovery of the arabino-pentosyl nucleosides. It was from this discovery that the development of the nucleoside-based drugs ara-A, ara-C, and AZT could be traced, and this development has been well documented [133, 134]. The 1967 symposium held in Rhode Island with the ambitious title *Drugs from the Sea* [135] promoted greater interest in the field and raised the promise for other potential pharmaceuticals. Perhaps the mindset was that the Porifera must hold further secrets? Certainly the phylum Porifera has fascinated and held the interest of researchers from the 1950s onward. Every year 25–30% of all MNP publications refer to this phylum. This same Porifera-centric selection showed up in the marine component of the collections of the Natural Products Branch of the NCI's Developmental Therapeutics Program undertaken between 1986 and 2004. From the 9,945 individual organisms tested no less than 4,600 were sponges [136]. Again, this is a figure that is out of all proportion to the species-abundance of this phylum and strongly supportive of anthropomorphic bias in the collection process. This is not surprising as sponges are well represented in depths readily accessible by snorkeling or SCUBA diving, are often colorful and easily spotted underwater, quite varied in structure, and range in size from thin encrusting films (not usually collected) through a very convenient size range for collection in enormous vases and cups.

There have been different foci of interest at different points in time and this too was possibly a result of different collection techniques as well as advances in separation technologies. For example, during the 1960s and 1970s there was about as much interest in the red algae (Rhodophycota) as there was in the sponges. In the semi-decade 1974–1978, publications on red algae ran at about the 20% mark compared with 25% for sponges [132]. This then steadily dropped down over the years to the current level of about 5%. Interest in the brown algae (Ochrophyta) shows a similar pattern (10% decaying to about 4%). Both the red and the brown algae are easily accessible by shore-wading and not reliant on snorkeling or SCUBA diving, so early focus on red algae can probably in part be ascribed to ease of collection as chemists first started to explore the marine environment. Another readily collected phylum is the Mollusca which has featured in around 7% of all publications each year since the early 1960s [132].

The type of chemistry that has been published through the years also shows definite influences. The metabolites published in the early years tended to be nonpolar, lower molecular weight compounds, in keeping with the chromatographic practices of the 1960s and 1970s, for example, halogenated monoterpenes and sesquiterpenes from red alga. In Faulkner's first review of marine natural products chemistry covering the period 1974–1976 [137], >90% compounds covered were nonnitrogenous and nonpolar in character. This percentage rapidly decreased with the advent of new chromatographic techniques and chromatographic phases in the 1970s and 1980s that were better suited to dealing with polar compounds. Simultaneously, greater emphasis was being placed on biological activity. In keeping with Lipinski's rules [138] such compounds are polar molecules, so the range and type of metabolites sought, isolated, and characterized was changing from less polar to more polar metabolites.

Another advantage of shore-wading had been the ability to collect large samples relatively easily. Initially snorkeling and then SCUBA diving markedly expanded the range of species available, but necessarily limited the scale at which collections could be made. But, introduction of new techniques such as ESMS and progressive changes in NMR techniques and field strengths saw marked reductions in the amount of compound required and significant increases in the complexity of the molecules that could be successfully studied. This effectively allowed work on much smaller field samples, perhaps at the 10 g scale rather than a kg scale with a marked expansion of collection options as very small samples only needed to be collected. For example, effective sampling from the myriad of thin, encrusting invertebrates that compete for space on a cliff face. Other, more recent advances in NMR technologies has seen the introduction of capillary probes [139] and small diameter cryoprobes [140] leading to NMR spectroscopy on the nanomole scale [141]. This has effectively reduced the field sample requirement to 50 mg or less [142]. These sources of bias need to be borne in mind when considering how the field of MNP chemistry developed and why it evolved as it has.

1.5 The Results Achieved

Terrestrial natural products chemistry has traditionally been the source of the modern pharmacopeia: the expectations for the marine counterpart were not dissimilar. Despite that amazing start provided by Bergman, the realization of the potential of marine natural products has been a long time in coming. Through the years many other potently bioactive compounds have been identified. Few have been developed, but many are serving useful roles as biomolecular probes. The first marine natural product to be marketed as a drug was ω -conotoxin MVIIA, in 2004 under the trade name Prialt, for the treatment of intractable pain. ω -Conotoxin MVIIA had originally been isolated from the Pacific marine snail *Conus magus* [114]. This development was followed in 2007 by the approval of Yondelis for soft tissue sarcomas. Yondelis, or ET-743, is a fused tris-isoquinoline isolated from the Caribbean tunicate *Ecteinascidia turbinata* [143, 144]. Until about 12 years ago the bioactivity most sought after was anticancer. Since then a much wider range of selective bioassays have been deployed and more often than not the natural products chemists have been working in collaboration with pharmacologists to achieve these results. Recent reviews have highlighted marine natural products of biological relevance [145–148]. Of particular interest is the source organism for these compounds. The largest collection and screening effort on marine organisms has been that carried out by the NCI [136]. Approximately 12,000 marine samples were collected from the Caribbean and the Indo-Pacific, and extracts from 9,945 individual organisms were tested in the NCI human cancer one dose/60-cell-line prescreen. Close to 50% of these samples were from the phylum Porifera. From this assembly, 620 organisms across 12 phyla were considered active with 407 being of sponge origin. When the data were reduced to a percentage “hit-rate,” eliminating the Porifera-centric character of the collection strategy, other marine phyla with

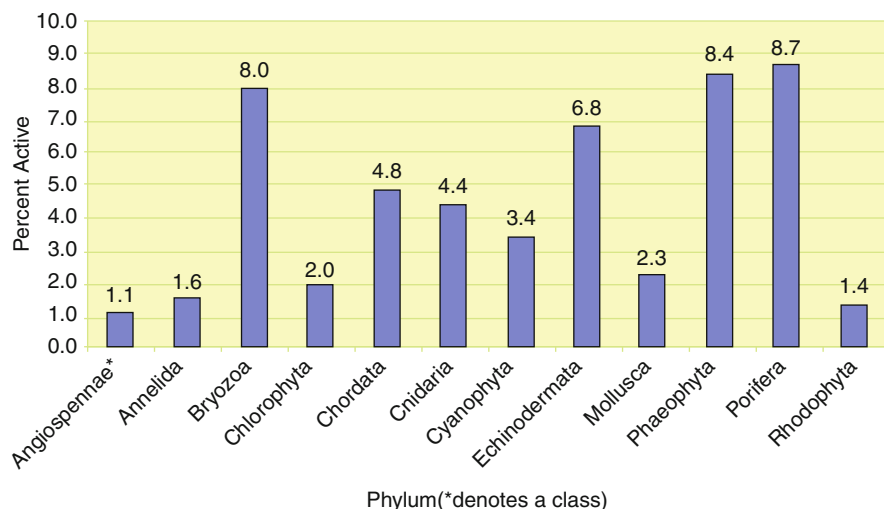


Fig. 1.13 Number of antileukemia “active” marine specimen extracts from marine phyla expressed as a percentage of the total number tested (Reproduced, with permission, from [136])

biological potential were revealed (see Fig. 1.13), suggesting that collection strategies in the future should be modified so as to be better balanced and include a wider range of marine phyla.

Over the past 30 years, many bioactive compounds have been isolated from marine organisms and a number have undergone extensive biological assessment and some are currently progressing through phase trials. These are potential candidates to join Prialt and Yondelis as drugs of marine origin. Others have been tested and for one reason or another were not suitable for advancement. A list of 40 bioactives and their biological origins has been compiled [73, 149–187] (Table 1.7 and Figs. 1.13 and 1.14) and serves to highlight the importance of marine phyla other than Porifera, if bioactivity is a consideration.

1.6 Macro or Micro: Which is the Producer?

Although isolated from macroorganisms such as sponges, bryozoans, or ascidians, it is by no means always certain that the parent organism is the producer of the (bioactive) compounds. Much progress has recently been made in assigning a definite microbial origin to many natural products. Take the case of swinholide A: originally isolated from the sponge *Theonella swinhoei*, centrifugation of macerated cell samples from the sponge showed that the macrolide was located within heterotrophic unicellular bacterial cells, but was absent from the sponge cells themselves and the co-occurring cyanobacterium *Aphanocapsa feldmanni* [188]. More recently, however, swinholide A has been found in field collections of

Table 1.7 A selection of bioactive marine compounds and their source phyla

Name	Phylum
Marinomycins [149]	Actinobacteria
Saliniketol A/B [150]	Actinobacteria
Salinosporamide A [151]	Actinobacteria
Thiocoraline [152]	Actinobacteria
Bryostatin 1 [100]	Bryozoa
Ascididemin [153]	Chordata
Aplidine [128]	Chordata
Diazonamide A [154]	Chordata
Didemnin B [127]	Chordata
<i>Yondelis</i> [143, 144]	Chordata
Meridianins [155]	Chordata
Vitilevuamide [156]	Chordata
Squalamine [157]	Chordata
Eleutherobin [158]	Cnidaria
Pseudopterosin [159]	Cnidaria
Apratoxin E [160]	Cyanophyta
Coibamide A [161]	Cyanophyta
Cryptophycins [162, 163]	Cyanophyta
Curacin A [164]	Cyanophyta
Largazole [165]	Cyanophyta
Symplostatin 1 [166]	Cyanophyta
Dolastatin 10 [167]	Mollusca
Indirubins [168]	Mollusca
Kahalalide F [73]	Mollusca
<i>Prialt</i> [114]	Mollusca
Spisulosine [169]	Mollusca
Bengamide [170, 171]	Porifera
Dictyodendrin [172]	Porifera
Dictyostatin [173]	Porifera
Discodermolide [174, 175]	Porifera
Dysidiolide [176]	Porifera
Halichondrin B [177]	Porifera
Hemiasterlin [178]	Porifera
Jaspamide [179, 180]	Porifera
Laulimalide [181]	Porifera
Nakijiquinone [182]	Porifera
Peloruside [183]	Porifera
Phorboxazole [184]	Porifera
Salicylhalamide A [185, 186]	Porifera
Variolin [187]	Porifera

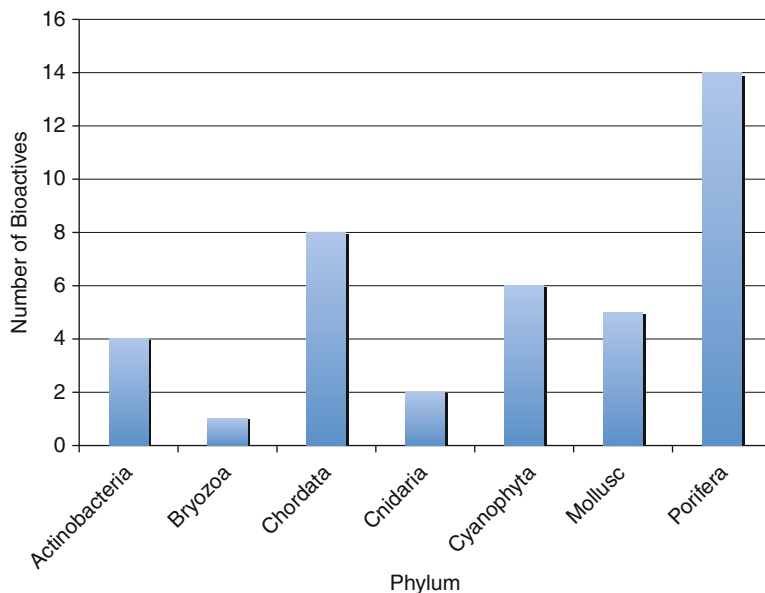


Fig. 1.14 The source phyla for a range of 40 bioactive marine metabolites

cyanobacteria [189], and it has been speculated that it may be produced by one component of this symbiont system, and stored by another [51, 189]. A comparable suggestion came from work on the phorbaxazoles and the phorbaxides with the comment that “they may actually be produced by cyanobacteria that either live within the host, or expressed from microbial genes that have been integrated into the host sponge.” [145]. Molecular genetics techniques were used to convincingly show that the patellamides A and C are biosynthesized by *Prochloron didemni*, the cyanobacterial symbiont that is hosted by the sponge *Lissoclinum patella* [190]. Molecular techniques also established that the bryostatins are likely produced by an unculturable endosymbiotic γ -proteobacterium “*Candidatus Endobugula sertula*” that is transmitted vertically between generations [101].

Natural products isolated from the higher marine organisms can also have a dietary origin. A good example would be dolastatin 10, isolated originally from an Indian Ocean sea hare (Mollusca) with a microbial origin suspected. The subsequent isolation of simplostatin, differing from dolastatin by a methyl group, from a cyanophyte suggested a possible Cyanophyta origin, which was subsequently confirmed by the isolation of dolastatin 10 from a *Symploca* species [166, 167, 191].

In some cases definite de novo biosynthesis of some natural products has been demonstrated, but it may also be true that although a particular metabolite isolated from a higher animal has not yet been found among the lower forms such as cyanophytes in the same ecosystem, this is merely an accident of the search process.

From a bioprospecting perspective the parent organism will remain the object of primary interest, but for development of a potential lead, the matter of supply is a vital question that must be answered right at the outset. It is at that point that questions on symbiont versus host-cell origin need to be answered, not during the search of the marine environment for new (bioactive) compounds.

1.7 Marine Biodiversity: Into the Future

The currently accepted number of marine species is of the order of 230,000–275,000 species and there are about 1,300–1,750 new species being added to that inventory each year, but these are mainly in the area of the Mollusca and Crustacea (Arthropoda). There is an enormous gulf between that accepted value and the estimated number of species which runs as high as 100 million depending on the authority consulted. Chapman has suggested a total of 11 million with dramatic rises in the invertebrates, most notably the arthropods, the fungi, bacteria, chromista, and protozoa with estimates in some of these taxa rising by several orders of magnitude [13]. Knowledge of the potential species remaining to be described is increasingly dependent on culture-independent molecular techniques. Although applied primarily to explore the diversity of Archaea and Bacteria assemblages, these techniques have not been restricted to prokaryote domains. The suggested 99,000 species of fungi [13] (described 45,000 [9]) is likely to rise to 1.5 million, an estimate considered “accurate” within a factor of 5 with the increase in described species likely to arise from ectoparasites and non-mycorrhizal endophytic species [192]. What proportion of these are marine is conjecture only, but Bouchet’s number of just 500 marine fungi seems very conservative.

Chromista and Protozoa diversities present uncertainties with only about 7,000 species in each taxon described (*Catalogue of Life*), but the numbers of projected species counts are listed in hundreds of thousands. Taking account of the large geographical areas still to be explored from a phycological perspective and the morphological similarities that mask genetic diversity, these suggested figures are not unreasonable. Culture-independent molecular techniques are also being widely used in studying protistan diversity [192].

Moving beyond the microbial world to the Animalia there are again important questions to ask on species biodiversity. A large part of the marine biodiversity is that of the symbionts – the commensals, associates, and parasites. How many copepods are there? How many marine helminthes? Reasoned answers suggest figures way beyond the currently accepted numbers. For example, the marine helminthes might number between 100 and 200,000 [17]. No comment has yet been made about the deep sea and what that area contributes to marine biodiversity. The deep sea was traditionally seen as a harsh environment inhabited only by those few and cosmopolitan species capable of living in the dark under enormous pressures and near freezing temperatures. But, following Grassle and Maciok’s 1992 seminal paper [193] on deep sea biodiversity, opinions have been markedly changed leading to comments such as “a riot of species in an environmental calm” [17] to describe the deep ocean and its accompanying biodiversity. Grassle and

Maciolo found 798 mainly new species of polychaetes and isopods in 233 box cores of 30×30 cm taken on a 176 km transect along a 2,100 m depth contour off the North Eastern coast of the USA. As there is 3×10^8 km² of ocean floor deeper than 1,000 m, it sparked a vigorous debate as to the actual numbers of species contributed by the deep sea ranging from 100 million downward. Until deepwater basins in the other oceans of the world are explored no consensus can be reached as to the contribution of the deep seas to overall marine biodiversity.

1.8 The “Neglected Phyla” and Marine Natural Products in the Future

Regardless of the actual numbers of marine species available, some conclusions can be reached based on the efforts over the past 75 years. Research on the phylum Porifera has dominated the effort and the subsequent chemistry. This focus has been productive and the chemistry novel and fascinating. It is not difficult to reach a conclusion for this fixation as succeeding generations of natural product chemists will naturally focus on areas that have proven successful in the past, and sponges are relatively easy to collect in good mass (>100 g), even for the inexperienced diver. This focus on sponges has led to a rather unequal coverage of the other major taxa and phyla. Some comparative data based on compounds isolated, papers published, and relative coverage of the major *macroorganisms*, which includes 12 of the phyla from Animalia, are presented in Table 1.8. The Cyanophyta have been included in this comparison as they too can be collected in bulk from the wild. Also included are the origins of 35 of the 40 compounds of interest listed in Table 1.7. Four of the remaining were of Bacteria origin and the fifth was isolated from a higher chordate (shark). Studies on the macrophyta have produced >90% of the marine natural products from just 1.4% of the accepted number of species in these phyla. These data, when coupled with the NCI’s study [136] (Fig. 1.13), suggest that studies that include Bryozoa, Cnidaria, Chordata (Ascidiaceae), Mollusca, Ochrophyta (Phaeophyceae), and Cyanophyta should be productive, particularly if the search for bioactivity is the objective. These phyla have been *neglected*. Furthermore, as no work has been reported on the other 18–21 phyla of the Animalia, one may be forgiven for coming to the conclusion that these are “forgotten” phyla.

Most of the biodiversity yet to be classified in the marine environment resides in the microscopic world of the Archaea, Bacteria, and the multicellular, but still microscopic members of the Eukaryota belonging to the Chromista and Protozoa taxa. The projected numbers of species still to be defined is estimated to be in the millions [13, 17, 52]. Despite the inherent difficulties of working with organisms from the Protozoa and Chromista, a significant body of work is evolving on the chemistry of the Ciliphora, Dinophyta, Euglenozoa, and from the Chrysophyceae (Ochrophyta), but as yet little or nothing has appeared on the chemistry of species in the Bacillariophyta, Radiolaria, and Foraminifera (Table 1.5). The chemistry of obligate marine, or at least marine-derived, fungi is of rapidly growing interest within the MNP community and already 289 species have been examined for new

Table 1.8 Comparative data for marine natural products from the macroorganisms

<i>Taxa</i>	Marine species	Species with new compounds	Coverage of phylum (%)	Compounds (% total)	Bioactive papers ^a (%)	Compounds of interest
Animalia						
Annelida	12,148	14	0.12	0.24	100	–
Arthropoda (Crustacea)	44,950	9	0.02	0.05	11	–
Brachiopoda	550	0	0.91	0	0	–
Bryozoa	5,700	28	0.49	0.86	14.1	1
Chordata (Ascidiaeae)	4,900	184	3.76	4.77	65.4	7
Cnidaria	9,795	415	4.24	19.49	22.5	2
Echinodermata	7,000	205	2.9	5.84	18.8	–
Hemichordata	106	3	2.77	0.14	17.6	–
Mollusca	52,525	201	0.38	5.22	12.2	5
Nematoda	12,000	2	0.017	0.01	0	–
Platyhelminthes	15,000	2	0.013	0.01	0	–
Porifera	5,500	878	15.96	35.03	63.4	14
Chromista						
Ochrophyta (Phaeophyceae)	1,600	161	10.1	5.81%	13.1	–
Plantae						
Chlorophyta	2,500	60	2.4	1.37%	11.5	–
Magnoliophyta	50	26	52.0	0.62%	27.6	–
Rhodophyta	6,200	195	3.10	7.85%	11.0	–
Bacteria						
Cyanophyta	1,000	73	0.73	3.03%	64.9	6
	181,524	2,456		90.34%		35

^aNumber of papers reporting bioactive compounds as percentage of all papers reporting compounds for each phylum

chemistry (Table 1.5). This interest seems destined to continue, and with the predicted burgeoning of the fungal species to 1.5 million, [13] there will be enormous scope in this aspect of marine natural products. Soil bacteria, especially members of the *Streptomyces* and *Micromonospora* genera of the Actinobacteria, have been the source of over 50% of the clinically useful microbial antibiotics. The first marine actinomycete was discovered in 1984 [53]. Since then at least three marine genera have been described [54–56]. These include the genus *Salinispora* [56] which has an obligate requirement for sodium ions. This discovery by Fenical and Jensen of the first *Salinispora* sp. has been followed by the identification of at least six actinomycete families [52] including marine-derived species related to the genus *Streptomyces*. The chemistry associated with these obligate marine actinomycetes has also been remarkable with discoveries of compounds such as salinosporamide, which is currently in Phase 1 trials as a 20S-proteasome

inhibitor [194]. These metabolites being isolated from the obligate marine actinomycetes show a high degree of halogenation, a feature considered desirable for induction of biological activity [195], and in the case of salinosporamide an essential feature of the mechanism of action [196, 197].

Every milliliter of seawater contains 10^6 microorganisms with marine sediment being even richer (10^9) [52]. This is a resource beyond comprehension. The terrestrial actinomycetes have been chemically prolific. There is every reason to believe that the marine counterparts will be equally prolific. Using a combination of metagenomics, genome analysis, and selective culture techniques, the potential for discovering other genera and new chemical entities from the microscopic world is enormous. If this is coupled with a fuller exploration of the “neglected” macroorganism phyla, the future viability, stability, and promise of marine natural products chemistry is assured.

1.9 Study Questions

1. Taking a particular organism group (e.g., molluscs) make a brief study of the differing taxonomic classifications that have been proposed for this group by taxonomists.
2. Could the differing taxonomic views for such a group have had an impact on the statistics of natural product occurrence in the group?
3. For a particular organism, or a small group of organisms, ascertain what is known about the dietary regime and predation pattern and determine if that coincides with what is known about the characterized constituents.
4. (a) As an ambitious young starting academic in the field of marine natural products, what considerations should you give to the selection of organisms (phylum, class, order, family, or genus) to study that would optimize your chances of finding new compounds and thus gaining publications?
(b) Suggest the collecting resources that you would need to access to effectively pursue your organisms of choice.
(c) Besides collecting resources, list the other resources or collaborations you think would be necessary in order to be successful.

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