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Bioactive Compounds from Marine Microalgae

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Abstract. Marine microalgae are relatively unexploited but rich resources for bioactive compounds. Toxins initially isolated from fish or shellfish were found to originate from microalgae, especially dinoflagellates. These toxins are useful tools to investigate the structure and function of ion channels on cell membranes or to elucidate the mechanism of tumor promotion based on their specific inhibitory action against protein phosphatases. The number of antifungal or antitumoral substances of microalgal origin is rapidly increasing. More importantly, structural similarities have been found between many bioactives found in marine invertebrates and those in freshwater blue-green algae. The similarities point to a great potential of marine blue-greens, the least explored resource, for producing bioactive compounds of medicinal value.

1. Introduction

The history of exploring the chemical diversity of the sea began only recently, when scientists met at the 'First International Conference on Drugs From The Sea' in 1967. Since then, extensive efforts have been made in the search for substances of medicinal value, primarily antitumoral and antiviral activities. The efforts have been rewarded with the discovery of numerous bioactive substances, featuring structural novelties and extreme potencies that have not been encountered from terrestrial sources [1]. Many of the chemicals are reportedly of promise for drug development. However, the question of supply presents a serious obstacle in transforming these chemicals into commercial products as they have their origins in sponges, ascidians, bryozoans, or opisthobranches [2][3]. The most representative examples of antitumoral drug candidates are shown in *Fig. 1* [4]. The molecules are too complex to be synthesized in quantity. Nor is it possible to supply them from natural sources without threatening the vulnerable marine ecosystem. Recently, ambitious projects to cultivate sponges, ascidians, and bryozoans have been launched and are hoped to open a door for production of at least some of the substances.

Compared with the size of the effort made in the search for bioactives from

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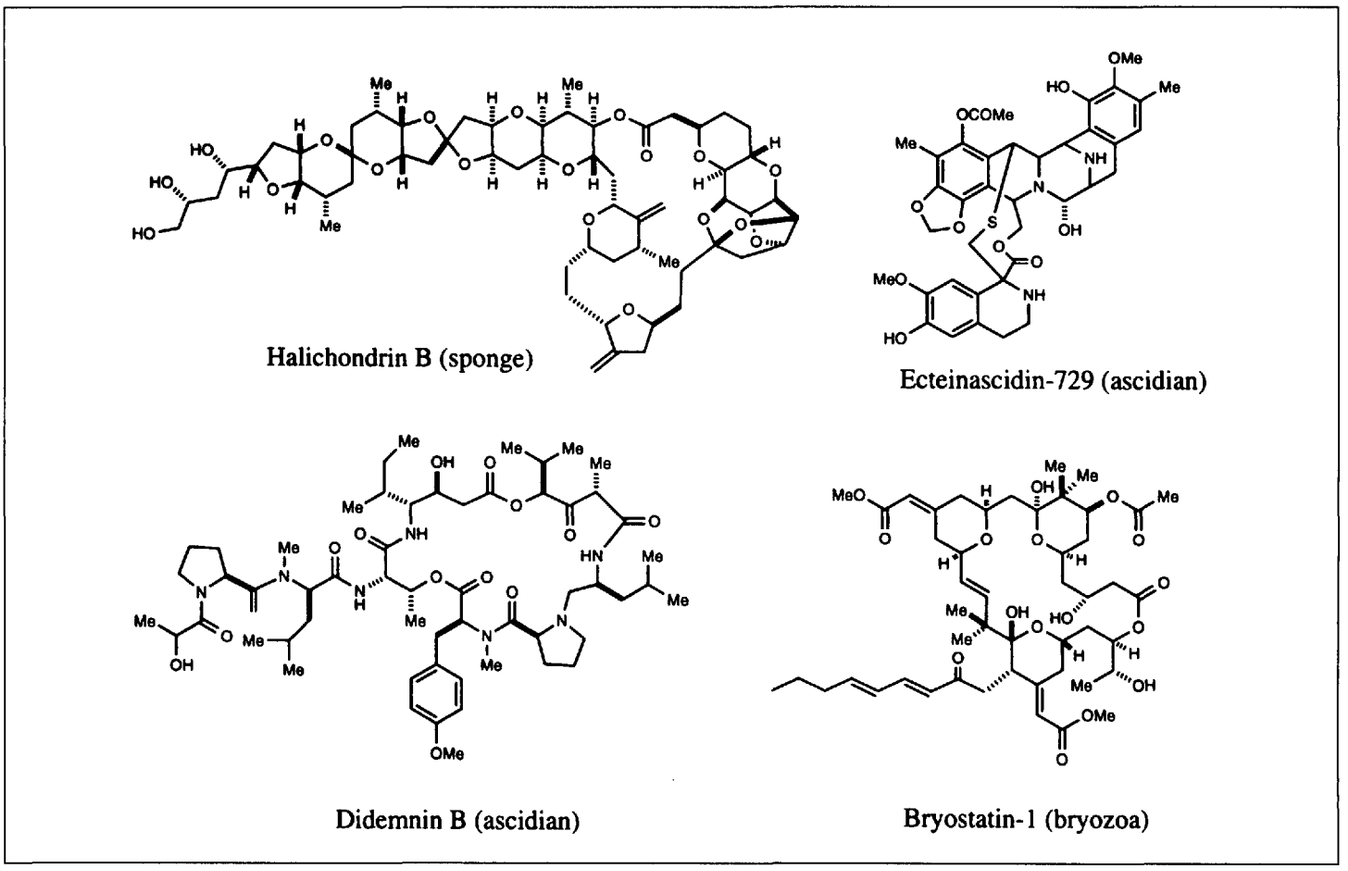

Fig. 1. *Representive lead compounds for antitumour drugs isolated from marine invertebrates*

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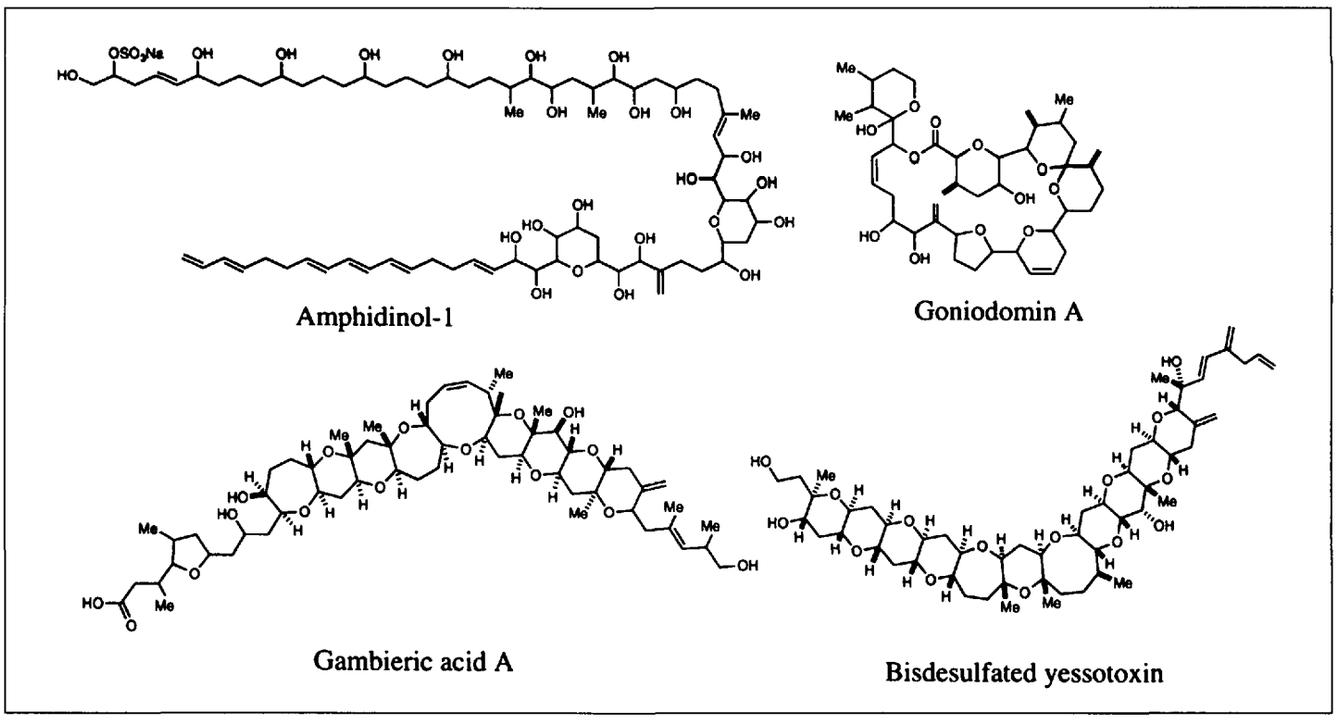

Fig. 2. *Examples of antifungal compounds of dinoflagellate origin*

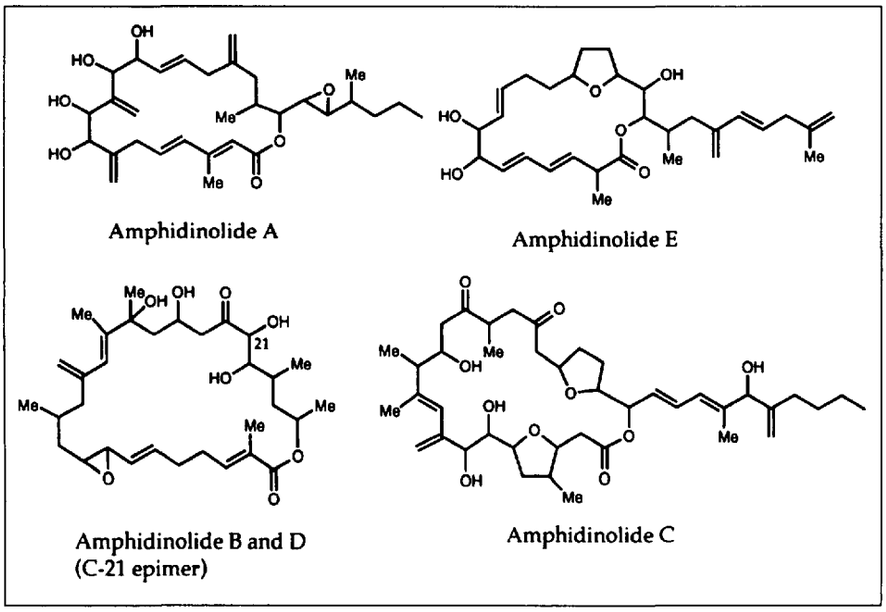

Fig. 3. *Examples of cytotoxic compounds isolated from a dinoflagellate* Amphidinium *sp.*

marine invertebrates, only a few attempts have been made to screen cultivable organisms for useful substances, probably due to the difficulty or laboriousness of culturing organisms. Scientists were also discouraged when they found that many of the bacterial products from 'marine environments' were as same as or similar to those already known from terrestrial organisms.

As the above historical aspects have already been reviewed in many articles, the authors would like to focus on the potential of marine microalgae, that are cultivable but relatively unexplored.

2. Bioactive Substances from Dinoflagellates

Dinoflagellates are a group of unicellular algae belonging to the Dinophyceae Class of the Dinophyta Division. They are important diet organisms for many zooplankton as well as for fish and shellfish. Some species form red tide blooms and cause massive fish kills. Some others produce toxins that are accumulated in fish or shellfish and cause human intoxication when these fish are consumed.

2.1. *Antifungal Compounds from Dinoflagellates*

In a systematic screening of various bloom-forming microalgae for antifungal substances, activity was detected in 7 of 12 species tested [5]. Eight of the nine active species were dinoflagellates (*Table*). Structures of some of the active substances were determined, and representative examples are shown in *Fig. 2*. Amphidinol-1 is characterized by a monosulfated polyhydroxy-polyene structure and is six times more active than amphotericin B [6]. Closely related substances, amphidinol-2 [7] and luteophanol A [8], were also reported recently from other *Amphidinium* species, suggesting a wide distribution of such compounds among dinoflagellates. Goniodomin A, a polyether macrolide isolated from *Goniodoma hiranoi*, inhibited growth of *Mortierella ramanniianus* and *Candida albicans* at a concentration of 0.5 µg/ml [9]. Gambieric acid, isolated from *Gambierdiscus toxicus*, probably is one of the most potent antifungals ever known, being 2000 times more potent than amphotericin B when compared by the paper-disk method against *Aspergillus niger* and *Penicillium funiculosum* [10]. Three analogs of comparative activity coexist in the same organism. Bisdesulfated yessotoxin is an artificial product pre-

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pared from a shellfish toxin, yessotoxin. Interestingly, eliminating the sulfate groups of the toxin resulted in the loss of acute mouse lethality and enhancement of antifungal potency to twice that of amphotericin B.

2.2. *Cytotoxic Compounds from Dinoflagellates*

A series of cytotoxic macrolides named amphidinolides A–S were isolated from a symbiotic dinoflagellate *Amphidinium* sp., associated with a marine flatworm [11]. Representative structures are shown in *Fig. 3*. The most prominent feature of these compounds is their extremely strong cytotoxicity: IC_{50} of amphidinolide B against L1210 and KB cells being 0.14 and 4.2 ng/ml, respectively. Occurrence of similar macrolides in other planktonic species is also known. The previously mentioned antifungal substances and some of the toxins that will be described later are also potent cytotoxins, suggesting a wide distribution of cytotoxic metabolites among dinoflagellates.

2.3. *Dinoflagellate Toxins*

Toxins are the most prominent and important bioactive metabolites of marine dinoflagellates. Through the food chain, they are accumulated in fish or shellfish and thus cause human intoxication. Red

Table. *Antifungal Activity of Extracts from Phytoplankton*

Phytoplankton	Fraction				
	H_2O layer	1-BuOH layer	Et_2O layer	Acetone extracts	XAD-2 MeOH
(Haptophyceae)					
Prymnesium parvum	–	–	–		–
(Euglenophyceae)					
Eutriptoella sp.	–	–	–		–
(Rahidophyceae)					
Chatonella antiqua (NIES-1)	–	+	–		–
Olisthodiscus luteus (NIES-15)	–	–	–	+	–
Heterosigma akasiwo (NIES-6)	–	–	–	–	–
(Dinophyceae)					
Alexandrium tamarense	–	+	–	–	–
Prorocentrum micans (NIES-12)	–	–	–	–	–
Scriptosiella trochoidea (NIES-369)	–	–	–	–	–
Gambierdiscus toxicus	–	+	–	–	+
Gymnodinium sanguineum (NIES-11)	–	+	–	–	–
Amphidinium carteri	–	+	–	–	–
Amphidinium klebsii	–	+	–	–	–

Test: Paper-disk method against *Aspergillus niger*.
Aliquots of the test solutions are corresponding to 100 ml of the cultures.
+: Positive; –: not detected; blank: not tested.

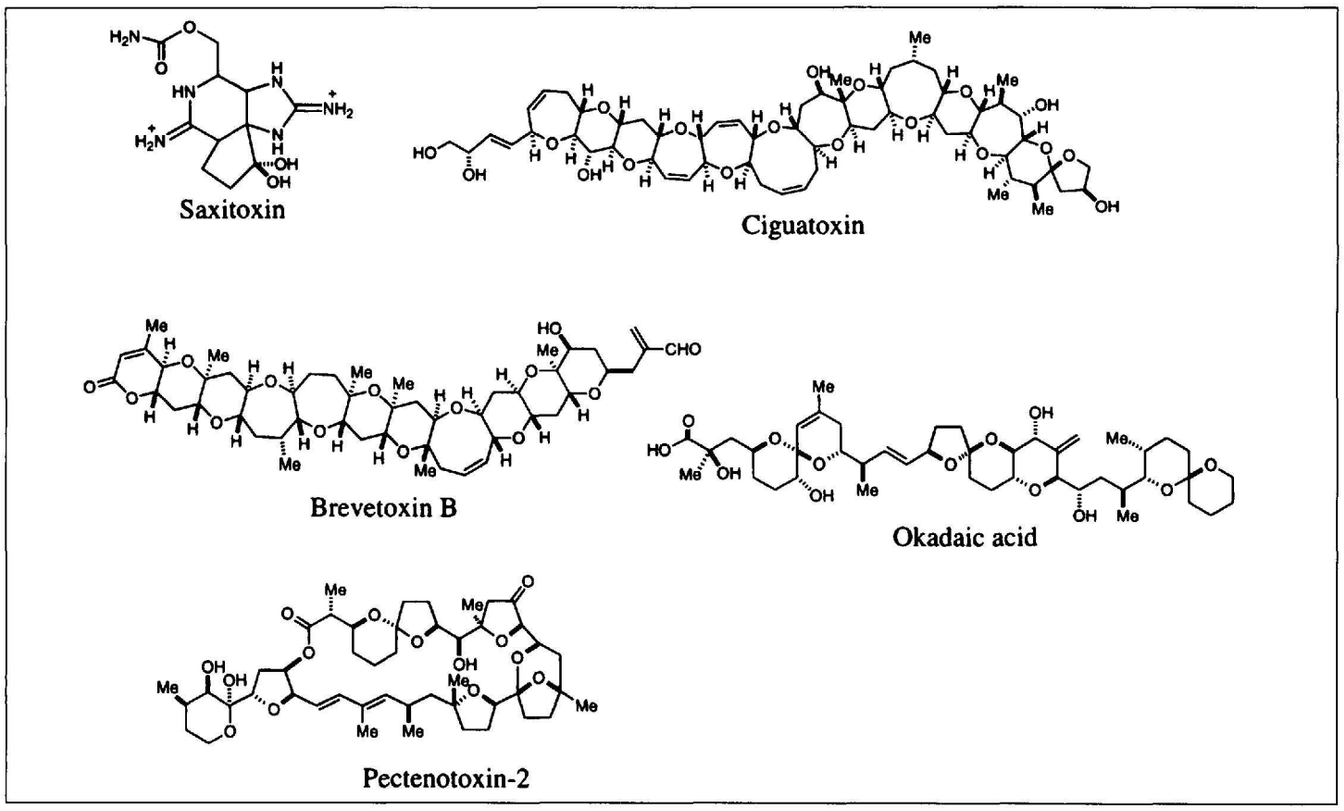

Fig. 4. *Marine toxins of dinoflagellate origin-I*

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tides of toxic species often cause devastating damage to the marine ecosystem. Chemically, toxins are the most exciting and challenging targets for natural product chemists because of their structural complexity, as exemplified by the molecules shown in *Figs. 4* and *5* [1]. The extremely potent and specific action of marine toxins make them suitable biochemical probes.

There are three groups of toxins which act on voltage-gated sodium channels: saxitoxin and its analogs, the brevetoxins, and the ciguatoxins. The saxitoxins are produced by *Alexandrium* spp., *Gymnodinium catenatum*, or *Pyrodinium bahamense* var. *compressum* and cause paralytic shellfish poisoning. Saxitoxin blocks

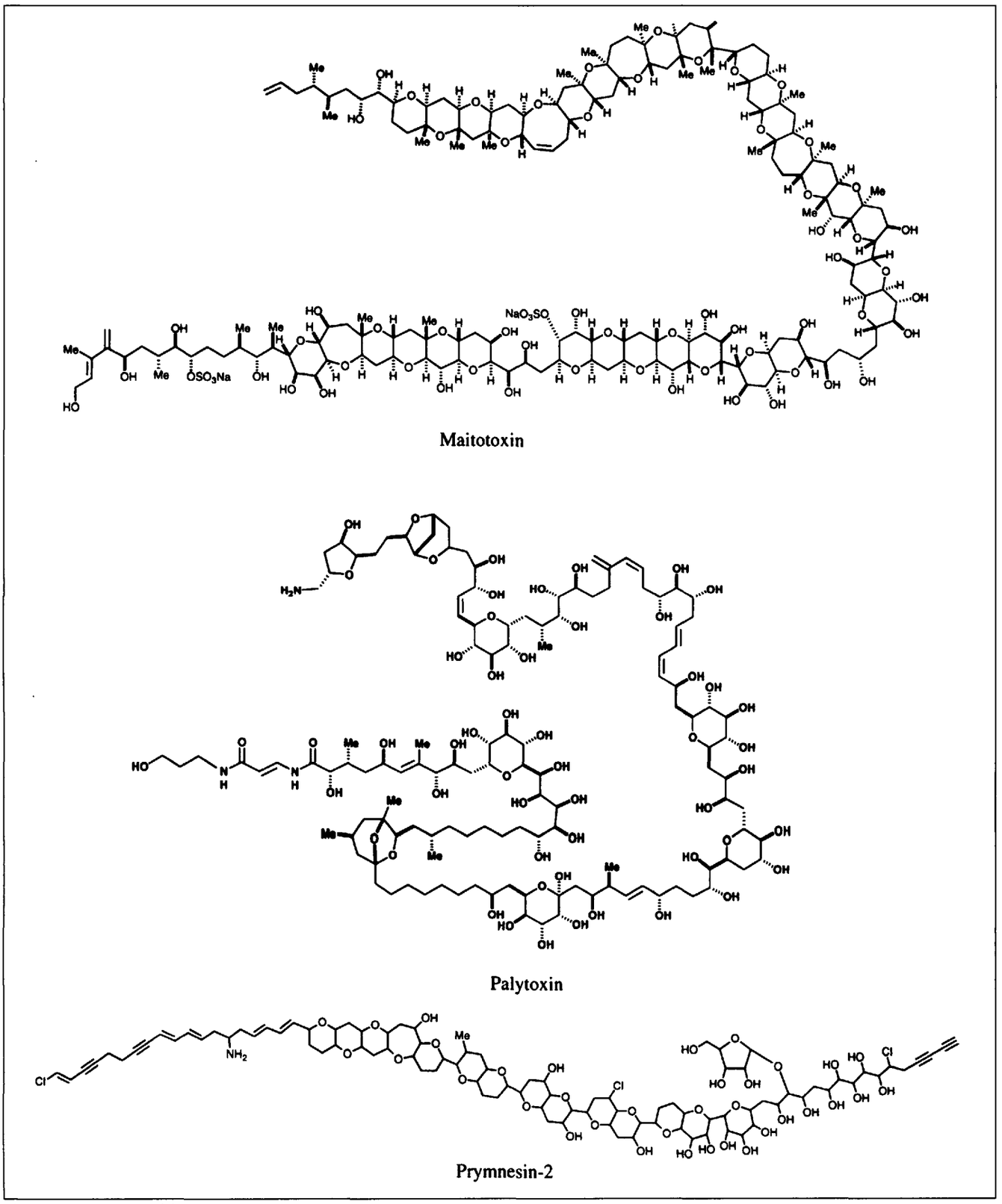

Fig. 5. *Marine toxins of dinoflagellate origin-II*

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sodium-ion influx into cells by binding the 'site-1' of the channel protein. Because of the high specificity and the ease of tritium labeling, the toxin has been extensively used to study the sodium-channel structure as well as biological phenomena mediated by the channel.

Brevetoxin B (*Fig. 4*) and its analogs, isolated from the red-tide dinoflagellate *Gymnodinium breve*, are polyether ladder toxins that are ichthyotoxic at nanomolar concentrations. Ciguatoxin (*Fig. 4*) and its analogs share the same structural features with the brevetoxins. The epiphytic dinoflagellate *Gambierdiscus toxicus* produces ciguatoxin precursors that are accumulated in many fish through the food chain, and thus causes human intoxication known as 'ciguatera'. Both brevetoxin B and ciguatoxin bind to the orphan receptor known as 'site-5' of voltage-gated sodium channels and cause inhibition of channel inactivation. These toxins make useful tools to explore the function of the channels.

Okadaic acid is a polyether carboxylic acid, first isolated from the marine sponge *Halichodria okadai* guided by cytotoxicity assays. The dinoflagellate origin of this acid and its analogs was soon revealed by the authors [1]. The most prominent feature of okadaic acid is its potent and specific action to inhibit protein phosphatases type 2A, 1, and 2B [12]. Because many biological events, including tumor promotion, are regulated by phosphorylation and dephosphorylation of signal proteins, the acid is extensively used in biochemical studies. Okadaic acid and its analogs produced by planktonic dinoflagellates *Dinophysis* spp. are accumulated by bivalve shellfish and cause worldwide human intoxication named diarrhetic shellfish poisoning. *Dinophysis fortii* produces another polyether macrolide, pectenotoxin-2, in addition to okadaic acid. Though pectenotoxin-2 shows potent cytotoxicity against cancer cells of human origin, its hepatotoxicity prohibits its potential use for therapeutic purpose.

The three toxins shown in *Fig. 5* are examples which show the extraordinarily complex structures of marine toxins. Maitotoxin is the largest (M_w 3422) and the most complex molecule so far elucidated. The lethality of the toxin to mice by intraperitoneal injection is 50 ng/kg, a value that is only exceeded by a few proteinaceous toxins of bacteria. Maitotoxin specifically induces calcium-ion influx through cell membranes. Almost all cell lines tested were sensitive to the toxin. Thus, maitotoxin has been used to explore the cell phenomena triggered by calcium-ion entry. Palytoxin is also an extremely complex and poisonous substance, first isolated from marine coelenterates (zoanthids) of the genus *Palytoa*. Its intravenous lethality ranges from 25 ng/kg in the rabbit to 450 ng/kg in the mouse. Palytoxin has been proposed to convert Na^+/K^+ ATPase into a cation-selective ion channel [13]. The toxin is produced by the epiphytic dinoflagellate *Ostreopsis siamensis*, accumulated in some species of fish and crabs through the food chain, and thus causes highly fatal poisoning [14]. Prymnesin-2, isolated from a red-tide flagellate *Prymnesium parvum*, is an extremely hemolytic as well as ichthyotoxic substance [15]. When compared on red blood cells of the dog, pyrmesin-2 was 50000 times more potent than plant saponin (*Merck*) in hemolytic activity. Prymnesin-2 is as potent as brevetoxin B in ichthyotoxicity, accounting for the massive fish kills observed in Europe and Israel in association with the blooms of this species.

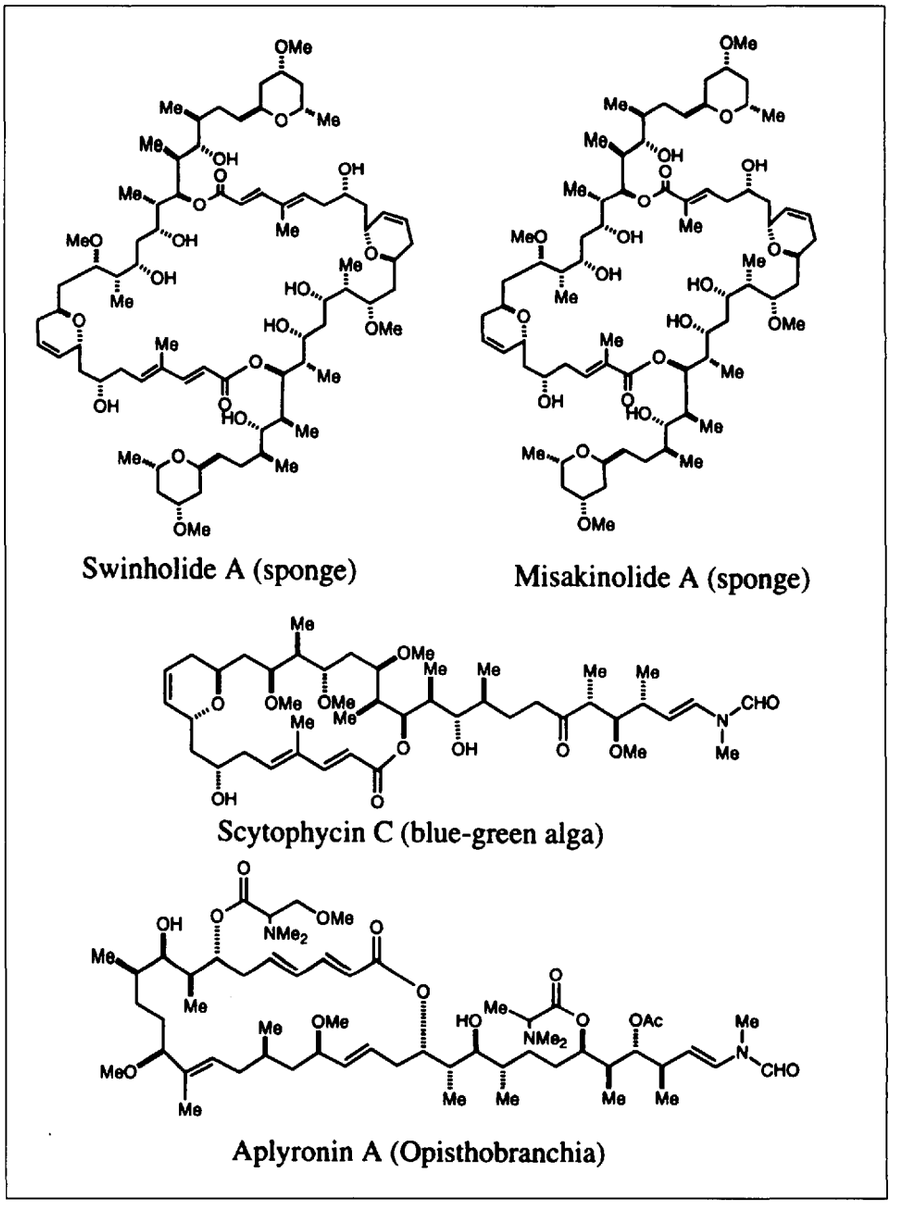

Fig. 6. *Structural resemblance between antitumour compounds isolated from invertebrates and that (Scytophycin C) from a fresh water blue-green alga*

3. Bioactive Metabolites of Blue-Green Algae (Cyanophytes)

Blue-green algae from freshwater environments have been extensively surveyed as one of the most promising sources of bioactive substances for medicinal uses. In contrast, marine species are the least explored resources, probably due to the difficulty of growing them in laboratories. In recent years, however, marine scientists have been paying more and more attention to marine blue-greens, expecting

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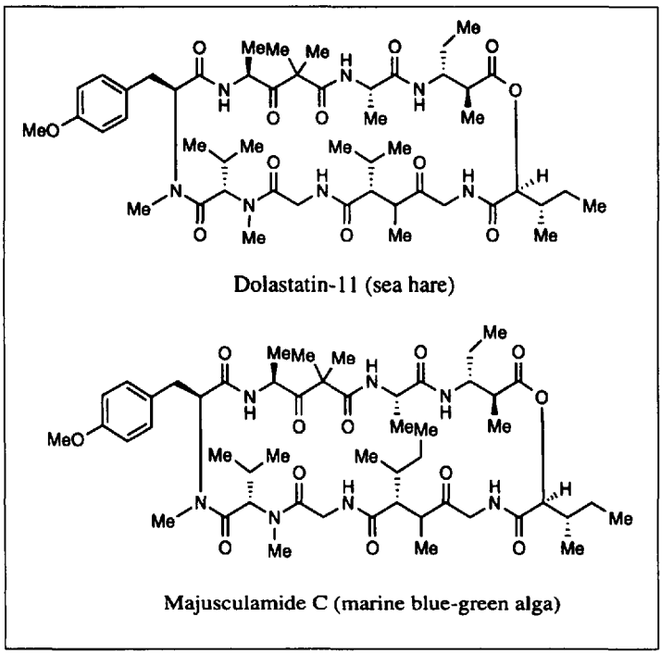

Fig. 7. *Examples of similar compounds from blue-greens and invertebrates-I*

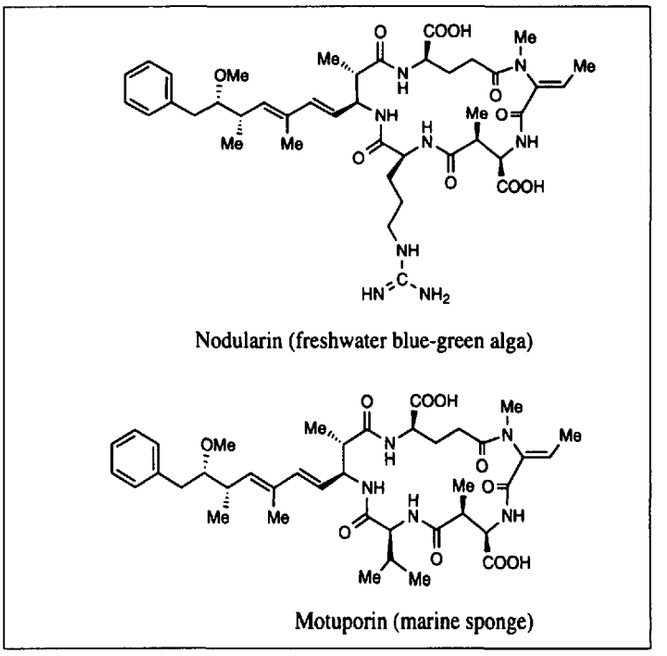

Fig. 8. *Examples of similar compounds from blue-greens and invertebrates-II*

the algae to be a rich source of medicinally useful substances. The notion is supported by the fact that many bioactive substances isolated from sponges, tunicates, or opisthobranches are strikingly similar in chemical structures to those isolated from freshwater blue-greens. Sponges may not only collect bioactives from microorganisms by filter feeding but also obtain various metabolites of microorganisms, including blue-green algae, that they harbor in their cavities or even in tissues. Typical examples are shown in *Fig. 6*. Swinholide A [16] and misakinolide A [17] are dimeric macrolide cytotoxins isolated from sponges. The structural resemblance is obvious between the macrolides of the marine sponges and a monomeric macrolide, scytophycin C, isolated from a freshwater blue-green alga [18]. The basic skeleton of a potent cytotoxin, aplyronin A, isolated from a sea hare [19], is virtually superimposable on that of scytophycin C, suggesting that the sea hare obtained the substance by feeding on macroalgae contaminated by blue-greens. We also see the close analogy between two cyclic peptides, dolastatin-11 [20] and majusculamide C [21], originating from another sea hare and a marine blue-green alga, respectively (*Fig. 7*). Likewise, nodularin from a freshwater blue-green [22] is similar to motuporin found in a marine sponge [23] (*Fig. 8*). This is only a small selection from many examples of the similarities of bioactive metabolites between invertebrates and blue-greens. Thus, we may hypothesize that some of the products found in marine invertebrate animals originate from blue-greens. At least, we may expect that marine blue-greens produce useful substances that are similar, if not the same, to those found in sponges or other marine animals. Assuming that marine species grow as fast and thick as their freshwater counterparts do, marine blue-greens may have a big advantage over dinoflagellates whose growth are slow and thin. Because blue-green algae are prokaryotic organisms, genetic engineering techniques may become applicable in future to increase target metabolites. In conclusion, the necessity of making more effort towards culturing microalgae and symbiotic microorganisms is stressed.

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