# Marine Toxins: An Overview

**Nobuhiro Fusetani and William Kem**

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**Abstract** Oceans provide enormous and diverse space for marine life. Invertebrates are conspicuous inhabitants in certain zones such as the intertidal; many are soft-bodied, relatively immobile and lack obvious physical defenses. These animals frequently have evolved chemical defenses against predators and overgrowth by fouling organisms. Marine animals may accumulate and use a variety of toxins from prey organisms and from symbiotic microorganisms for their own purposes. Thus, toxic animals are particularly abundant in the oceans. The toxins vary from small molecules to high molecular weight proteins and display unique chemical and biological features of scientific interest. Many of these substances can serve as useful research tools or molecular models for the design of new drugs and pesticides. This chapter provides an initial survey of these toxins and their salient properties.

## 1 Introduction

The world's oceans cover more than 70% of the earth's surface and represent greater than 95% of the biosphere. According to the World Conservation Monitoring Center, ~1.75 million species of living organisms have been described by systematists, but these probably represent just a fraction of the total number of existent species. Estimates of the total number occupying the Earth vary considerably, from 3.5 to 100 million species (Groombridge et al. 2000, cited in Müller et al. 2003). Because marine species are relatively inaccessible compared with terrestrial species, it is likely that currently described marine organismic diversity (<500,000 species) is greatly underestimated several-fold. The phyletic diversity of animals (and bacteria) in the marine biosphere is exceptional: all but one of the 35 principal animal phyla are represented in aquatic environments and 8 of these phyla are exclusively aquatic. Most marine invertebrates that are sessile and soft-bodied have evolved chemical means to defend against predators and overgrowth of competing species. In fact, high percentages of them are poisonous and venomous (Halstead 1965, 1967, 1970; Hashimoto 1979). It should be noted that normally non-toxic

animals can become poisonous by sequestering toxins from their prey organisms. For example, many bivalves accumulate toxins from dinoflagellates (see Sect. 3) while ciguateric fishes accumulate ciguatoxins and related toxins through food chains (see also Sect. 3 and Chap. 3). Tetrodotoxins are found in many marine organisms ranging from algae to various fishes; perhaps they are accumulated from bacteria and/or through food chains (see also Sect. 10 and Chap. 3). Opisthobranch mollusks sequester defensive substances from prey organisms including seaweeds, sponges and ascidians (Faulkner 1992). Other marine invertebrates, especially sponges and ascidians, often contain highly cytotoxic compounds that probably originate from symbiotic microbes, namely bacteria, cyanobacteria and dinoflagellates (Brewley and Faulkner 1998; Piel 2004).

A variety of marine animals possess peptides and proteins that are used either for defensive means, catching prey, or both. Two prominent examples are cone shells and sea anemones, whose venoms are complex mixtures of highly toxic peptides with different modes of action (see also Chap. 2 for discussion of the cone shell peptides). It should be also noted that antimicrobial peptides of several types are found in a wide range of marine animals (Andreu and Rivas 1998; Bulet et al. 2004).

## 2 Cyanobacteria

Freshwater cyanobacterial (blue–green algal) blooms of the genera *Anabaena*, *Aphanizomenon*, and *Microcystis* have been known to produce neuro- and hepatotoxic compounds that cause mass mortalities of birds, wild animals and livestock (van Apeldoorn et al. 2007). Though marine cyanobacteria also form blooms, these have not yet presented serious health and economic problems. It is now known that marine species, especially *Lyngbya majuscula*, produce an amazing variety of bioactive metabolites, including highly cytotoxic compounds (Gerwick et al. 2001; Burja et al. 2001). Many cytotoxic compounds found in marine sponges and gastropods are also of cyanobacterial origin (Burja et al. 2001; Yamada and Kigoshi 1997).

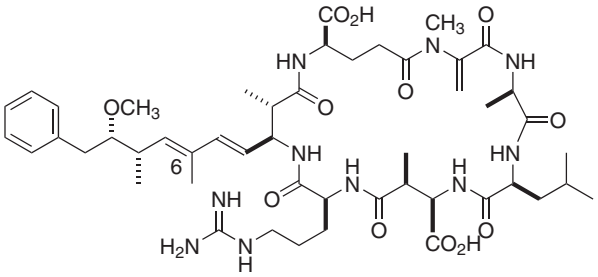
### *2.1 Microcystins*

The hepatotoxic cyclic heptapeptides produced by freshwater cyanobacteria are collectively referred to as microcystins, since they were first isolated from *Microcystis aeruginosa* (van Apeldoorn et al. 2007). Currently 64 microcystins are known; they have the general structure cyclo-(D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha), where X and Z are variable L-amino acids. The first investigated and most prominent member is microcystin-LR (**1**), displaying a mouse $LD_{50}$ of 50–60 µg/kg (i.p.). Microcystins are powerful cancer promoters that act by inhibiting protein phosphatases 1 and 2A (see Chap. 8).

A similar cyclic pentapeptide named nodularin (**2**) having toxicity and mode of action comparable to those of microcystin-LR was isolated from the brackish

water cyanobacterium *Nodularia spumigena*. Seven variants of nodularins are known at present. An analogue of nodularin named motuporin has been found in the marine sponge *Theonella swinhoei*. It is likely produced by symbiotic cyanobacteria (see Chap. 8).

It should be noted that microcystins affect zooplankton community composition (Hanson et al. 2005).



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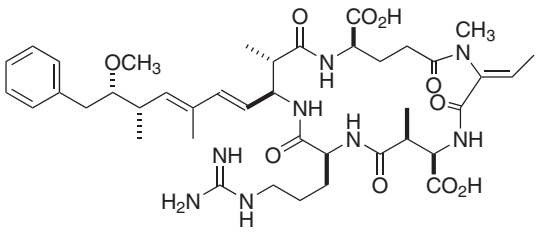

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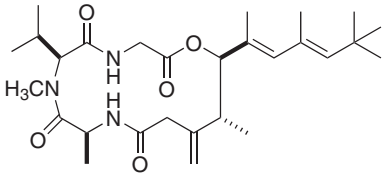
## 2.2 Antilatoxin and Kalkitoxin

Among a large variety of bioactive metabolites produced by *L. majuscula,* particularly interesting are antilatoxin (**3**) and kalkitoxin (**4**), ichthyotoxins that respectively activate and inhibit voltage-gated $Na^+$ channels (Gerwick et al. 2001). It is intriguing that antilatoxin binds the site of $Na^+$ channels different from that of brevetoxins (Al-Sabi et al. 2006).



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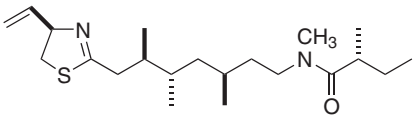


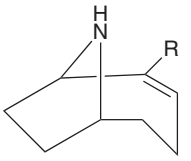
**4**

## 2.3 Alkaloids

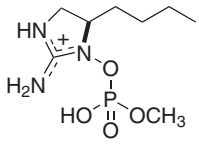
### 2.3.1 Anatoxins

Anatoxin-*a* (**5**) and homoanatoxin-*a* (**6**) are fast-acting neurotoxins produced by cyanobacteria of the genera *Anabaena, Oscillatoria, Cylindrosperum*, and *Aphanizomenon* (van Apeldoorn et al. 2007). They transiently stimulate nicotinic acetylcholine (ACh) receptors by binding directly at the ACh binding sites but ultimately block neuromuscular transmission. Their mouse $LD_{50}$ values were 375 and 250 µg/kg, respectively.

Interestingly, anatoxin-*a*(S) (**7**) produced by *Anabaena* spp. inhibits cholinesterase and its mouse $LD_{50}$ is 31 mg/kg (i.p.).




**5: R=Ac**
**6: R=$COCH_2CH_3$**



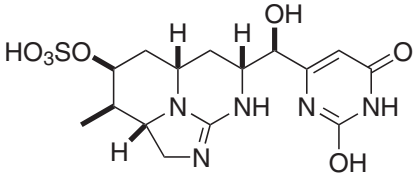

**7**

### 2.3.2 Saxitoxins

Saxitoxins originally discovered from marine bivalves that were infested by dinoflagellates are also produced by cyanobacteria of the genera *Anabaena, Aphanizomenon*, and *Lyngbya* (see Sect. 3.1).

### 2.3.3 Cylindrospermopsins

Cylindrospermopsin (**8**), an unusual alkaloid possessing a tricyclic guanidium moiety, was first isolated from an Australian strain of *Cylindrospermopsis raciborskii*. Several variants have been reported from various cyanobacteria (van Apeldoorn et al. 2007). Cylindrospermopsin blocks protein synthesis and induces kidney and liver failure (Froscio et al. 2008).




**8**

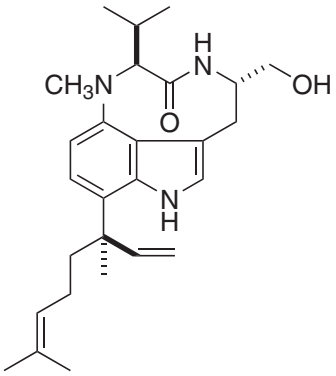
### 2.3.4 Lyngbyatoxins

*Lyngbya majuscula* is a causative agent of "swimmers' itch," a skin dermatitis well-known in Hawaii, and lyngbyatoxins and aplysiatoxins are responsible for this syndrome. Lyngbyatoxin A (**9**) is actually teleocidin A-1 produced by actinomycetes of the genus *Streptomyces* and is a potent tumor promoter (see Chap. 8). It was also reported to be a causative agent of turtle poisoning (Ito et al. 2002).

## *2.4 Polyketides*

### 2.4.1 Aplysiatoxins

Aplysiatoxin (**10**) and debromoaplysiatoxin (**11**) were originally isolated from the digestive glands of the Hawaiian sea hare *Stylochaelus longicauda* and were later found to originate from *L. majuscule,* a prey of the sea hare (van Apeldoorn et al. 2007). Some variants of aplysiatoxins are known from marine cyanobacteria (Burja et al. 2001). These compounds are inflammatory agents and tumor promoters (see Chap. 8). They were also reported as causative agents of human intoxication from ingestion of the Hawaiian red alga *Gracilaria coronopifolida* (Nagai et al. 1996).

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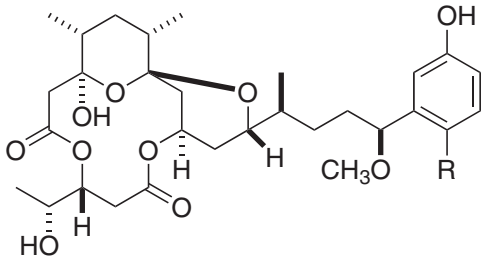
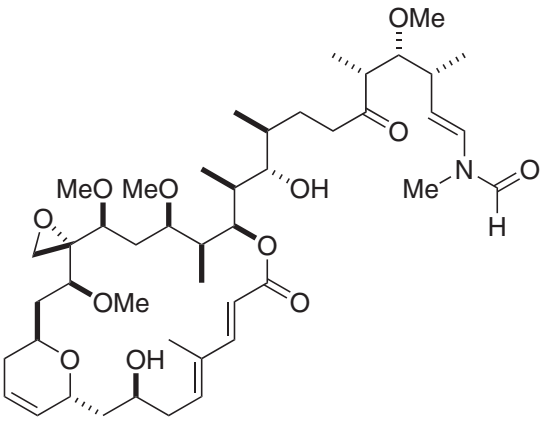



**10: R=Br**
**11: R=H**

### 2.4.2 Other Cyanobacterial Toxins

A large number of cytotoxic polyketides have been reported from marine cyanobacteria of the genera *Lyngbya, Symploca*, and *Scytonema* (Gerwick et al. 2001; Burja et al. 2001). Scytophycin (**12**), an unusual macrolide isolated from *Scytonema pseudohofmanni*, inhibits actin polymerization (Burja et al. 2001; see Chap. 7).



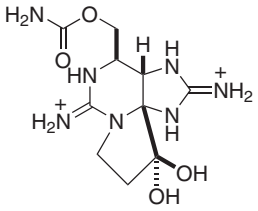

**12**

## 3 Dinoflagellate (Pyrrophyta) Toxins

Dinoflagellates, aquatic photosynthetic eukaryotes, are classified as belonging to the kingdom Protoctista (Protista) (Camacho et al. 2007). They produce an array of highly toxic metabolites, many of which are unprecedented in terrestrial secondary metabolites, are involved in human intoxications from ingestion of sea food and cause mortality of marine animals (Yasumoto and Murata 1993; Daranas et al. 2001; Cembella 2003).

### *3.1 Saxitoxins*

Saxitoxins, highly potent neurotoxins, cause paralytic shellfish poisoning from ingestion of bivalves (see Chap. 3). Bivalves such as mussels, scallops and oysters become toxic by sequestering saxitoxins from dinoflagellates, *Alexanidrium catenella, A. tamarense, Gymnodinium catenatum,* and *Pyrodinium bahamense* var. *compress*a. In addition to saxitoxin (**13**) which was first isolated from the Alaska batter clam *Saxidomas giganteus*, more than 30 variants have been reported from bivalves, dinoflagellates, and cyanobacteria of the genera *Anabaena, Aphanizomenon, Nostoc,* and *Oscillatoria* (Llewellyn 2006). Saxitoxin is one of the most toxic variants with an $LD_{50}$ value of 10 µg/kg in mice (i.p.). It inhibits volatage-gated $Na^+$ channels by binding at the external surface of the pore (Site 1) (see Chap. 3).




**13**

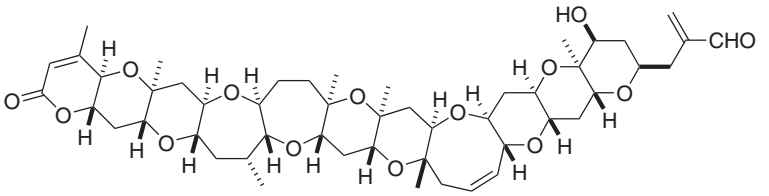
## *3.2 Polyethers*

Since the discovery of brevetoxin B from *Karenia brevis* (formally, *Gymnodinium breve* and *Ptichodiscus brevis*), a wide array of polyethers have been isolated from dinoflagellates as well as mollusks that prey on dinoflagellates and toxin containing fish (Daranas et al. 2001).

### 3.2.1 Brevetoxins

Brevetoxin B (**14**), an ichthyotoxic constituent of the red-tide forming dinoflagellate *K. brevis,* was the first isolated "ladder-shaped polyether" from nature. This was followed by the more potent polyether, brevetoxin A (**15**). Though not highly toxic to mice ($LD_{50}$ values of ~50μg/kg), both are potent ichthyotoxins ($LC_{50s}$ 3–15ng/ml for zebrafish). Fifteen brevetoxins have been isolated from *K. bevis* (Baden et al. 2005). Brevetoxins activate voltage-gated $Na^+$ channels by binding at Site 5 (see Chaps., 3 and 6).

Brevetoxins and their metabolites are involved in neurotoxic shellfish poisoning (NSP), in which some symptoms are similar to those of ciguatera fish poisonig (CFP) (see Sects. 3.2.2 and 8.4). Like ciguatoxins, brevetoxins accumulate in fish (Naar et al. 2007).

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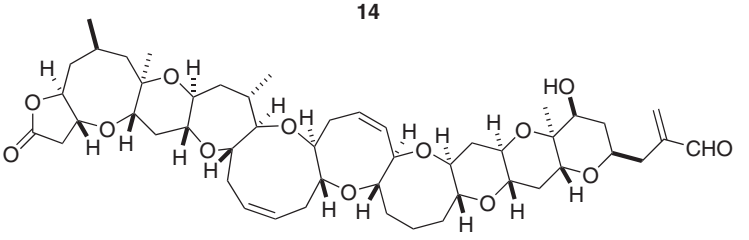
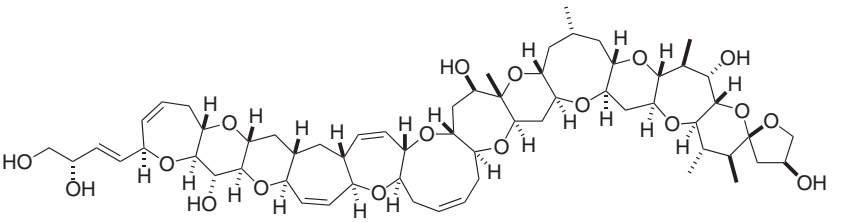



**15**

### 3.2.2 Ciguatoxins

Ciguatera is a food poisoning that results from eating subtropical and tropical fish at certain times and places. Ciguatoxin was named for a toxic principle first isolated from a Pacific red snapper (*Lutjanus bohar),* which frequently causes this disorder (Hashimoto 1979; Nicholson and Lewis 2006). Later, it was isolated in a purity sufficient for chemical analysis from the moray eel *Gymnothrax javanicus* and its structure was shown to be a complex ladder-shaped polyether (**16**) (Yasumoto and Murata 1993;

Yasumoto 2005; Nicholson and Lewis 2006). Actually, ciguatoxin is a metabolite of ciguatoxin 4B (**17**); the latter compound is synthesized in the dinoflagellate *Gambierdiscus toxicus*. Ciguatoxin 4B is metabolized to ciguatoxin in fishes as it passes up the food chain from herbivorous fish that feed upon seaweeds containing the dinoflagellate to carnivorous fish (Yasumoto 2005). So far more than 30 ciguatoxin variants have been isolated or detected from fishes or this dinoflagellate. Ciguatoxin ($LD_{50}$ 0.25 µg/kg in mice) is about 40 times more toxic that tetrodotoxin and activates voltage-gated $Na^+$ channels after binding to Site 5 (see Chaps. 3 and 6).



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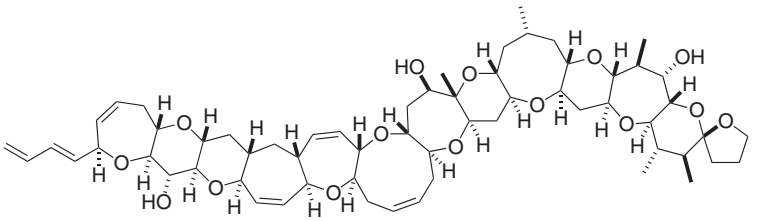
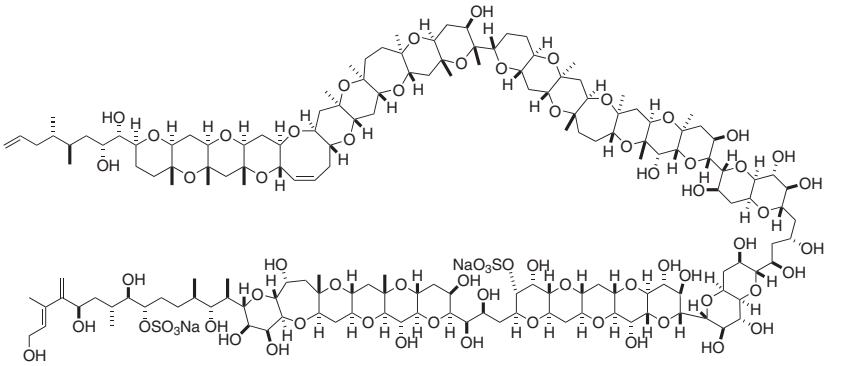


**17**

### 3.2.3 Maitotoxin

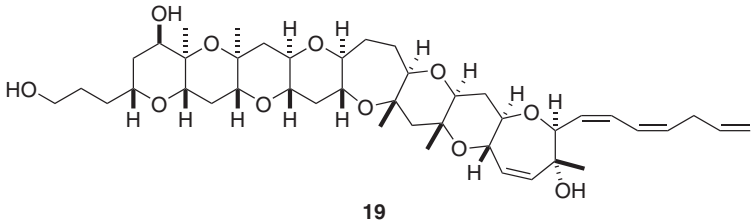
Maitotoxin (**18**) was named after the Tahitian name for the surgeonfish *Ctenochaetus striatus* which frequently causes ciguatera in Tahiti (Murata and Yasumoto 2000). Again, the fish accumulates this *Gambierdiscus toxicus* toxin from its prey. Maitotoxn is the most complex polyether to be isolated and the most toxic natural product known (mouse $LD_{50}$ 50 ng/kg). It increases membrane permeability to $Ca^{2+}$ by an as yet unknown mechanism.



**18**

### 3.2.4 Gambierol

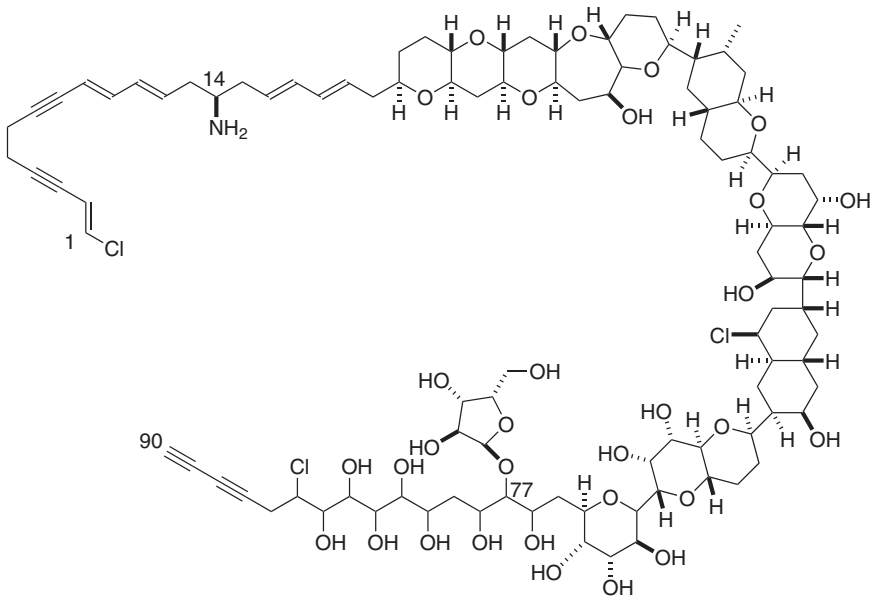
*G. toxicus* produces a variety of polyethers with potent biological activities, among which gambierol (**19**) is intriguing because it strongly inhibits voltage-gated $K^+$ channels with an $IC_{50}$ value of 1.8 ng/ml and is toxic to mice with $LD_{50}$ 50 µg/kg (Ghiaroni et al. 2005). Gambierol's mechanism of action is discussed in Chap. 6.



**19**

### 3.2.5 Prymnesins

Although produced by the haptophyte *Prymnesium parvum*, prymnesin-2 (**20**) should be mentioned here (Murata and Yasumoto 2000). It is a causative agent of massive fish kills in brackish waters. In fact, its minimal icthyotoxic concentration is 3 nM against the medaka *Tanichthys albonubes*. It is also highly hemolytic. *Prymnesium* extracts' modes of action are discussed in Chap. 6.



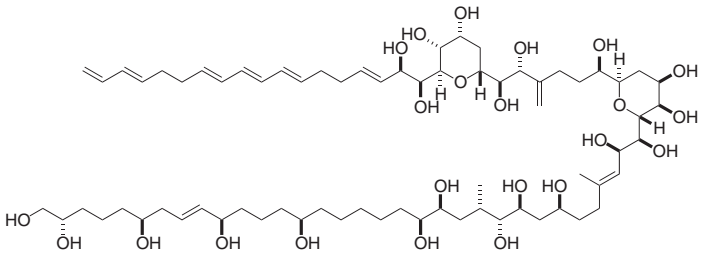
**20**

### 3.2.6 Other Polyethers

Many other polyether compounds, most of which are of dinoflagellate origin, are involved in human intoxications from ingestion of mollusks. Therefore, they are described in Sect. 8.

## *3.3 Long-Chain Polyketides*

Dinoflagellates of the genus *Amphidinium* produce long-chain polyketides, e.g. amphidinol-3 (**21**), that are highly hemolytic (Yasumoto and Murata 1993). Symbiotic dinoflagellates of the genus *Symbiodinium* produce long-chain polyhydroxylated macrolides, including zooxanthellatoxins, zooxanthellamides, and symbiodinolide (**22**), that activate voltage-gated $Ca^{2+}$ channels (Moriya et al. 1998; Onodera et al. 2005; Kita et al. 2007).



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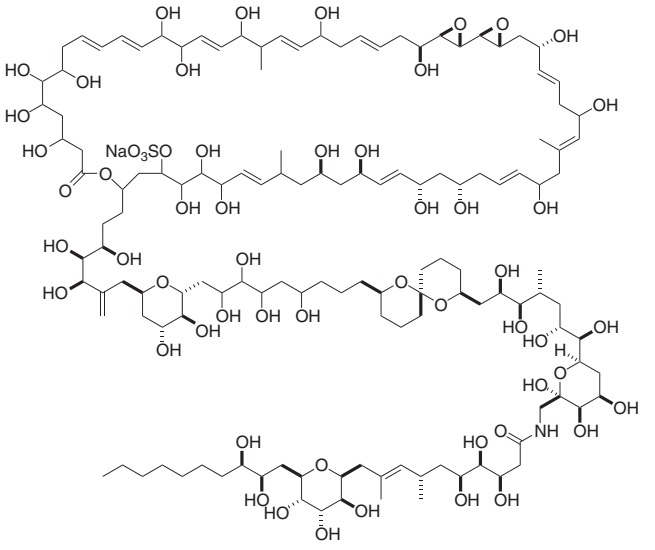
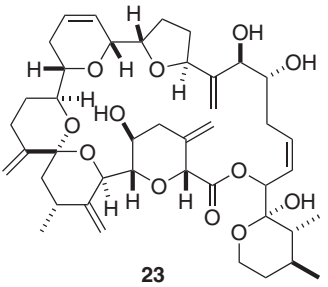


**22**

### *3.4 Macrolides*

Among an array of macrolides produced by dinoflagellates, goniodomin A (**23**) isolated from *Alexandrium hiranoi* (formerly, *Goniodoma pseudogonyaulax*) was reported to be highly ichthyotoxic and to stimulate actomyosin ATPase activity (Daranas et al. 2001). Prorocentrolide (**24**), produced by *Prorocentrum* spp., is a unique macrolide possessing a cyclic imine moiety that is a fast-acting toxin with $LD_{50}$ 400 μg/kg (i.p.) in mice (Torigoe et al. 1988).



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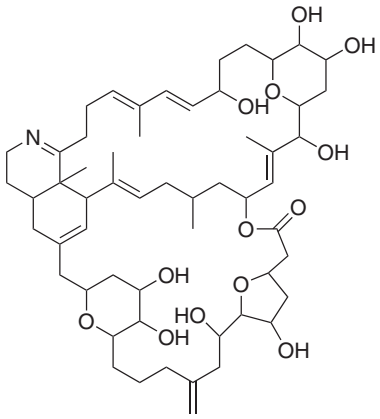
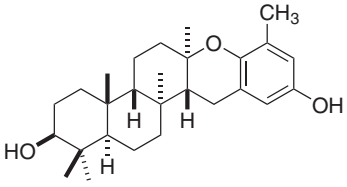


**24**

## 4 Macroalgal Toxins

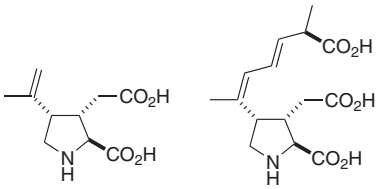
A wide variety of seaweeds have been consumed in Asian countries and, to a lesser extent in some parts of Europe and North America, but only small numbers of human intoxications were reported (Hashimoto 1979). Coralline algae of the genus *Jania* were reported to contain tetrodotoxins (Yasumoto et al. 1986a). Brown algae often contain meroditerpenoids (Blunt and Munro 2007), of which 2β,3α-epitaondiol (**25**) and others isolated from *Stypopodium flabelliforme* were reported to be voltage-gated $Na^+$ channel modifiers (Al-Sabi et al. 2006).



**25**

## 4.1 Kainic and Domoic Acids

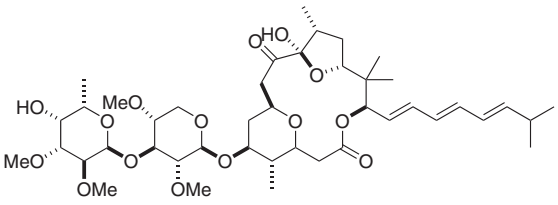
Several seaweeds have been used as anhelminthics in China and Japan (Hashimoto 1979); α-kainic (**26**) and domoic acids (**27**) were isolated from the red algae *Digenea simplex* and *Chondria armata*, respectively. These amino acids are potent agonists of glutamate receptors (see Chap. 5). Domoic acid is also a causative agent of amnesic shellfish poisoning as mentioned later (Sect. 8.5).




**26** **27**

## 4.2 Polycavernosides

Human intoxications from ingestion of red algae of the genus *Gracilaria* have been reported in several places (Hashimoto 1979; Nagai et al. 1996; Paquette and Yotsu-Yamashita 2007). As mentioned earlier, aplysiatoxin and debromoaplysiatoxin were causative agents in the Hawaiian intoxications (Nagai et al. 1996). Polycavernoside A (**28**) and other congeners were isolated from the *G. edulis* that caused fatal human intoxication in Guam and Philippines (Paquette and Yotsu-Yamashita 2007). This compound is toxic to mice with $LD_{99}$ values of 0.2–0.4 mg/kg. Polycavernoside A triggers an intial extracellular $Ca^{2+}$ entry produced by a mechanism other than activation of L-type voltage-gated $Ca^{2+}$ channels or muscarinic receptors (Cagide et al. 2007). Polycavernosides are believed to be of cyanobacterial origin, perhaps *Lyngbya* spp.

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## 4.3 Other Macroalgal Toxins

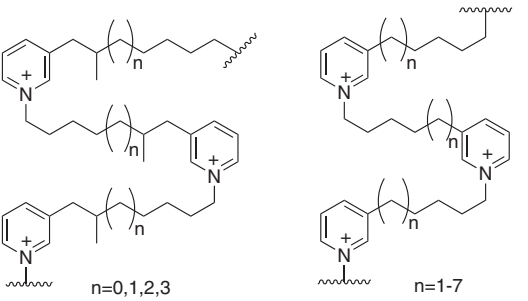
A large number of ichthyotoxic terpenoids have been isolated from macroalgae (Blunt and Munro 2007), but their toxicities toward mammals have not been evaluated.

# 5 Sponge Toxins

Sponges, often referred to as the most primitive multicellular animals, harbor large numbers of symbiotic microorganisms that are frequently thought to be the real producers of bioactive sponge metabolites (Piel 2004). Although a large number of such metabolites have been isolated from marine sponges, few of them have been evaluated for toxicity toward mammals. Significant numbers of cytotoxic compounds of sponge origin target tubulin or actin as mentioned in Chap. 7, while others are potent tumor promoters that inhibit protein phosphatases 1 and 2A as described in Chap. 8.

## *5.1 Polyalkylpyridiniums*

Sponges of the order Haplosclerida often contain 3-alkylpyridinium salts (Andersen et al. 1996; Sepčić 2000), some of which were reported to be toxic to mammals. The first example of this class is halitoxin (**29**) isolated from *Haliclona* spp. which is actually a mixture of oligomeric/polymeric 3-alkylpyridinium salts (Schmitz et al. 1978; Turk et al. 2007). Halitoxin is hemolytic, ichthyotoxic and toxic to mice ($LD_{50}$ 1.4 mg/kg, i.v.). A similar polymeric mixture (**30**) from the Mediterranean *Reniera* (*Haliclona*) *sarai* and referred to as poly-APs (polymeric alkylpyridinium salts) largely consists of two major polymers with molecular weights of 5 and 19 Da. It showed a wide range of biological activities, including hemolytic, lethal (above 2.7 mg/kg i.p. in rats), and acetylcholinesterase inhibitory (Sepčić et al. 1997a, b). More significantly, it forms transient pores in cell membranes like halitoxin (McClelland et al. 2003).

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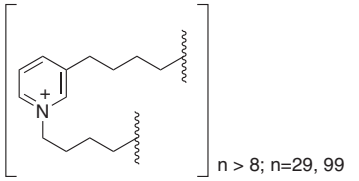


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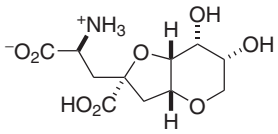
## 5.2 Kainate Receptor Agonists

Several agonists of glutamate receptors including dysiherbaine (**31**) have been isolated *Dysidea harbasea* (Sanders et al. 2006) as will be described in Chap. 6.



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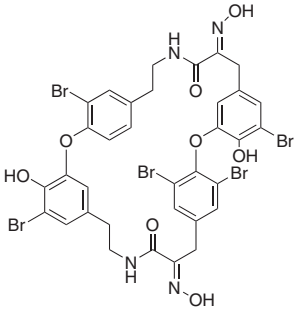
## 5.3 Other Sponge Toxins

Bastadins [e.g. bastadin 5 (**32**)], bromotyrosine-derived metabolites isolated from *Ianthella basata,* are selective modulators of sarcoplasmic reticulum $Ca^{2+}$ channels and behave either as full or partial agonists (Zucchi and Ronca-Testoni 1997). Penaramides (**33**), novel acylated polyamines isolated from *Penares* aff. *incrustans*, inhibit binding of ω-conotoxin GVIA to N-type $Ca^{2+}$ channels at μM levels (Ushio-Sata et al. 1996). Cyclostellettamines A–F [A (**34**)], cyclic 3-alkylpyridines isolated from the marine sponge *Stelletta maxima*, are muscarinic acetylcholine receptor antagonists (Fusetani et al. 1994).

Finally, a variety of isocyanoterpenoids and related terpenoids are known from sponges, especially *Acanthella* spp. They show a wide range of activities including ichthyotoxic and cytotoxic, but their modes of action as well as toxicity toward mammals are unknown (Chang 2000).



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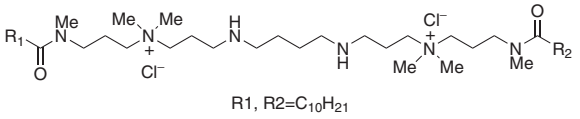

R1, R2=$C_{10}H_{21}$

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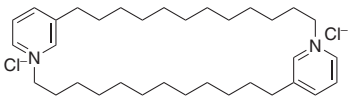

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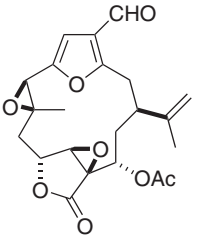
# 6 Cnidarian (Coelenterate) Toxins

Soft corals have been intensively investigated for their secondary metabolites, and a large number of terpenoids, especially diterpenoids have been isolated (Blunt and Munro 2007). Cembranoids, a typical class of soft coral diterpenes, exhibit a wide range of biological activities (Coll 1992). Lophotoxin (**35**) and related cembranoids

isolated from gorgonians of the genera *Lophogorgia* and *Pseugopterogogi*a are irreversive nicotinic receptor antagonists; lophotoxin is lethal to mice with an $LD_{50}$ value of 8 mg/kg and blocks the binding of cobra a-toxin to nicotinic receptors of BC3H-1 cells with $IC_{50}$ values of 1–2 μM (Fenical et al. 1981; Culver et al. 1985). Cnidarians possess cnidocysts (previously called nematocysts) that contain peptidic and proteinaceous toxins; these will be described in Sects. 6.2 and 6.3.

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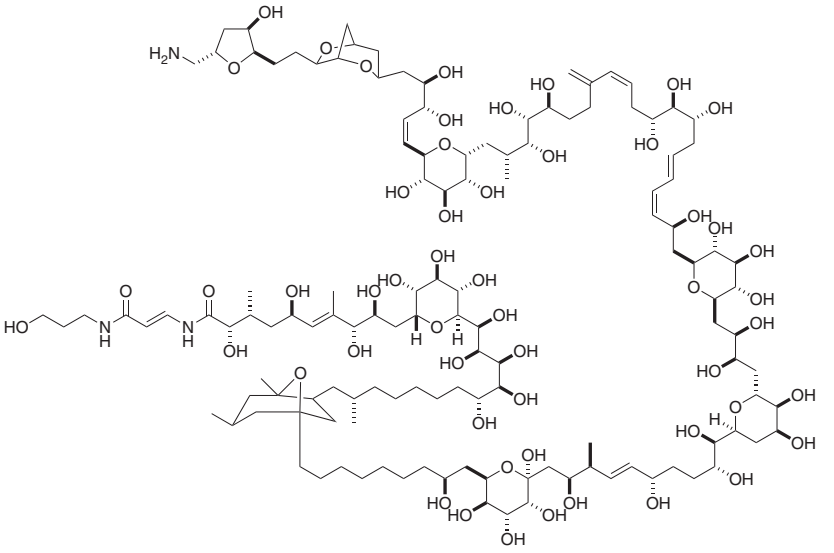
## *6.1 Palytoxins*

Palytoxin (**36**), is a highly unusual metabolite occurring in zoanthids of the genus *Palythoa* (Hashimoto 1979; Moore 1985; Uemura 1991). It is highly toxic with a mouse $LD_{50}$ of 0.45 μg/kg (i.p.). Its mode of action has been extensively studied and it has been demonstrated that palytoxin binds to the cell membrane Na, K-ATPase and converts it from an ion pump into an ion channel, which greatly increases membrane $Na^+$ permeability and results in a large inward current (Artigas and Gadsby 2003; Hilgemann 2003). It should be noted that palytoxin is a novel skin tumor pomoter (Wattenberg 2007; also, see Chap. 8).

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Palytoxin and its analogues have been reported to be responsible for such human intoxications as clupeotoxism (Onuma et al. 1999), xanthid crab poisoning (Yasumoto et al. 1986b) and filefish poisoning (Hashimoto 1979). Palytoxin analogues have been isolated also from the red alga *Chondria armata* (Maeda et al. 1985) and the dinoflagellates of the genus *Osteropsis* (Usami et al. 1995; Riobo et al. 2006). Palytoxin is sequestered by a wide range of coral reef animals from zoanthid colonies (Gleibs and Mebs 1999).

## *6.2 Cnidarian Peptides*

The phylum Cnidaria (the three classes are: anthozoans, schyphozoans, and hydrozoans) capture their prey and repel predators via cnidae, also called cnidocysts or nematocysts. Many forms of cnidae have been described and the "cnidome" of each cnidarian species usually consists of several anatomically distinct forms. Each cnidocyst is elaborated within the Golgi apparatus of a cnidocyte, the cell which makes this unique structure. When cnidocytes are activated by a combination of chemical and mechanical stimuli, an arrow-like tube within the cnidocyst rapidly everts to make contact with the organism that stimulated the discharge. Depending on the cnidocyte, the tubule may be used to ensnare the prey (adhesive spirocyst) or, if barbed, to actually penetrate the skin of the victim and inject a paralyzing venom (most of the >30 different types of cnidocytes are thought to have this role). All members of this large, almost entirely marine phylum possess cnidae and most of these are predicted to contain venoms composed of a variety of peptides and proteins (peptides with MW >10,000). While there may be some relatively non-toxic cnidarian species, these would be quite exceptional.

Cnidarian peptides have not yet been investigated in as much detail as the *Conus* peptides, because they are usually of larger molecular size, which makes structure determination and subsequent synthesis for biological evaluation quite challenging. Furthermore, cnidarian toxins are often diffusely distributed over the entire surface of the body rather than being localized within a discrete organ, and this makes toxin collection and purification more difficult. It is likely that the myriad types of toxins produced by cnidarians will ultimately be found to be as diverse as those of the cone shells, a genus producing an amazingly diverse group of peptide toxins.

### 6.2.1 $Na^+$ Channel Modulating Toxins

These were the first cnidarian peptides purified. ATXs I–III isolated from a European species (*Anemonia sulcata*; Béress et al. 1975) and anthopleurin A from a Pacific species (*Anthopleura xanthrogrammica*, Norton et al. 1976) were initially investigated for their cardiotonic (inotropic) and nerve action potential modifying activities. These "long" peptides consist of a single peptide chain of 45–48 amino acid residues cross-linked by three disulfide bonds. High resolution NMR studies

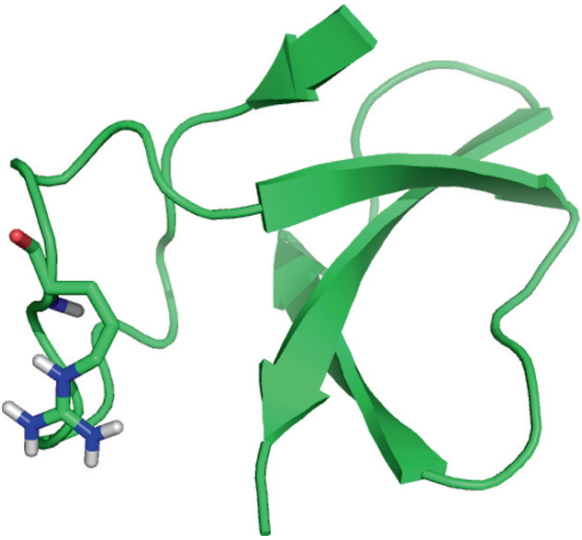


**Fig. 1** Ribbon diagram of the solution structure of ShI, a peptide modulating Na channel inactivation from the Carribbean mat sea anemone, *Stichodactyla helianthus* (Kem et al., 1989). The folded structures of the so-called "long" sea anemone neurotoxins are stabilized by several segments of β-pleated sheets and three disulfide bonds; all contain a relatively flexible loop that may also interact with the Na channel (Fogh et al. 1990; Wilcox et al. 1993)

have yielded a number of similar solution structures; the ShI structure (Fig. 1) was of particularly high resolution (Fogh et al. 1990).

Although the existence of 40+ homologous long toxin sequences might seem adequate for assessing the structure-activity relationship for these toxins, most of these sequences are from closely related sea anemone species. Future studies of related toxins from other sea anemone families are expected to provide a better perspective as to the structural variability of these peptides.

These toxins act by slowing the repolarization phase of nerve and muscle action potentials by inhibiting the process of $Na^+$ channel inactivation (Murayama et al. 1972; Salgado and Kem 1992). When a nerve action potential, which may now last hundreds of milliseconds (like a myocardial action potential) reaches the nerve terminal, massive release of neurotransmitter results, causing hyperexcitability and convulsions. The cardiotonic actions of the long peptides are of potential therapeutic interest; however, the positive inotropic effect is transitory, leads to calcium-loading of the myocardial cell and is often accompanied by arrhythmias. Currently these $Na^+$ channel toxins are mainly used as molecular probes for studying the gating mechanisms of these channels.

While most sea anemone $Na^+$ channel modulating peptides are "long" toxins, representatives of another subfamily, the "short" toxins are found in a few species. These contain 30–32 residues and 4 disulfide bonds. They lack homology with the long toxins. So far, these peptides are only known to affect crustacean and insect nerve

action potentials in a manner similar to the long sea anemone toxins (summarized by Honma et al. 2003).

Other anthozoans, particularly hexacorals, represent an almost unstudied group with respect to their $Na^+$ (and other) channel toxins. However, a 12 kDa peptide isolated from an unidentified *Goniopora* species (Hashimoto and Ashida 1973) was shown to delay $Na^+$ channel inactivation (Muramatsu et al. 1985; Gonoi et al. 1986). While its effects were similar in many respects to those of the anemone toxins, its sequence was not homologous to them (Ashida et al. 1987).

### 6.2.2 Sea Anemone $K^+$ Channel Peptide Toxins

Currently the most actively investigated ion channel modulating peptides are those which affect the gating of various $K^+$ channels, which are more diverse than $Na^+$ channels, in function as well as in number. While $K^+$ channels are important during the repolarization phase of an action potential, they also regulate excitability by controlling resting membrane potential and conductance; they also regulate calcium fluxes in non-electrically excitable cells such as T-lymphocytes as well as electrogenic cells. The initial "short" $K^+$ channel toxins characterized were BgK (Aneiros et al. 1993) and ShK (Castenada et al. 1995), both isolated from Carribbean sea anemones. These basic peptides consist of a 35–37 residue chain whose folded structure is stabilized by 4 disulfide bonds. Each peptide contains two small alpha-helical segments that flank an essential lysyl sidechain that enters and blocks the pore. The mechanism of channel block is nearly identical to what had previously been demonstrated for a scorpion toxin, charybdotoxin (Pennington et al. 1996a; Dauplais et al. 1997). These anemone toxins mainly block "delayed rectifier" K channels that are activated during the repolarization phase of action potential, but they also block an intermediate conductance $Ca^{2+}$-activated $K^+$ channel found in certain blood cells. Although they are minor constituents of sea anemone extracts, their highly basic character and thermal stability (even at 100°C) facilitated their isolation and subsequent sequence determinations. They were readily synthesized by solid-phase methods (Pennington et al. 1995); this allowed detailed structure-activity analyses, including so-called "alanine" scans, to identify residues critical for ion channel binding (Pennington et al. 1996a, b; Alessandri-Haber et al. 1999). The solution structure of synthetic ShK (Fig. 2) could then be determined by NMR methods (Tudor et al. 1996). Analogs of ShK are currently in development for treating autoimmune diseases because they are able to preferentially inhibit the proliferation of antigen-activated T-lymphocytes. By blocking Kv1.3 channels the membrane potential of the lymphocyte is reduced and this indirectly reduces the $Ca^{2+}$ influx of required to stimulate proliferation (Kalman et al. 1998; Kem et al. 1999; Beeton et al. 2006).

Anemone peptides affecting other types of $K^+$ channels are also known and these will be described in Chap. 4.

**Fig. 2** Ribbon diagram of the solution structure of ShK, a peptide blocking certain K channels from the Carribbean mat sea anemone, *Stichodactyla helianthus*. A short stretch of α-helix is present, but no β-pleated sheet structure is present (Tudor et al. 1996)

### 6.2.3 A Protein Toxin that Blocks $Ca^{2+}$ Channels

A coral toxin (MW~19,000) blocking Ca channels has been isolated from an unidentified Red Sea *Goniopora* species (Qar et al. 1986). Systematic screening of extracts from other anthozoan species likely will provide other $Ca^{2+}$ channel blockers and modulators.

## *6.3 Cnidarian Protein Toxins*

### 6.3.1 Actinoporins

Two hemolytic sea anemone toxins, one from European Atlantic and Mediterranean coasts (equinatoxin) and one from the Gulf of Mexico (*Stichodactyla* cytolysin II) were initially isolated (Macek and Lebez 1988; Kem and Dunn 1988). Edman and mass spectrometric sequence analyses and comparison of Florida-collected cytolysin II and Cuba-collected Sticholysin I showed that they are the same molecule ((Blumenthal and Kem 1983; Huerta et al. 2001; Stevens et al. 2002). These toxins are small (~21 kDa) proteins and though they lack disulfide bonds, their folded structures are very stable due to an extensive BB-pleated sheet secondary structure that involves most of their peptide bonds. Because these sea anemone (order Actinaria) toxins structurally resemble bacterial porins by having a predominantly β-sheet

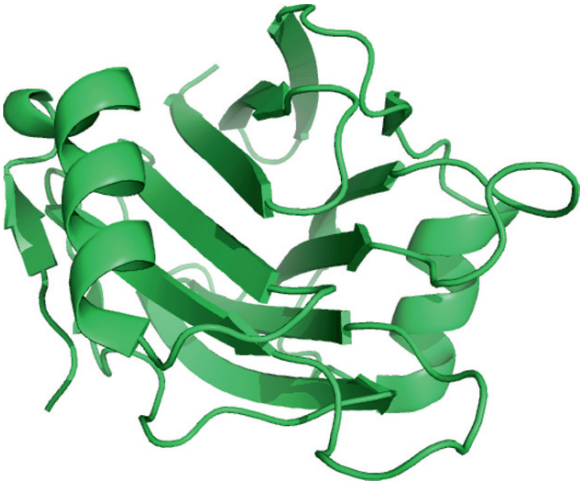


**Fig. 3** Ribbon diagram of the solution structure of equinatoxin II, a cytolytic pore-forming protein (actinoporin) occurring in the European sea anemone *Actinia equine*. Although most of the folded structure of this type of toxin is stabilized by β-pleated sheet secondary structure, a helical segment near the N-terminus is essential for membrane insertion and pore formation in association with three other monomers (Hinds et al. 2002)

secondary structure and form stable, relatively large transmembrane pores, they are now commonly referred to as "actinoporins" (Kem 1988). Four monomers of the toxin aggregate to form a functional pore. One of the most interesting properties of the actinoporins, their ability to insert into lipid bilayers membranes and behave like membrane proteins, is greatly facilitated by the presence of small amounts of sphingomyelin. A high resolution X-ray crystallographic structure of equinatoxin monomer reveals an α-helical segment near the N-terminus that other studies (Fig. 3) implicate in membrane insertion and pore formation (Athaniasiadis et al. 2001). X-ray diffraction and EM studies of 2D crytals of sticholysin II adsorbed to a lipid layer have provided a low resolution model of the tetrameric pore (Mancheno et al. 2003).

### 6.3.2 Scyphozoan and Hydrozoan Toxins

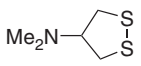
In spite of many attempts, these proteins have been very difficult to isolate as stable, active toxins. However, recent studies with certain scyphozoan and hydrozoan toxins have been successful (Nagai et al. 2000; 2002). These relatively large proteins were sequenced by molecular biological methods. Two additional toxins from the cubomedusan *Chironex fleckeri,* whose sting can be lethal to swimmers, were recently isolated and sequenced (Brinkman and Burnell 2007). These homologous proteins (MW ~45,000) have certain common features.

The first hydrozoan toxin that was isolated and sequenced was a lytic protein called "hydralysin" (Zhang et al. 2003). It was reported that this toxin is not located within cnidocytes. Further cloning revealed several other hydralysins of similar molecular size (27–31 kDa) and sequence (Sher et al. 2005). Recently a new method for isolating toxins from undischarged cnidocysts of fire corals *Millepora* spp., another hydrozoan group, was reported. Three different toxic fractions were resolved. The smallest MW fraction was partially sequenced and its entire sequence inferred by analysis from its cDNA (Iguchi et al. 2007). MCTx-1 is an acidic protein of 222 amino acid residues that lacks phospholipase $A_2$ activity but is lethal ($IC_{50}$ 79 ng/ml) to cultured mouse leukemia cells and to crayfish ($LD_{50}$ 160 ug/ml). Interestingly, it displays some homology with extracellular matrix proteins called dermatopontins. The successful use of recombinant DNA methods in these investigations will hopefully inspire others to "fish out" sequences of other schyphozoan and hydrozoan toxins in the near future, which will facilitate the production of antibodies for treating victims of their stings and provide insights into the mechanisms of action and evolution of these protein toxins.

# 7 Worm Toxins

## *7.1 Annelid Alkaloids*

The polychaete *Lumbriconereis brevicirra s*ecrets a toxic mucus when disturbed; its active constituent is nereistoxin (**37**), a dimethylamine possessing a 1,2-dithiolane ring. Its structure was proposed in 1962 and subsequently confirmed by synthesis (Okaichi and Hashimoto 1962; Hagiwara et al. 1965). It is toxic to mice ($LD_{50}$ 30 mg/kg i.v.) and also displays ichthyotoxic and insecticidal properties. It inhibits nicotinic acetylcholine receptors (nAChRs). Depending on the particular nAChR involved, it (1) competes with acetylcholine at postsynaptic membrane nicotinic acetylcholine receptor sites, and (2) directly blocks the nAChR ion channel. A family of insecticides that includes cartap (the first member, initially developed in Japan) is based on the nereistoxin structure (Hashimoto 1979).

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## *7.2 Annelid Peptides and Proteins*

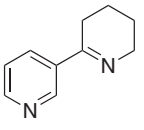
Arenicins are novel antimicrobial peptides that were recently isolated from coelomocytes of the lugworm *Arenicola marina*. Two isoforms (MW~2,800) were sequenced. Arenicins act upon Gram-positive and Gram-negative bacteria and

fungi (Ovchinnikova et al. 2004). The interesting solution structure of arenicin and its membrane actions have been the subject of recent papers (Lee et al. 2007; Andra et al. 2008).

Glycerotoxin, a very large (~320 kDa) protein from the blood worm (*Glycera convoluta*, from Atlantic coast of France) specifically stimulates the exocytotic release of neurotransmitter from nerve terminals and is thus a useful tool for understanding the presynaptic processes that are involved in neurotransmitter release (see Chap. 6).

## *7.3 Nemertine Alkaloids*

Bacq, a Belgian pharmacologist, discovered the existence of toxins in nemertines, a phylum of carnivorous marine worms that currently includes ~1,000 described species. Many species that belong to the class Hoplonemertinea contain pyridine alkaloids that function as toxins and feeding repellents (Kem 1971; Kem et al. 2001, 2003). Hoplonemertines paralyze their prey, releasing an alkaloidal venom from a proboscis that simultaneously punctures the skin of the prey with a mineralized stylet. These alkaloid toxins allow the worm to rapidly subdue its prey (usually annelids or crustaceans) since they target nicotinic acetylcholine receptors important for body movements.

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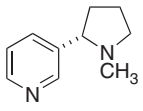


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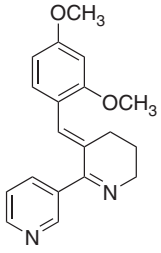


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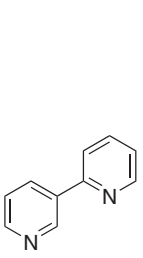
Anabaseine, 2-(3-pyridyl)-3,4,5,6-tetrahydropyridine (**38**) was the first alkaloid to be isolated and identified (Kem et al. 1971; Kem et al., 1997). The skin and proboscis of a Pacific hoplonemertine, *Paranemertes peregrina*, contains very high concentrations of this compound (Kem 1971). Anabaseine, like nicotine (**39**), stimulates all known nAChRs to some degree. While nicotine preferentially stimulates a brain nicotinic acetylcholine receptor (alpha4beta2) associated with smoking pleasure, anabaseine preferentially stimulates the neuromuscular nAChR and another brain nAChR, the so-called alpha7 receptor (Kem et al. 1997). A 3-benzylidene anabaseine derivative, GTS-21 (**40**), was shown to improve learning and memory in animal experiments and clinical trials (Kem et al. 2008; Kem et al., 2004; 2006). It is the first compound found to selectively stimulate the $\alpha 7$ type nAChR.

Another hoplonemertine found in the northwestern Atlantic as well as Pacific oceans, *Amphiporus angulatus*, contains at least 15 different pyridine alkaloids (Kem et al. 1976). This is one of the largest hoplonemertines known, attaining a biomass of about one gram fresh weight; most hoplonemertines are less than 0.1 g fresh weight. While anabaseine is only a trace constituent of this species, the related 2,3'-bipyridyl (**41**) readily paralyzes crustaceans. The 3-methyl-2,3'-bipyridyl was recently identified (Kem et al., submitted). All eight C-methylated 2,3'-bipyridyls were synthesized and tested for toxicity and ability to inhibit barnacle larval settlement (Kem et al. 2003). Nemertelline (**42**), the most abundant alkaloid, is a tetrapyridyl with similarities to the tripyridyl tobacco alkaloid nicotelline (Thesing and Muller 1956; Cruskie et al., 1995), whose structure is identical to nemertelline without the nemertelline A ring. The biological activities of nemertelline are not yet well understood, though it has been demonstrated to inhibit the settlement of barnacle larvae at relatively high concentrations (Kem and Soti 2001).

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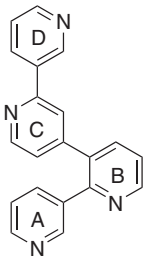



**42**

Although the toxins of only a few readily accessible species have been examined in any detail, it is already clear that hoplonemertines contain a multitude of heterocyclic compounds. Future investigations of a wider variety of species should yield many new structures and interesting biological activities.

## *7.4 Nemertine Peptide Toxins*

Nemertines that are not hoplonemertines also capture their prey with a long proboscis that wraps around the prey and prevents its escape. Heteronemertines such as the large *Cerebratulus* and *Parbolasia* species are known to produce peptide neurotoxins and/or cytolysins, some of which have been sequenced (Kem, 1976; Blumenthal and Kem, 1976; 1980; Blumenthal et al., 1981). *Cerebratulus lacteus (Cl)*, a relatively common intertidal nemertine along the eastern seaboard of North America produces at least four homologous neurotoxins (MW ~6,000), referred to as B toxins because they elute from a gel column after the larger A-type cytolysins. The B peptides are toxic to crustaceans but not mammals. These are very basic peptides consisting largely of α-helical segments that are folded together and

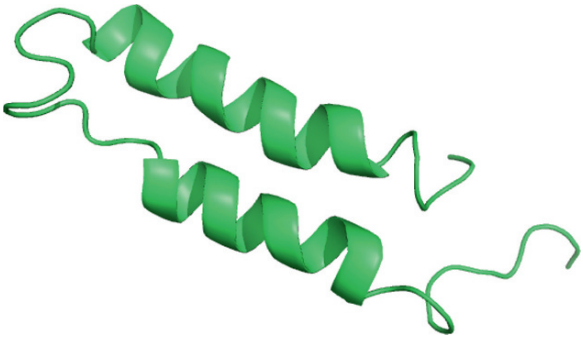


**Fig. 4** Ribbon diagram of the solution structure of neurotoxin B-IV, a peptide occurring in the Atlantic heteronemertine *Cerebratulus lacteus*. Two long α-helices connected by a hairpin B-turn and stabilized by four disulfide bonds constitute the scaffold of this elongated toxin, which induces spontaneous action potentials in crustacean nerves (Tudor et al. 1996)

stabilized by four disulfide bonds (Kem 1976; Kem et al. 1990). The folded structure of the most abundant crustacean paralyzing toxin Cl-BIV (Fig. 4) is rather unique for neurotoxins affecting voltage-gated ion channels, consisting primarily of two α-helices separated by a hairpin turn (Barnham et al. 1997).

The *Cerebratulus* B-neurotoxins transiently excite but then block the generation of action potentials in crustacean nerves and thereby cause spastic followed by flaccid paralysis. Their targets are likely sodium channels, as TTX inhibits their contractile action on the isolated crayfish nerve-opener muscle preparation (Kem, unpublished results).

The *Cerebratulus* A toxins are small (~10 kDa) cytolytic proteins localized in the integumentary (skin and proboscis) tissues. Cl-AIII is the most abundant and well characterized isotoxin (Kem and Blumenthal 1978). These peptides are also very basic and contain several disulfide bonds to stabilize their folded structures. A homologous, cytolytic protein was isolated from a very large (>2 m) Antarctic species, *Parborlasia corrugans*. Whereas the Cl-A isotoxins were difficult to chromatographically resolve, separation of the various *Parborlasia* isotoxins was not possible due to their similarity in size, ionization or other characteristics (Berne et al. 2003). Cloning techniques will probably be required to satisfactorily resolve and sequence these toxins. These heteronemertine cytolysins presumably permeabilize membranes by forming pores or by acting as potent detergents. Both the A and B types of heteronemertine toxins may be used offensively as well defensively, even if there is no special skin puncturing mechanism available for facilitating their entry into a victim. *Cerebratulus* feeds on clams as well as annelids; it displays an uncanny ability to slip its proboscis between the two shells of a clam and cause it to open.

Paleonemertines, a third class, also possess cytolytic proteins that have not yet been isolated (Kem 1971, 1994).

### 7.5 Other Worm Toxins

Two species of flatworms (Miyazawa et al. 1987; Ritson-Williams et al. 2006) and a polychaete (Yasumoto et al. 1986) have been reported to contain tetrodotoxins. Arrowworms (phylum Chetognaths) are abundant members of planktonic predators, among which six species are known to possess tetrodotoxin (Thuesen et al. 1988). Flatworms and arrowworms are likely to use tetrodotoxin to catch prey organisms; arrow worms may sequester tetrodotoxin from *Vibrio alginolyticus* living associated with these worms (Thuesen and Kogure 1989).

## 8 Mollusks

Bivalve and gastropod mollusks often accumulate a variety of toxic compounds in digestive glands (mid-gut glands) from prey organisms and cause human intoxications known as shellfish poisonings mentioned below (Halstead 1967; Hashimoto 1979; Camacho et al. 2007). In addition, the trumpet shell *Charonia sauliae* and the Japanese ivory shell *Babylonia japaonica*, carnivorous gastropods, and the blue-ringed octopus *Octopus maculosa* accumulate tetrodotoxin (Hashimoto 1979; Mosher and Fuhrman 1984). Cone snails of the genus *Conus* hunt prey organisms, fishes, mollusks and worms, using complex mixtures of toxic peptides as described in Chap. 2.

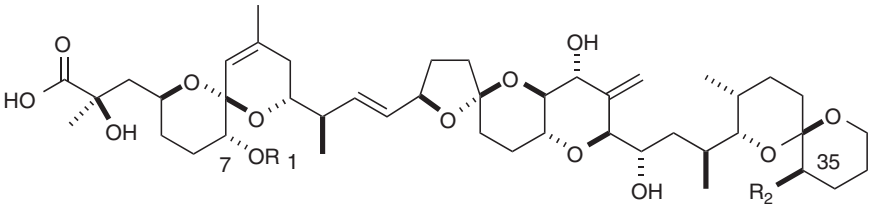
### 8.1 Paralytic Shellfish Poison (PSP)

Bivalves accumulate saxitoxin and its derivatives which are sequestered from dinoflagellates and cause paralytic shellfish poisoning as mentioned in Sect. 2.

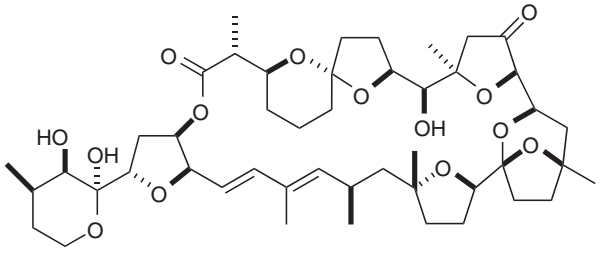
### 8.2 Diarrhetic Shellfish Poison (DSP)

Since the first outbreak of diarrhetic shellfish poisoning (DSP) reported from northern Japan in 1976, large numbers of DSP incidents have been reported from Europe, Japan, and other countries. Okadaic acid (**43**) and its derivatives named dinophysistoxins-1 (**44**) and −3 (**45**) as well as pectenotoxins [pectenotoxin-2 (**46**)] were isolated from mussels and dinoflagellates of the genus *Dinophysis* (Daranas et al. 2001; Ciminello and Fattorusso 2004; Camacho et al. 2007) these polyethers except for pectenotoxins inhibit protein phosphatases 1 and 2A strongly. Although

the toxicities of okadaic acid and various dinophysistoxins are not strong ($LD_{50}$ values of 160–500 µg/kg, i.p. in mice), their potent tumor promotion activity is of serious concern from the viewpoint of public health as mentioned in Chap. 8. The pectenotoxins cause similar symptoms and are as toxic as okadaic acid, but pectenotoxin-2 is not a tumor promoter; rather, it inhibits actin polymerization (Burgess and Shaw 2001; also, see Chap. 7). It should be noted that more than 20 okadaic acid analogues have been isolated from mollusks, sponges and dinoflagellates of the genera *Dinophysis, Prorocentrum* (Daranas et al. 2001).

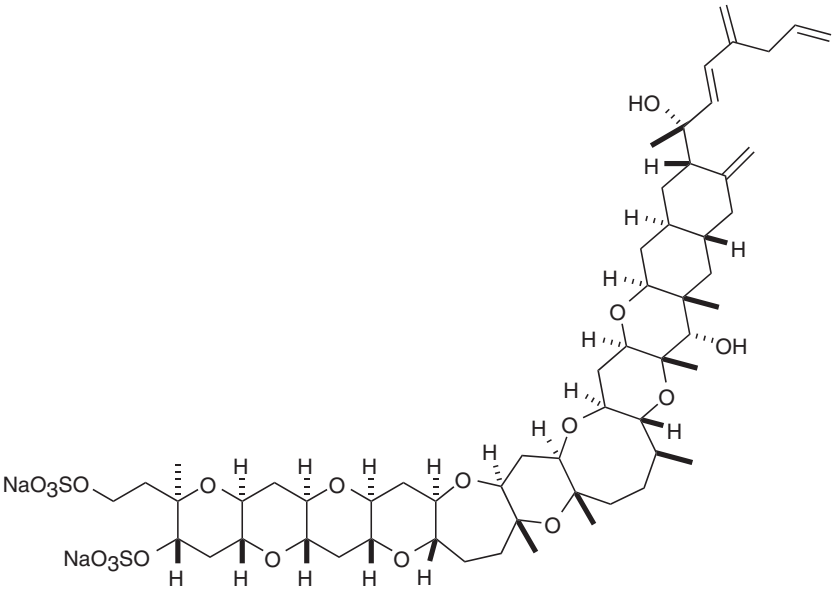



**43:$R_1$=$R_2$=H**
**44:$R_1$=H, $R_{2=Me}$**
**45:$R_1$=acyl, $R_2$=Me**




**46**

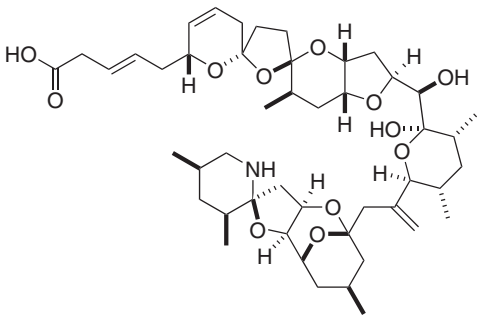
Yessotoxin (**47**) is a ladder-shaped polyether originally isolated as a causative agent of DSP from the Japanese scallop *Patinopecten yessoensis* (Bowden 2006). More than 22 analogues of yessotoxins have been isolated either from shellfish or the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum*. They kill mice within hours at doses of 100–200 µg/kg and are highly cytotoxic. At cellular levels, accumulation of a 100 kDa fragment of E-cadherin was observed when treated with yessotoxin. It also produces $Ca^{2+}$ influx through some types of $Ca^{2+}$ channels. However, yessotoxins are no longer considered as causative agents of DSP due to their low oral toxicity and lack of diarrhetic acitivity in mice (Ciminello and Fattorusso 2004).



47

## 8.3 *Azaspiracid Shellfish Poison (AZP)*

A human intoxication similar to DSP occurred after ingestion of mussels in Europe in 1995. It was named azaspiracid shellfish poisoning (AZP) after azaspiracids, the causative agents isolated from mussels; 11 azaspiracids have been known at present (Virariño et al. 2007). Azaspiracid-1 (**48**), a novel nitrogen-containing polyether, is lethal to mice at i.p. doses of 110–200 µg/kg, highly cytotoxic ($IC_{50}$ values of $10^{-9}$ to $10^{-8}$ M), and teratogenic (Ronzitti et al. 2007). Azaspiracids cause severe damage in the epithelium of several organs and induce accumulation of an E-cadherin fragment (as the case of yessotoxins).



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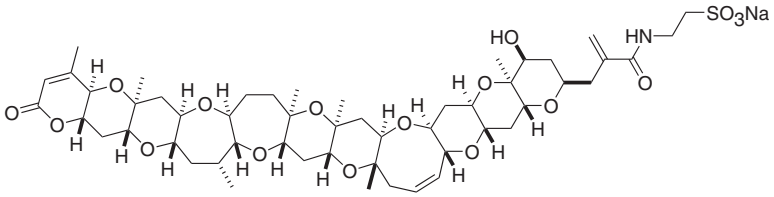
## 8.4 Neurotoxic Shellfish Poison (NSP)

During *Karenia brevis* blooms bivalves often become poisonous and cause human intoxications similar to ciguatera, as mentioned earlier. The causative agents are thought to be brevetoxins and their metabolites such as brevetoxin B1 (**49**) (Yasumoto 2005).



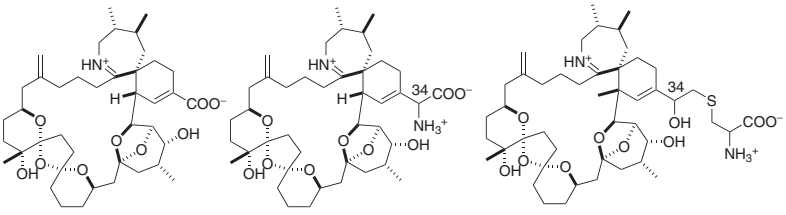

**49**

## 8.5 Amnesic Shellfish Poison (ASP)

A unique food poisoning characterized by the short-term memory loss occurred by ingesting mussels in Canada in 1987 and was named amnesic shellfish poisoning (ASP) (Mos 2001; Sobel and Painter 2005). The causative agent was identified as domoic acid and it was shown to originate from the diatom *Pseudonitzschia multiseries*. Domoic acid and several congeners are produced by several diatoms of the same genus (Daranas et al. 2001). Mass mortalities of marine animals occur from eating domoic acid containing prey (Mos 2001).

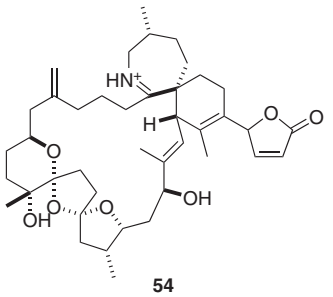
## 8.6 Pinnatoxins and Related Cyclic Imines

Food poisonings from ingestion of bivalves of the genus *Pinna* are known in China and southern Japan. In fact, four novel cyclic imines named pinnatoxins A–D were isolated from the digestive glands of *P. muricata* collected in Okinawa (Kita and Uemura 2005; Molgo et al. 2007). Pinnatoxin A (**50**) is lethal to mice with an $LD_{99}$ value of 180 µg/kg (i.p.), while pinnatoxins B and C (**51**, **52**) are more toxic ($LD_{99}$ 20 µg/kg). Similar toxins named pteriatoxins A–C [A (**53**)] have been isolated from the Okinawan bivalve *Pteria penguin*.




**50** **51: 34*S*** **52: 34*R*** **53**

Spirolides [spirolide A (**54**)] were originally discovered from the digestive glands of mussels and scallops near aquaculture sites along the eastern shore of Nova Scotia, and were later found in the dinoflagellate *Alexandrium ostenfeldii* (Gill et al. 2003; Molgo et al. 2007). Spirolides are fast-acting toxins that may target the muscarinic and nicotinic receptors. Gymnodimine (**55**) is a causative agent of the food poisoning resembling neurotoxic shellfish poisoning occurred from ingestion of New Zealand oysters (Molgo et al. 2007). It is produced by the dinoflagellate *Karenia selliformis*. Gymnodimine is lethal to mice with an $LD_{50}$ value of 96 μg/kg (i.p.) and broadly targets nicotinic acetylcholine receptors (Molgo et al. 2007).

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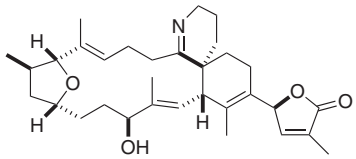
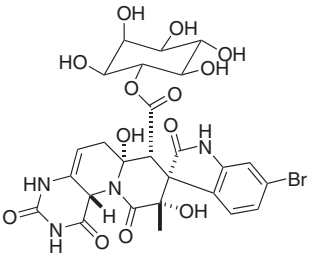


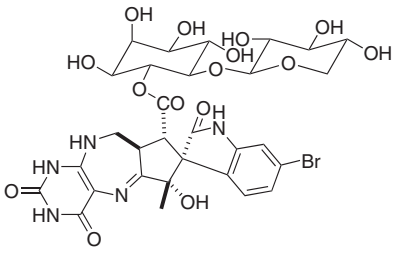

**55**

## *8.7 Surugatoxins*

The Japanese ivory shell *Babylonia japaonica* is a carnivorous gastropod and is known to accumulate toxic substances such as tetrodotoxin from food (Hashimoto 1979). It caused an unusual food poisoning characterized by failing eyesight. Surugatoxin (**56**) was originally isolated as a causative agent from the digestive glands, but further examination led to isolation of neosurugatoxin (**57**), which is 100 times more active than surugatoxin, inducing mydriasis in mice. The conversion of surugatoxin to neosurugatoxin was experimentally demonstrated (Kosuge et al. 1982). Neosurugatoxin is a relatively potent antagonist at nicotinic acetylcholine receptors (Hayashi et al. 1984). Interestingly, neosurugatoxin was produced by a Coryneform bacterium isolated from sea sediments inhabited by *B. japonica* (Kosuge et al. 1985).



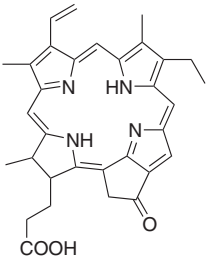

**56**




**57**

## *8.8 Other Toxins*

Several Japanese abalones (genus *Haliotis)* accumulate pyrophaephorbide *a* (**58**) in their viscera in early spring and an unusual food poisoning from eating such viscera was reported in northern Japan (Hashimoto 1979). Pyropheophorbide *a* is a photodynamic agent derived from chlorophyll *a*. Thus, abalone poisoning is a rare photosensitization disease.



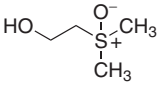

**58**

A protein toxin fraction named cephalotoxin that potently paralyses crabs is found in the posterior salivary glands of octopuses and cuttlefishes (Ghiretti 1959; Songdahl and Shapiro 1974). The α- and β-cephalotoxins purified from *Octopus vulgaris* are glycoproteins with respective molecular weights of 91,200 and 33,900 (Cariello and Zanetti 1977). Their mechanism of action is not yet known. A cytolytic protein displaying only weak crab paralytic activity occurs in the posterior salivary gland of the Atlantic squid, *Loligo pealii* (Kem and Scott 1980). The blue-ringed octopus *Hapalochlaena maculosa* is unique among octopi in that its posterior salivary glands contain TTX, which is secreted as a venom when it bites an attacker. An additional toxin, hapalotoxin, is apparently more effective in immobilizing prey (Savage and Howden 1977).

# 9 Arthropods, Bryozoans and Echinoderms

Crabs belonging to the family Xanthidae cause human intoxications in Indo-Pacific regions, while horseshoe crabs sporadically cause food poisonings in South-East Asian countries (Halstead 1967; 1984; Hashimoto 1979). While the xanthid crabs contain palytoxin, saxitoxins, and/or tetrodotoxins (Halstead 1984; Yasumoto 2005), horseshoe crabs often contain either saxitoxins or tetrodotoxins (Halstead 1984; Miyazawa and Noguchi 2001). The origins of these toxins are unknown, except for tetrodotoxins in xanthid crabs that presumably originate from red algae of the genus *Jania* (Yasumoto 2005). It was also reported that the starfish *Asteropecten polyacanthus* contains tetrodotoxin (Mosher and Fuhrman 1984).

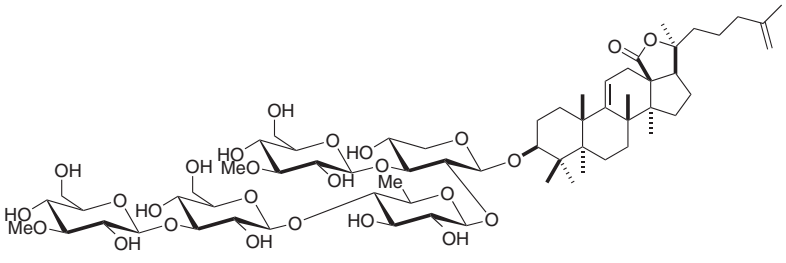
"Dogger Bank itch" is an allergic contact dermatitis disease popular among North Sea fishermen caused by sensitization to an allergen from the marine bryozoan *Alcyonidium gelatinosum*. The allergen was identified as (2-hydroxyethyl) dimethylsulfoxonium (**59**) (Carlé and Christophersen 1980). This compound was also found in a sponge (Warabi et al. 2001).



**59**

## *9.1 Holothurins and Asterosaponins*

The first animal saponin named holothurin was isolated from the sea cucumber *Holothuria vagabunda* in 1955 (Yamanouchi 1955) and the sea cucumber saponins have been generally called "holothurins." The complete structure of a holothurin [holotoxin A (**60**) from *Sticopus japonicus*] was elucidated in 1978 (Hashimoto 1979). Holothurins are triterpenoid saponins composed of a lanosterol-derived aglycone and several sugars (Minale et al. 1993) and show a wide range of biological activities such as hemolytic, ichthyotoxic, antimicrobial (Hashimoto 1979). Asterosaponins [thornasteroside A (**61**) from the crown-of-thorns starfish *Acanthaster planci*] are steroidal saponins derived from starfishes and exhibit a variety of biological activities similar to the holothurins (Hashimoto 1979; Minale et al. 1993). In recent decades a large number of saponins have been isolated from sponges, cnidarians and brittle stars, in addition to sea cucumbers and starfishes (Blunt and Munro 2007).



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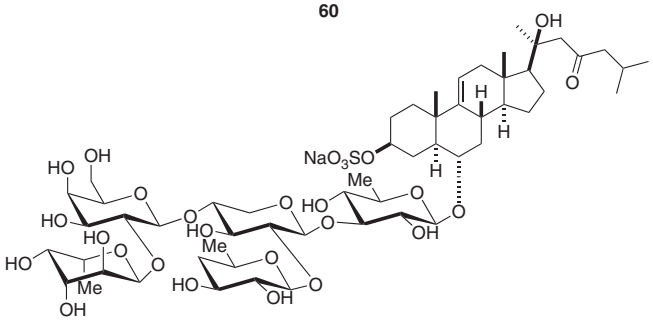


**61**

### 9.2 Protein Toxins

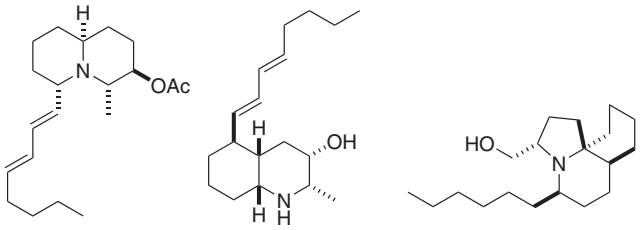
From some sea urchins a venomous secretion is elicited when globiferous pedicellariae, flower-shaped structures mounted on stalks found between the spines, are mechanically and chemically stimulated at the same time. Some urchins (*Diadema* species, for example) also have long slender spines that are reputed to be poisonous, but this has not yet been unequivocally demonstrated. Pedicellarian toxins are largely, if not totally, peptidic substances. Investigations on several poisonous European and IndoPacific sea urchins have reported a variety of physiological actions of pedicellarian venom, including hemolysis, inflammation, algesia (pain), neurotoxicity and cardiovascular depression. It is not yet clear that these activities are due to the same peptides. Most activities are retained on concanavalin A chromatographic columns and can be released by the addition of specific sugars to the eluting solution. Two D-galactose-specific sea urchin lectins (SUL-1 and -2) with smooth muscle stimulatory activity were isolated from *T. pileolus* (Nakagawa et al. 1991). SUL-1 (MW~32,000) is mitogenic and induces dendritic cell maturation (Takei and Nakagawa 2006). These lectins could become useful probes for investigating the processes involved in the exocytotic secretion of neurotransmitters and other signaling molecules (Takei et al. 1993).

While most starfish chemically defend themselves by means of their integumerntary saponins, the crown of thorns starfish also contain secretes interesting basic peptide toxins (Shiomi et al. 1988a; Ota, 2006). The most lethal, isolated protein (MW~25,000) displayed a mouse i.p. $LD_{50}$ of 0.43 mg/kg. It potently damaged the mouse liver (Shiomi et al. 1988b). More recently the hepatotoxic plancitoxins were thoroughly purified and characterized by cloning. Both homologs were sequenced and shown to be homologous to deoxyribonucleases; their DNAase activities were then demonstrated. Thus, plancitoxin I is the first known example of a toxic DNAase (Shiomi et al. 2004).

## 10 Urochordates and Fishes

Ascidians (tunicates) contain a variety of cytotoxic metabolites, some of which may be toxic to mammalians (Blunt and Munro 2007). Pictamine (**62**), a quinolizidine alkaloid isolated from the ascidian *Clavelina picta* and lepadin B (**63**), a decahydroquinoline alkaloid from *C. lepadiformis* block neuronal nicotinic acetylcholine receptors at μM levels (Tsuneki et al. 2005), whereas lepadiformine (**64**), a rare decahydro-1*H*-pyrrolo[2,1-*j*]quinoline isolated from *Clavelina* spp. inhibits cardiac muscle $K^+$ channels (Sauviat et al. 2006).

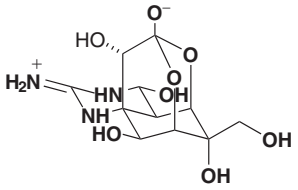
Subtropical and tropical fishes accumulate ciguatoxins and cause ciguatera as mentioned Sect. 3.2.2 (also see Chap. 3). Some other types of fish poisonings have been known, but their causative agents remain to be fully elucidated (Hashimoto 1979).




62 63 64

## 10.1 Tetrodotoxins

It is well known that tetrodotoxin (**65**) is widely distributed in puffer fish of the family Tetraodontidae and is believed to be originated from bacteria of the genera *Vibrio, Pseudoalteromonas* and others (Yasumoto 2005). At moment, more than ten variants of tetrodotoxin have been isolated from a wide range of organisms including xanthid crabs, horseshoe crabs, frogs, and newts in addition to puffers and other marine animals that mentioned earlier (Miyazawa and Noguchi 2001). Interestingly, freshwater puffer fish contain saxitoxins, but not terodotoxins (Ngy et al. 2008). Tetrodotoxins bind to Site 1 on $Na^+$ channels and block sodium influx into muscle and nerve cells (see Chap. 3).




65

## 10.2 Peptide and Protein Toxins

### 10.2.1 Fish Integument Toxins

Integumentary secretions of soapfishs, another teleost family, contain hemolytic peptides called grammistins (Hashimoto and Oshima 1972; Hashimoto 1979; Sugiyama et al., 2005). These are linear peptides of 12–28 residues that contain many basic amino acids and possess alpha-helical secondary structures. These peptides display antimicrobial activities against a wide variety of bacteria. Several grammistins were recently cloned (Kaji et al. 2006). Pardaxins (named after the genus *Pardachirus*) are surface-active anti-microbial and hemolytic peptides that are secreted from the skin of the Red Sea sole when it is stressed; these were purified

and shown to form pores in liposomes (Lazarovici et al. 1986; Thompson et al. 1986 also, see Chap. 6).

### 10.2.2 Fish Spine Toxins

Quite a few families of fish have members that possess poisonous spines. Perhaps the most well known are the scorpion fish including the Pacific stonefishs and lionfish. During the past 15 years the proteinaceous toxins isolated from three stonefish, *Synanceja horrida (*stonustoxin), *S. trachynis* (trachnilysin) and *S. verrucosa* (verrucotoxin and neoverrucotoxin) have been purified and extensively characterized (Poh et al. 1991; Ueda et al. 2006; Ghadessy et al. 1996). They are large (~150 kDa) but relatively stable proteins, containing two subunits of similar size. The cloned α- and β-subunits (71 and 79 kDa, respectively) of stonustoxin display ~50% sequence homology. There are 7 and 8 cysteine residues, respectively, in these subunits, and 10 of the 15 residues participate in intrachain disulfide linkages. The remaining five cysteines are uncombined. The stonefish toxins are pore-forming proteins, explaining their ability to lyse erythrocytes (Chen et al. 1997; Khoo 2002). The synaptic effects of trachnilysin will be described further in Chap. 6.

Many other fish contain poisonous spines that inflict painful wounds in fisherman and bathers. Oriental catfish (*Plotosus lineatus*) spine venom contains hemolytic, edema-forming and lethal proteins. The hemolytic protein fraction is of large size (~180 kDa) compared with the lethal proteins (~12 kDa). One lethal protein has been purified and partially characterized (Shiomi et al. 1986). Sting rays also possess poisonous spines and broken spines are often embedded in the surfaces of their predators; their proteinaceous toxins have yet to be purified and characterized.

## 11 Concluding Comments

Investigations of marine toxins during the past few decades have provided a remarkable diversity of molecules new to science. Besides stimulating chemical interest in unique structures posing many synthetic challenges, these substances often possess such unique targets that they can serve not only as useful research tools, but in some cases can either be useful drug candidates in their naturally occurring forms or as leads for designing analogs that possess even more selective actions. Traditionally, small, non-peptide molecules (MW < ~500) have served as lead compounds for drug design, due to their superior bioavailability and ease of synthesis, but this approach is rapidly being enlarged to include much larger molecules including peptides and nucleic acids. Who would have thought that an extremely lethal, large bacterial protein like botulinum toxin would become a very useful drug for treating various muscle spasms (Cooper 2007). Similarly a sea anemone peptide of 35 residues (ShK, Fig. 1) that blocks an important $K^+$ channel (Kv1.3) in T-lymphocytes has reasonable pharmacokinetic properties and has

become a drug candidate for treating host-graft (transplantation) immune rejection and certain autoimmune diseases (Beeton et al. 2006).

Regardless of whether a natural product or its analogue is to be used as a molecular probe or as a drug, target selectivity will generally be of the utmost importance. If the toxin is to be used as a probe in an in vitro system, where additional sites of action are not present, the degree of selectivity is much less stringent. On the other hand, for use as in vivo probe or as a drug administered to the whole organism, the natural product may require considerable structural manipulation (engineering) to improve target selectivity, reduce toxicity and improve pharmacokinetic properties. Natural products including toxins often preferentially interact with one or a few receptor subtypes, but it would be rare for a toxin to affect only a single receptor subtype, as this would limit its ability in nature to neutralize a wide variety of prey or predators.

Historically, marine natural products research has lagged behind the investigation of terrestrial natural products, due to the limited accessibility of most marine organisms. However, the chemical diversity of natural toxins among marine animals is likely to be much higher than for terrestrial animal toxins, as the phyletic diversity of marine animals is greater. Since the chemical diversity of marine organisms is only beginning to be appreciated, it can be anticipated that future studies will reveal a plethora of new compounds of scientific and potential therapeutic interest.

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## References

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