Shellfish Poisons

M. Kita, D. Uemura

Abstract. In our ongoing search for bioactive metabolites from marine organisms, novel shellfish poisons have been isolated. Pinnatoxins, which are amphoteric polyether compounds, were purified from the Okinawan bivalve *Pinna muricata*. Pinnatoxins show acute toxicity against mice and activate Ca^{2+} channels. Two novel alkaloids, pinnamine and pinnaic acid, were also obtained from *P. muricata*. Pinnaic acid inhibits cytosolic phospholipase ($cPLA_2$). Pteriatoxins, which are pinnatoxin analogs, were isolated from the Okinawan bivalve *Pteria penguin*. A nanomole-order structure determination of pteriatoxins was achieved by the detailed analysis of 2D-NMR and ESI-TOF MS/MS. This review covers the isolation, structure determination, bioactivity, synthesis, and biogenesis of these shellfish poisons and related compounds.

2.1 Introduction

Seafood products are important both nutritionally and economically, but human intoxication resulting from the ingestion of shellfish occurs worldwide. The most important diseases, which are caused by red tides, are paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), and ciguatera fish poisoning (CFP; Yasumoto and Murata 1993; Todd 1994; Lehane 2001). Severe cases of many different types of seafood poisonings can result in fatalities. A number of toxins from poisonous shellfish have been isolated, e.g., okadaic acid (Tachibana et al. 1981), saxitoxin (Schantz et al. 1957), and pinnatoxins (Uemura et al. 1995; Chou et al. 1996a). These compounds have attracted interest not only from pharmacologists but also from biochemists and chemists due to their extraordinary biological activities and complex structures. These bioactive marine metabolites are quite useful candidates for drugs or biological probes for physiological studies. For the purpose of preventing disease and safeguarding consumer health, it is quite important to clarify the structure and biological functions of these poisonous metabolites.

Fascinating compounds with unique chemical structures and biological activities have been found in marine organisms. However, the true origins or progenitors of these metabolites are not entirely clear. The possible primary producers of the secondary metabolites have been suggested to be microalgae, bacteria, and fungi. These metabolites are carried through

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symbiosis, association, food chain, and other forms of nutrient dependency (Shimizu 1993, 1996, 2003; Daranas et al. 2001). For instance, palytoxin (PTX) is a potent toxic polyol compound isolated from the zoanthid Palythoa sp. (Cha et al. 1982). The presence of related toxins in animals from distinct geographical areas was questioned due to seasonal and regional variations (Moore and Scheuer 1971; Moore and Bartolini 1981; Uemura et al. 1981). In 1995, a PTX analog, osteocin, was isolated from the dinoflagellate Ostreopsis siamensis. Its true origin was suggested from microorganisms (Usami et al. 1995). Therefore, related shellfish poisons may also be biosynthesized by marine microorganisms.

Shellfish poisons and related compounds have been well represented, with many fine reviews (Kuramoto et al. 1999, 2003, 2004; Brett 2003; Ciminiello et al. 2003; Ciminiello and Fattorusso 2004; Jeffery et al. 2004; Suenaga 2004; Kita and Uemura 2005). In our ongoing search for bioactive metabolites from marine organisms, novel shellfish poisons such as pinnatoxins, pteriatoxins, pinnamine, and turbotoxins have been isolated. In this chapter, details of our latest work on shellfish poisons are described, along with up-to-date topics.

2.2 Pinnatoxins, Ca^{2+} Channel-Activating Polyether Toxins from the Okinawan Bivalve *Pinna muricata*

Shellfish of the genus *Pinna* live mainly in shallow waters of the temperate and tropical zones of the Indian and Pacific Oceans (Rosewater 1961). The adductor muscle of this bivalve is eaten in Japan and China, and food poisoning resulting from its ingestion occurs frequently. Although this poisoning has been shown to be caused by bacterial infection or neurotoxins, the true causative agent is ambiguous. Chinese investigators have reported that a toxic extract, referred to as pinnatoxin, from *P. attenuata* is a Ca^{2+} channel activator (Zheng et al. 1990). We have successfully isolated pinnatoxin A (**1**), a mixture of pinnatoxins B (**2**) and C (**3**), and pinnatoxin D (**4**) from *P. muricata* (Fig. 2.1) as a major cause of food poisoning (Uemura et al. 1995; Chou et al. 1996a,c; Takada et al. 2001a).

2.2.1 Isolation and Structure of Pinnatoxin A

The viscera (45 kg) of *P. muricata* collected in Okinawa, Japan, were extracted with 75% ethanol. The extract was filtered and the concentrated filtrate was washed with ethyl acetate and concentrated. The oily residue was successively chromatographed on TSK-G3000S polystyrene gel, Sephadex LH-20, DEAE Sephadex A-25, and an ODS-AQ column, using a

bioassay-guided (intraperitoneal injection against mice) fractionation. Final purification was achieved by reverse-phase HPLC to give pinnatoxin A (3.5 mg), a mixture of B and C (1.2 mg), and D (2.0 mg).

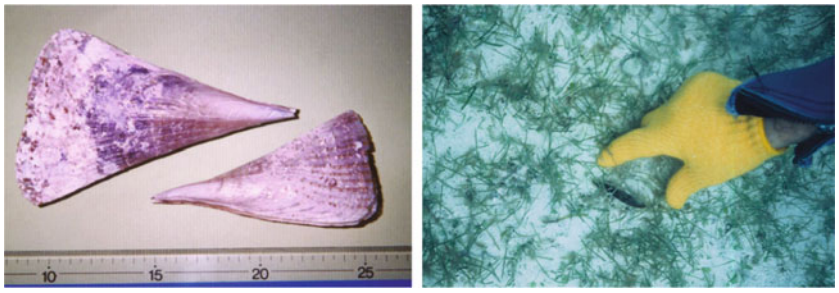
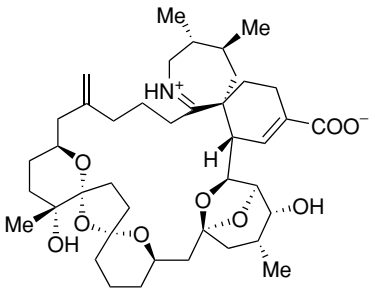


Fig. 2.1. The Okinawan bivalve *Pinna muricata*

The structures and stereochemistry of pinnatoxins have been clarified by extensive NMR experiments and positive ion ESI MS/MS spectra (Satake et al. 1991; Naoki et al. 1993). Pinnatoxins consist of a 20-membered ring, i.e., with 5,6-bicyclo, 6,7-azaspiro, and 6,5,6-triketal moieties in their structure. In particular, they contain a carboxylate anion and an iminium cation or an ammonium cation.



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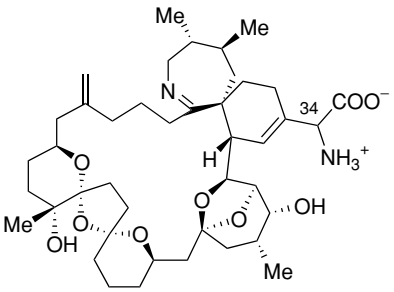
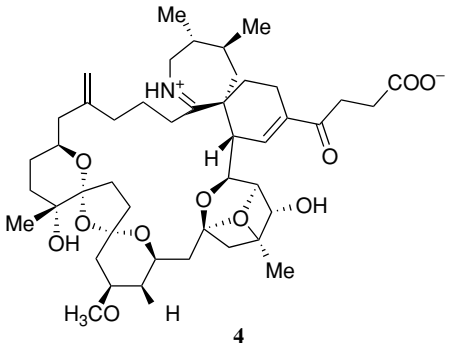


2 34*S* isomer
3 34*R* isomer



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2.2.2
Structure of Pinnatoxins B and C

The molecular formula of both pinnatoxins B (**2**) and C (**3**) was determined to be $C_{42}H_{64}N_2O_9$ by ESI MS, which reflects a 29 MS unit (CH_3N) increase compared with that of pinnatoxin A (**1**). A positive ninhydrin test on a TLC plate for **2** and **3** suggested the presence of an amino group. Although they were obtained in small amounts, the structures and relative stereochemistries of the macrocycles in **2** and **3** were successively established as follows. A detailed analysis of the DQF-COSY and HOHAHA spectra of **2** and **3** supported almost all the carbon–carbon connectivities (Takada et al. 2001a). On the positive ion ESI MS/MS analysis, a series of prominent fragment ions was generated by a cyclohexane ring-opening reaction, the retro-Diels–Alder reaction, which was followed by bond cleavage of carbocycles (Fig. 2.2).

Reduction of the imino group in **2** and **3** with $NaBH_4$ followed by oxidative cleavage with $NaIO_4$ provided aldehyde **6** (Scheme 1). The same product was obtained from pinnatoxin A methyl ester (**5**) by reduction of both the iminium and carboxylic acid functionalities, followed by oxidation of the resulting alcohol. Thus, pinnatoxins **1**–**3** had the same relative stereochemistry in the macrocyclic core.

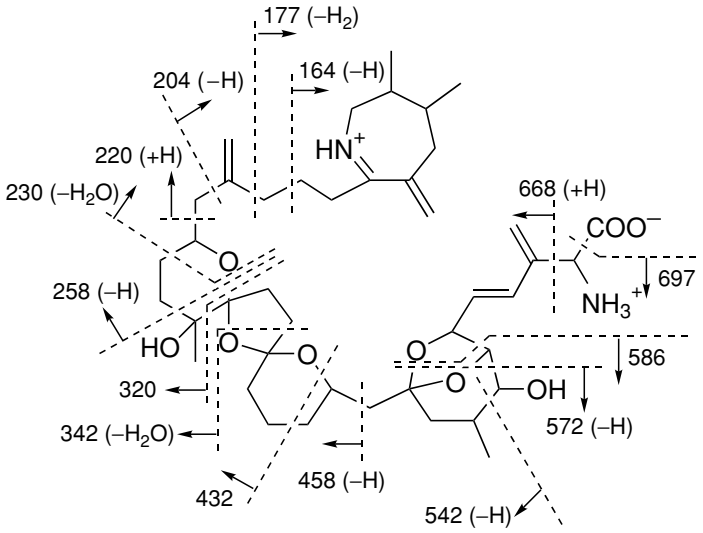
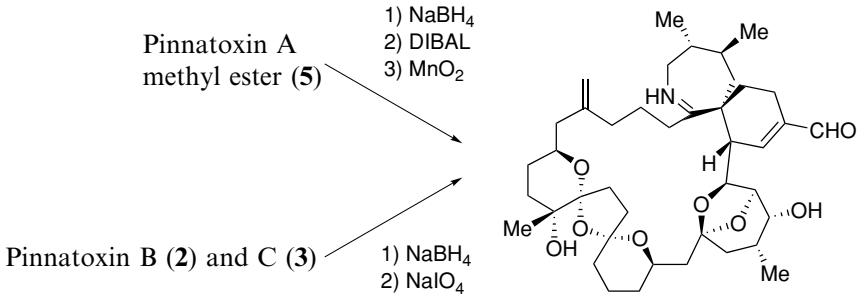


Fig. 2.2. Fragmentation patterns of pinnatoxins B (**2**) and C (**3**)



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Scheme 1

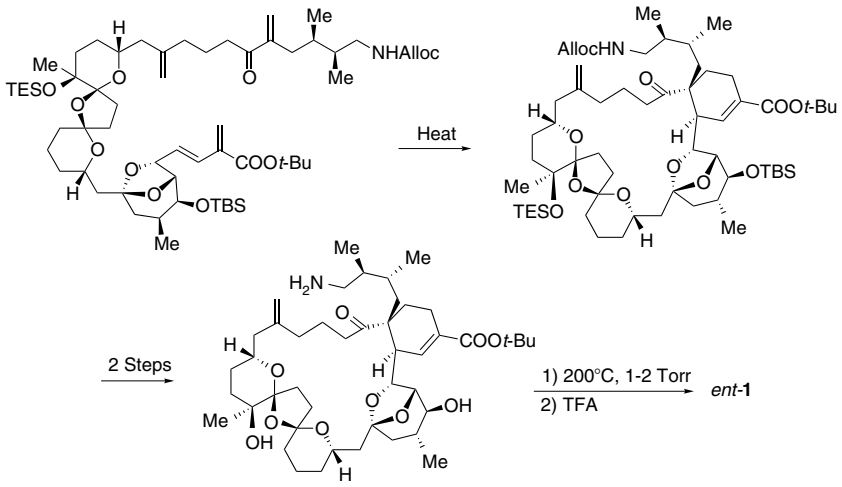
2.2.3 Biological Activity of Pinnatoxins

Pinnatoxin A (**1**) showed potent acute toxicity against mice (LD_{99} 180 μg kg^{-1}, i.p.) with characteristic neurotoxic symptoms. Pinnatoxin A activated Ca^{2+} channels. Pinnatoxins B (**2**) and C (**3**), the most toxic constituents in the pinnatoxin series, have been isolated from *P. muricata* (as a 1:1 mixture; Takada et al. 2001a). The LD_{99} values of **2** and **3** were 22 μg kg^{-1}, which makes them as potent as tetrodotoxin. Although pinnatoxin D (**4**) showed weaker acute toxicity than the other pinnatoxins (LD_{50} > 10 μg $mouse^{-1}$ unit), **4** showed the strongest cytotoxicity against mouse leukemia cell line P388 (IC_{50} 2.5 μg ml^{-1}).

2.2.4 Biogenesis and Synthesis of Pinnatoxins

The backbone of pinnatoxins and their analogs could be configured from C1 to C34 in a single carbon chain, in a polyketide biogenetic pathway (Fig. 2.3; Uemura et al. 1995). This biosynthetic proposal entails an intramolecular Diels–Alder reaction to construct a G-ring as well as the macrocycle, followed by imine formation to establish a 6,7-spiro-ring system. Due to the structural similarity of the imine moiety adjacent to the spirocyclic core, other macrocyclic imines represented by pinnatoxin may also be biosynthesized via the same intramolecular Diels–Alder reaction.

Recently, Kishi's group achieved the total synthesis of **1** and *ent*-**1**, utilizing a biomimetic intramolecular Diels–Alder reaction (Scheme 2; McCauley et al. 1998). Interestingly, natural **1** showed significant acute toxicity, while its antipode *ent*-**1** was nontoxic (Nagasawa 2000). This investigation also confirmed the stereochemistry of **1**, including its absolute stereochemistry.



Scheme 2

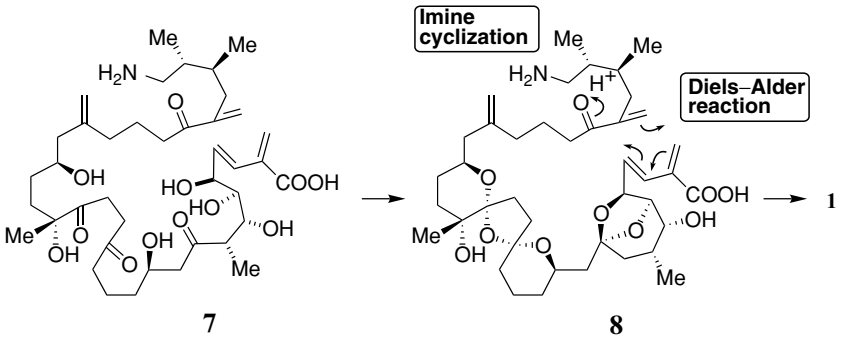


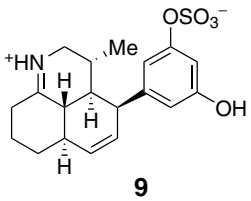
Fig. 2.3. Proposed biogenesis of pinnatoxin A (**1**)

2.2.5 Symbioimine, a Potential Antiresorptive Drug

Recently, we isolated a unique amphoteric iminium compound, named symbioimine (**9**), from the symbiotic marine dinoflagellate *Symbiodinium* sp. (Kita et al. 2004). This dinoflagellate is found in a wide range of marine invertebrates and produces several bioactive large polyol compounds, such as zooxanthellatoxins (ZTXs; Nakamura et al. 1993, 1995a,b) and zooxanthellamides (Onodera et al. 2003, 2004). A number of bioactive large polyol and polyether compounds, represented by PTX, halichondrin, ciguatoxin, and maitotoxin, have been reported (Uemura 1991;

Yasumoto 2001; Murata and Yasumoto 2000) and called "super-carbon -chain compounds" (Uemura 1991).

The structure of **9**, which consists of a characteristic 6,6,6-tricyclic iminium ring, was deduced by spectroscopic analysis and X-ray crystallographic analysis.



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Symbioimine (**9**) inhibited osteoclastogenesis of the mouse monocytic cell line RAW264, which can differentiate into osteoclasts following treatment with a receptor activator of nuclear factor-κB ligand (RANKL; EC_{50} = 44 μg ml^{-1}; Kita et al. 2004). RANKL induces the formation of osteoclast-like multinucleated cells in cultures of bone marrow cells. Symbioimine (**9**) inhibited an increase in the sRANKL-induced TRAP activity of preosteoclast cells. Meanwhile, it did not affect cell viability, even at 100 μg ml^{-1}. Thus, symbioimine (**9**) is a potential antiresorptive drug for the prevention and treatment of osteoporosis in postmenopausal women. Symbioimine (**9**) also inhibited cyclooxygenase 2 (COX-2) activity (EC_{50} ~ 10 μM; Kita et al. 2005; Kita and Uemura 2005). Although the real ecological role of **9** in the symbiotic dinoflagellate is unknown yet, it can be hypothesized that it serves as a defense material which prevents their host animal's digestion.

A plausible biogenetic pathway for **9** could involve an intramolecular *exo*-transition state Diels–Alder reaction followed by imine cyclization, the same as that proposed for pinnatoxins (Fig. 2.4).

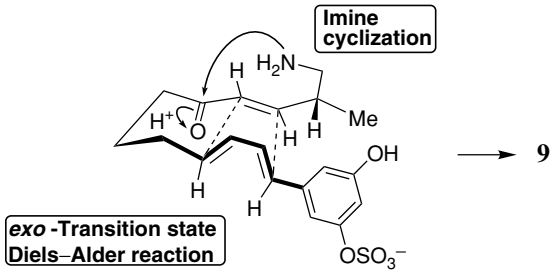


Fig. 2.4. Proposed biogenesis of symbioimine (**9**)

2.3
Pteriatoxins, Pinnatoxin Analogs from the Okinawan Bivalve *Pteria penguin* – Nanomole- Order Structure Determination

2.3.1
Isolation of Pteriatoxins

In our study of shellfish poisons, we observed that a moray eel vomits the viscera of the Okinawan bivalve *Pteria penguin* (Fig. 2.5). The aqueous 75% EtOH extract of viscera (82 kg) of *P. penguin*, which showed acute toxicity, was partitioned between EtOAc and H_2O. Guided by acute toxicity against mice, the aqueous fraction was chromatographed on TSK-G3000S polystyrene gel (50% EtOH), DEAE Sephadex A-25 (0.02 M phosphate buffer), CM Sephadex C-25 (0.2 M phosphate buffer), reversed-phase HPLC (Develosil 300 ODS, MeCN/H_2O/TFA), and reversed-phase HPLC (Develosil 300 C8, MeCN/H_2O/TFA) to isolate successfully pteriatoxins A (**10**) and B, C (**11**, **12** as a 1:1 mixture) as extremely toxic and minor components (Takada et al. 2001b). Since there was too little of these toxins to weigh, the weights of pteriatoxins A (20 μg) and B, C (8 μg) were estimated by comparison of the signal-to-noise (S/N) ratio in ^{1}H NMR spectra of 67 μM okadaic acid with those of pteriatoxins. Pteriatoxins (**10**–**12**) showed significant acute toxicity against mice, with LD_{99} values of 100 and 8 μg kg^{-1}, respectively. The toxic symptoms of pteriatoxins resemble those of pinnatoxins.

Fig. 2.5. The Okinawan bivalve *Pteria penguin*

2.3.2 Structure of Pteriatoxins

The molecular formula of pteriatoxin A (**10**) was determined by ESI MS to be $C_{45}H_{70}N_2O_{10}S$. As mentioned above, the positive ion ESI MS/MS of pinnatoxins showed a series of prominent fragment ions generated by G ring-opening reactions, followed by bond cleavage. The positive ion ESI MS/MS of **10** showed the same series of prominent fragment ions as the carbocyclic moiety in pinnatoxins (Fig. 2.6). The observation of fragment ion peaks (*m*/*z* 787, 744) suggested the presence of an α-amino acid moiety in the side-chain. Furthermore, the chemical shifts of H-35 (δ_H 2.80) and H-1′ (δ_H 3.04, 3.13) suggested the presence of a sulfide bond between C-35 and C-1′. The chemical shift of H-34 (δ_H 4.22) suggested the presence of an allylic hydroxyl group at C-34. Therefore, the gross structure of pteriatoxin A was determined, as shown in **10**.

The molecular formula of both **11** and **12** was determined by ESI MS to be $C_{45}H_{70}N_2O_{10}S$. Analysis of the ^{1}H NMR spectrum showed duplicate signals (1:1) for a set of protons (H-3, H-4, H-28 to H-37, H-40, H-41), suggesting the presence of epimeric isomers. Analysis of the ^{1}H NMR, COSY, and HOHAHA spectra suggested nine partial structures, as depicted in Fig. 2.7. The positive ion ESI MS/MS of **11** and **12** showed the same series of prominent fragment ions as the macrocyclic moiety in pinnatoxins and pteriatoxin A (**10**). Therefore, pteriatoxins B (**11**) and C (**12**) were also assumed to have the same polyether macrocycles as pinnatoxins. The observation of fragmentation ion peaks (*m*/*z* 787, 744, 710) suggested the presence of a cysteine moiety in the side-chain. Furthermore, the observation of another fragment ion peak (*m*/*z* 712), which was not observed in **10**, suggested the presence of a hydroxymethyl group. Therefore, the gross structure of pteriatoxins B and C was determined, as shown in **11** and **12**. The position of duplicate signals in the ^{1}H NMR spectrum suggested that pteriatoxins B (**11**) and C (**12**) are also C-34 epimers, like **2** and **3**.

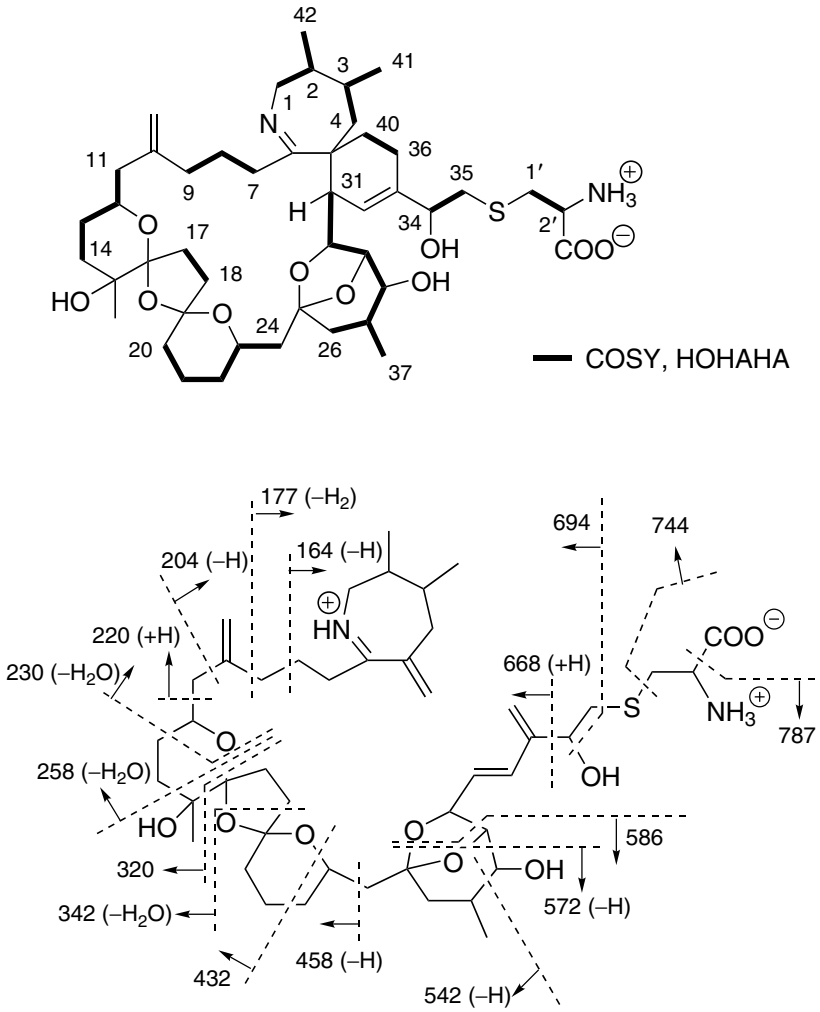


Fig. 2.6. Partial structures and fragmentation pattern of pteriatoxin A (**10**)

As described above, the absolute stereochemistries of a series of pinnatoxins have been confirmed by total synthesis (McCauley et al. 1998; Nagasawa 2000). By comparing the chemical shifts and coupling patterns in the ^{1}H NMR spectra of pteriatoxins with those of pinnatoxins, the same relative stereochemistry in the carbocyclic moieties was suggested for both series of compounds (Takada et al. 2001b). As a result, though the isolated yields of pteriatoxins were too small to deduce their structures by usual NMR analysis, a nanomole-order structure determination of pteriatoxins was achieved by the detailed analysis of ESI MS/MS.

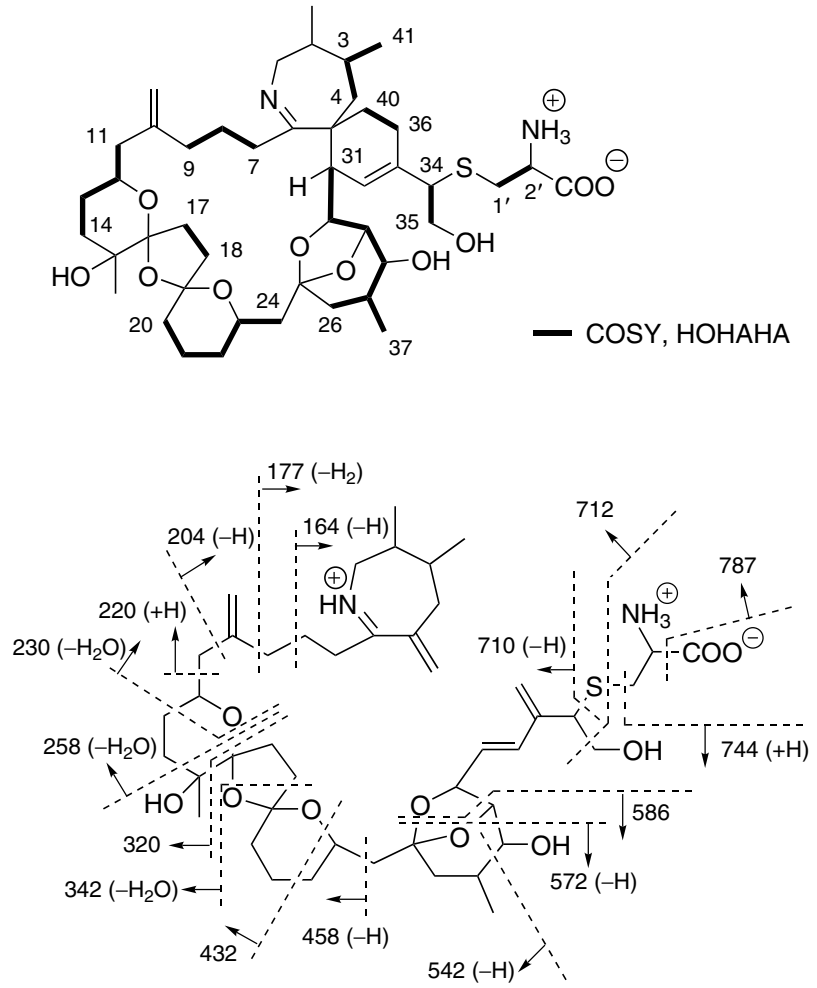


Fig. 2.7. Partial structures and fragmentation pattern of pteriatoxins B (**11**) and C (**12**)

All extracts from the digestive glands of several *Pinna* spp, including *P. muricata*, *P. attenuata*, *P. atropupurea*, and the commonly eaten shellfish *Atrina pectinata*, produced the same symptoms of poisoning in mice. These data suggested that *Pinna* shellfish might become toxic as a result of feeding on toxic organisms, such as dinoflagellates. Also, the presence of pinnatoxin analogs in both shellfish *Pinna* spp and *Pteria* spp suggested that the toxins are biosynthesized by microorganisms that are in the food chain of or in a symbiotic relationship with these shellfish.

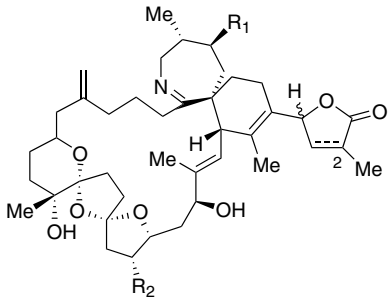
2.3.3 Other Macrocyclic Iminium Toxins Related to Pinnatoxins

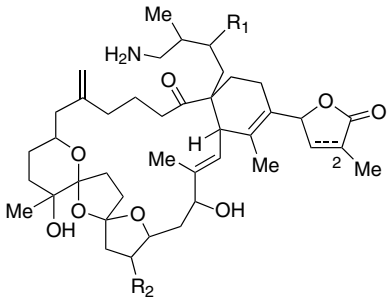
Several shellfish poisons containing nitrogen atoms, especially iminium moieties, have been isolated. Spirolides, a class of macrocyclic imines, were identified in extracts of the digestive glands of mussels and scallops from the Atlantic coast of Nova Scotia, Canada (Hu et al. 1995). The marine dinoflagellate *Alexandrium ostenfeldi* (Paulsen) Balech and Tangen was identified as the cause of spirolide toxicity in Nova Scotia in the early 1990s (Hu et al. 1996a, 2001; Cembella et al. 2000). Seven compounds, spirolides A–D (**13**–**16**) and 13-desmethyl C (**17**), which showed toxicity against mice, and the keto amine derivatives E and F (**18**, **19**), have been isolated and structurally characterized from the extracts of both shellfish and cultured dinoflagellate from Nova Scotia. The spirolide family contains a 5:5:6 trispiroketal ring system. Recently, the relative stereochemistry of spirolides B (**14**), D (**16**), and 13-desmethyl C (**17**), except for one chiral center, has been determined from 2D-NMR data analysis and a molecular modeling method, which showed that these compounds have the same relative stereochemistry as pinnatoxins in the region of their common structure (Falk et al. 2001).

Other marine toxins possessing a cyclic imine moiety elsewhere in the carbon skeleton are known as gymnodimine, centrimine, and prorocentrimine. Gymnodimine (**20**) was isolated from the New Zealand oyster *Tiostrea chilensis* and the dinoflagellate *Gymnodinium* sp. (Seki et al. 1995). The minimum lethal dose (MLD) of **20** was 450 μg kg^{-1}; and this compound also showed potent ichthyotoxicity against the fish *Tanichthys albonubes* at 0.1 ppm. The absolute stereostructure of gymnodimine (**20**) has been established by X-ray crystal structure analysis of the *p*-bromobenzamide derivative **22** derived from gymnodamine (**21**; Stewart et al. 1997). Gymnodimine B, which contains an exocyclic methylene at C-17 and an allylic hydroxyl group at C-18, was also isolated from the same dinoflagellate (Miles et al. 2000; Munday et al. 2004).

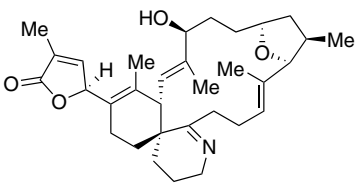
Prorocentrolide (**23**), a toxic marine macrolide that incorporates a hexahydroisoquinoline moiety, was isolated from the cultured dinoflagellate *Prorocentrum lima* (Torigoe et al. 1988; Hu et al. 1996b). This dinoflagellate produces DSP toxins, such as okadaic acid and dinophysistoxins (Tachibana et al. 1981; Yasumoto et al. 1985; Hu et al. 1992). Recently, a prorocentrolide derivative, spiro-prorocentrimine (**24**), was isolated from a cultured benthic *Prorocentrum* sp. in Taiwan and its relative stereochemistry was established by X-ray crystallographic analysis (Lu et al. 2001). Compound **24** is much less toxic than other cyclic iminium toxins.

It should be noted that both the keto amine derivatives spirolide E and F (**18**, **19**; Hu et al. 1996a), in which this ring is open, and the reduced form of gymnodamine (**21**; Stewart et al. 1997) were inactive. Although the pharmacological action of these iminium compounds has not been fully defined yet, the cyclic imine functionality may be essential and may act as a pharmacophore of macrocyclic iminium compounds, e.g., pinnatoxins and spirolides.





13 R_1 = H, R_2 = CH_3, $\Delta^{2,3}$
14 R_1 = H, R_2 = CH_3
15 R_1 = CH_3, R_2 = CH_3, $\Delta^{2,3}$
16 R_1 = CH_3, R_2 = CH_3
17 R_1 = CH_3, R_2 = H, $\Delta^{2,3}$
18 R_1 = H, R_2 = CH_3, $\Delta^{2,3}$
19 R_1 = H, R_2 = CH_3



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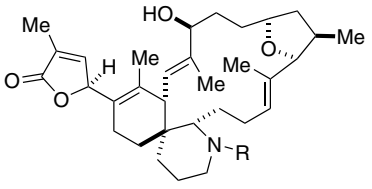
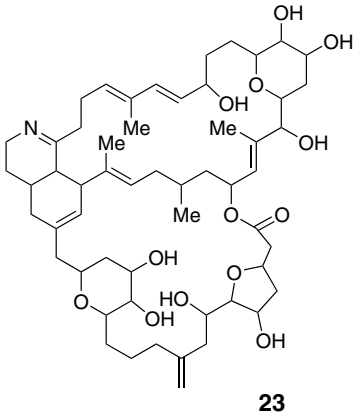


21 : R = H
22 : R = *p*-BrC_6H_4CO-



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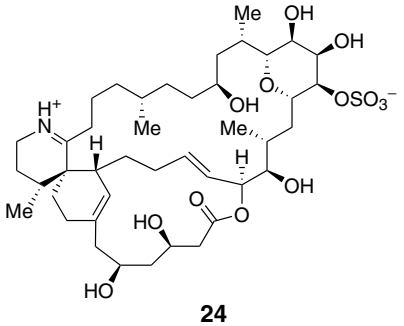

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2.4
Turbotoxins, Diiodotyramine Derivatives from the Japanese Gastropod *Turbo marmorata*

In Japan, the gastropod *Turbo marmorata* (Fig. 2.8) is eaten after removal of the viscera, which cause intoxication. Yasumoto and coworkers studied the toxic components of *T. marmorata* and obtained several toxic fractions from this animal. They indicated the occurrence of saxitoxin and a minor toxin, iodomethyltrimethylammonium salt, in the water-soluble fraction of *T. marmorata* (Kanno et al. 1976; Yasumoto and Kotaki 1977; Kotaki et al. 1981). Although they mentioned the presence of other toxins in this animal (Kotaki and Yasumoto 1977), not all of them have been identified. We have isolated two diiodotyramine derivatives, turbotoxins A (**25**) and B (**26**), from the Okinawan gastropod *T. marmorata* (Kigoshi et al. 1999, 2000).

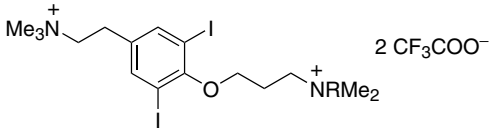
Fig. 2.8. The Okinawan gastropod *Turbo marmorata*

2.4.1
Isolation and Structure of Turbotoxins

The 75% aqueous ethanol extract of the viscera (4.5 kg, 36 individuals) of *T. marmorata* was partitioned between ethyl acetate and water. The aqueous layer was chromatographed using bioassay-guided (intraperitoneal mouse lethality) fractionation, to give two toxic fractions. The early toxic fraction was purified by HPLC to give turbotoxin A (**25**; 2.0 mg; LD_{99} 1.0 mg kg^{-1}) and the late toxic fraction was purified in the same way to give turbotoxin B (**26**; 0.9 mg; LD_{99} 4.0 mg kg^{-1}). Compounds **25** and **26** were isolated as trifluoroacetate salts because of the solvent system used for chromatographic purification.

Based on the HRFAB MS and 2D-NMR analyses including COSY, HSQC, and HMBC spectra, the structure of turbotoxin A (**25**) was confirmed as a diiodotyramine derivative. Turbotoxin B (**26**) was found to be

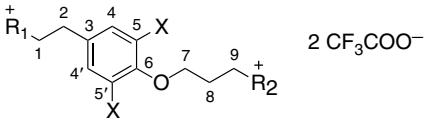
a demethylated analogue of **25**, based on its molecular formula and NMR data, i.e., a trimethylammonium group at C9 in **25** was demethylated to a dimethylamino group in **26**. Turbotoxins A (**25**) and B (**26**) are structurally related to dakaramine (Xynas and Capon 1989) and dibromotyramine derivatives, such as aplysamine-1 (Hamann et al. 1993), moloka'iamine (Tsukamoto et al. 1996), and ceratinamine (Yasumoto and Endo 1974a,b), all of which were isolated from marine sponges.



25 R = Me
26 R = H

2.4.2 Structure–Activity Relationship

To investigate the structure–toxicity relationships of turbotoxins, turbotoxins (**25**, **26**) and their 12 analogues **27–38** were synthesized (Fig. 2.9; Kigoshi et al. 2000). The relationships between the structure of turbotoxins and their acute toxicity (intraperitoneal mouse lethality) are shown in Table 2.1. Compound **28** shows the weakest toxicity among the compounds **25–28**, indicating that the quaternary ammonium group is responsible for the toxicity. The toxicities of turbotoxin B (**26**; 4.0 mg kg^{-1}) and isomer **27** (8.0 mg kg^{-1}) are weaker than that of **25** and stronger than that of **28**. This finding shows that the number of the quaternary ammonium groups is important to the toxicity of turbotoxins.



Turbotoxin A (**25**) X = I, $R_1 = R_2 = NMe_3$
Turbotoxin B (**26**) X = I, $R_1 = NMe_3$, $R_2 = NHMe_2$
27 X = I, $R_1 = NHMe_2$, $R_3 = NMe_3$
28 X = I, $R_1 = R_2 = NHMe_2$
29 X = Br, $R_1 = R_2 = NMe_3$
30 X = Cl, $R_1 = R_2 = NMe_3$
31 X = H, $R_1 = R_2 = NMe_3$
32 X = Me, $R_1 = R_2 = NMe_3$
33 X = I, $R_1 = R_2 = NMe_2Bn$
34 X = I, $R_1 = NMe_2Bn$, $R_2 = NMe_3$
35 X = I, $R_1 = NMe_3$, $R_2 = NMe_2Bn$
36 X = I, $R_1 = R_2 = NMe_2Ar$
37 X = I, $R_1 = NMe_2Ar$, $R_2 = NMe_3$
38 X = I, $R_1 = NMe_3$, $R_2 = NMe_2Ar$
Ar = 4-phenylbenzyl

Fig. 2.9. Natural and artificial analogues of turbotoxins

Table 2.1. Acute toxicity of turbotoxins and their analogs

compound	acute toxicity (LD_{99}, mg/kg-1)
Turbotoxin A (**25**)	1.0
Turbotoxin B (**26**)	4.0
27	8.0
28	100
Bromo analog **29**	4.0
Chloro analog **30**	8.0
Hydro analog **31**	12
Methyl analog **32**	8.0
Dibenzyl analog **33**	4.0
Monobenzyl analog **34**	2.0
Monobenzyl analog **35**	0.5
Diphenylbenzyl analog **36**	>32
Monophenylbenzyl analog **37**	32
Monophenylbenzyl analog **38**	8.0

Upon intraperitoneal injection into ddY mice (n >4)

Comparison of the toxicities of turbotoxin A (**25**), bromo analogue **29**, chloro analogue **30**, and hydro analogue **31** (1.0, 4.0, 8.0, 12.0 mg kg^{-1}) indicates the importance of iodine atoms in the toxicity of **25**. The toxicity of methyl analogue **32** (8.0 mg kg^{-1}) is more potent than that of hydro analogue **31** and is the same as that of chloro analogue **30**, indicating the importance of the steric bulkiness of the substituents at C-5 and C-5′ positions.

The effect of the substituents of the quaternary ammonium moieties was also examined. While the toxicities of dibenzyl and monobenzyl analogues **33** and **34** were weaker than that of **25**, monobenzyl analogue **35** exhibited toxicity stronger than that of **25**. The benzyl substituent at the *N*-9 ammonium group increases its toxicity twofold. The phenylbenzyl analogues, **36–38**, exhibited weaker toxicities than turbotoxin A (**25**) and its benzyl analogues, **33–35**. These facts indicated that the bulky 4-phenylbenzyl group prevents the phenylbenzyl analogs from binding to target molecules.

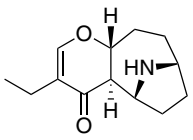
The target biomolecule of turbotoxin A was investigated; and it was found that turbotoxin A (**25**) inhibits acetylcholinesterase, with an IC_{50} of 28 μM. Donepezil hydrochloride (Aricept), a drug for Alzheimer's disease, also inhibits degradation of acetylcholine and activates the central cholinergic system, with an IC_{50} of 5.7 nM (Sugimoto et al. 1995). X-ray crystallographic studies of complexes of acetylcholinesterase with small

molecules, such as decamethonium bromide, tacrine, and edrophonium bromide, indicated that the aromatic gorge exists at the bottom of the active site (Harel et al. 1993). There as yet no data of relationships between the toxicity and affinity to acetylcholinesterase of turbotoxin analogues. The benzyl group in **35** might, however, be stacked against the aromatic gorge to increase its toxicity. Preliminary neuropharmacological experiments were affected for turbotoxin A (**25**); and **25** was proved not to interact with the peripheral nervous system.

2.5 Pinnamine and Pinnaic Acids, Alkaloidal Marine Toxins from *Pinna muricata*

2.5.1 Pinnamine

As described above, pinnatoxins (**1**–**4**) are Ca^{2+} channel activators that have been isolated from the Okinawan bivalve *Pinna muricata*. In a continuation of this work, we isolated a novel marine alkaloid, pinnamine (**39**), from aqueous ethanol extracts of *P. muricata*. Pinnamine exhibited significant acute toxicity against mice, with an LD_{99} of 0.5 mg kg^{-1}, and produced characteristic toxic symptoms, such as scurrying around (Takada et al. 2000).



39

The structure of pinnamine (**39**) was determined by spectroscopic analysis to be an alkaloid containing a dihydropyrone ring. The relative stereostructure of pinnamine was determined by an analysis of coupling constants and NOE experiments (Fig. 2.10); and the absolute stereostructure was determined by an analysis of the circular dichroism spectrum (Djerassi et al. 1962; Snatzke 1965a–c). The structure and toxic symptoms of pinnamine resemble those of anatoxin (Devlin 1976), called Very Fast Death Factor, and atropine (Leete et al. 1954), a representative suppressor of the parasympathetic nervous system. This indicates that the toxic expression of pinnamine, similar to that of atropine, may result from excitability of the cerebrum.

Recently, we reported an enantioselective synthesis of pinnamine (**39**; Kigoshi et al. 2001). Synthetic pinnamine was found to correspond uniquely to natural **39** by comparison of their spectral data, including

their CD spectra, and acute toxicity data. Further biological studies of pinnamine are currently underway.

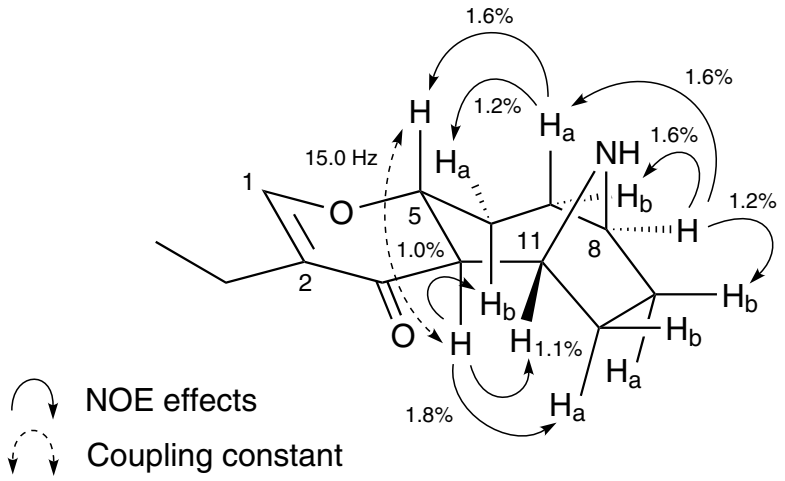


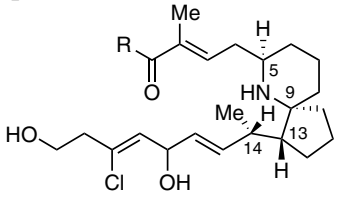
Fig. 2.10. A plausible conformation of pinnamine

2.5.2 Pinnaic Acids: cPLA$_2$ Inhibitors

Specific inhibitors of phospholipase A_2 (PLA_2) have been considered as potential drugs for the treatment of inflammation and other disease states, since PLA_2 is linked to the initial step in the cascade of enzymatic reactions that lead to the generation of inflammatory mediators (van den Bosch 1980; Arita et al. 1989). Marine natural products such as manoalide (Scheuer and de Silva 1980) and luffariellolide (Albizati et al. 1987) have been reported to be potent PLA_2 inhibitors (Potts et al. 1992a,b). A cytosolic 85-kDa phospholipase ($cPLA_2$; Kramer et al. 1990; Kramer and Sharp 1995) exhibits specificity for the release of arachidonic acid from membrane phospholipids (Kim et al. 1990). Therefore, compounds that inhibit $cPLA_2$ activity have been targeted as anti-inflammatory agents.

The 80% EtOH extract of the viscera (10 kg) of. *P. muricata* (3,000 individuals) was partitioned between EtOAc and water. The water layer was fractionated by column chromatography on TSK-G3000S polystyrene gel, Sephadex LH-20, DEAE Sephadex A-25, ODS-AQ, and silica gel to obtain two novel fatty acids, i.e., pinnaic acid (**40**; 1 mg) and tauropinnaic acid (**41**; 4 mg; Chou et al. 1996b).

The structure of **40** was determined by an analysis of NMR spectral data. Tauropinnaic acid (**41**) has a 6-azaspiro[4.5]decane unit and a taurine moiety. The relative stereochemistry of **41** was deduced from phase-sensitive NOE correlations. Furthermore, the gross structure of **40** was elucidated by a detailed comparison of the EI-MS fragment peaks with the corresponding peaks of **41**.



40 R = OH
41 R = $NHCH_2CH_2SO_3H$

Pinnaic acid (**40**) and tauropinnaic acid (**41**) inhibited $cPLA_2$ activity in vitro, with IC_{50} values of 0.2 and 0.09 mM, respectively. Inhibitors of $cPLA_2$ have rarely been reported. Therefore, though the activity of pinnaic acids was not as strong, we have great interest in the mode of action of these compounds.

2.5.3 Halichlorine: an Inhibitor of VCAM-1 Induction

Adhesion molecules are involved in the process of adhesion between cells and the extracellular matrix in the formation of multicellular bodies. In addition, it is known that the activity of adhesive molecules is very important for the maintenance of function and performance. A recent study suggested that the clinical application of adhesion molecules as anti-inflammatory agents and immunosuppressive agents may some day be possible, provided that the function of the adhesive molecules can be controlled (Kock et al. 1995).

A simple model of multistage adhesion between leukocyte and vascular cells is shown in Fig. 2.11. This process can be classified into four stages, i.e., (1) rolling, (2) triggering, (3) strong adhesion, and (4) transmigration. Vascular cell adhesion molecule-1 (VCAM-1; Osborn et al. 1988) is affected during (3), the phase of strong adhesion. Drugs that block the induced expression of VCAM-1 may be useful for treating atherosclerosis, coronary artery diseases, angina, and noncardiovascular inflammatory diseases.

The marine sponge *Halichondria okadai* Kadota (200 kg) was immersed in EtOH at room temperature. The ethanolic extract was filtered, concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl acetate extract was partitioned between 70% aqueous methanol

and hexane. The aqueous methanol layer was separated by column chromatography on TSK-G3000S, then on ODS, and then on SiO_2. Finally, the active fraction was purified by preparative TLC on SiO_2 to give halichlorine (**42**; 70.8 mg; Kuramoto et al. 1996).

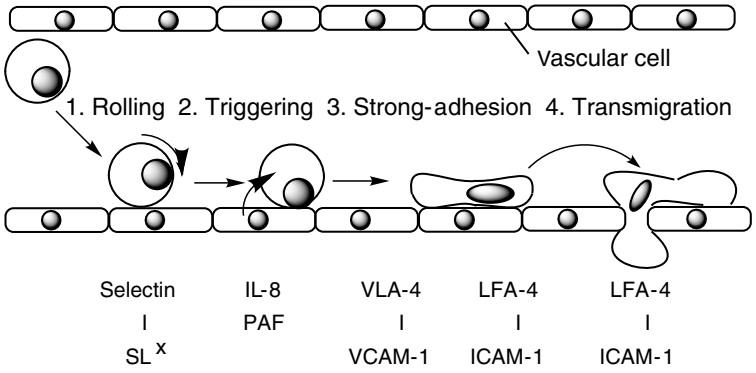
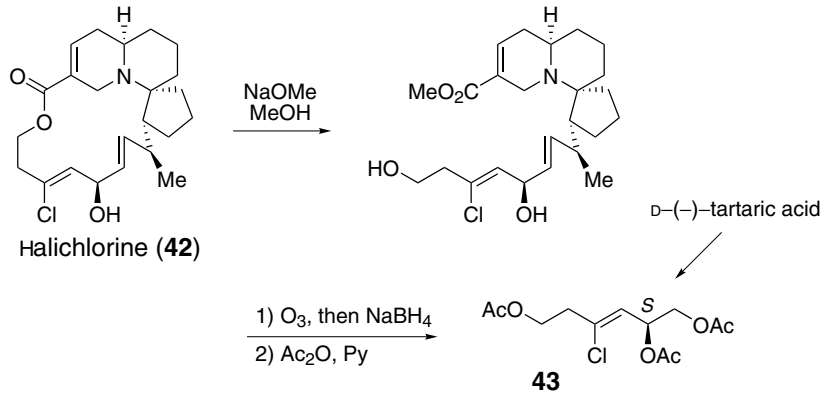


Fig. 2.11. A model of adhesion between leukocyte and vascular cells

The gross structure of **42** was elucidated by an analysis of MS, IR, and extensive 2D-NMR spectra. Halichlorine consists of a sterically hindered 15-membered lactone, an azabicyclo[4.4.0] ring, and a 5.6-spiro ring moiety. The relative stereochemistry of **42** was confirmed mainly by the coupling constants and NOESY spectral data. The Bohlmann band (Nikon 1957; Bohlmann, 1958) in the IR spectrum suggested the conformation around the tertiary amine functionality. Furthermore, oxidative degradation of **42**, as well as asymmetric synthesis of the degradation product **43** from D-(−)-tartaric acid, allowed us to determine the absolute stereochemistry of halichlorine (Scheme 3; Arimoto et al. 1998).



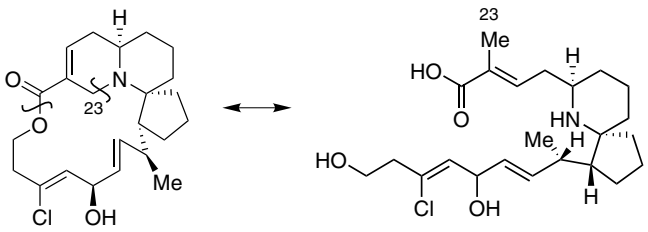
Scheme 3

Halichlorine inhibits the induction of VCAM-1 at IC_{50} 7 μg ml^{-1}. Although VCAM-1 and ICAM belong to the same immunoglobulin superfamily, halichlorine does not affect ICAM (IC_{50} > 100 μg ml^{-1}; Boschelli et al. 1995). It is largely unknown why halichlorine affects only VCAM-1. Additional research is needed to clarify the functions and mechanisms of action of VCAM-1.

These architecturally novel alkaloids have attracted the attention of synthetic chemists. (Clive et al. 2005) The Danishefsky group has achieved the total synthesis of pinnaic acid (Carson et al. 2001a,b) and halichlorine (Trauner and Danishefsky 1999; Trauner et al. 1999) in an asymmetric manner. We recently reported a racemic total synthesis of **40** (Hayakawa et al. 2003). Detailed comparison of the ^{1}H-NMR spectra of both synthetic and natural samples supported Danishefsky's revision of the configuration at C14. We also synthesized the tricyclic azadecaline core of halichlorine (**42**, Hayakawa et al. 2004). Further studies of the detailed chemistry of pinnaic acids and halichlorine, including biogenetic pathways and structure–activity relationships, are in progress.

2.5.4 Biogenesis of Pinnaic Acid

Interestingly, the structure of pinnaic acid (**40**) from the bivalve *P. muricata* has been shown to be closely similar to those of halichlorine (**42**) from the marine sponge *H. okadai*. Each carbon atom has been tentatively numbered according to the supposed biogenetic formation of the N–C23 bond (Fig. 2.12). These results suggest that both of these bioactive metabolites may be produced by symbiotic marine microorganisms.



42 from *Halichondria okadai* **40** from *Pinna muricata*

Fig. 2.12. Biogenesis of pinnaic acids and halichlorine

2.6 Conclusions

The study of natural resources may lead to the further discovery of novel bioactive compounds. As described in this chapter, it is still important to clarify the structure and biological activity of shellfish poisons to prevent disease and safeguard consumer health. Further chemical and biological studies on these shellfish poisons should contribute a deeper understanding of their roles in nature. New natural products continue to fascinate us because of the extremely unexpected structure of molecules, their biologically relevant mode of action, and their sacred and inviolable roles in the ecosystem. However, a number of unexpected and unforeseen compounds may have been naturally secreted. To overcome difficulties in the isolation and characterization of such compounds, a new chemical approach for dynamic ecological systems will be required, together with new methods in organic chemistry and spectral analysis. Intensive studies involving the comprehensive evaluation of these molecules may lead to the creation of a new field in bioscience.

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References

Albizati KF, Holman T, Faulkner DJ, Glaser KB, Jacobs RS (1987) Luffariellolide, an anti-inflammatory sesterterpene from the marine sponge *Luffariella* sp. Experientia 43:949–950

Arimoto H, Hayakawa I, Kuramoto M, Uemura D (1998) Absolute stereochemistry of halichlorine; a potent inhibitor of VCAM-1 induction. Tetrahedron Lett 39:861–862

Arita H, Nakano T, Hanasaki K (1989) Thromboxane A2: its generation and role in platelet activation. Prog Lipid Res 28:273–301

Bohlmann, F (1958) Lupinen-Alkaloide, VIII. Zur Konfigurationsbestimmung von Chinolizidin-Devivaten. Chem Ber 91:2157

Boschelli DH, Karmer JB, Khatana SS, Sorenson RJ, Connor DT, Ferin MA, Wright CD, Lesch ME, Imre K, Okonkwo GC, Schrie DJ, Conroy MC, Ferguson E, Woelle J, Saxena U (1995) Inhibition of E-selectin-, ICAM-1-, and VCAM-1-mediated cell adhesion by benzo[*b*]thiophene-, benzofuran-, indole-, and naphthalene-2-carboxamides: identification of PD 144795 as an anti-inflammatory agent. J Med Chem 38:4597–4614

Brett MM (2003) Food poisoning associated with biotoxins in fish and shellfish. Curr Opin Infect Dis 14:461–465

Carson MW, Kim G, Hentemann MF, Trauner D, Danishefsky SJ (2001a) Concise stereoselective routes to advanced intermediates related to natural and unnatural pinnaic acid. Angew Chem Int Ed Engl 40:4450–4452

Carson MW, Kim G, Danishefsky SJ (2001b) Stereochemistry of natural and unnatural pinnaic acids: a remarkable long-range stereochemical effect in the reduction of 17-oxo precursors of the pinnaic acids. Angew Chem Int Ed Engl 40:4453–4456

Cembella AD, Lewis NL, Quilliam MA (2000) The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. Phycologia 39:67–74

Cha JK, Christ WJ, Finan JM, Fujioka H, Kishi Y, Klein LL, Ko SS, Leder J, McWhorter WW Jr, Pfaff KP, Yonaga M, Uemura D, Hirata Y (1982) Stereochemistry of palytoxin 4. Complete structure. J Am Chem Soc 104:7369–7371

Chou T, Kamo O, Uemura D (1996a) Relative stereochemistry of pinnatoxin A, a potent shellfish poison from *Pinna muricata*. Tetrahedron Lett 37:4023–4026

Chou T, Kuramoto M, Otani Y, Shikano M, Yazawa, K, Uemura D (1996b) Pinnaic acid and tauropinnaic acid: two novel fatty acids composing a 6-azaspiro[4.5]decane unit from the Okinawan bivalve *Pinna muricata*. Tetrahedron Lett 37:3871–3874

Chou T, Haino T, Kuramoto M, Uemura, D (1996c) Isolation and structure of pinnatoxin D, a new shellfish poison from the Okinawan bivalve *Pinna muricata*. Tetrahedron Lett 37:4027–4030

Ciminiello P, Fattorusso E (2004) Shellfish toxins – chemical studies on northern Adriatic mussels. Eur J Org Chem 2004:2533–2551

Clive DLJ, Yu M, Wang J, Yeh VSC, Kang S (2005) Synthetic Chemistry of Halichlorine and the Pinnaic Acids. Chem Rev 105:4483–4514

Daranas AH, Norte M, Fernández JJ (2001) Toxic marine microalgae. Toxicon 39:1101–1132

Devlin JP, Edwards OE, Gorham PR, Hunter NR, Pike RK, Stavric B (1976) Anatoxin A, a toxic alkaloid from anabaena-flos-aquae NRC-44H. Can J Chem 55:1367–1371

Djerassi C, Records R, Bunnenberg E, Mislow K, Miscowitz A (1962) Inherently dissymetric chromophores. Optical rotatory dispersion of α, β-unsaturated ketones and conformational analysis of cyclohexenones. J Am Chem Soc 84:870–872

Falk M, Burton IW, Hu T, Walter JA, Wright JLC (2001) Assignment of the relative stereochemistry of the spirolides, macrocyclic toxins isolated from shellfish and from the cultured dinoflagellate *Alexandrium ostenfeldii*. Tetrahedron 57:8659–8665

Hamann MT, Scheuer PJ, Kelly-Borges M (1993) Biogenetically diverse, bioactive constituents of a sponge, order Verongida – bromotyramines and sesquiterpene-shikimate derived metabolites. J Org Chem 58:6565–6569

Harel M, Schalk I, Ehret-Sabatier L, Bouet F, Goeldner M, Hirth C, Axelsen PH, Silman I, Sussman JL (1993) Quaternary ligand-binding to aromatic residues in the active-site gorge of acetylcholinesterase. Proc Natl Acad Sci USA 90:9031–9035

Hayakawa I, Arimoto H, Uemura D (2003) Synthesis of (±)-pinnaic acid. Heterocycles 59:441–444

Hayakawa I, Arimoto H, Uemura D (2004) Synthesis of the tricyclic core of halichlorine. Chem Commun 1222–1223

Hu TM, Doyle J, Jackson D, Marr J, Nixon E, Pleasance S, Quilliam MA, Walter JA, Wright JLC (1992) Isolation of a new diarrehetic shellfish poison from Irish mussels. J Chem Soc Chem Commun 39:39–41

Hu T, Curtis JM, Walter JA, Wright JLC (1995) Spirolide-B and spirolide-D, 2 novel macrolides isolated from the digestive glands of shellfish. J Chem Soc Chem Commun 2159–2161

Hu T, Curtis JM, Walter JA, Wright JLC (1996a) Characterization of biologically inactive spirolides E and F: identification of the spirolide pharmacophore. Tetrahedron Lett 37:7671–7674

Hu T, DeFreitas ASW, Curtis JM, Oshima Y, Walter JA, Wright JLC (1996b) Isolation and structure of prorocentrolide B, a fast-acting toxin from *Prorocentrum maculosum*. J Nat Prod 59:1010–1014

Hu T, Burton IW, Cembella AD, Curtis JM, Quilliam MA, Walter JA, Wright JLC (2001) Characterization of spirolides A, C, and 13-desmethyl C, new marine toxins isolated from toxic plankton and contaminated shellfish. J Nat Prod 64:308–312

Jeffery B, Barlow T, Moizer K, Paul S, Boyle C (2004) Amnesic shellfish poison. Food Chem Toxicol 42:545–557

Kanno K, Kotaki Y, Yasumoto T (1976) Toxicity studies on marine snails. 6. Distribution of toxins in mollusks associated with coral reefs. Bull Jpn Soc Sci Fish 42:1395–1398

Kigoshi H, Kanematsu K, Uemura D (1999) Turbotoxins A and B, novel diiodotyramine derivatives from the Japanese gastropod *Turbo marmorata*. Tetrahedron Lett 40:5745–5748

Kigoshi H, Kanematsu K, Yokota K, Uemura D (2000) Turbotoxins A and B, novel diiodotyramine derivatives from the Japanese gastropod *Turbo marmorata*. Tetrahedron 56:9063–9070

Kigoshi H, Hayashi N, Uemura D (2001) Stereoselective synthesis of pinnamine, an alkaloidal marine toxin from *Pinna muricata*. Tetrahedron Lett 42:7469–7471

Kim DK, Kudo I, Fujimori Y, Mizushima H, Masuda M, Kikuchi R, Ikizawa K, Inoue K (1990) Detection and subcellular localization of rabbit platelet phospholipase A2 which preferentially hydrolyzes an arachidonoyl residue. J Biochem 108:903–906

Kita M, Kondo M, Koyama T, Yamada K, Matsumoto T, Lee KH, Woo JT, Uemura D (2004) Symbioimine exhibiting inhibitory effect of osteoclast differentiation, from the symbiotic marine dinoflagellate *Symbiodinium* sp. J Am Chem Soc 126:4794–4795

Kita M, Ohishi N, Washida K, Kondo M, Koyama T, Yamada K, Uemura D (2005) Symbioimine and neosymbiomine, amphpteric iminium metabolites form the symbiotic dinoflagellate *Symbiodinium* sp. Bioorg Med Chem 13:5253 –5258

Kita M, Uemura D (2005) Iminium alkaloids from marine invertebrates: structure, biological activity, and biogenesis. Chem Lett 34:454–459

Kock AE, Halloran MM, Haskell CJ, Sah MR, Polverini PJ (1995) Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. Nature 376:517–519

Kotaki Y, Yasumoto T (1977) Toxicity studies on marine snails. 8. Identification of iodomethyltrimethyl-ammonium as a minor toxin of green turban shell. Bull Jpn Soc Sci Fish 43:1467

Kotaki Y, Oshima Y, Yasumoto T (1981) Studies on paralytic shellfish poisoning in tropical waters. 1. Analysis of paralytic shellfish toxins of marine snails. Bull Jpn Soc Sci Fish 47:943–946

Kramer RM, Sharp JD (1995) Recent insights into the structure, function and biology of cPLA2. Agents Actions Suppl 46:65–67

Kramer RM, Johansen B, Hession C, Pepinsky RB (1990) Structure and properties of a secretable phospholipase A2 from human platelets. Adv Exp Med Biol 275:35–53

Kuramoto M, Chou T, Yamada K, Chiba T, Hayashi Y, Uemura D (1996) Halichlorine, an inhibitor of VCAM-1 induction from the marine sponge *Halichondria okadai* Kadota. Tetrahedron Lett 37:3867–3870

Kuramoto M, Arimoto H, Uemura D (1999) Structures and functions of marine bioactive alkaloids. J Synth Org Chem Jpn 57:105–115

Kuramoto M, Arimoto H, Uemura D (2003) Studies in bioactive marine alkaloids. J Synth Org Chem Jpn 61:1099–1105

Kuramoto M, Arimoto H, Uemura D (2004) Bioactive marine alkaloids. A review. Mar Drugs 1:39–54

Leete E, Marion L, Spenser ID (1954) The biogenesis of alkaloids. 12. The mode of formation of the tropine base of hyoscyamine. Can J Chem 32:1116–1123

Lehane L (2001) Paralytic shellfish poisoning: a potential public health problem. Med J Aust 175:29–31
Lu CK, Lee GH, Huang R, Chou HN (2001) Spiro-prorocentrimine, a novel macrocyclic lactone from a benthic *Prorocentrum* sp. of Taiwan. Tetrahedron Lett 42:1713–1716
McCauley JA, Nagasawa K, Lander PA, Mischke SG, Semones MA, Kishi Y (1998) Total synthesis of pinnatoxin A. J Am Chem Soc 120:7647–7468
Miles CO, Wilkins AL, Stirling DJ, MacKanzie AL (2000) New analogue of gymnodimine from a *Gymnodinium* species. J Agric Food Chem 48:1373–1376
Moore RE, Bartolini GJ (1981) Structure of palytoxin. J Am Chem Soc 103:2491–2494
Moore RE, Scheuer PJ (1971) Palytoxin – new marine toxin from a coelenterate. Science 172:495
Munday R, Towers NR, Mackenzie L, Beuzenberg V, Holland PT, Miles CO (2004) Acute toxicity of gymnodimine to mice. Toxicon 44:173–178
Murata M, Yasumoto T (2000) The structure elucidation and biological activities of high molecular weight algal toxins: maitotoxin, prymnesins and zooxanthellatoxins. Nat Prod Rep 17:293–314
Nagasawa K (2000) Total synthesis of pinnatoxin A. J Synth Org Chem Jpn 58:877–886
Nakamura H, Asari T, Murai A (1993) Structure of periodate oxidation products with characteristic partial structures of zooxanthellatoxin-A, a potent vasoconstrictive polyol from a symbiotic dinoflagellate. J Org Chem 58:313–314
Nakamura H, Asari T, Murai A, Kan Y, Kondo T, Yoshida K, Ohizumi Y (1995a) Zooxanthellatoxin-A, a potent vasoconstrictive 62-membered lactone from a symbiotic dinoflagellate. J Am Chem Soc 117:550–551
Nakamura H, Asari T, Fujimaki F, Maruyama K, Murai A, Ohizumi Y, Kan Y (1995b) Zooxanthellatoxin-B, vasoconstrictive congener of zooxanthellatoxin-A from a symbiotic dinoflagellate *Symbiodinium* sp. Tetrahedron Lett 36:7255–7258
Naoki H, Murata M, Yasumoto T (1993) Negative FAB tandem mass spectrometry for structural study on polyether compounds; structural verification of yessotoxin. Rapid Commun Mass Spectrom 7:179–182
Nikon A (1957) A relationship between conformation and infrared absorption in 1,2-halohydrins. J Am Chem Soc 79:243–247
Onodera K, Nakamura H, Oba Y, Ojika M (2003) Zooxanthellamide A, a novel polyhydroxy metabolite from a marine dinoflagellate of *Symbiodinium* sp. Tetrahedron 59:1067–1071
Onodera K, Nakamura H, Oba Y, Ojika M (2004) Zooxanthellamide B, a novel large polyhydroxy metabolite from a marine dinoflagellate of *Symbiodinium* sp. Biosci Biotechnol Biochem 68:955–958
Osborn L, Hession C, Tizard R, Vassallo C, Huhovoskyi S, Chi-Rosso G, Hobb R (1988) Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59:1203–1211
Potts BCM, Faulkner DJ, Carvalho MS de, Jacobs RS (1992a) Chemical mechanism of inactivation of bee venom phospholipase A2 by the marine natural products manoalide, luffariellolide, and scalaradial. J Am Chem Soc 114:5093–5100
Potts BCM, Faulkner DJ, Jacobs RS (1992b) Phospholipase A2 inhibitors from marine organisms. J Nat Prod 66:1701–1717
Rosewater J (1961) The family Pinnidae in the Indo-Pacific. Indo-Pac Mollusca 1:53/501/632
Satake M, Murata M, Yasumoto T, Fujita T, Naoki H (1991) Amphidinol, a polyhydroxypolyene antifungal agent with an unprecedented structure, from a marine dinoflagellate, *Amphidinium klebsii*. J Am Chem Soc 113:9859–9861
Schantz EJ, Mold JD, Stanger DW, Shavel J, Riel FJ, Bowden JP, Lynch JM, Wyler RS, Riegel B, Sommer H (1957) Paralytic shellfish poison. 4. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. J Am Chem Soc 79:5230–5235
Scheuer PJ, de Silva ED (1980) Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (polejaeff). Tetrahedron Lett 21:1611–1614

Seki T, Satake M, Mackenzie L, Kasper HF, Yasumoto T (1995) Gymnodimine, a new marine toxin of unprecendented structure isolated from New-Zealand oysters and the dinoflagellate, *Gymnodinium* sp. Tetrahedron Lett 36:7093–7096

Shimizu Y (1993) Microalgal metabolites. Chem Rev 93:1685–1698

Shimizu Y (1996) Microalgal metabolites: A new perspective. Annu Rev Microbiol 50:431–465

Shimizu Y (2003) Microalgal metabolites. Curr Opin Microbiol 6:236–243

Snatzke G (1965a) Circulardichroismus-VIII: modifizierung der octantenregel für, α, β-ungesättigte ketone: theorie. Tetrahedron 21:413–419

Snatzke G (1965b) Circulardichroismus-IX: modifizierung der octantenregel für, α, β-ungesättigte ketone: transoid enone. Tetrahedron 21:421–438

Snatzke G (1965c) Circulardichroismus-X: modifizierung der octantenregel für, α, β-ungesättigte ketone: cisoide enone, dienone und arylketone. Tetrahedron 21:439–448

Stewart M, Blunt JW, Munro MHG, Robinson WT, Hannah DJ (1997) The absolute stereochemistry of the New Zealand shellfish toxin gymnodimine. Tetrahedron Lett 38:4889–4890

Suenaga K (2004) Bioorganic studies on marine natural products with bioactivity, such as antitumor activity and feeding attractance. Bull Chem Soc Jpn 77:443–451

Sugimoto H, Iimura Y, Yamanishi Y, Yamatsu K (1995) Synthesis and structure–activity relationships of acethylcholinesterase inhibitors 1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride and related compounds. J Med Chem 38:4821–4829

Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Engen DV, Clardy J, Gopichand Y, Schmitz FJ (1981) Okadaic acid, a cyto-toxic polyether from 2 marine sponges of the genus *Halichondria*. J Am Chem Soc 103:2469–2471

Takada N, Iwatsuki M, Suenaga K, Uemura D (2000) Pinnamine, an alkaloidal marine toxin, isolated from *Pinna muricata*. Tetrahedron Lett 41:6425–6428

Takada N, Uemura N, Suenaga K, Chou T, Nagatsu A, Haino T, Yamada K, Uemura D (2001a) Pinnatoxins B and C, the most toxic components in the pinnatoxin series from the Okinawan bivalve *Pinna muricata*. Tetrahedron Lett 42:3491–3494

Takada N, Uemura N, Suenaga K, Uemura D (2001b) Structural determination of pteriatoxins A, B and C, extremely potent toxins from the bivalve *Pteria penguin*. Tetrahedron Lett 42:3495–3497

Todd ECD (1994) Emerging diseases associated with seafood toxins and other water-borne agents. Ann NY Acad Sci 740:77–94

Torigoe K, Murata M, Yasumoto T, Iwashita T (1988) Prorocentrolide, a toxic nitrogenous macrocycle from a marine dinoflagellate, *Prorocentrum lima*. J Am Chem Soc 110:7876–7877

Trauner D, Danishefsky SJ (1999) Studies towards the total synthesis of halichlorine: asymmetric synthesis of the spiroquinolizidine subunit. Tetrahedron Lett 40:6513–6516

Trauner D, Schwarz JB, Danishefsky SJ (1999) Total synthesis of (+)-halichlorine: an inhibitor of VCAM-1 expression. Angew Chem Int Ed Engl 38:3542–3545

Tsukamoto S, Kato H, Hirota H, Fusetani N (1996) Ceratinamine: an unprecedented antifouling cyanoformamide from the marine sponge *Pseudoceratina purpurea*. J Org Chem 61:2936–2937

Uemura D (1991) Antitumor polyethers. In: Scheuer PJ (ed.) Bioorganic Marine Chemistry. Springer, Berlin Heidelberg New York, pp. 1–31

Uemura D, Ueda K, Hirata Y (1981) Further studies on palytoxin. 2. Structure of palytoxin. Tetrahedron Lett 22:2781–2784

Uemura D, Chou T, Haino T, Nagatsu A, Fukuzawa S, Zheng SZ, Chen H (1995) Pinnatoxin A: a toxic amphoteric macrocycle from the Okinawan bivalve *Pinna muricata*. J Am Chem Soc 117:1155–1156

Usami M, Satake M, Ishida S, Inoue A, Kan Y, Yasumoto T (1995) Palytoxin analogs from the dinoflagellate *Ostreopsis siamensis*. J Am Chem Soc 117:5389–5390

van den Bosch H (1980) Intracellular phospholipases A. Biochim Biophys Acta 604:191–246

Xynas R, Capon R (1989) Two new bromotyrosine-derived metabolites from an Australian marine sponge *Aplysina* sp. Aust J Chem 42:1427–1433
Yasumoto T (2001) The chemistry and biological function of natural marine toxins. Chem Rec 1:228–242
Yasumoto T, Endo M (1974a) Toxicity study on a marine snail, *Turbo afgyrostoma*. 3. Identification of (3-meyhylthiopropyl)trimethylammonuim chloride. Bull Jpn Soc Sci Fish 40:217–221
Yasumoto T, Endo M (1974b) Toxicity study on a marine snail *Turbo argyrostoma* – occurrence of candicine. Bull Jpn Soc Sci Fish 40:841–845
Yasumoto T, Kotaki Y (1977) Occurrence of saxitoxin in a green turban shell. Bull Jpn Soc Sci Fish 43:207–211
Yasumoto T, Murata M (1993) Marine toxins. Chem Rev 93:1897–1909
Yasumoto T, Murata M, Oshima Y, Sano M, Matsumoto GK, Clardy J (1985) Diarrhetic shellfish toxins. Tetrahedron 41:1019–1025
Zheng SZ, Huang FL, Chen SC, Tan XF, Zuo JB, Peng J, Xie RW (1990) The isolation and bioactivities of pinnatoxin. Zhongguo Haiyang Yaowu (Chin J Mar Drugs) 9:33–35