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Bioactive Heterocyclic Alkaloids of Marine Origin

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Abstract Many kinds of alkaloids with extraordinary structures and significant biological activities have been isolated from marine organisms. This work features the structures, biological activities, and biogenesis of novel heterocyclic marine alkaloids, which control biologically and physiologically intriguing phenomena. Pinnatoxins and pteriatoxins, potent shellfish poisons, were isolated from the Okinawan bivalve *Pinna* sp. and *Pteria* sp. Norzoanthamine hydrochloride, isolated from the colonial zoanthid *Zoanthus*

sp., suppresses decreases in bone weight and strength in ovariectomized mice. Symbioimine, an amphoteric iminium metabolite from the dinoflagellate *Symbiodinium* sp., inhibits osteoclast differentiation. Other novel alkaloids, such as pinnamine, pinnaic acids, halichlorine, and zamamistatin, are also described.

Keywords Heterocyclic marine alkaloids · Shellfish poison · Anti-osteoporosis · Super-carbon-chain compound

1 Introduction

Alkaloids are nitrogen-containing compounds that occur naturally not only in plants but also in microorganisms, marine organisms, and animals. Many kinds of alkaloids with extraordinary structures and significant biological activities have been isolated from marine organisms [1, 2]. They continue to provide lead structures in the search for new drugs or biological probes for physiological studies. As new and more complicated diseases are encountered worldwide, the importance of novel bioactive alkaloids has increased due to their potential application in chemotherapy.

Many kinds of bioactive nitrogenous compounds, such as peptides, indols, oxazoles, and thiazoles, have been identified from marine invertebrates [3, 4]. The true origins or progenitors of these metabolites have been suggested to be microorganisms, i.e., microalgae, bacteria, and fungi. These microorganisms are carried through symbiosis, association, a food chain, and other forms of nutrient-dependency with host animals [5–7]. Consequently, the isolation of bioactive metabolites from cultured marine microorganisms, such as symbiotic dinoflagellates and bacteria, as well as from their host animals, has been well investigated. Several alkaloidal metabolites isolated from cyanobacteria have been suggested to help to inhibit predation by marine herbivores, such as fish and sea urchins. However, the real role of most marine bioactive alkaloids in the ecosystem has not been well clarified.

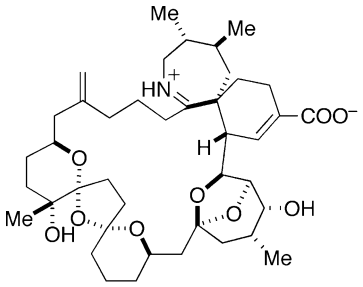
In our ongoing search for bioactive metabolites from marine organisms, several novel heterocyclic alkaloids, such as pinnatoxins, norzoanthamine, pinnaic acids, zamamistatin, and symbioimine, have been isolated. This work features the structures, biological activities, and biogenesis of these bioactive heterocyclic marine alkaloids, along with up-to-date topics.

2
Pinnatoxins and Pteriatoxins, Potent Macrocyclic Iminium Toxins from an Okinawan Bivalve

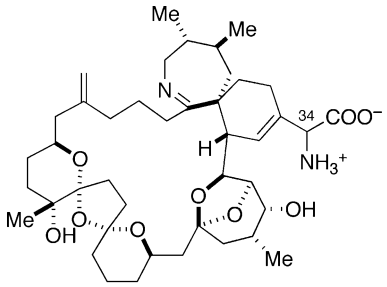
2.1
Isolation and Structure of Pinnatoxins

Shellfish of the genus *Pinna* live mainly in shallow waters of the temperate and tropical zones of the Indian and Pacific Oceans [8]. The adductor muscle of this bivalve is eaten in Japan and China, and food poisoning resulting from its ingestion occurs frequently. Although it has been suggested that this poisoning is caused by bacterial infection or neurotoxins, the true causative agent was ambiguous. Chinese investigators have reported that a toxic extract from *P. attenuata*, referred to as pinnatoxin, is a Ca^{2+} channel activator [9]. We have successfully isolated pinnatoxin A (**1**), a mixture of pinnatoxins B and C (**2**, **3**), and pinnatoxin D (**4**) from *P. muricata* as a major cause of food poisoning [10–13].

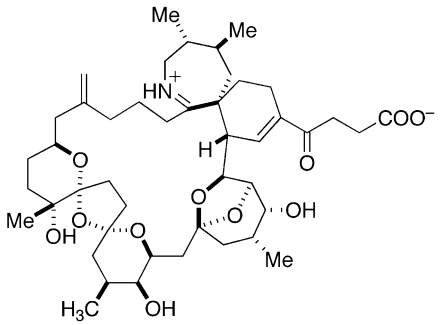
The structures and stereochemistries of pinnatoxins have been clarified by extensive analysis using NMR experiments and positive ion ESI MS/MS. Pin-



Pinnatoxin A (**1**)



Pinnatoxin B (**2**) : 34*S* isomer
Pinnatoxin C (**3**) : 34*R* isomer



Pinnatoxin D (**4**)

natoxins 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 or ammonium cation. Recently, Kishi's group achieved the total synthesis of **1** and *ent*-**1** [14], and the absolute stereochemistry of pinnatoxin A (**1**) has been confirmed. Interestingly, while natural **1** showed significant acute toxicity, its antipode *ent*-**1** was not toxic [15].

Pinnatoxins B (**2**) and C (**3**), the most toxic constituents in the pinnatoxin series, were isolated as a 1 : 1 mixture [13]. The molecular formula of both pinnatoxins B (**2**) and C (**3**) was determined to be $C_{42}H_{64}N_2O_9$ by ESIMS, which reflects a 29 MS unit (CH_3N) increase compared to that of pinnatoxin A (**1**). In positive ion ESI MS/MS analysis, a series of prominent fragment ions

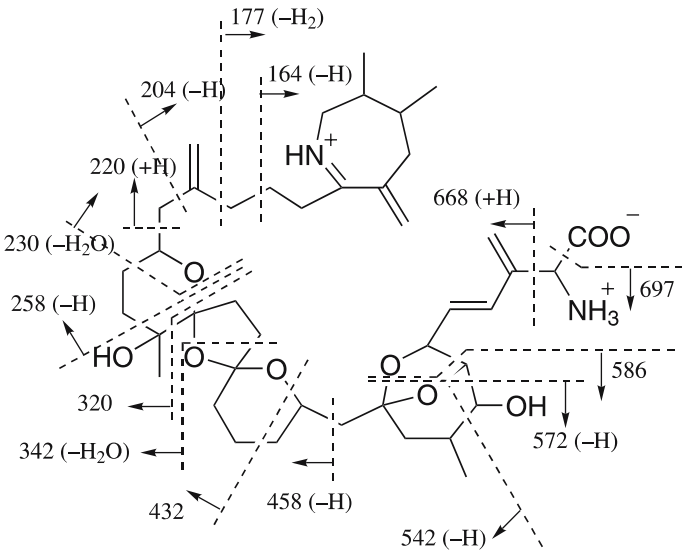
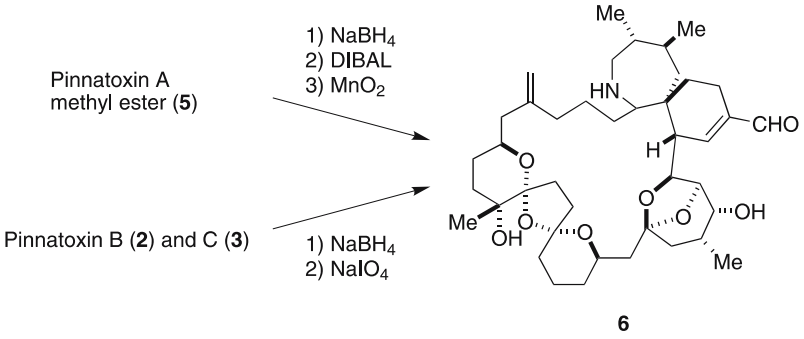


Fig. 1 ESI-MS/MS fragmentation pattern of pinnatoxins B (**2**) and C (**3**)



Scheme 1

were generated by a cyclohexane ring-opening reaction (retro-Diels–Alder reaction), followed by bond cleavage of carbocycles (Fig. 1).

The stereochemistry of the macrocyclic moiety in pinnatoxins B (**2**) and C (**3**) was determined as follows. Reduction of the imino group in **2** and **3** with $NaBH_4$ followed by oxidative cleavage with $NaIO_4$ provided aldehyde **6** (Scheme 1). Aldehyde **6** was also obtained by the reduction of iminium and a carboxylic acid moiety in pinnatoxin A methyl ester (**5**) followed by oxidation of the resulting alcohol. Since the spectroscopic data of **6** derived from **2** and **3** and that from **5** were identical, the relative stereochemistry of the macrocyclic core in **2** and **3** was confirmed to be the same as that in pinnatoxin A (**1**).

2.2 Biological Activity and Biogenesis of Pinnatoxins

Pinnatoxin A (**1**) showed potent acute toxicity against mice (LD_{99} 180 μg/kg (*i.p.*)) with characteristic neurotoxic symptoms. Pinnatoxin A activated Ca^{2+} channels. Pinnatoxins B (**2**) and C (**3**) were the most toxic constituents in the pinnatoxin series, which makes them as potent as tetrodotoxin (LD_{99} 22 μg/kg). Although pinnatoxin D (**4**) showed weaker acute toxicity than the other pinnatoxins (LD_{50} > 10 μg/MU), **4** showed the strongest cytotoxicity against the murine leukemia cell line P388 (IC_{50} 2.5 μg/ml).

The backbone of pinnatoxins and their analogues could be configured from C1 to C34 in a single carbon chain, in a polyketide biogenetic pathway (Fig. 2) [10]. This biosynthetic proposal entails an intramolecular Diels–Alder reaction to construct a cyclohexene ring (G-ring) followed by imine formation to establish a 6,7-spiro-ring system. Because of the structural similarity of the imine moiety adjacent to the spirocyclic core, other macrocyclic imines, i.e. pteriatoxins, spirolides, gymnodimine, may also be biosynthesized via the same intramolecular Diels–Alder reaction. Indeed, Kishi and his coworkers achieved the total synthesis of **1** using a biomimetic intramolecular Diels–Alder reaction as shown in Scheme 2 [14].

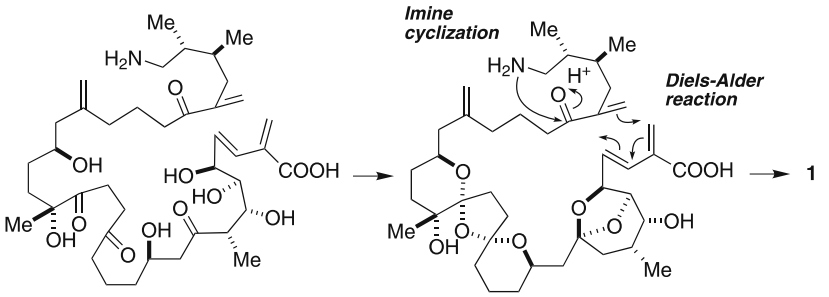
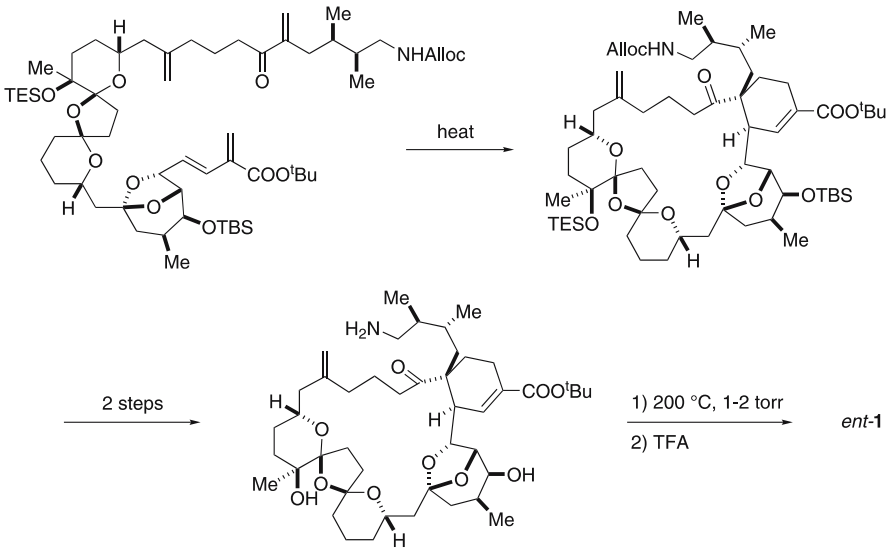


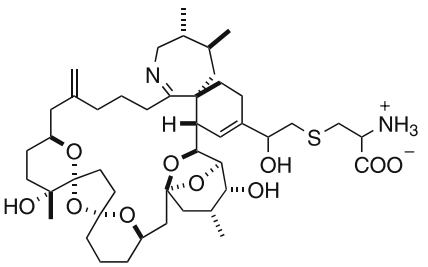
Fig. 2 Proposed biogenesis of pinnatoxin A (**1**)



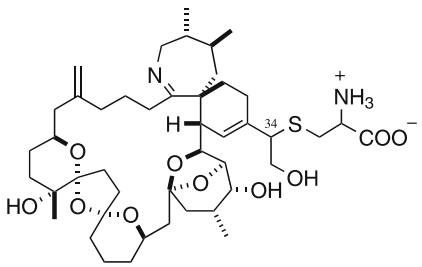
Scheme 2

2.3
Pteriatoxins, Pinnatoxin Analogs from the Okinawan Bivalve *Pteria penguin*

In our study of shellfish poisons, we observed that a moray eel vomits the viscera of the Okinawan bivalve *Pteria penguin*. We found that the aqueous EtOH extract of viscera of *P. penguin* showed potent acute toxicity against mice along with severe convulsion. Guided by this toxicity, pteriatoxins A (**7**), B, and C (**8**, **9**: a 1 : 1 mixture) were isolated as extremely toxic and minor components [16]. Although the isolated yields of pteriatoxins were too low (less than 20 μg) to deduce their structures by usual NMR analysis, a nano-mole-order structure determination of pteriatoxins was achieved by a detailed analysis of ESI MS/MS. As a consequence, pteriatoxins were determined to



Pteriatoxin A (**7**)



Pteriatoxin B (**8**)
Pteriatoxin C (**9**)

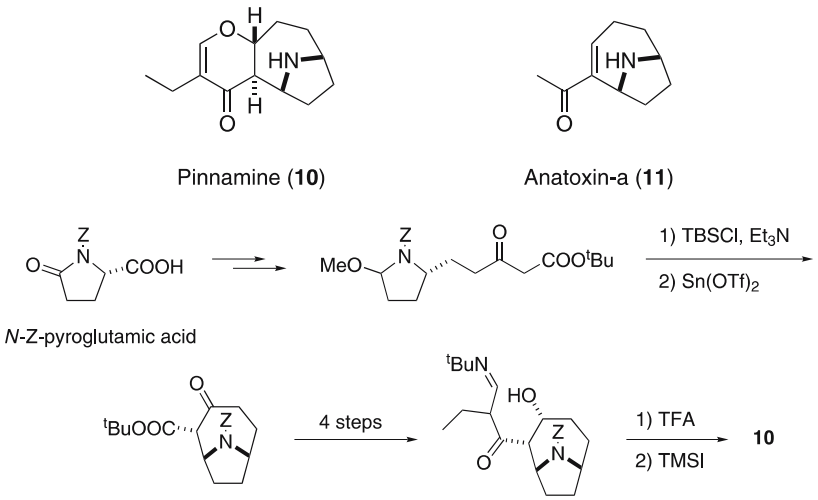
be pinnatoxin analogs containing a cysteine moiety. The position of duplicate signals in the ^{1}H NMR spectrum suggested that pteriatoxins B (**8**) and C (**9**) are C-34 epimers, like **2** and **3**.

Pteriatoxins A (**7**), B, and C (**8**, **9**) showed significant acute toxicity against mice with LD_{99} values of 100 and 8 μg/kg, respectively. The toxic symptoms of pteriatoxins were also similar to those of pinnatoxins. Extracts from the digestive glands of several species that are closely related to *Pinna* sp., including *P. muricata*, *P. attenuata*, *P. atropupurea*, and the commonly eaten shellfish *Atrina pectinata*, all produced the same symptoms of poisoning in mice. Thus, these shellfish may become toxic as a result of feeding on common toxic organisms such as dinoflagellates.

2.4
Pinnamine, an Alkaloidal Marine Toxin from *Pinna muricata*

In a continuation of our work on pinnatoxins, a novel marine alkaloid, pinnamine (**10**), was isolated from the Okinawan bivalve *P. muricata*. Pinnamine exhibited acute toxicity against mice, with characteristic toxic symptoms, such as scurrying around and convulsion (LD_{99} 0.5 mg/kg) [17]. The structure of pinnamine (**10**) was determined to be a unique alkaloid containing a 9-azabicyclo[4.2.1]nonane moiety and a dihydro-γ-pyrone ring. The absolute stereostructure was determined by an analysis of the circular dichroism spectrum [18].

The structure and toxic symptoms of pinnamine resemble those of anatoxin-a (**11**) [19, 20], a potent postsynaptic depolarizing neurotoxin known as very fast death factor (VFDF), and atropine [21], a representative suppressor of the



Scheme 3

parasympathetic nervous system. Thus, the toxic expression of pinnamine, similar to that of atropine, may result from excitability of the cerebrum.

Recently, an enantioselective synthesis of pinnamine (**10**) has been achieved (Scheme 3) [22]. The 9-aza-bicyclo[4.2.1]nonane moiety in **10** was constructed by convergence of the β-keto ester into the silyl enol ether followed by Lewis acid treatment. Synthetic pinnamine was found to correspond uniquely to the natural compound based on a comparison of their spectral data including their CD spectra.

3
Norzoanthamine, A Significant Inhibitor of Osteoclast

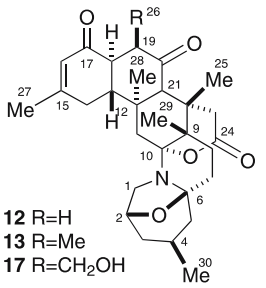
3.1
Structure of Zoanthamines

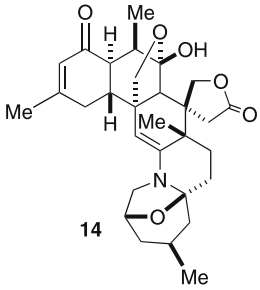
Norzoanthamine (**12**), zoanthamine (**13**), and its homologues **14–21** were isolated from the colonial zoanthid *Zoanthus* sp. [23–26]. The relative stereochemistry of norzoanthamines was determined by X-ray analysis. The absolute stereochemistry of norzoanthamine was established by a modified Mosher's method [27].

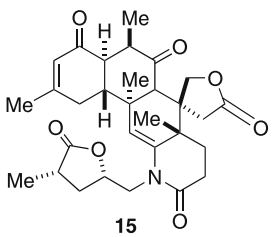
Interestingly, equilibration was observed between the lactone structure and iminium structure on norzoanthamine (**12**). The NMR spectrum of norzoanthamine hydrochloride in CD_3OD implied the presence of an iminium structure (**22**, $\delta_{C\text{-}10} = 193.3$) but not a lactone structure **12** in norzoanthamine (Scheme 4). The zwitter iminium structure was also demonstrated by transformation into methyl ester **23** by the treatment of **12** with $CH_3I - Ag_2O$. On the other hand, hydrolysis of **23** with aqueous HCl led to the recovery of **12**. Recently, zooxathellamine (**21**) was isolated from the cultured symbiotic dinoflagellate *Symbiodinium* sp. [28]. As with norzoanthamine (**12**), **21** also adopted a zwitter ion structure with carboxylate and iminium moieties in D_2O, but had a lactone structure in either $CDCl_3$ or CD_3OD.

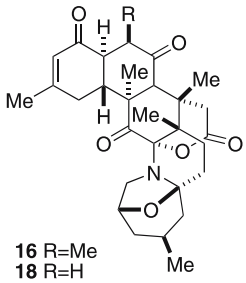
3.2
Biological Activities of Zoanthamines

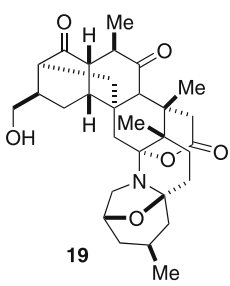
IL-6 (Interleukin-6) is known to stimulate osteoclast formation, and the suppression of secretion can be effective in the prevention of osteoporosis. Norzoanthamine (**12**) and norzoanthamine hydrochloride (**22**) inhibit IL-6 induction at values of 13 and 4.7 μg/ml, respectively [29]. Meanwhile, the effect of norzoanthamine hydrochloride (**22**) on bone weight and strength was tested in ovariectomized mice, an animal model of postmenopausal osteoporosis (Fig. 3). Norzoanthamine hydrochloride (**22**) (0.08 mg/kg/day, p.o.) significantly suppressed the decrease in femoral weight caused by ovariec-

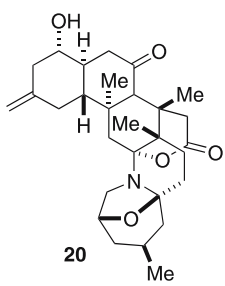


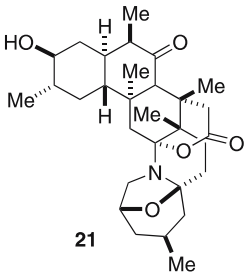








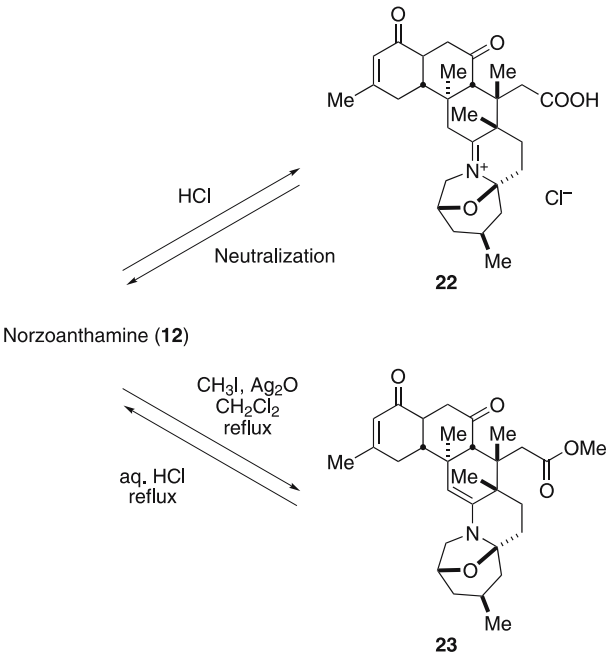




Norzoanthamine (**12**)
Zoanthamine (**13**)
Zoanthenamine (**14**)
Zoanthamide (**15**)
Zoanthaminone (**16**)
Oxyzoanthamine (**17**)
Norzoanthaminone (**18**)
Cyclozoanthamine (**19**)
Epinorzoanthamine (**20**)
Zooxathellamine (**21**)

tomy [30, 31]. The primary spongiosa did not significantly increase, and the morphology of the metaphysis remained nearly normal. It is known that uterine hypertrophy is a serious side effect of 17β-estradiol [32]. In contrast, norzoanthamine hydrochloride (**22**) did not have an estrogen-like side effect on reproductive organs. Thus, the action mechanism of norzoanthamine hydrochloride is suggested to differ from that of estrogen.

Osteoporosis is caused by an imbalance between bone resorption and bone formation, which results in bone loss and fractures after mineral flux occurs [33]. The frequency of fracture is significantly increased in patients with osteoporosis, and hip fracture in elderly patients with osteoporosis is a very serious problem because it often limits their quality of life [34]. In addition to preventing the loss of bone mass, maintenance of the mechanical strength of bone tissue is a very important point to consider in the development of anti-



Scheme 4

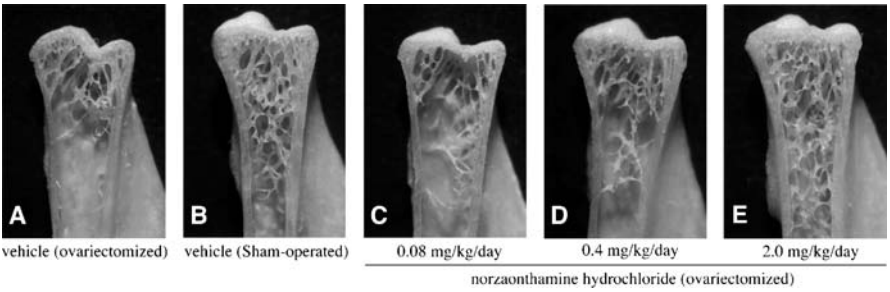


Fig. 3 Effect of norzoanthamine hydrochloride (**22**) on humeralis morphology in ovariectomized mice

osteoporotic drugs. From this point of view, norzoanthamine hydrochloride (**22**) is considered to be a potent candidate.

3.3 Biogenesis of Zoanthamines

On the basis of their molecular formulas, zoanthamines have been regarded as terpenoids, but the biogenetic pathway of zoanthamines remains unclear. We have proposed a polyketide biogenetic pathway for zoanthamines, as

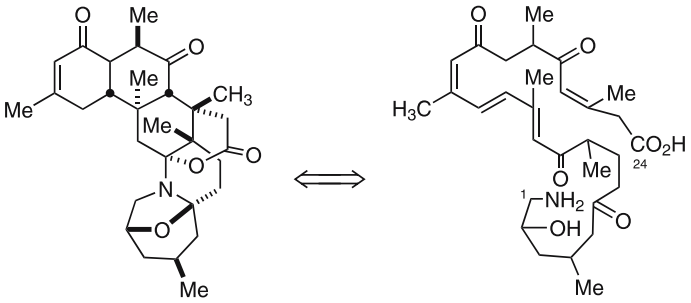


Fig. 4 Proposed biogenesis of zoanthamine

shown in Fig. 4 [23]. A single carbon chain precursor (C1 to C24) could be cyclized to give a complex polycyclic structure, like **13**. Meanwhile, feeding experiments with a labeled compound for zooxathellamine (**21**) have been examined, and a polyketide biosynthetic pathway has been supported [28].

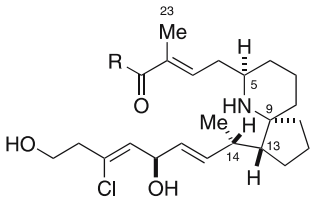
Recently, a total synthesis of norzoanthamine (**12**) has been achieved using an intramolecular Diels–Alder reaction as a key step for constructing the requisite chiral triene [35]. This synthesis may be a powerful tool for advancing the study of norzoanthamine as a therapeutic drug.

4
Pinnaic Acids and Halichlorine, Novel Marine Azaspirocycles

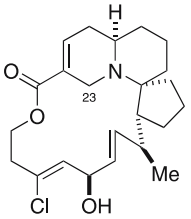
4.1
Pinnaic Acids, Potent $cPLA_2$ Inhibitors

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 [36, 37]. Marine natural products such as manoalide [38] and luffariellolide [39] have been reported to be potent PLA_2 inhibitors [40, 41]. A cytosolic 85-kDa phospholipase ($cPLA_2$) exhibits specificity for the release of arachidonic acid from membrane phospholipids [42–44]. Therefore, compounds that inhibit $cPLA_2$ activity have been targeted as anti-inflammatory agents.

Pinnaic acid (**24**) and tauropinnaic acid (**25**) were isolated from the viscera of *P. muricata* [45]. Both **24** and **25** have a unique 6-azaspiro[4.5]decane moiety. The gross structure of **24** was also confirmed by a comparison of the EI-MS fragment peaks with the corresponding peaks of **25**. The relative stereochemistry of **25** was deduced from phase-sensitive NOE correlations.



Pinnaic acid (**24**) : R = OH
Tauropinnaic acid (**25**) : R = $NHCH_2CH_2SO_3H$



Halichlorine (**26**)

Pinnaic acid (**24**) and tauropinnaic acid (**25**) inhibited $cPLA_2$ activity in vitro with IC_{50} values of 0.2 mM and 0.09 mM, respectively. The activities of pinnaic acids were not so strong, but were still interesting, since $cPLA_2$ inhibitors are rare.

4.2
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. The activity of adhesive molecules is very important for the maintenance of function and performance [46]. The clinical application of adhesion molecules as anti-inflammatory agents and immunosuppressive agents are possible, provided that the function of the adhesive molecules can be controlled.

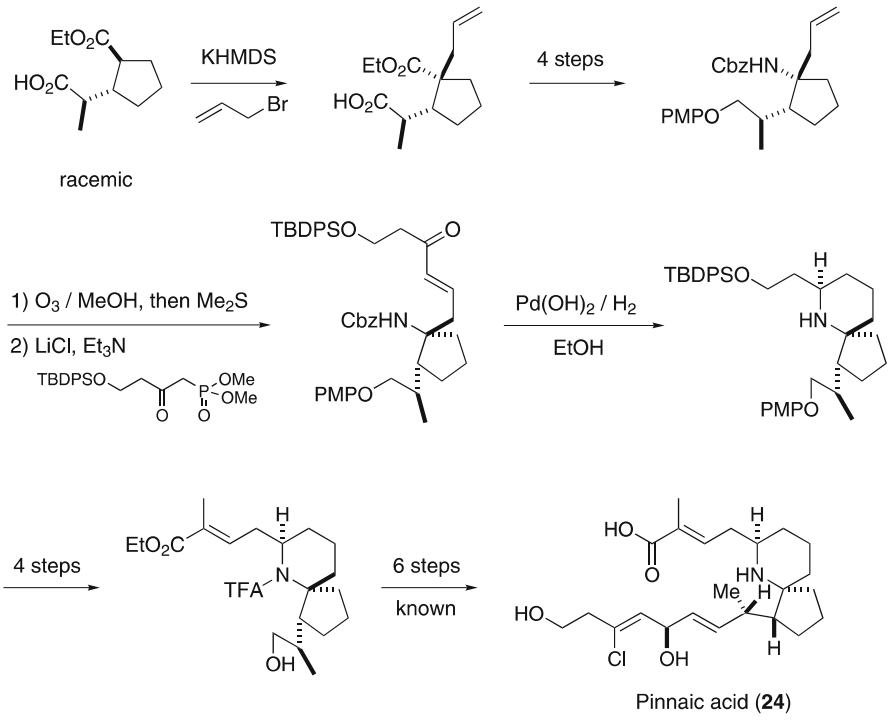
A simple model of multistage adhesion between leukocyte and vascular cells is proposed. This process can be classified into four stages, i.e., (1) rolling, (2) triggering, (3) strong adhesion, and (4) transmigration. VCAM-1 (vascular cell adhesion molecule-1) is affected during the phase of strong adhesion [47]. Thus, drugs that block the induced expression of VCAM-1 may be useful for treating atherosclerosis, coronary artery diseases, angina, and noncardiovascular inflammatory diseases.

Halichlorine (**26**) was isolated from the marine sponge *Halichondria okadai* Kadota [48]. 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 **26** was confirmed mainly by the coupling constants and NOESY spectral data. Furthermore, an oxidative degradation product of **26** was synthesized from D-(–)-tartaric acid to determine the absolute stereochemistry of halichlorine [49]. Halichlorine inhibits the induction of VCAM-1 at IC_{50} 7 μg/ml. Although VCAM-1 and ICAM (intercellular adhesion molecule 1) belong to the same immunoglobulin superfamily, halichlorine does not affect ICAM ($IC_{50} > 100$ μg/ml).

4.3
Biogenesis and Synthesis of Pinnaic Acids and Halichlorine

The structure of pinnaic acid (**24**) from the bivalve *P. muricata* has been shown to be closely similar to that of halichlorine (**26**) from the marine sponge *H. okadai*. Each carbon atom has been tentatively numbered according to the supposed biogenetic formation of the N-C23 bond. Thus, these two bioactive metabolites may each be biosynthesized by symbiotic marine microorganisms.

Both pinnaic acids and halichlorine have attracted the attention of synthetic chemists. To date, 15 research groups have published synthetic studies [50]. The Danishefsky group has achieved the total synthesis of pinnaic acid [51, 52] and halichlorine [53, 54] in an asymmetric manner. Since pinnaic acid is a zwitterionic molecule, the NMR spectrum is quite sensitive to the measurement conditions. Recently, a racemic total synthesis of pinnaic acid (**24**) has been achieved (Scheme 5) [55]. A detailed comparison of the ^{1}H-NMR spectra of both synthetic and natural samples supported Danishefsky's revision of the configuration at C14.



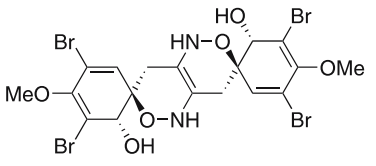
Scheme 5

5
Zamamistatin, a Significant Antibacterial Bromotyrosine Derivative from a Marine Sponge

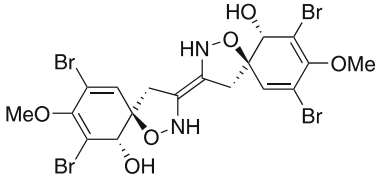
Bacteria and diatoms are present soon after immersion in seawater, resulting in a biofilm that covers the surface. The establishment of this microfouling biofilm layer is soon followed by macrofouling by barnacles, mussels, and algae. To prevent such macrofouling, several metallic compounds, such as bis(tributyltin)oxide (TBTO), have been used in antifouling paints. However, since their use is restricted to prevent environmental pollution the development of environmentally acceptable antifouling agents has been expected. From marine algae, which are known to be one of the largest producers of biomass in the marine environment, several substances with potent antifouling activity have been isolated, such as fatty acids, lipopeptides, amides, alkaloids, terpenoids, lactones, pyrroles, and steroids [56].

We have especially searched for compounds to prevent microfouling, which would consequently prevent such macrofouling [57]. Antibacterial activity against the marine bacteria *Rhodospirillum salexigens* SCRC 113 strain with adhering properties was selected as a bioassay to identify such compounds. In our continuing search for such compounds, zamamistatin, a novel bromotyrosine derivative, was isolated from the Okinawan sponge *Pseudoceratina purpurea* [58]. Zamamistatin exhibited significant antibacterial activity against *R. salexigens* (21 mm, 1.6 μg/disk).

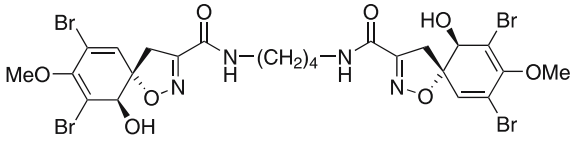
The molecular formula of zamamistatin was determined to be $C_{18}H_{18}Br_4N_2O_6$. Observation of only nine carbon signals by ^{13}C NMR and its optical rotation value suggested that zamamistatin was an optically active dimer with a C_2 symmetrical structure. On the basis of the analysis of 2D-NMR spectra, the structure of zamamistatin was elucidated to be **28**, possess-



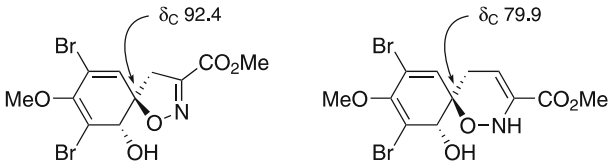
Zamamistatin (**27**)



28



Aerothionin (**29**)



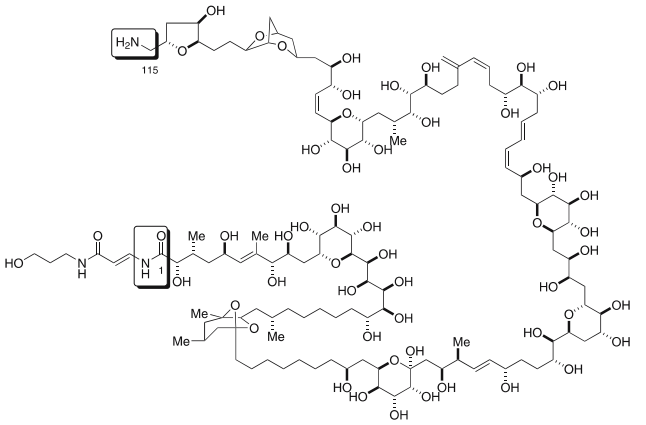
30 **31**

ing an *exo*-type dimer of an isooxazoline ring like aerothionin **29** [59, 60]. Recently, however, zamamistatin has been re-isolated and its structure has been revised [61]. A dihydro-1, 2-oxazine methyl ester **30** and a isoxazoline methyl ester **31** [62] were synthesized as model compounds. The spiro-carbon signal (C-6) for **30** and **31** appeared at δ_C 92.4 and 79.9, respectively, while the carbon signal in the natural compound appeared at δ_C 74.3. Thus, the structure of natural zamamistatin has been revised to be **27**, an *endo*-type dimer of an aza-oxa-spiro[6.6] unit possessing a dihydro-1,2-oxazine ring moiety.

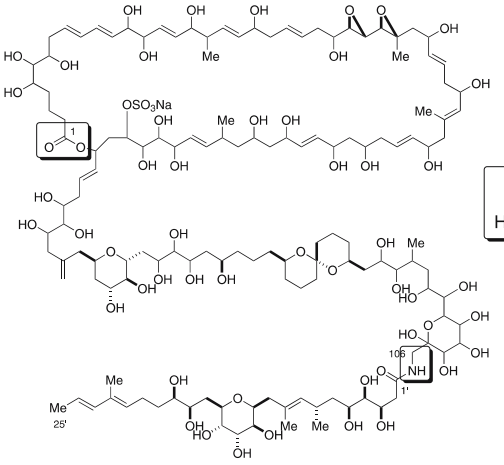
6
Symbioimines, Potential Antiresorptive Drugs

Large polyol and polyether compounds, such as palytoxin, halichondrin, ciguatoxin, and maitotoxin, are characteristic marine secondary metabolites. These compounds are composed of a long carbon backbone functionalized by oxygen, and have been called "super-carbon-chain compounds" [63]. Interestingly, several super-carbon-chain compounds consist of a single chain starting from a carboxylic acid (C-terminus) and an amine moiety (N-terminus, which are sometimes acylated), thus they can be considered to be huge amino acids, i.e., palytoxin (**32**) (115 straight carbon chain) [64], zooxanthellatoxin-A (**33**) (106 carbons) [65], and azaspiracid 1 (**34**) (40 carbons) [66, 67] (Fig. 5). This concept may also be applicable to pinnatoxins and zoanthamines, which could be biosynthesized from a single carbon chain precursor possessing a terminal amino group, as shown in Figs. 2 and 4.

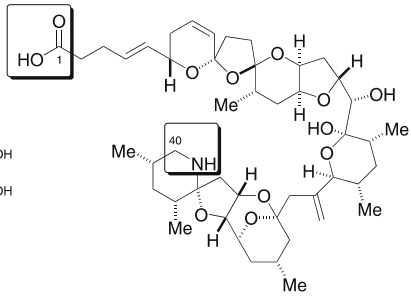
The symbiotic marine dinoflagellate *Symbiodinium* sp., which is a type of zooxanthellae, is found in a wide range of marine invertebrates, and produces several large bioactive polyol compounds, such as zooxanthellatoxins and zooxanthellamides [65, 68–70]. In our continuing search for biologically active compounds, two unique amphoteric iminium compounds were isolated from this dinoflagellate: symbioimine (**35**) and neosymbioimine (**36**) [71–73].



Palytoxin (**32**)

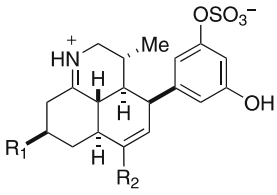


Zooxanthellatoxin A (**33**)



Azaspiroacid 1 (**34**)

Fig. 5 Super-carbon-chain compounds with a terminal amino group



35 : $R_1 = R_2 = H$ (Symbioimine)
36 : $R_1 = R_2 = Me$ (Neosymbioimine)

6.1
Structure of Symbioimines

IR spectrum of symbioimine (**35**) showed absorption bands for hydroxyl (3450 cm^{-1}), iminium (1690 cm^{-1}), and sulfate (1240, 1140, 1050 cm^{-1}) groups. The ^{13}C NMR signal at 188.0 (C-5) implied the presence of an iminium functionality in this water-soluble amphoteric compound. Its structure, which consists of a characteristic 6,6,6-tricyclic iminium ring possessing an aryl sulfate moiety, was deduced by 2D-NMR analysis (Fig. 6).

The relative stereochemistry of **35** was deduced as follows. The large magnitudes of $J_{1a,2}$ = 12.0 Hz, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 11.1 Hz, $J_{4,9}$ = 11.9 Hz, $J_{7a,8a}$ = 12.9 Hz, and $J_{8a,9}$ = 12.9 Hz suggested that all seven of these protons, H-1a, H-2, H-3, H-4, H-7a, H-8a, and H-9a, were oriented in *anti* arrangements with respect to the tricyclic ring. Thus, three six-membered rings may show *trans* ring fusion with each other and that the methyl group (C-19) may be oriented in a pseudo-equatorial conformation with respect to the six-membered iminium ring with a twist-boat conformation. NOEs were observed for H-4/H-14 and H-4/H-18, suggesting that the aryl moiety may be oriented in a pseudo-axial conformation with respect to the cyclohexene ring with a twist-boat conformation. Finally, the stereostructure of **35** was confirmed by X-ray crystallographic analysis. The absolute stereochemistry of **35** was confirmed to be 2*R*, 3*R*, 4*S*, 9*R*, 12*S*, based on the value of the Flack parameter 0.03(13).

Neosymbioimine (**36**) was found to be a congener of **35** possessing a 6,6,6-tricyclic iminium ring, an aryl sulfate moiety, and three methyl groups.

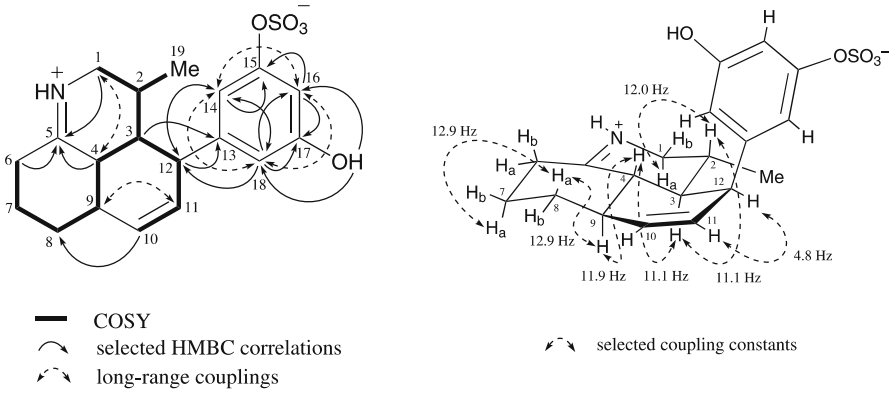
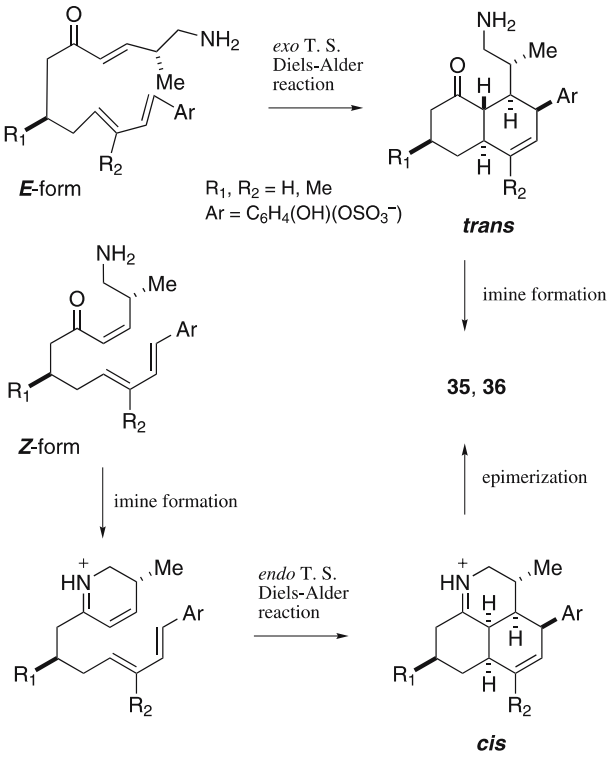


Fig. 6 Planar and stereo structure of symbioimine (**35**) based on 2D-NMR analysis



Scheme 6

6.2
Biogenesis of Symbioimines

The unique structures of symbioimines, including a 6,6,6-tricyclic iminium ring, can be explained by the plausible biogenetic pathway shown in Scheme 6, [72, 74]. An intramolecular *exo* transition-state Diels–Alder reaction followed by imine cyclization could form the carbon framework of **35** and **36** stereospecifically, as in the case of pinnatoxins (Fig. 2). Since the 6,6,6-tricyclic iminium ring moiety of neosymbioimine (**36**) is composed of 15 carbons including three methyl groups, a sesquiterpene biosynthesis pathway can be considered. However, due to its inconsistency with the products in the normal sequential head-to-tail condensation of two molecules of isopentenyl diphosphate with dimethylallyl diphosphate, we proposed the polyketide synthesis pathway for construction of the characteristic C1–C12 moiety in **35** and **36.** From this point of view, the tricyclic ring in symbioimines can be considered to be biosynthesized by a cyclic imine formation, *endo*-transition state Diels–Alder reaction to provide *cis*-6,6,6-tricyclic ring stereospecifically, followed by epimerization. Studies of the biosynthetic path-

ways of symbioimines using isotope-labeled precursor incorporation studies are currently underway.

6.3 Biological Activities of Symbioimines

Symbioimine (**35**) inhibited osteoclastogenesis of the murine monocytic cell line RAW264, which can differentiate into osteoclasts following treatment with receptor activator of nuclear factor-κB ligand (RANKL) (EC_{50} = 44 μg/mL) [71]. RANKL induces the formation of osteoclast-like multinucleated cells in cultures of bone marrow cells [75]. Symbioimine (**35**) inhibited an increase in sRANKL-induced TRAP activity of preosteoclast cells. Meanwhile, it did not affect cell viability even at 100 μg/mL. Thus, symbioimine is a potential antiresorptive drug for the prevention and treatment of osteoporosis in postmenopausal women.

Symbioimine (**35**) also significantly inhibited cyclooxygenase-2 (COX-2) activity (32%) at 10 μM. Meanwhile, it had only weak inhibitory ability (5%) toward COX-1 at 10 μM [72]. The overexpression of COX-2 has been observed in many kinds of tumors, and its role in carcinogenesis and angiogenesis has been extensively investigated [76, 77]. Several COX-2-selective inhibitors, such as rofecoxib, celecoxib, and sulindac, have been developed. Because of its moderate subtype specificity, symbioimine (**35**) may be useful for the development of new nonsteroid anti-inflammatory drugs (NSAID) to treat COX-associated diseases, such as inflammatory diseases and cancer.

7 Conclusion

Along with the development of new analytical instruments and techniques over the past 30 years, a variety of marine alkaloids have been isolated and characterized from natural resources. Further chemical and biological studies on these marine alkaloids should contribute to a deeper understanding of their roles in nature. Also, intensive studies involving the comprehensive evaluation of these molecules may lead to the creation of a new field in bioscience.

References

1. Kuramoto M, Arimoto H, Uemura D (2003) Studies in bioactive marine alkaloids. J Synth Org Chem Jpn 61:1099
2. Kuramoto M, Arimoto H, Uemura D (2004) Bioactive marine alkaloids. A review. Mar Drugs 1:39

3. Gerwick WH, Tan LT, Sitachitta N (2001) Alkaloids of marine cyanobacteria. In: Cordell GA (ed) The Alkaloids, vol 57. Academic Press, San Diego, p 75
4. Fusetani N, Matsunaga S (1993) Bioactive sponge peptides. Chem Rev 93:1793
5. Shimizu Y (1993) Microalgal metabolites. Chem Rev 93:1685
6. Shimizu Y (1996) Microalgal metabolites: A new perspective. Annu Rev Microbiol 50:431
7. Daranas AH, Norte M, Fernández JJ (2001) Toxic marine microalgae. Toxicon 39:1101
8. Rosewater J (1961) The family Pinnidae in the Indo-Pacific. Indo-Pacific Mollusca 1:53/501/632
9. Zheng SZ, Huang FL, Chen SC, Tan XF, Zuo JB, Peng J, Xie RW (1990) The Isolation and bioactivities of pinnatoxin. Zhongguo Haiyang Yaowu (Chinese Journal of Marine Drugs) 9:33
10. 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
11. Chou T, Kamo O, Uemura D (1996a) Relative stereochemistry of pinnatoxin A, a potent shellfish poison from *Pinna muricata*. Tetrahedron Lett 37:4023
12. 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
13. 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
14. McCauley JA, Nagasawa K, Lander PA, Mischke SG, Semones MA, Kishi Y (1998) Total synthesis of pinnatoxin A. J Am Chem Soc 120:7647
15. Nagasawa K (2000) Total synthesis of pinnatoxin A. J Synth Org Chem Jpn 58:877
16. 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
17. Takada N, Iwatsuki M, Suenaga K, Uemura D (2000) Pinnamine, an alkaloidal marine toxin, isolated from *Pinna muricata*. Tetrahedron Lett 41:6425
18. 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
19. Carmichael WW, Blggs DF, Gorham PR (1975) Toxicology and pharmacological action of anabaena flos-aquae toxin. Science 187:542
20. Devlin JP, Edwards OE, Gorham PR, Hunter NR, Pike RK, Stavric B (1977) Anatoxin A, a toxic alkaloid from anabaena-flos-aquae NRC-44H. Can J Chem 55:1367
21. 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
22. Kigoshi H, Hayashi N, Uemura D (2001) Stereoselective synthesis of pinnamine, an alkaloidal marine toxin from *Pinna muricata*. Tetrahedron Lett 42:7469
23. Fukuzawa S, Hayashi Y, Uemura D, Nagastu A, Yamada K, Ijyuin Y (1995) The isolation and structures of five new alkaloids, norzoanthamine, oxyzoanthamine, norzoanthaminone, cyclozoanthamine and epinorzoanthamine. Heterocycl Commun 1:207
24. Rao CB, Anjaneyula ASR, Sarma NS, Venkatateswarlu Y, Rosser RM, Faulkner DJ, Chen MHM, Clardy J (1984) Zoanthamine; a novel alkaloid from a marine zoanthid. J Am Chem Soc 106:7983
25. Rao CB, Anjaneyula ASR, Sarma NS, Venkatateswarlu Y, Rosser RM, Faulkner DJ, Chen MHM, Clardy J (1985) Alkaloids from a marine zoanthid. J Org Chem 50:3757

26. Rahman AU, Alvi KA, Abbas SA, Choudhary MI, Clardy J (1989) Zoanthaminone, a new alkaloid from a marine zoanthid. Tetrahedron Lett 30:6825
27. Kuramoto M, Hayashi K, Fujitani Y, Yamaguchi K, Tsuji T, Yamada K, Ijyuin Y, Uemura D (1997) Absolute configuration of norzoanthamine, a promising candidate for an osteoporotic drug. Tetrahedron Lett 38:5683
28. Nakamura H, Kawase Y, Maruyama K, Murai A (1998) Studies on polyketide metabolites of a symbiotic dinoflagellate, *Symbiodinium* sp: A new C30 marine alkaloid, zooxanthellamine, a plausible precursor for zoanthid alkaloids. Bull Chem Soc Jpn 71:781
29. Kuramoto M, Hayashi K, Yamaguchi K, Yada M, Tsuji T, Uemura D (1998) Structure-activity relationship of norzoanthamine exhibiting significant inhibition of osteoporosis. Bull Chem Soc Jpn 71:771
30. Yamaguchi K, Hayama T, Makita T, Tsuji T (1997) Time course of bone biomechanical parameters in ovariectomized mice. J Bone Miner Metab 15:138
31. Kuramoto M, Yamaguchi K, Tsuji T, Uemura D (2000) Zoanthamines, antiosteoporotic alkaloids. In: Fusetani N (ed) Drugs from the Sea. Karger, Basel, p 98
32. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J (1996) Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925
33. Turner CH, Burr DB (1993) Basic biomechanical measurements of bone: A tutorial. Bone 14:595
34. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G (1996) Increased bone formation in osteocalcin-deficient mice. Nature 382:448
35. Miyashita M, Sasaki M, Hattori I, Sakai M, Tanino K (2004) Total synthesis of norzoanthamine. Science 305:495
36. van den Bosch H (1980) Intracellular phospholipases A. Biochim Biophys Acta 604:191
37. Arita H, Nakano T, Hanasaki K (1989) Thromboxane A2: its generation and role in platelet activation. Prog Lipid Res 28:273
38. Scheuer PJ, de Silva ED (1980) Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (polejaeff). Tetrahedron Lett 21:1611
39. Albizati KF, Holman T, Faulkner DJ, Glaser KB, Jacobs RS (1987) Luffariellolide, an anti-infmammatory sesterterpene from the marine sponge *Luffariella sp*. Experientia 43:949
40. Potts BCM, Faulkner DJ, de Carvalho MS, 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
41. Potts BCM, Faulkner DJ, Jacobs RS (1992b) Phospholipase A2 inhibitors from marine organisms. J Nat Prod 66:1701
42. 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
43. 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
44. Kramer RM, Sharp JD (1995) Recent insights into the structure, function and biology of cPLA2. Agents Actions Suppl 46:65
45. 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

46. 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
47. 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
48. 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
49. Arimoto H, Hayakawa I, Kuramoto M, Uemura D (1998) Absolute stereochemistry of halichlorine; a potent inhibitor of VCAM-1 induction. Tetrahedron Lett 39:861
50. Clive DLJ, Yu M, Wang J, Yeh VSC, Kang S (2005) Synthetic chemistry of halichlorine and the pinnaic acids. Chem Rev 105:4483
51. Carson MW, Kim G, Hentemann MF, Trauner D, Danishefsky SJ (2001) Concise stereoselective routes to advanced intermediates related to natural and unnatural pinnaic acid. Angew Chem Int Ed 40:4450
52. Carson MW, Kim G, Danishefsky SJ (2001) 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 40:4453
53. Trauner D, Danishefsky SJ (1999) Studies towards the total synthesis of halichlorine: asymmetric synthesis of the spiroquinolizidine subunit. Tetrahedron Lett 40:6513
54. Trauner D, Schwarz JB, Danishefsky SJ (1999) Total synthesis of (+)-halichlorine: an inhibitor of VCAM-1 expression. Angew Chem Int Ed 38:3542
55. Hayakawa I, Arimoto H, Uemura D (2003) Synthesis of (±)-pinnaic acid. Heterocycles 59:441
56. Bhadury P, Wright PC (2004) Exploitation of marine algae: biogenic compounds for potential antifouling applications. Planta 219:561
57. Yamada A, Kitamura H, Yamaguchi K, Fukuzawa S, Kamijima C, Yazawa K, Kuramoto M, Wang GYS, Fujitani Y, Uemura D (1997) Development of chemical substances regulating biofilm formation. Bull Chem Soc Jpn 70:3061
58. Takada N, Watanabe R, Suenaga K, Yamada K, Ueda K, Kita M, Uemura D (2001) Zamamistatin, a significant antibacterial bromotyrosine derivative, from the Okinawan sponge *Pseudoceratina purpurea*. Tetrahedron Lett 42:5265
59. Moody K, Thomson RH, Fattorusso E, Minale L, Sodano G (1972) Aerothionin and homoaerothionin: two tetrabromo spirocyclohexadienylisoxazoles from *Verongia* sponges. J Chem Soc Perkin Trans 1, p 18
60. Ogamino T, Obata R, Nishiyama S (2006) Symmetric synthesis of aerothionin, a marine dimeric spiroisoxazoline natural product, employing optically active spiroisoxazoline derivative. Tetrahedron Lett 47:727
61. Hayakawa I, Teruya T, Kigoshi H (2006) Revised structure of zamamistatin. Tetrahedron Lett 47:155
62. Nishiyama S, Yamamura S (1985) Total synthesis of (+/–)-aerothionin, (+/–)-homoaerothionin, and (+)-aerophobin-1. Bull Chem Soc Jpn 58:3453
63. Uemura D (1991) Antitumor polyethers. In: Scheuer PJ (ed) Bioorganic Marine Chemistry. Springer, Berlin Heidelberg New York, p 1
64. 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

65. Nakamura H, Asari T, Murai A, Kan Y, Kondo T, Yoshida K, Ohizumi Y (1995) Zooxanthellatoxin-A, a potent vasoconstrictive 62-membered lactone from a symbiotic dinoflagellate. J Am Chem Soc 117:550
66. Satake M, Ofuji K, Naoki H, James KJ, Furey A, McMahon T, Silke J, Yasumoto T (1998) Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. J Am Chem Soc 120:9967
67. Nicolaou KC, Koftis TV, Vyskocil S, Petrovic G, Ling T, Yamada YM, Tang W, Frederick MO (2004) Structural revision and total synthesis of azaspiracid-1, part 2: Definition of the ABCD domain and total synthesis. Angew Chem Int Ed 43:4318
68. 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
69. 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
70. Onodera K, Nakamura H, Oba Y, Ohizumi Y, Ojika M (2005) Zooxanthellamide Cs: vasoconstrictive polyhydroxylated macrolides with the largest lactone ring size from a marine dinoflagellate of *Symbiodinium* sp. J Am Chem Soc 127:10406
71. 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
72. Kita M, Ohishi N, Washida K, Kondo M, Koyama T, Yamada K, Uemura D (2005) Symbioimine and neosymbioimine, amphoteric iminium metabolites from the symbiotic dinoflagellate Symbiodinium sp. Bioorg Med Chem 13:5253
73. Kita M, Uemura D (2005) Iminium alkaloids from marine invertebrates: structure, biological activity, and biogenesis. Chem Lett 34:454
74. Kita M, Sakai E, Uemura D (2006) Pursuit of novel bioactive marine metabolites. J Synth Org Chem Jpn 64:471
75. Soda H, Shim N, Nakagawa N, Yamaguchi K, Kiosk M, Mochizuki S, Tomoyasu A, Yano K, Got M, Murakami A, Stud E, Morinaga T, Hibachi K, Fukazawa N, Takahashi N, Soda T (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597
76. Warner TD, Giuliano F, Vojnovic I, Nukasa A, Mitchell JA, Vane JR (1999) Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. Proc Natl Acad Sci USA 96:7563
77. Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV (2000) Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. Cancer Res 60:293