Bioactive Alkaloids from the Sea: A Review

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Abstract: In our ongoing search for bioactive substances from marine organisms, novel alkaloids have been isolated. Pinnatoxins and pinnamine, potent shellfish poisons, were purified from the Okinawan bivalve *Pinna muricata*. Pinnatoxins activate Ca²⁺ channels. Halichlorine was isolated from the marine sponge *Halichondria okadai*. This compound inhibits the induction of VCAM-1. Drugs that block VCAM-1 may be useful for treating coronary artery diseases, angina, and noncardiovascular inflammatory diseases. Pinnaic acids, which are cPLA₂ inhibitors, were also obtained from *P. muricata*. Interestingly, the structures of pinnaic acids are closely related to that of halichlorine. Norzoanthamine hydrochloride, isolated from the colonial zoanthid *Zoanthus* sp., suppresses decreases in bone weight and strength in ovariectomized mice, and could be a good candidate for an osteoporotic drug. Ircinamine, purified from the marine sponge *Ircinia* sp., has a reactive thioester. Aburatubolactams, inhibitors of superoxide anion generation, were isolated from *Streptomyces* sp. This article covers the bioactive marine alkaloids that have been recently isolated by this research group.

Keywords: Ca²⁺ channel, VCAM-1, cPLA₂, anti-Osteoporosis, Superoxide anion.

Introduction
Alkaloids are nitrogen-containing compounds that occur naturally not only in plants but also in microorganisms, marine organisms, and animals. Although it is not clear why alkaloids show significant biological activity, they are often useful as drugs or biological probes for physiological studies. As new and more complicated diseases are encountered worldwide, the importance of bioactive alkaloids has increased due to their potential application in chemotherapy. As the application of alkaloids has expanded, the definition of alkaloids has become less restricted.

Results and Discussion

Ca²⁺ Channel-Activating Shellfish Poisons (Pinnatoxins)

Shellfish of the genus Pinna live mainly in shallow waters of the temperate and tropical zones of the Indian and Pacific Oceans [1]. The adductor muscle of this bivalve is eaten in Japan and China, and food poisoning resulting from its ingestion occurs frequently [2]. Chinese investigators have reported that a toxic extract from P. attenuata, referred to as pinnatoxin, is a Ca²⁺ channel activator [2]. We isolated pinnatoxin A (1), a mixture of B and C (2, 3), and D (4) from P. muricata (Fig. 1) [3-7].

Fig. 1. Structures of Pinnatoxin.

![Pinnatoxin structures](image)

The structures and stereochemistry of pinnatoxins were clarified by extensive NMR experiments and positive ion ESI MS/MS spectra [3-8]. 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, pinnac acids contain a carboxylate anion and an iminium cation or an ammonium cation. Recently, Kishi’s group achieved the total synthesis of 1 [9]. This investigation also supported the stereochemistry of 1.

Pinnatoxin A (1) showed potent acute toxicity against mice (LD₅₀ 2.7 µg/MU (i.p.)). It was noted that the toxicity of 2, 3 (LD₅₀ 0.99 µg/MU) was as potent as that of tetrodotoxin. Although the acute toxicity of 4 (LD₅₀ > 10 µg/MU) was weaker than that of the other pinnatoxins, 4 showed the
strongest cytotoxicity against the murine leukemia cell line P388 (IC_{50} 2.5 \mu g/ml). Pteratoxins A (5), B, and C (6, 7: a 1:1 mixture) were also isolated from the Okinawan bivalve *Petria penguin* [10]. Pteratoxins (5, 6, and 7) showed significant acute toxicity against mice (LD_{99} 100; 8 mg/kg) (Fig. 2).

**Fig. 2.** Structures of Pteratoxin.

Extracts from the digestive glands of several *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. These data suggest that *Pinna* shellfish may become toxic as the result of feeding on toxic organisms such as dinoflagellates [10].

Interestingly, the backbone of pinnatoxins and their analogues could be configured from C1 to C34 in a single carbon chain. Marine organisms usually produce super carbon chain molecules with a terminal amino group, e.g., palytoxin. In this study, we proposed a polyketide biogenetic pathway for pinnatoxins, shown in Fig. 3.

An Inhibitor of VCAM-1 (Vascular Cell Adhesion Molecule-1) Induction (Halichlorine)

A recent study suggested that adhesion molecules may some day be used clinically as anti-inflammatory agents and immunosuppressive agents, provided that the function of the adhesive molecules can be controlled [11].

A simple model of multistage adhesion between leukocyte and vascular cells is shown in Fig. 4. VCAM-1 [12] is affected during 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 [12].
Halichlorine (7) was isolated from the marine sponge *H. okadai* Kadota [13]. The gross structure of 7 was elucidated by an analysis of MS, IR and extensive 2D NMR spectra, as shown in Fig. 5 [14,15]. Halichlorine consists of a sterically hindered 15-membered lactone, an azabicyclo [4.4.0] ring, and a [5.6]-spiro ring moiety. Oxidative degradation of 7, as well as asymmetric synthesis of the degradation product, allowed us to determine the absolute stereochemistry of halichlorine [16]. The first total synthesis by Danishefsky and co-workers [17,18] also supported our conclusions regarding the structure of 7. Halichlorine inhibits the induction of VCAM-1 at IC$_{50}$ 7 µg/ml. Although VCAM-1 and ICAM belong to the same immunoglobulin superfamily, halichlorine does not affect ICAM (IC$_{50}$ > 100 µg/ml) [10,19]. It is largely unknown why halichlorine affects only VCAM-1. Thus, additional research will be needed to clarify the functions and mechanisms of action of VCAM-1.
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 [20-22]. Marine natural products such as manoalide [23] and luffariellolide [24] have been reported to be potent PLA$_2$ inhibitors [25,26]. A cytosolic 85-kDa phospholipase (cPLA$_2$) [27,28] exhibits specificity for the release of arachidonic acid from membrane phospholipids [29]. Therefore, compounds that inhibit cPLA$_2$ activity have been targeted as anti-inflammatory agents.

Pinnaic acids (8, 9) were isolated from the viscera of *P. muricata* (Fig. 6) [30]. The structure of 9 was determined by an analysis of NMR spectral data. Taupinnaic acid (9) has a 6-azaspiro[4.5]decane unit and a taurine moiety. Furthermore, the gross structure of 8 was elucidated by a detailed comparison of the EI-MS fragment peaks with the corresponding peaks of 9. Synthetic studies of pinnaic acid (*vide infra*) unambiguously established that the relative stereochemistry of pinnaic acid is similar to that of 7 [31-33].

Pinnaic acid (8) and taupinnaic acid (9) inhibited cPLA$_2$ activity *in vitro* with IC$_{50}$ values of 0.2 mM and 0.09 mM, respectively. Although the activity of pinnaic acids was moderate, inhibitors
of cPLA$_2$ have not yet been reported. Therefore, it is necessary to clarify the mechanism of action of these inhibitors. As described above, pinnaic acids are closely related to halichlorine. Therefore, each carbon atom has been tentatively numbered according to the supposed biogenetic formation of the N-C23 bond (Fig. 7). It is possible that these bioactive compounds from marine organisms are produced by symbiotic microorganisms.

**Fig. 7.** Biogenesis of pinnaic acids and halichlorine.

These architecturally novel alkaloids have attracted the attention of synthetic chemists; to date, 14 research groups have published synthetic studies of these alkaloids. The Danishefsky group has achieved the total synthesis of pinnaic acid [31,32] and halichlorine [17,18] in an asymmetric manner. Since pinnaic acid is a zwitterionic molecule, the NMR spectrum is quite sensitive to the measurement conditions. We recently reported a racemic total synthesis of 8 (Scheme 1) [33], and our detailed comparison of the $^1$H-NMR spectra of both synthetic and natural samples supported Danishefsky’s revision of the configuration at C14.

**Scheme 1.** Synthesis of (±)-pinnaic acid [33].
A Significant Inhibitor of Osteoporosis (Norzoanthamine)

Osteoporosis is caused by an imbalance between bone resorption and bone formation, which results in bone loss and fractures after mineral flux occurs. 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. Therefore, 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 novel anti-osteoporotic drugs [34].

Norzoanthamine (10) [35] and its homologues (11-18) [35-38] were isolated from the genus Zoanthus sp. The relative stereochemistry of norzoanthamines was determined by X-ray analysis. Furthermore, the absolute stereochemistry of norzoanthamine was determined by an advanced version of Mosher’s method, as shown in Fig. 8 [39]. IL-6 is known to stimulate osteoclast formation, and the suppression of IL-6 secretion can be effective in the prevention of osteoporosis. Norzoanthamine and norzoanthamine hydrochloride inhibit IL-6 induction at values of 13 and 4.7 µg/ml, respectively [39-41]. Furthermore, norzoanthamine and norzoanthamine hydrochloride, both of which counteract decreases in bone weight and strength in ovarieectomized mice, could be good candidates for osteoporotic drugs [34,42].

**Fig. 8.** Structures of Norzoanthamines.
The effect of norzoanthamine hydrochloride on bone weight and strength was tested in ovariectomized mice, an animal model of postmenopausal osteoporosis [39-41,43]. Norzoanthamine hydrochloride (0.08 mg/kg/day, p.o.) significantly suppressed the decrease in femoral weight caused by ovariectomy without an increase in uterine weight (Fig. 9). Such data suggested that the mode of action of norzoanthamine hydrochloride differs from that of estrogen [44]. Furthermore, the failure load and yield energy of the femur were maintained by the administration of norzoanthamine hydrochloride at a dose of 0.4 mg/kg/day (p.o.) (Fig. 10). Finally, the thickness of the cortical bone was measured from a photograph of the ground bone. Ovariectomy caused a decrease in humeralis trabeculae (Fig. 11). Norzoanthamine hydrochloride significantly suppressed this decrease in a dose-dependent manner (Fig. 11C, D, E). In ovariectomized mice treated with norzoanthamine hydrochloride, the primary spongiosa did not significantly increase, and the morphology of the metaphysis remained nearly normal.

Fig. 9. Effects of norzoanthamine hydrochloride on uterine and femoral weight.

Fig. 10. Effects of norzoanthamine hydrochloride on failure load and yield energy.
Fig. 11. Effect of norzoanthamine hydrochloride on humeralis morphology in ovariectomized mice.

Based on their molecular formulas, zoanthamines have been regarded as terpenoids; however, the biogenetic pathway of zoanthamines remains unclear. As described above, marine organisms usually produce super carbon chain molecules with a terminal amino group. Here, we propose a polyketide biogenetic pathway for zoanthamines, as shown in Fig. 12.

Fig. 12. Proposed biogenesis of zoanthamines.

Interestingly, Nakamura’s group isolated zooxathellamine (19, Fig. 8) from a symbiotic dinoflagellate *Symbiodinium* sp. [45]. The absolute stereochemistry of 19 was the same as that of norzoanthamine. The structural similarity of 19 and zoanthid alkaloids suggests that these zoanthamines may have an algal origin. Furthermore, a feeding experiment with a labeled compound suggested the biosynthetic pathway of 19. This pathway was similar to that which we suggested previously.

*Other Alkaloids from Marine Organisms (Pinnamine, Ircinamine)*

As described above, pinnatoxins, are Ca\(^{2+}\) channel activators that have been isolated from the Okinawan bivalve *P. muricata*. In a continuation of this work, we isolated pinnamine (20), which
produced characteristic toxic symptoms, such as scurrying around [46]. The gross structure of \( \text{20} \) was clarified by a detailed analysis of NMR and CD spectra (Fig. 13) [47-50]. The absolute stereochemistry of \( \text{20} \) was also supported by a synthetic study [51]. Pinnamine exhibited significant acute toxicity against mice, with an LD\(_{99}\) of 0.5 mg/kg.

Fig. 13. Structures of pinnamine and ircinamine.

Ircinamine (\( \text{21} \)) was isolated from the marine sponge \( \text{Ircinia} \) sp., and its structure was elucidated by spectroscopic analysis and reductive transformation (Fig. 13) [52]. Ircinamine has a unique structure with an amphibolous pyrroline ring moiety and a reactive thioester unit. Although \( \text{21} \) has only moderate activity toward P388 (LD\(_{50}\) 24.6 µg/ml), marked biological activity is expected based on the reactivity of the thioester moiety [53].

These compounds contain unique structural features and may be biosynthesized through unusual biogenetic pathways.

Inhibitors of Superoxide Anion Generation from Marine Microorganisms (Aburatubolactams)

As described above, extremely bioactive, structurally novel compounds have been found in marine organisms. However, their practical use in drugs is considerably limited because of the extraordinarily low amount of physiologically active compounds obtainable from these marine organisms. Therefore, research has necessarily focused on the metabolites of such marine microorganisms, which most likely are the true producers of the active compounds.

Aburatubolactams (\( \text{22}, \text{23}, \text{24} \)) [54] were isolated from the cultured broth of a \( \text{Streptomyces} \) sp., SCRC-A20, in a study that monitored the inhibition of superoxide anion generation [55,56]. Superoxide anions are thought to be closely associated with inflammation, cancer, and aging [57,58]. The structures of these compounds were mainly determined by NMR analysis. Fortunately, a single crystal of aburatubolactam A was obtained from MeOH. The structure of the 20-membered macrocyclic structure is thought to contain diene amide and dienone functionalities. The structures of other aburatubolactams were deduced by a detailed comparison of the NMR spectra with that of aburatubolactam A (Fig. 14).
Aburatubolactams (22, 23, 24) inhibited TPA-induced superoxide anion generation by human neutrophils (IC₅₀ 26, 6.3, 2.7 µg/ml, respectively). The mechanism of action and the in vivo behavior of aburatubolactams are currently under investigation.

Aburatubolactams, which possess an acyl tetramine structure, are biogenetically related to ikarugamycin [59] from a terrestrial actinomycete, alteramide A [60] from a marine bacterium, and cyrindramine [61] from a marine sponge. These results suggest that microorganisms may be the true producers of most marine metabolites.

**Conclusions**

As described above, bioactive alkaloids have been isolated from marine organisms. These structures were clarified by spectroscopic analysis and synthetic methods. The biogenesis of these compounds was proposed based on comparisons with their analogs. Additional biological activities of these compounds in vivo are currently under investigation in our laboratories.

Thanks to the development of new analytical instruments and techniques, numerous compounds have been isolated and elucidated from natural resources over the past 30 years. The study of natural resources may lead to the further discovery of novel bioactive compounds.
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