Highlight Review

Iminium Alkaloids from Marine Invertebrates: Structure, Biological Activity, and Biogenesis

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Abstract

The discovery of new natural products frequently triggers a breakthrough in basic sciences. This review features marine iminium alkaloids, which control biologically and physiologically intriguing phenomena. Pinnatoxins, potent shellfish poisons purified from the Okinawan bivalve *Pinna muricata*, activate Ca^{2+} channels. 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. The latter two compounds are good candidates for antiosteoporotic drugs.

♦ Introduction

Alkaloids are nitrogen-containing compounds that occur naturally not only in plants but also in microorganisms, marine organisms, and animals. 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 because of their potential application in chemotherapy.

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.[1] The possible primary producers of the secondary metabolites have been suggested to be microalgae, bacteria, and fungi, and they are carried through symbiosis, association, a food chain, and other forms of nutrient dependency. For instance, palytoxin (PTX) is a potent toxic polyol compound isolated from the zoanthid *Palythoa* sp., the bioorganic origin of which was questioned because of seasonal and regional variations.[2] In 1995, a PTX analogue, osteocin, was isolated from the dinoflagellate *Ostreopsis siamensis*, and it has been suggested that its true origin is also microorganisms.[3]

Numerous bioactive nitrogenous compounds, such as peptides, indols, oxazoles, and thiazoles, have been isolated from marine invertebrates. Since there have been many very fine reviews on these compounds,[4] they are not described here. However, it is still not clear why alkaloids show significant biological activity. This question may never be answered for in vivo systems. In the eco-system, the alkaloidal metabolites of cyanobacteria help to inhibit predation by marine herbivores, such as fish and sea urchins.[4a] Recently, several novel bioactive compounds possessing an imino group, such as pinnatoxins and symbioimine, have been reported. This review features the structure, biological activity, and biogenesis of these bioactive iminium alkaloids.

♦ Macrocyclic Iminium 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.[5] The adductor muscle of this bivalve is eaten in Japan and China, and food poisoning resulting from its ingestion occurs frequently.[6] Chinese investigators have reported that a toxic extract from *P. attenuata*, referred to as pinnatoxin, is a Ca^{2+} channel activator.[7a] We have isolated pinnatoxin A (**1**), a mixture of pinnatoxins B and C (**2**, **3**), and pinnatoxin D (**4**) from *P. muricata* (Figure 1).[7]

The structures and stereochemistry of pinnatoxins have been clarified by extensive NMR experiments and positive ion ESI MS/MS spectra.[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, they contain a carboxylate anion and an iminium cation or an ammonium cation. Pinnatoxin A (**1**) showed potent acute toxicity against mice (LD_{99} 180 μg/kg (ip)) with characteristic neurotoxic symptoms. Recently, Kishi's group achieved the total synthesis of **1** and *ent*-**1**.[9] Interestingly, while natural **1** showed significant acute toxicity, its antipode *ent*-**1** showed no toxicity.[10] This investigation also clarified the stereochemistry of **1**, including its absolute stereochemistry.

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).[7e] The LD_{99} values of **2** and **3** have been reported to be 22 μg/kg, which makes them as potent as tetrodotoxin. Although they were obtained in small amounts (≈0.3 mg), the structures and relative stereochemistries of the macrocycles in **2** and **3** were successively confirmed as follows. On the positive ion ESI MS/MS analysis, a series of prominent fragment ions were generated by a cyclohexane ring-opening reaction, the retro-Diels–Alder reaction, followed by bond cleavage of carbocycles (Figure 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 spectroscopic data of **6** derived from **2** and **3** were the same as those of the pinnatoxin A methyl ester

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(**5**), which was obtained by the reduction of iminium and a carboxylic acid moiety followed by oxidation of the resulting alcohol. Thus, the relative stereochemistry of the macrocyclic core in **2** and **3** was the same as that in pinnatoxin A (**1**).

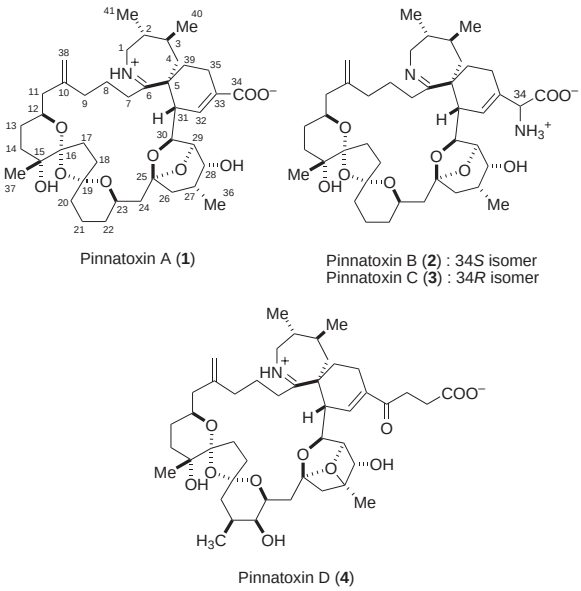


Figure 1. Structure of pinnatoxins.

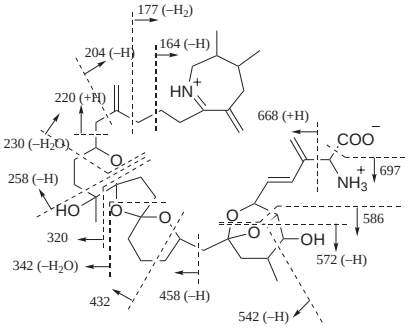


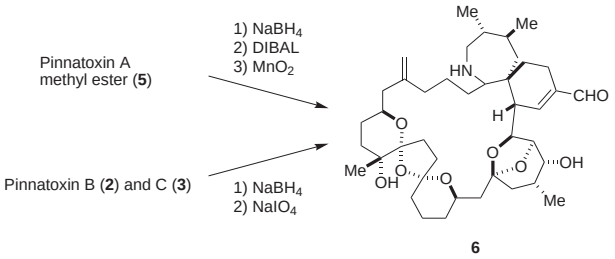
Figure 2. Fragmentation patterns of pinnatoxins B (**2**) and C (**3**).

Although pinnatoxin D (**4**) showed weaker acute toxicity than the other pinnatoxins ($LD_{50} > 10\,\mu g/MU$), **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 (Figure 3). Based on 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.

♦ *Other Macrocyclic Iminium Toxins Related to Pinnatoxins*

Several shellfish poisons containing an iminium moiety have been isolated (Figure 4). In our study of shellfish poisons,



Scheme 1.

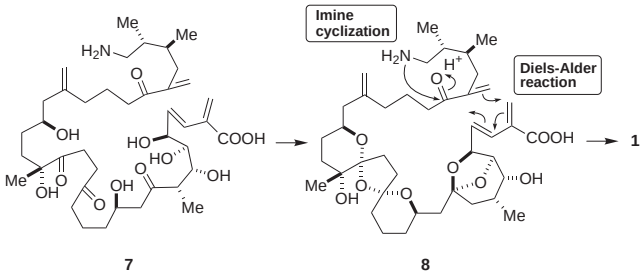


Figure 3. Proposed biogenesis of pinnatoxin A (**1**).

we observed that a moray eel vomits the viscera of the Okinawan bivalve *Pteria penguin*. Pteriatoxins A (**9**), B, and C (**10**, **11**: a 1:1 mixture) were successively isolated from this shellfish species.[11] Based on the analysis of 2D-NMR spectra and positive ion ESI MS/MS spectra, they were determined to be pinnatoxin analogs that contained a cysteine moiety. Pteriatoxins (**9**–**11**) showed significant acute toxicity against mice with LD_{99} values of 100 and 8 μg/kg, respectively. The toxic symptoms of pteriatoxins resemble those of pinnatoxins. 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. Also, based on the presence of pinnatoxin analogs in both shellfish *Pinna* sp. and *Pteria* sp., the pinnatoxin series may be biosynthesized by microorganisms that are in the food chain of or in a symbiotic relationship with these shellfish.

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.[12] The marine dinoflagellate *Alexandrium ostenfeldi* (Paulsen) Balech and Tangen was identified as the cause of spirolide toxicity in Nova Scotia in the early 1990's.[12,13] Seven compounds, spirolides A–D (**12**–**15**) and 13-demethyl C (**16**), which show toxicity against mice, and the keto amine derivatives E and F (**17** and **18**), have been isolated and structurally characterized from shellfish extracts and cultured dinoflagellate isolates from Nova Scotia. The spirolide family contains a 5:5:6 trispiroketal ring system. Recently, the relative stereochemistry of spirolides B (**13**), D (**15**), and 13-demethyl C (**16**), 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.[14]

Other marine toxins possessing a cyclic imine moiety else-

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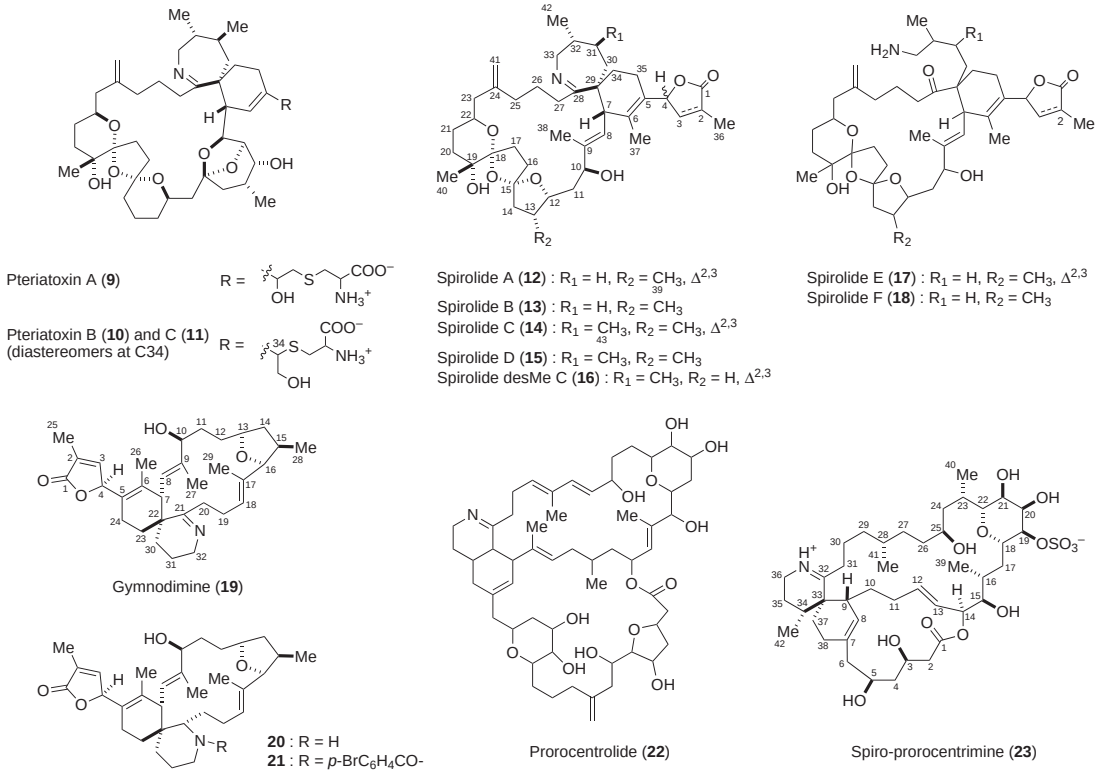


Figure 4. Structures of polyether toxins containing imine moieties.

where are known as gymnodimine, centrimine, and proro-centrimine. Gymnodimine (**19**) was isolated from the New Zealand oyster *Tiostrea chilensis* and the dinoflagellate *Gymnodinium* sp., and its structure was established.[15] The MLD (minimum lethal dose) of **19** was 450 μg/kg, and this compound also showed potent ichthyotoxicity against the fish *Tanichthys albonubes* at 0.1 ppm. The absolute stereostructure of gymnodimine (**19**) has been established by an X-ray crystal structure analysis of the *p*-bromobenzamide derivative **21** derived from gymnodamine (**20**).[16] 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.[17]

Prorocentrolide (**22**), a toxic marine macrolide that incorporates a hexahydroisoquinoline moiety, was isolated from the cultured dinoflagellate *Prorocentrium lima*.[18] This dinoflagellate produces diarrhetic shellfish poisoning toxins such as okadaic acid and dinophysistoxins (DTXs).[19] Recently, the prorocentrolide derivative spiro-prorocentrimine (**23**) was isolated from a cultured benthic *Prorocentrium* sp. in Taiwan, and its relative stereochemistry was established by the X-ray crystallographic analysis.[20] Compound **23** was much less toxic than other cyclic iminium toxins.

It should be noted that both the keto amine derivatives spirolide E and F (**17** and **18**),[12e] in which this ring has been opened, and the reduced form of gymnodamine (**20**)[16] were inactive. Although the pharmacological action of these iminium compounds has not yet been fully defined, the cyclic imine functionality may be essential and may act as a pharmacophore of macrocyclic iminium compounds, e.g., pinnatoxins and spirolides.

♦ *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 antiosteoporotic drugs.[21]

Norzoanthamine (**24**),[22a] zoanthamine (**25**), and its homologues were isolated from *Zoanthus* sp.[22] The relative stereochemistry of norzoanthamines was determined by X-ray analysis. Furthermore, the absolute stereochemistry of norzoanthamine was determined by a modified Mosher's method, as shown in Figure 5.[23a]

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.[23] Furthermore, norzoanthamine and norzoanthamine hydrochloride, both of which counteract decreases in bone weight and strength in ovariectomized mice, may be good candidates for antiosteoporotic drugs.[24]

The effect of norzoanthamine hydrochloride on bone weight and strength was tested in ovariectomized mice, an animal model of postmenopausal osteoporosis.[23,25] Norzoanthamine hydrochloride (0.08 mg/kg/day, p.o.) significantly suppressed the decrease in femoral weight caused by ovariectomy without an in-

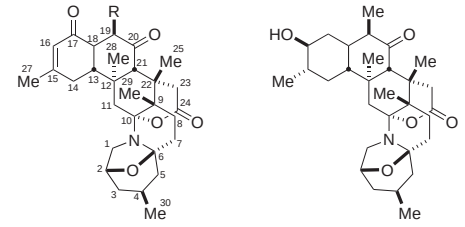
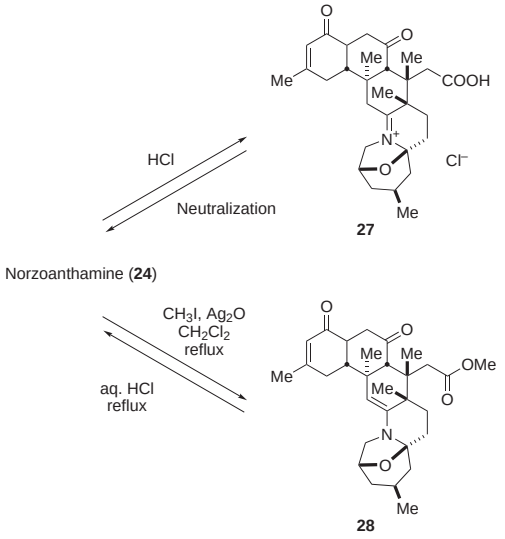


Figure 5. Structure of norzoanthamine derivatives.



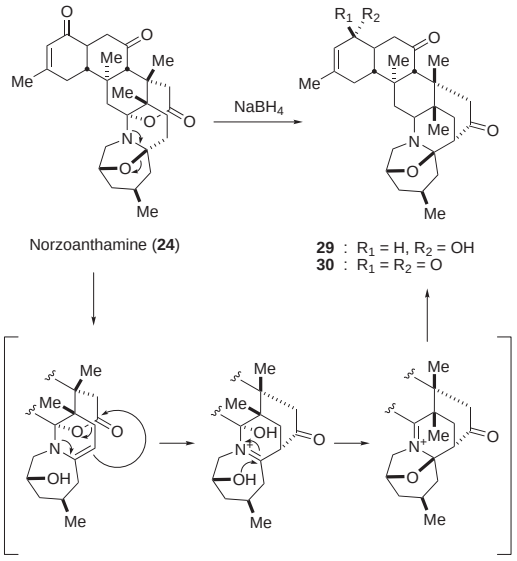
Scheme 2.

crease in uterine weight. These results suggest that the mode of action of norzoanthamine hydrochloride differs from that of estrogen.[26] In ovariectomized mice treated with norzoanthamine hydrochloride, the primary spongiosa did not significantly increase, and the morphology of the metaphysis remained nearly normal.

Equilibration between the lactone structure and iminium structure has been examined.[23b] The NMR spectrum of norzoanthamine hydrochloride in CD_3OD implied the presence of an iminium structure (**27**, $\delta_{C\text{-}10} = 193.3$) but not a lactone structure **24** in norzoanthamine (Scheme 2). The zwitter iminium structure was also demonstrated by transformation into methyl ester **28** by the treatment of **24** with CH_3I–Ag_2O. On the other hand, hydrolysis of **28** with aqueous HCl led to the recovery of **24**. Zooxathellamine (**26**), isolated from the cultured symbiotic dinoflagellate *Symbiodinium* sp., also adopted a zwitter ion structure with carboxylate and iminium moieties in D_2O based on their ^{13}C chemical shifts, but had a lactone structure in either $CDCl_3$ or CD_3OD.[27]

During a structural study of norzoanthamine (**24**), we found an intriguing rearrangement reaction.[23] Reduction of **24** with $NaBH_4$ in MeOH gave two derivatives, deoxydihydronorzoanthamine (**29**) and deoxynorzoanthamine (**30**). The proposed mechanism of this rearrangement reaction is shown in Scheme 3.

Based on their molecular formulas, zoanthamines have been regarded as terpenoids; however, the biogenetic pathway of



Scheme 3.

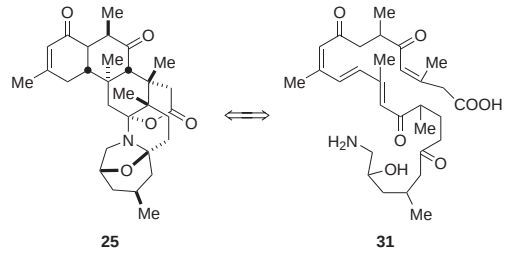


Figure 6. Proposed biogenesis of zoanthamines.

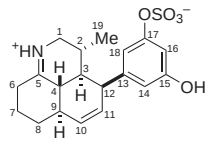
zoanthamines remains unclear. As described above, marine organisms usually produce super-carbon-chain molecules with a terminal amino group. We propose here a polyketide biogenetic pathway for zoanthamines, as shown in Figure 6. Furthermore, a feeding experiment with a labeled compound suggested a biosynthetic pathway for zooxathellamine (**26**). This pathway was similar to that which we suggested previously.

Recently, a total synthesis of norzoanthamine (**24**) has been achieved using an intramolecular Diels–Alder reaction as a key step to construct the requisite chiral triene.[28] This synthesis may be a powerful tool for advancing the study of norzoanthamine as a therapeutic drug.

♦ *A Potential Anti-osteoclast Differenciation Drug (Symbioimine)*

The symbiotic marine dinoflagellate *Symbiodinium* sp., which is a type of zooxanthellae, is found in a wide range of marine invertebrates and produces several bioactive large polyol compound, such as zooxanthellatoxins (ZTXs)[29] and zooxanthellamides.[30] In our continuing search for biologically active compounds, a unique amphoteric iminium compound, named symbioimine (**32**), was isolated from this dinoflagellate (Figure 7).[31]

IR spectrum of **32** showed absorption bands for hydroxy (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



Symbioimine (**32**)

Figure 7. Structure of symbioimine (**32**).

the presence of an iminium functionality in this water-soluble amphoteric compound. Crystallization of **32** from water gave well-formed, monocyclic colorless crystals as a monohydrate. Its structure, which consists of a characteristic 6,6,6-tricyclic iminium ring, was deduced by spectroscopic analysis and X-ray crystallographic analysis (Figure 8).

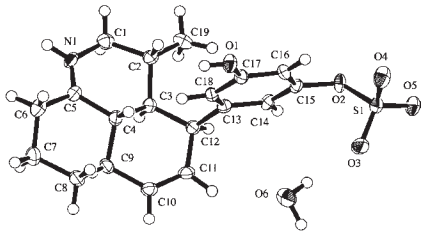


Figure 8. ORTEP drawing of symbioimine (**32**). The dihedral angle (deg) between C3–C12–C11 and the aromatic ring = 86.73(9); Selected torsion angles (deg): C3–C12–C13–C18 = 53.0(5), C11–C12–C13–C18 = −74.4(4).

Symbioimine (**32**) 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) ($EC_{50} = 44\,\mu g/mL$).[31] RANKL induces the formation of osteoclast-like multinucleated cells in cultures of bone marrow cells. Meanwhile, it did not affect cell viability even at 100 μg/mL. Thus, symbioimine (**32**) is a potential antiresorptive drug for the prevention and treatment of osteoporosis in postmenopausal women. Symbioimine (**32**) also inhibited cyclooxygenase 2 (COX-2) activity ($EC_{50} \approx 10\,\mu M$).[32] Although the real ecological role of **32** in the symbiotic dinoflagellate has been unknown, a hypothesis is suggested that it serves as a defense material which prevents their host animal's digestion.

A plausible biogenetic pathway for **32** includes an intramolecular *exo*-transition-state Diels–Alder reaction followed by imine cyclization, as with pinnatoxins (Figure 9). Studies of the biosynthetic pathways of symbioimine (**32**) using isotope-labeled precursor incorporation studies are in progress.

Large polyol and polyether compounds, such as palytoxin, halichondrin, ciguatoxin, and maitotoxin, have been widely

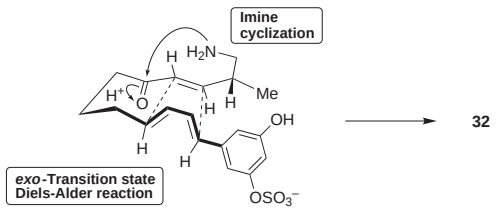


Figure 9. Proposed biogenesis of symbioimine (**32**).

studied in natural product chemistry.[33] These compounds are composed of a long carbon backbone functionalized by oxygen, and have been called "super-carbon-chain compounds."[33a] Recently, a novel super-carbon-chain compound that is a congener of ZTXs has been isolated from the symbiotic dinoflagellate *Symbiodinium* sp. and named symbiodinolide.[32] Although the super-carbon-chain compounds with iminium moiety have not been identified, several super-carbon-chain compounds consist of carbon chains starting from a carboxylic acid (C-terminus) and an amine moiety (N-terminus, which are sometimes acylated), i.e., palytoxin (115 straight carbon chain), ZTX-A (106 carbons), and azaspiracid 1[34,35] (40 carbons). Although the actual role and origin of the nitrogen atom in these nitrogenous polyketide derivatives have not been clarified, these compounds are expected to play an important role in vivo.

♦ Conclusion

Thanks to the development of new analytical instruments and techniques, numerous compounds have been isolated from natural resources over the past 30 years. The study of natural resources may lead to the further discovery of novel bioactive compounds. As described above, iminium alkaloids have been isolated not only from terrestrial organisms, but also as marine metabolites, and most have been shown to be produced by microorganisms. Although the effect of norzoanthamine hydrochloride was shown in vivo, most of the etiological aspects of other iminium alkaloids have not been clarified. Further chemical and biological studies on these iminium alkaloids should contribute a deeper understanding of their roles in nature.

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