Marine Toxins

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Contents

I. Introduction

Marine toxins have drawn scientists' attention due to their involvement in human intoxication and the socioeconomic impacts brought by those incidents. Elucidation of chemical structures is imperative not only for understanding the molecular basis of mechanism of actions but also for designing proper countermeasures such as detection, determination, and therapeutic methods. Many of the toxins were found to be useful tools for probing biological or pharmacological phenomena, as exemplified by tetrodotoxin in sodium channel studies[1] and okadaic acid in protein phosphatase studies.[2] Thus, chemical modification followed by structure–activity relationship studies provides an attractive target for chemists.

This article is intended to review structures and actions of marine toxins involved in human intoxication or massive fish kills. Bioactive compounds structurally related to toxins, especially polyether toxins, are also included. Attention is also paid to etiological studies, as most natural products chemists are concerned about the origins of bioactive substances. Although the majority of marine toxins have been found to be produced by microalgae, especially dinoflagellates, it is now clear that bacteria are responsible for production of some toxins. Readers who want to learn about the historical aspects and details of marine toxin studies should consult previously published books.[3–5]

II. Tetrodotoxin Derivatives

A. Biogenesis

Tetrodotoxin (TTX, 1; Chart I) is one of the best-known marine toxins because of its frequent involvement in fatal food poisoning, its unique chemical structure, and its specific action of blocking sodium channels of excitable membranes. The toxin, which derives its name from the pufferfish family (Tetraodontidae), occurs widely in both the terrestrial and marine animal kingdom.[1,6] The marked fluctuation of toxin concentration in TTX-containing animals according to individual, region, and season led some scientists to suspect an exogenous origin of the toxin in those animals. Incapability of puffers to develop toxicity when raised with artificial baits further supported the idea. The primary source of the toxin was traced from fish to a dietary alga and finally to an epiphytic, or symbiotic, bacterium.[7] The bacterium was first assigned to *Pseudomonas* sp. then to *Alteromonas* sp. and finally placed in a new species, *Shewanella alga*.[8] The toxin was isolated in a pure form and unambiguously identified by FABMS, fluorometric HPLC, dose–survival responses in mice, and degradation to 2-amino-6-(hydroxymethyl)-8-hydroxyquinazoline.[7] Subsequently, a broad spectrum of bacteria has been reported to produce the toxin.[9–11] The amounts of the toxin produced by the bacteria, however, were so small that identification of the toxin in bacterial cultures was often made on the basis of rather poor evidence. The toxin amount in the bacteria was also

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Takeshi Yasumoto was born in Okinawa, Japan, completed his B. S., M.S., and Ph.D. in Marine Biochemistry, Faculty of Agriculture, the University of Tokyo. He did his postdoctoral work at the Department of Chemistry, the University of Hawaii, from 1966 to 1968. He was an Assistant Professor in Marine Biochemistry from 1960 to 1969 at the University of Tokyo, Associate Professor in Food Chemistry from 1969 to 1977 at Tohoku University, Professor of Food Hygiene from 1977 to 1992, and is currently Professor of Bioorganic Chemistry at the Faculty of Agriculture, Tohoku University. He has been a visiting professor to University of Napoli, 1998, University of Hawaii, 1988, and Norwegian College of Veterinary Medicine, 1988. Some of his honors are as follows: award for young scientists from Japanese Society of Scientific Fisheries, 1977; Yoneda Memorial Lecturer in 34th Toxin Symposium, 1987; Award for Excellence in Research, IVth International Conference on Toxic Phytoplankton, Sweden, 1989; award from the Japan Society for Bioscience, Biotechnology, and Agrochemistry, 1992. His research interest is in bioorganic chemistry of marine toxins and other bioactive marine natural products.

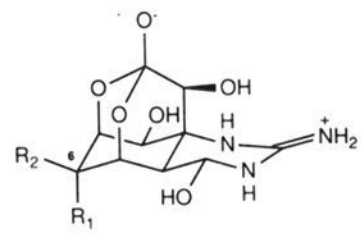
Michio Murata was born in Osaka, Japan in 1958. He graduated in 1981 and was awarded his Ph.D. degree in 1986 from Tohoku University. He worked at Suntory Institute for Bioorganic Research (1983–1985) directed by Koji Nakanishi, where he was involved in marine natural products chemistry; e.g. symbiosis-inducing substances between sea anemone and anemone fish, or antimolting hormones of crustacean. In 1985 he moved to Faculty of Agriculture, Tohoku University, and worked with Takeshi Yasumoto as a research associate. His main achievements have been those on structural determination of marine toxins by using modern NMR techniques; e.g. ciguatoxin, maitotoxin, or diarrhetic shellfish toxins. He spent his postdoctoral period in John Daly's laboratory, National Institutes of Health. Since February 1993 he has been working in Department of Chemistry, University of Tokyo, as an associate professor.

far too small to allow chemists to conduct biosynthetic studies.

B. Tetrodotoxin Analogs

Detection of TTX analogs occurring in puffers, newts, and a frog was facilitated by a highly sensitive TTX analyzer, which separates analogs on a reversed-phase column and detects fluorescent products produced upon heating with sodium hydroxide solution.[12,13] Structures of six new analogs **2**–**7** (Chart I) were determined.[14–18] Biosynthesis of TTX supposedly involves arginine, analogous with saxitoxin, and a C5 unit derived from either amino acids, isoprenoids, shikimates, or branched sugars. The occurrence of **2** and **3** analogs renders an isoprenoid unit a favorable candidate, because it possesses both sp^2 carbons available for Diels–Alder-type condensation and a methyl that remains in **3**. Two epimers of 11-norTTX **5** and **6** are likely to be decarboxylation products of a hypothetical 11-COOH derivative. Those analogs found in puffers and newts were not found in a Costa Rican frog, *Atelops chiriquiensis*, which contained **1** and chiriquitoxin (**7**).[17] Interestingly, **4** was more active than **1** in blocking sodium channels.[20] The potency of chiriquitoxin was comparable to that of TTX.[21] Other analogs were less potent than TTX. On the basis of the structure–activity relationship, a pocket-shaped model has been proposed for the binding site in the sodium channel protein. The charge groups in a clevis of channel protein supposedly act as anchoring points by interacting with the toxins' functional groups orienting in different directions.[21]

Chart I



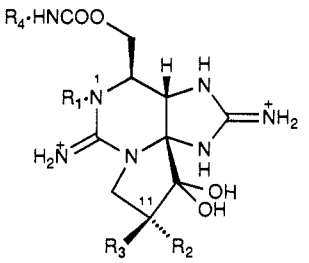
	R_1	R_2
1 TTX	OH	$^{11}CH_2OH$
2 6-epi-TTX	CH_2OH	OH
3 11-deoxyTTX	OH	CH_3
4 11-oxoTTX	OH	CHO
5 11-norTTX-6(*R*)-ol	H	OH
6 11-norTTX-6(*S*)-ol	OH	H
7 Chiriquitoxin	OH	$CH(OH)_R^{}CH(NH_2)_S^{}COOH$

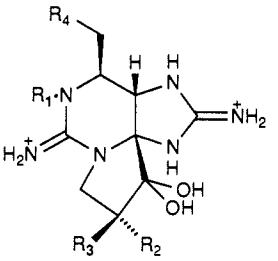
III. Saxitoxin and Derivatives

A. Biogenetic Origins

Saxitoxin and its derivatives are well known for their involvement in highly fatal poisoning, termed paralytic shellfish poisoning (PSP). Among all seafood poisoning, PSP poses the most serious threat to public health, and the economic damage caused by accumulation of the toxins in shellfish is immeasurable. A number of dinoflagellate species are known to produce the toxins: *Alexandrium* spp. (formerly *Gonyaulax* or *Protogonyaulax*), *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum*.[23] Some strains of the freshwater blue-green alga, *Aphanizomenon flos-aquae*, also produce saxitoxin and neosaxitoxin.[24–27] The origin of saxitoxin in PSP is suggested to be bacterial, analogous with TTX.[27] However, the claim has been made on rather poor evidence and needs further confirmation by more sophisticated methods. Saxitoxin has also been found in a macroalga, *Jania* sp., and this red alga is apparently the source of saxitoxin in crabs.[28]

Chart II





		R_1	R_2	R_3	R_4
8	STX	H	H	H	H
9		H	H	H	SO_3^-
10	GTX2	H	OSO_3^-	H	H
11		H	OSO_3^-	H	SO_3^-
12	GTX3	H	H	OSO_3^-	H
13		H	H	OSO_3^-	SO_3^-
14	NeoSTX	OH	H	H	H
15		OH	H	H	SO_3^-
16	GTX1	OH	OSO_3^-	H	H
17		OH	OSO_3^-	H	SO_3^-
18	GTX4	OH	H	OSO_3^-	H
19		OH	H	OSO_3^-	SO_3^-

	R_1	R_2	R_3	R_4
20	H	OSO_3^-	H	OH
21	H	H	OSO_3^-	OH
22	H	H	H	H
23	H	OSO_3^-	H	H
24	H	H	OSO_3^-	H
25	OH	H	H	OH

B. Structures

Like in the case of TTX, discovery of new saxitoxin analogs was accelerated by the use of a fluorometric HPLC analyzer, which measures fluorescence derived from oxidation products.[29,30] Saxitoxin derivatives (Chart II) found in the earlier stage of the studies are characterized by the presence of 11-*O*-sulfate (**10–13** and **16–19**), *N*-sulfate (**9**, **11**, **13**, **15**, **17**, and **19**), and oxygen at N-1 (**14–19**).[31] Newer members of the saxitoxin family **20–25** do not possess the carbamoyl moiety of **8**.[32–35] Interestingly, oxidation at N-1 and formation of 11-*O*-sulfate are suggested to occur at an early stage of biosynthesis.[35] The biosynthetic pathways for saxitoxin will be discussed by Shimizu in this issue.[36]

IV. Ciguatera Toxins

A. Ciguatera (Seafood Poisoning)

Ciguatera is a seafood poisoning prevalent in circumtropical areas. The poisoning is caused by ingestion of coral reef fish that have become toxic through diet. There are two groups of compounds implicated in the poisoning; the main responsible toxins are ciguatoxin and its congeners, and the other is maitotoxin. Both groups are produced by the epiphytic dinoflagellate *Gambierdiscus toxicus*[37,38] and transferred to herbivorous fish and subsequently to carnivores through the food chain. Ciguatoxin is regarded as the principal toxin responsible for human illnesses, while other toxic constituents have been recently found in association with the poisoning (see section IV.E). The clinical symptoms are diverse.[39] Neurologic disturbances are prominent; reversal of thermal sensation, called "dry-ice sensation", is one of the most characteristic symptoms of ciguatera. Other illnesses include joint pain, miosis, erethism, cyanosis, and prostration. Gastrointestinal disorders are nausea, vomiting, and diarrhea. Cardiovascular disturbances are low blood pressure and bradycardia.

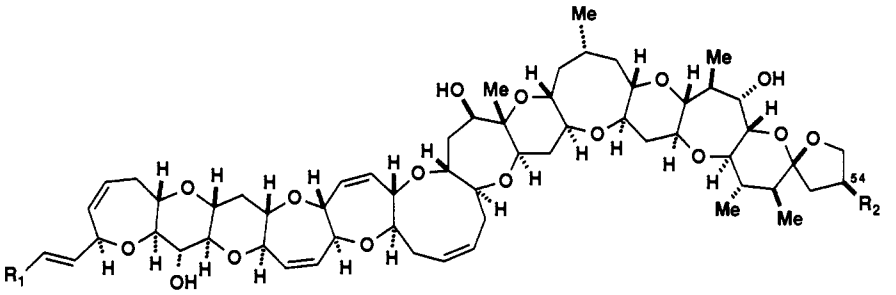
B. Ciguatoxins

Ciguatoxin (**26**) was first isolated in 1980 by Scheuer's group at the University of Hawaii and characterized to be a polyether compound.[40] An inadequate amount of material, however, prevented this group from determining its structure.

The structure of ciguatoxin was finally elucidated by Yasumoto's group in 1989.[41a] For that study toxin was extracted from moray eels (*Gymnothorax javanicus*) collected in French Polynesian waters.[42] With no more than 0.35 mg of **26** and 0.75 mg of a congener (CTX 4B, **27**; formerly gambiertoxin 4b), their structures were successfully solved to be the polycyclic ethers **26** and **27** (Chart III), respectively.[41] Since 1989, chemical studies on ciguatera toxins have made rapid strides. Ciguatoxin congeners have been isolated either from toxic fish (54-deoxy-ciguatoxin)[43] or from cultured *G. toxicus* (CTX 3C, **28**).[44] Furthermore dozens of ciguatoxin analogs have been found in fish and in the dinoflagellates,[45] although only a few of them have been identified with regard to structures.

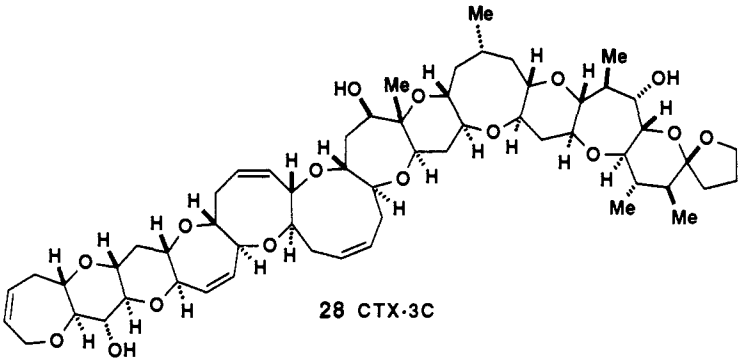
The moray eel, which is placed near the top in the coral ecosystem, tends to contain more polar (more oxygenated) congeners while the dinoflagellate produces less polar ones. Ciguatoxin (**26**) itself, the most oxygenated member of this class of toxins is absent in the dinoflagellates. These data suggest that less polar congeners produced by *G. toxicus* are precursors to the more polar toxins found in fish, the latter formed by oxidative enzyme systems in the fish. Interestingly, toxicity of oxidized metabolites is often increased, as is the case with ciguatoxin which is 11 times more toxic than its plausible precursor **27**.

Chart III



26 Ciguatoxin R_1= -CH(OH)-CH_2OH; R_2=OH

27 CTX-4B R_1= -CH=CH_2; R_2= H

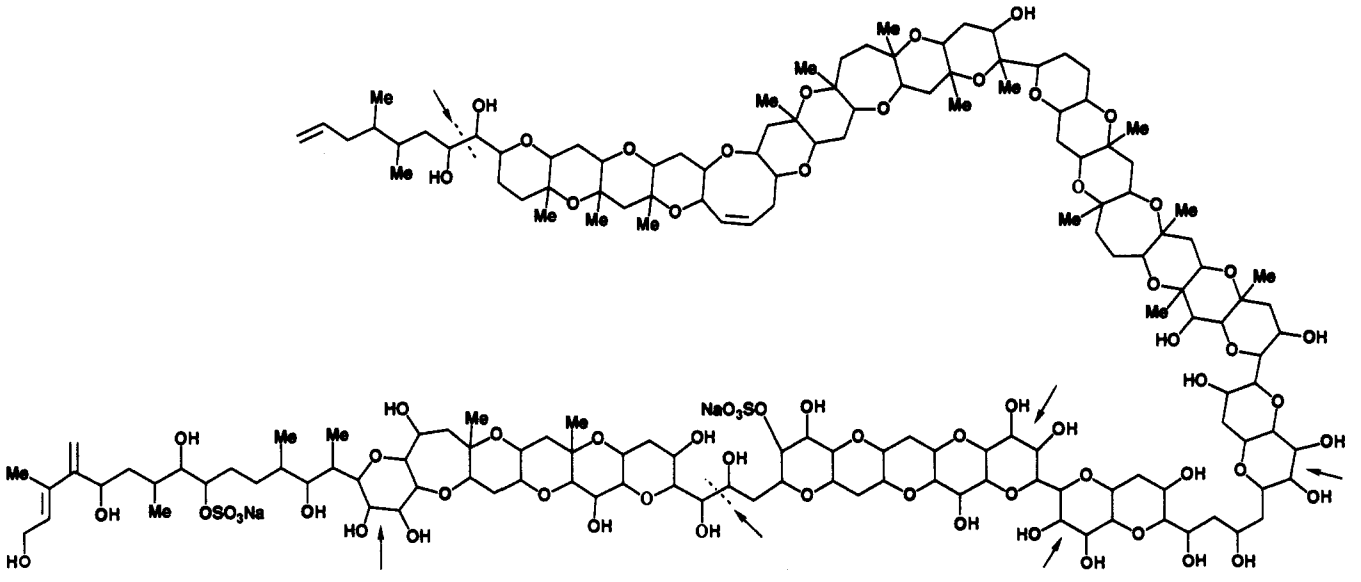


28 CTX-3C

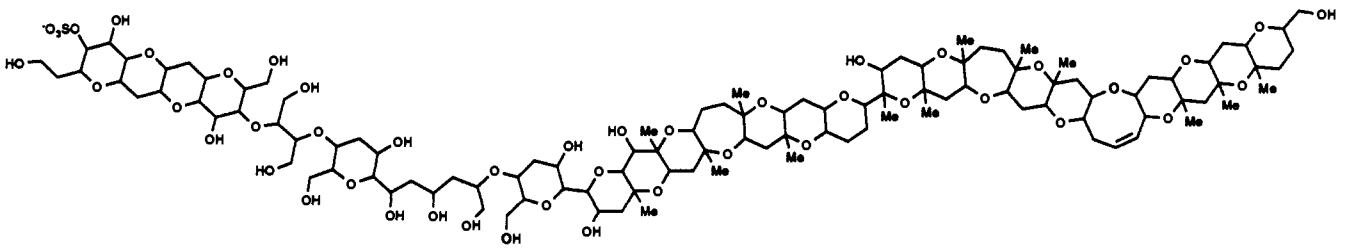
C. Maitotoxin

Maitotoxin has attracted much attention for the following three reasons: First of all, it has a molecular weight of 3422 Da (as the disodium salt), which exceeds that of any other known natural products,[46] except for biopolymers. Secondly, it presumably plays a role in diversifying ciguatera symptoms, particularly in the

Chart IV



29 Maitotoxin (arrows denote cleavage sites by $NaIO_4$)



30 Fragment-B of Maitotoxin

poisoning caused by herbivorous fish. Finally, it has extremely potent bioactivity. The lethality against mice (LD_{50} is ca. 50 ng/kg, ip), for example, suggests that it might be the most potent nonproteineceous toxin.[46] Thus its structural determination has been regarded as one of the most exciting challenges in natural products chemistry.

Very recently, a total structure has been proposed for MTX (**29**) on the basis of extensive spectroscopic analysis.[47] Periodate reaction of **29** led to three degradation products (fragments A, B, and C). Multidimensional NMR experiments were applied either to the whole MTX molecule or to the fragments. Among them, fragment B **30** was the largest with the molecular weight of 2328 Da (as the sodium salt). This exceeded the molecular size of all natural products and biomolecules that had been totally identified for gross structure by spectroscopic methods without further degradation. The NMR spectra of fragment B was complicated by the presence of 160 protons. All feasible NMR spectra with 2 μmol of the material were measured, but a few parts of the structure remained unassigned. Further structural conclusions on **30** were obtained with negative FAB MS/MS experiments.[47] It provided invaluable information for assigning the sizes of the ether rings and the sequence around the acyclic ethers formed as a result of periodate degradation.

Assembly of the three fragments led to elucidation of the entire structure of maitotoxin (**29**, Chart IV).[47] MTX is a polyether having the composition $C_{164}H_{256}O_{68}S_2Na_2$ and constructed from a C142 carbon chain containing 32 ether rings, 28 hydroxyl groups, and two sulfate esters functionalities.

D. Mode of Action of Maitotoxin

More than 50 papers have been published about the pharmacology and biochemistry on maitotoxin (MTX). In earlier studies, the primary action was reported to be increased Ca^{2+} influx, which could be blocked by verapamil, suggesting that MTX acted on a voltage-sensitive Ca^{2+} channel.[48] Lately, diverse actions of MTX have been reported;[49] e.g. muscle contractions, stimulation of hormones/neurotransmitter release, activation of phospholipase C and A2, and activation of protein kinases, some of which do not appear to be directly linked with simple elevation of intracellular Ca^{2+} concentration. Its poor specificity to tissues or cell lines implies that the primary target of MTX is not a physiological receptors but a ubiquitous membrane component.

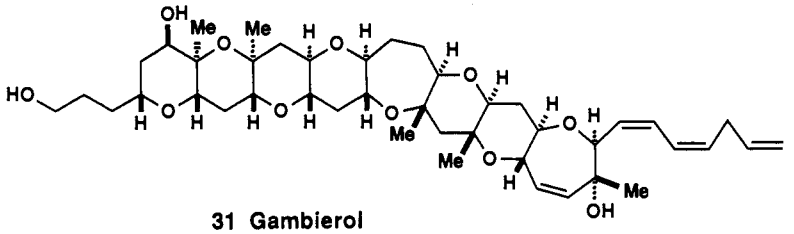
Recent electrophysiological studies have revealed that the channel activated by MTX has ion selectivity and passes more Ca^{2+} than Na^+, the ratio between Ca^{2+} and Na^+ being about 50:1.[50] A proposed inhibitor to the receptor-mediated calcium channel, SK&F 96365, reportedly blocked most of MTX's actions, thereby implying that MTX probably acted through a receptor-mediated channel.[51] The structural elucidation (**29**) should be of help to disclose the mode of action of this unique toxin.

E. Other Ciguatera-Related Bioactive Compounds

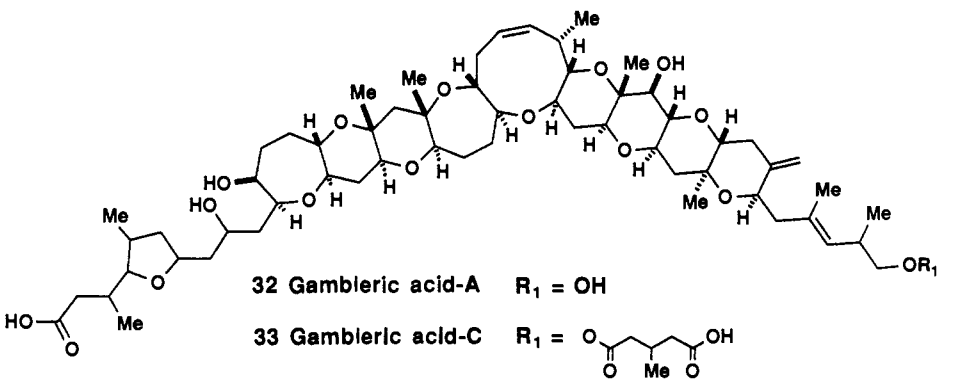
Gambierol (**31**, Chart V) is another example of brevetoxin-type metabolites from dinoflagellates.[52] It was isolated as a toxic constituent from the ciguatera-causative organism *G. toxicus* and showed toxicity against mice. The mice symptoms resemble those shown by ciguatoxins, inferring the possibility that it is also implicated in ciguatera.

From the culture medium of *G. toxicus*, potent antifungal agents, gambieric acids A–D (gambieric acid A, **32** and C **33**, Chart V), were isolated.[53,54] The antifungal potency of **32** exceeds that of amphotericin

Chart V



31 Gambierol



32 Gambieric acid-A R_1 = OH

33 Gambieric acid-C R_1 =

B by a factor of 2000, making it the most potent antifungal agent known.

Palytoxin has been found in the trigger fish *Melichtys vidua* in Micronesia, which was described as a ciguateric species.[55] This suggests that a certain number of poisonings surmised as ciguatera might be attributable to palytoxin.

V. Brevetoxins

A. Massive Fish Mortality by *Gymnodinium breve*

Along the Florida coast, the dinoflagellate *Gymnodinium breve* (*Ptychodiscus brevis*) often forms blooms, leading to mass mortality of fish. Large blooms of this organism (red tides) can kill hundreds of tons of fish a day. The blooms sometimes cause human irritation of eyes and throat in the coastal area,[56] and the contamination of shellfish, occasionally result in human poisoning cases.

The toxic principle was identified to be a highly oxygenated metabolite with, at the time it was discovered in 1981, an unprecedented structure.[57] Brevetoxin B (**35**), thereby, became the first example of a polyether compound produced by a dinoflagellate.

B. Brevetoxin A and B

Brevetoxin B (**35**, Chart VI) is the first member of this unique class of natural products.[57] Its structural feature is a ladderlike skeleton consisting of trans-fused polyether rings.[57] The toxin was isolated from the cultured cells of *Gymnodinium breve* as one of ichthyotoxic constituents. The structure **35** was determined by X-ray crystallography.[57]

Brevetoxin A (**34**) is the most potent ichthyotoxin among the toxins produced by *G. breve*.[58a] Its lethality against zebrafish is reportedly 3 ppb.[58b] The structure of **34** was also elucidated by X-ray.[59] The intriguing structural feature in **34** is its fused middle-membered rings. NMR signals due to nuclei in rings E, F, and G were extremely broad,[60] indicating that the rate constant for conformational changes fell within the range of NMR time scale (1–100 ms). The X-ray data have revealed that two conformers for ring E exist, even in the crystal state.[61]

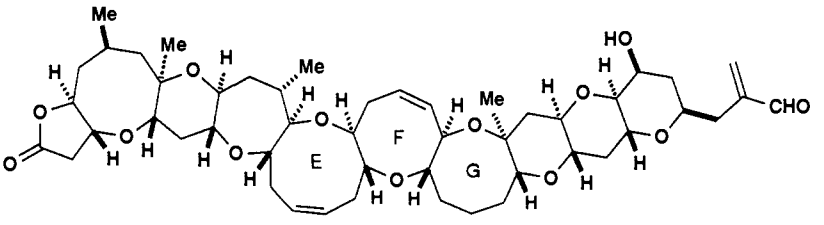
C. Mode of Actions for Brevetoxins and Ciguatoxins

Pharmacological studies on brevetoxins and ciguatoxin have disclosed the primary site of their action to be a voltage-sensitive sodium channel (VSSC).[62,63] Brevetoxin B was first reported to activate a VSSC in neuroblastoma cells in the presence of veratridine. This action was blocked by tetrodotoxin.[62] A binding assay using a radioligand of brevetoxin (PbTx-3, tritiated derivative of brevetoxin B at 42-aldehyde) indicated that both toxins shared the same binding site on the VSSC.[64]

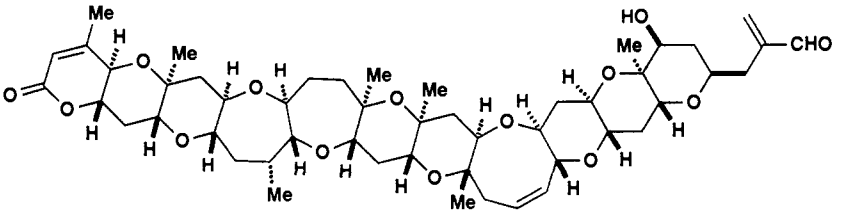
With respect to the pharmacological/toxicological actions of ciguatoxin (CTX), a great number of studies were carried out because of its central role in human illnesses. In earlier studies, the primary action of CTX had been thought to be inhibition of choline esterase,[65] until Rayner revealed that CTX stimulated sodium ion influx into cells.[66]

As shown in Figure 1, six groups of polyether compounds classified as brevetoxin-type have been reported. All of these compounds have been isolated from dinoflagellates or from shellfish which fed on them. An intriguing point is that these polyethers possess seven-, eight-, or nine-membered ring(s) in the middle of the molecule (the hinge part) which possibly undergo slow conformational changes. Ciguatoxins and gambieric acids possess a 9,7-bicyclic system[67] (rings F and G of ciguatoxin) and brevetoxin B bears 7,7-bicycle. During alteration in conformation, the molecule can flip around the hinge part as shown in Figure 2. It is speculated that these slow conformational changes have something to do with the binding to a VSSC and then lead to alteration of the gating mechanism (or the inactivation mechanism) of the channel.[67]

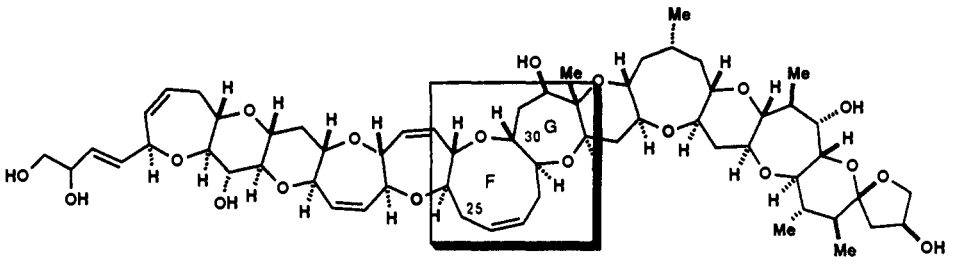
Chart VI



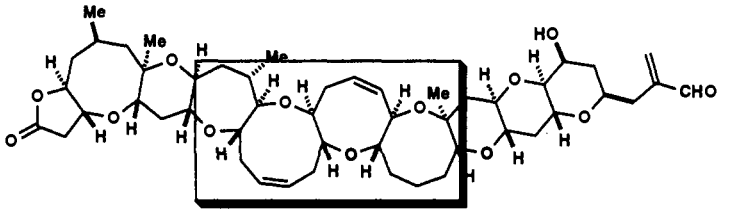
34 Brevetoxin-A



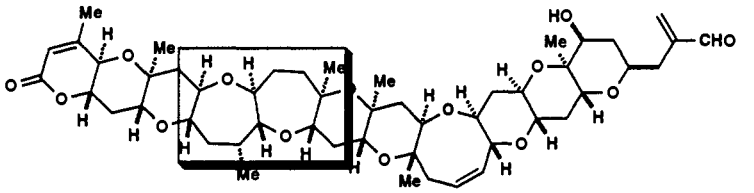
35 Brevetoxin-B



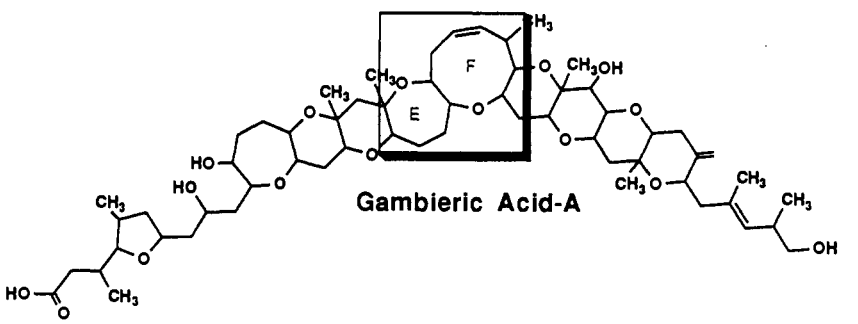
Ciguatoxin



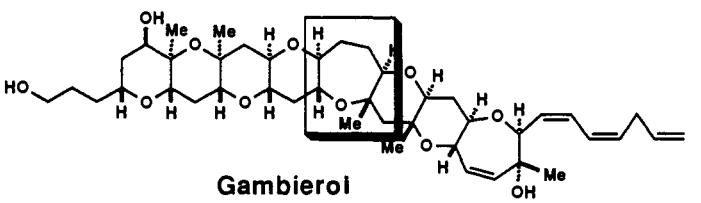
Brevetoxin-A



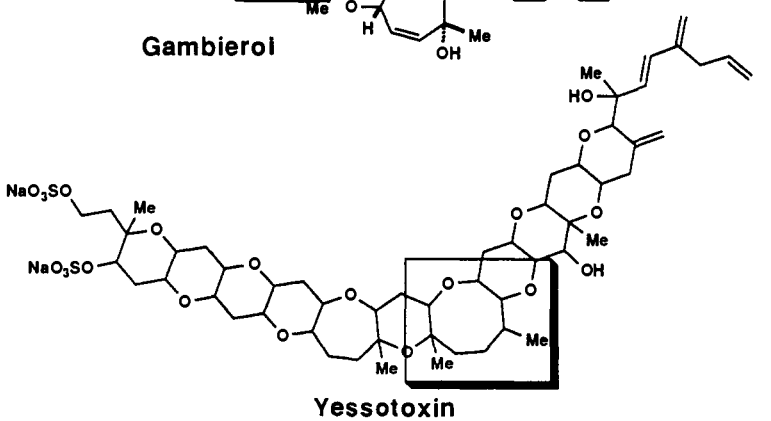
Brevetoxin-B



Gambieric Acid-A



Gambieroi



Yessotoxin

Figure 1. Brevetoxin-type polyether compounds with a flexible part in the middle of the molecule.

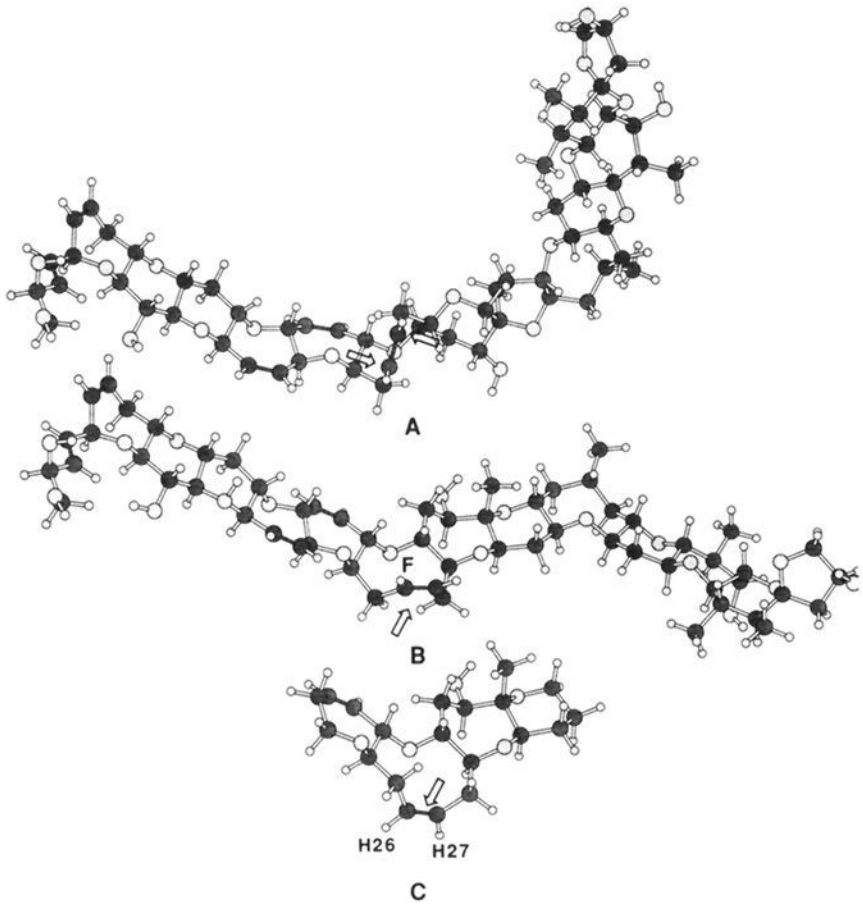


Figure 2. Three conformers of ciguatoxin (**26**). MM2-type calculation (Dreiding) output the grand-state conformers (**B** and **C**), and one of the possible transition-states (**A**) that appeared during the conformational alteration in the middle of the molecule at 9-membered ring F. As is the case with brevetoxin-A,[61] a conformational change at ring F between **B** (H26/H27 pointing up) and **C** (down) gives little changes in the shape of the whole molecular as seen in **C** (Modeling was done by Prof. Minoru Isobe of Nagoya University; unpublished data).

VI. Diarrhetic Shellfish Toxins

A. Diarrhetic Shellfish Poisoning

Diarrhetic shellfish poisoning (DSP) was discovered in 1976[68] when a mussel poisoning case occurred in Northeastern Japan. DSP is associated with eating bivalves such as mussels, scallops, or clams which have accumulated dinoflagellate toxins.[69] The causative organisms have been identified as several dinoflagellates in the genus *Dinophysis*.[70] Appearance of the dinoflagellates, even at a low density (e.g. hundreds of cells per liter) leads to toxification of the shellfish. DSP has wide distribution over the world; particularly in Japan and in Northwestern part of Europe,[71] the poisoning is a serious problem both to public health and to the shellfish industry. The prominent human symptoms are gastrointestinal disorders such as diarrhea, nausea, vomiting, and abdominal pain.[68]

B. Okadaic Acid and Its Derivatives

Okadaic acid (**36**, Chart VII) and its analogs are the toxins responsible for most human DSP-related illnesses. The acid was first isolated from the sponge *Halichondria okadai*[72] and subsequently was found in dinoflagellates *Prorocentrum lima*[73] and *Dinophysis* sp.[69] Recently **36** has been shown to be the causative toxin of DSP in Europe.[71]

Diarrhetic shellfish toxin was first isolated from the digestive glands of mussels and named dinophysistoxin 1 (DXT1)[74] after the genus of the causative dinoflagellate. By spectral comparison with okadaic acid, DTX1 was identified to be 35-(*R*)-methylokadaic acid (**37**).[74,75] A series of congeners substituted with various fatty acids, 7-*O*-acyl-35-(*R*)-methylokadaic acid (DTX3, **38**, Chart VII), were isolated as the toxic principle of poisonous scallops from Northeastern Japan.[72] Recently 31-demethyl-35-methylokadaic acid (DTX2) has been isolated from Irish mussels.[77] The total synthesis of **36** was accomplished by Isobe's group in 1986.[78]

C. Mode of Action of Okadaic Acid and Its Derivatives

Since okadaic acid (**36**) was discovered to act as an inhibitor of protein phosphatases,[79] numerous biochemical/pharmacological studies have been carried out using **36** as a probe. All the biological activities of **36** are now considered to be explainable by its inhibitory action against protein phosphatases. Against the four groups of protein phosphatases (PPs) according to Cohen's classification, okadaic acid inhibits PP2A at the lowest concentration (K_i of 30 pM), PP1 at the next lowest concentration, and PP2B at the highest concentration; it shows no effect on PP2C.

Okadaic acid and DTX1 have been reported to be non-phorbol ester type cancer promoters.[80] In contrast to phorbol esters (e.g. TPA), which activate protein kinase C, okadaic acid inhibits dephosphorylation of proteins, predominantly serine/threonine residues. Both types of tumor promoters eventually cause the accumulation of essentially the same phosphorylated proteins, some of which are involved in tumor promotion.[81]

The structure–activity relationship of **36** has been investigated by several groups.[82] Alteration of the C1 carboxylic acid or 24-OH greatly reduces the activity. Other structural changes (e.g. hydrogenation at C14=C15 or deoxidation at C2) which affect the pseudocyclic conformation formed by interaction between C1 carboxylic acid and C24 hydroxyl group also reduce the potency. Further investigation should reveal the molecular basis of the inhibition mechanism against the phosphatase.

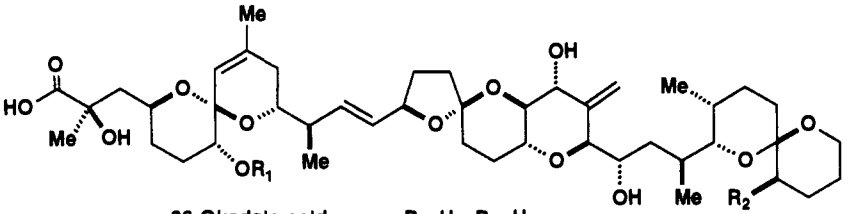
D. Pectenotoxins and Yessotoxin

Pectenotoxin 1 (PTX1, **39**, Chart VII) was isolated as one of diarrhetic shellfish toxins from the digestive glands of the scallop *Patinopecten yessoensis* found in Northeastern Japan.[76] The structure was elucidated to be a novel polyether lactone by X-ray crystallography.[76] The structures of four pectenotoxin homologs have been elucidated so far.[83] Structural alteration among them resides at C43, where all stages of oxidation from methyl to carboxylic acid are found (PTX2, CH_3; PTX1, CH_2OH, **39**; PTX3, CHO; and PTX6, COOH). The toxin was found in the same dinoflagellate *Dinophysis fortii*, as that of dinophysistoxin 1.[70]

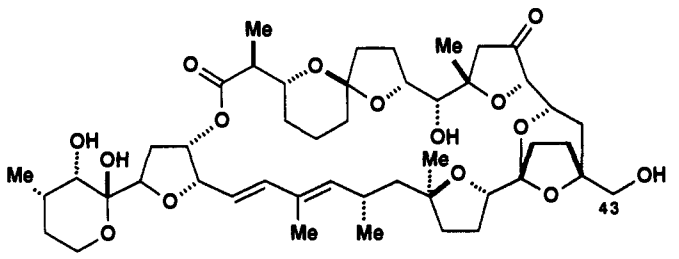
Histopathological investigations have revealed that PTX1 is hepatotoxic and induces rapid necrosis of hepatocytes. The pathological action of PTX1 resembles that of phalloidin.[84]

Yessotoxin (**40**, Chart VII) was isolated from scallops and elucidated to be a brevetoxin-type polyether.[85,86] Its structural features are the presence of two sulfate

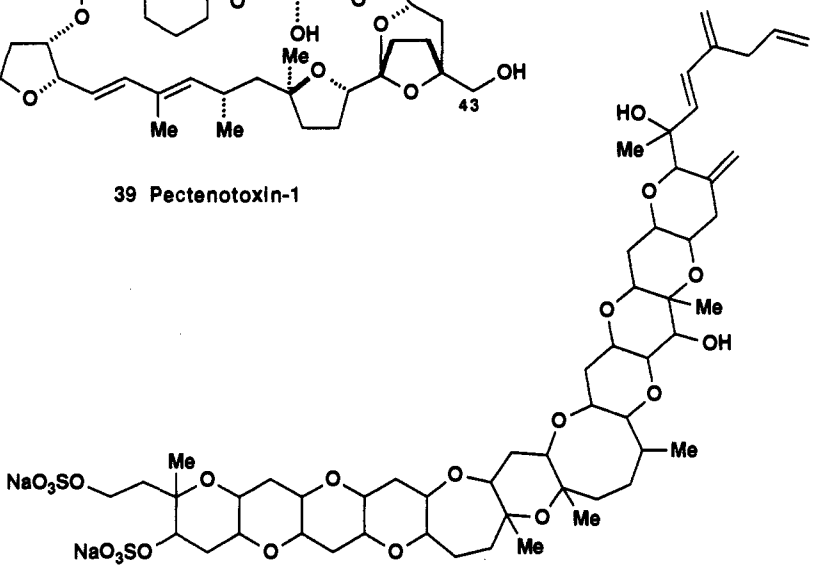
Chart VII



36 Okadaic acid R_1=H R_2=H
37 Dinophysistoxin-1 R_1=H R_2=CH_3
38 Dinophysistoxin-3 R_1=fatty-acid esters R_2=CH_3

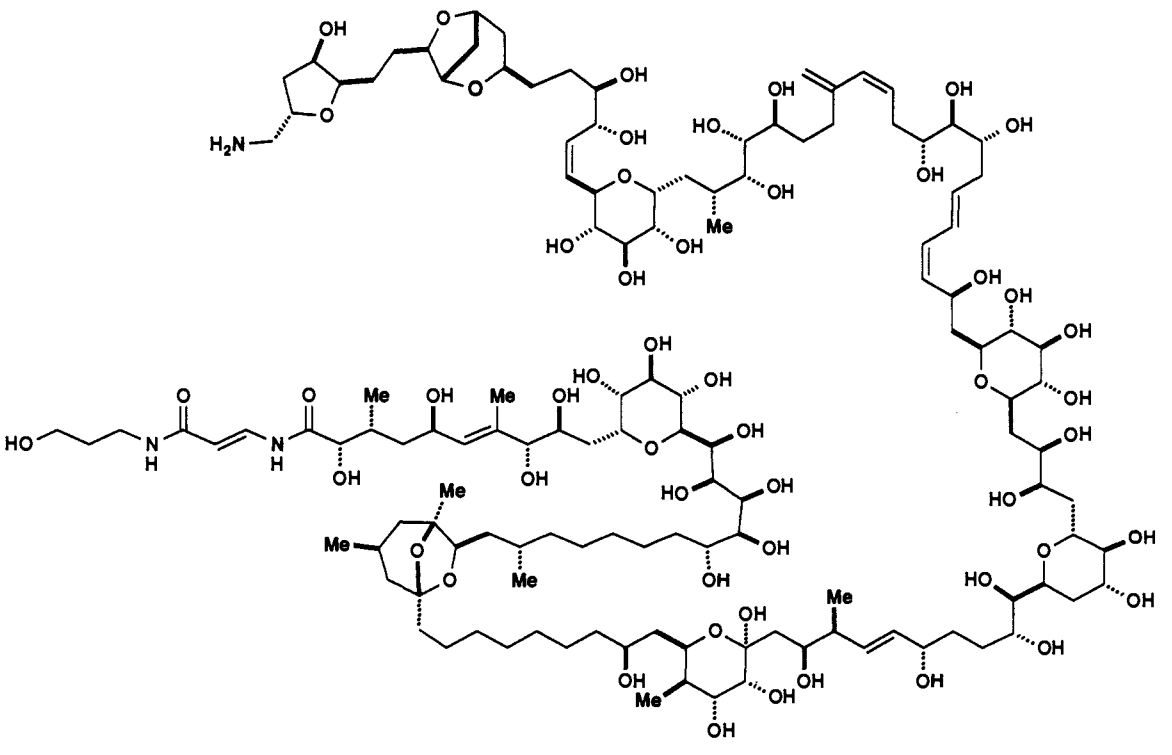


39 Pectenotoxin-1



40 Yessotoxin

Chart VIII



41 Palytoxin

esters and a C9 side chain. The toxin is believed to be produced by a microalga, as are other DSP toxins, although in this case the origin remains unknown.

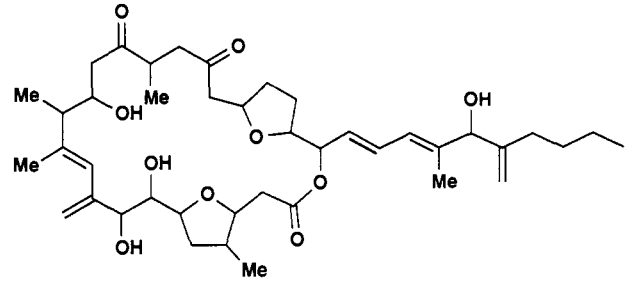
VII. Palytoxin

A. Structure and Distribution

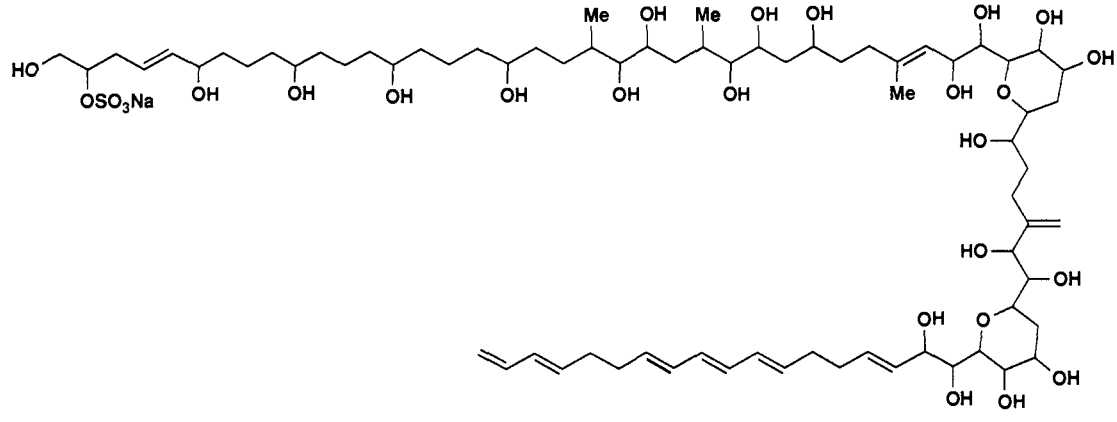
Palytoxin (41, Chart VIII) was first discovered as the toxic principle of the Hawaiian legendary "limu-make-O-Hana (deadly seaweed of Hana)" (actually the soft coral *Palythoa toxica*).[90] Historical achievements done by three chemistry laboratories, Moore's,[87] Uemura/Hirata's,[88] and Kishi's groups,[89] have fully elucidated its complex structure and made 41 one of the best-known natural products. All stereochemistry of 41 was rigorously determined by comparison of synthetic fragments with the natural product. Palytoxin had been the most complex and largest natural product with a molecular weight of 2677 Da ($C_{129}H_{223}O_{54}N_3$), until the structure of maitotoxin was elucidated.

Palytoxin and/or its analogs have not only been found in *Palythoa* soft corals but in wide variety of other

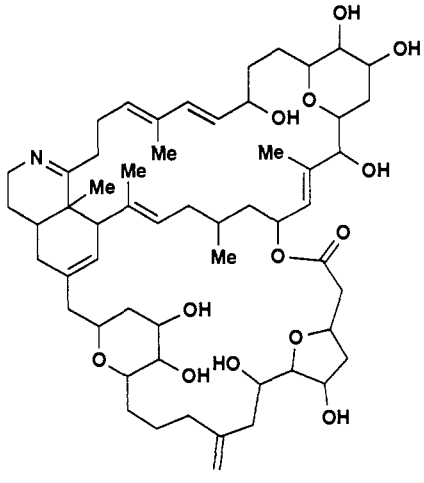
Chart IX



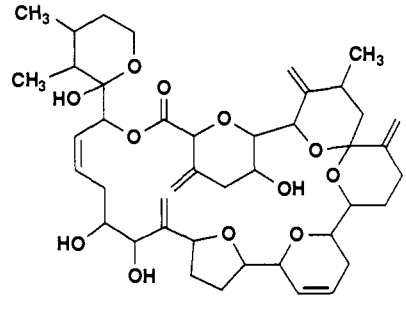
42 Amphidinolide-C



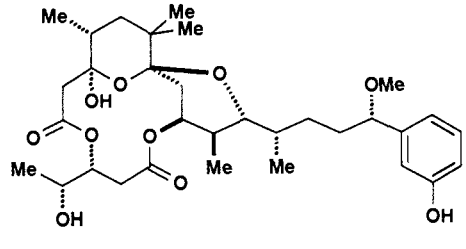
43 Amphidinol



44 Prorocentrolide



45 Goniodomine-A



46 Debromoaplysiatoxin

organisms, a seaweed *Chondria armata*,[91] crabs belonging to the genera *Demania* and *Lophozozymus*,[92] a triggerfish *Melichtys vidua*,[93] and a file-fish *Alutera scripta*.[3]

In addition to its possible implication in ciguatera, 41 is responsible for human mortality associated with eating the toxic crab *Demania reynaudii* in the Philippines.[92]

B. Mode of Action of Palytoxin

Extensive pharmacological/biochemical researches have been carried out on palytoxin[94] such as membrane depolarization, Na^+ or Ca^{2+} influx, stimulation of arachidonic acid release, stimulation of neurotransmitter release, inhibition of Na^+/K^+-ATPase, induction of contraction of smooth muscle, tumor-promoting, and so on. While it is proposed that palytoxin acts through Na^+/K^+-ATPase,[94] detailed mechanism of its action is still largely unknown. The primary mode of action accounting for its variable biological effects is not fully clarified yet.

VIII. Other Miscellaneous Toxins

A. Other Polyether Toxins and Bioactive Polyoxygenated Compounds

In the course of screening microalgae for toxin production, a wide variety of bioactive metabolites have been isolated from dinoflagellates, some of which were possibly implicated in poisonings.

Three groups of macrolides named amphidinolides [the structure of amphidinolide C (**42**) is shown in Chart IX] have been isolated from the dinoflagellate *Amphidinium* sp. that is symbiotic to the flatworm *Amphiscolops breviviridis*.[95] Some of the macrolides exhibit extremely potent cytotoxicity against L1210 around 100 pg/mL.

Amophidinol (**43**, Chart IX), which belongs to a new class of polyhydroxypolyene compounds, has been isolated from the dinoflagellate *Amphidinium klebsii*[97] and shows potent antifungal and hemolytic activity.

Prorocentrolide (**44**, Chart IX) has been isolated from the ciguatera-associated dinoflagellate *Prorocentrum lima*, the producer of okadaic acid.[73] The structure was disclosed to be a new type of nitrogenous polyether lactone.[98]

Goniodomin A (**45**, Chart IX) has been isolated from the tide pool dinoflagellate *Gonyaudoma pseudogonyaulax* as an antifungal substance and elucidated to be a novel polyether lactone.[99]

B. Neosurugatoxin and Prosurugatoxin

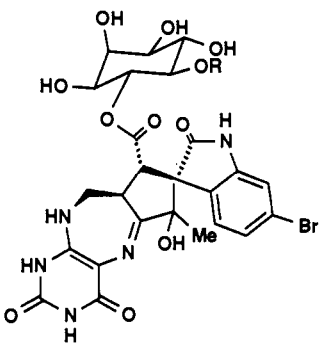
The Japanese ivory shell *Babylonia japonica*, which is widely consumed in Japan, caused intoxication in 26 persons in 1965. Reported symptoms were visual defects, including dimness of vision and abnormal dilation of pupil, thirst, numbness of lips, speech disorders, constipation, and dysuria. The causative toxins, designated neosurugatoxin (**47**) and prosurugatoxin (**48**) (Chart X), were isolated from the digestive glands.[100-102] Their structures were confirmed by synthesis.[103] Both **47** and **48** were over 5000 times more active as ganglion blocking agents than existing drugs such as mecamylamine or hexamethonium. Since **47** and **48** specifically block only nicotinic receptors in the ganglion, both toxins are excellent tools for studying the neurosystem or brain. Surugatoxin, which had been reported by the same research group prior to **47** and **48**, was found to be an artifact produced during purification.[100] Interestingly, the origin of **47** and **48** has been found to be a bacterium belonging to the Coryneform group.[102]

C. Macroalgal Toxins

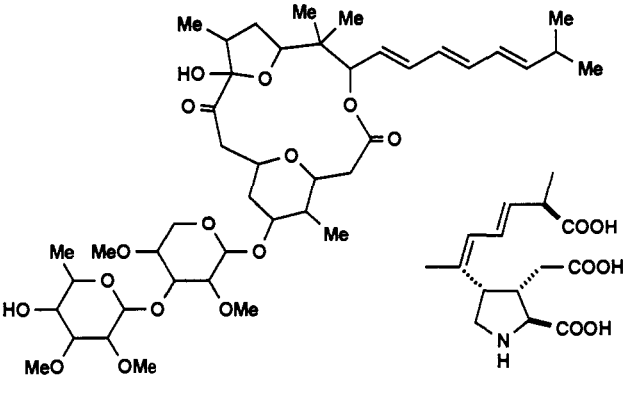
1. Polycavernoside A

In contrast to frequent involvement of microalgae in various forms of seafood poisoning, cases due to ingestion of macroalgae are rare. Human intoxication due to ingestion of the red alga *Polycavernosa tsudai* (formerly *Gracilaria edulis*) occurred in Guam in 1991. Thirteen people became ill, three of whom died. A novel glycosidic macrolide, polycavernoside A (**49**), was obtained from the alga and could be responsible for the poisoning.[104] The content of **49** in the alga was low, but it caused in mice symptoms comparable with those observed in human patients. A macrocycle, trioxatridecane, in the aglycon is reminiscent of trioxadodecane of the aplysiatoxins.[105,106] The methylated fucose of **49** suggests its algal origin, but the sudden and transient occurrence of **49** remains unexplained. Previous outbreaks of fatal poisoning caused by two other *Gracilaria*, *G. chorda* and *G. verrucosa*, also remain unexplained as to the nature of the toxin(s).[107,108]

Chart X

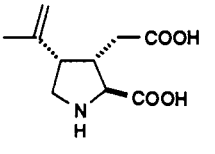


47 Neosurugatoxin R = β-xylose
48 Prosurugatoxin R = H



49 Polycavernoside-A

50 Domoic acid



51 Kainic acid

2. Toxic Substances of Chondria armata

The red alga *C. armata* is a folk medicine used as an anthelmintic. In addition to two palytoxin analogs,[91] domoic acid (**50**, Chart X) and its seven derivatives were isolated from the alga.[109] No incident of poisoning due to this alga is known. However, **50** produced by the diatoms *Nitzchia pungens f. multiseries* and *Pseudonitzschia australis* has caused fatal food poisonings, after accumulating in shellfish.[110,111] This recently described poisoning was termed amnesic shellfish poisoning. A related neurotoxic amino acid, kainic acid (**51**, Chart X), also occurs in red algae,[112,113] but with no intoxication episode.

3. Debromoaplysiatoxin

The toxin **46** has been isolated from the blue-green alga *Lyngbya majuscula*[114] as a potent inflammatory substance and is responsible for incidents of severe dermatitis among swimmers that have come in contact with the alga in Hawaii and Okinawa. Interestingly **46** is reported to have tumor promoting activity which is identical to that shown by phorbol esters (TPA-type).[115]

IX. Conclusion

Incidents of seafood poisoning and massive fish kills are rapidly increasing in both frequency and geographical distribution. Increased public awareness and improved detection methods may explain the increase in reported incidents. However, much blame has to be placed on the deteriorating marine environment and the spreading of resistant cysts of noxious species by ships. Thus, we see more problems appearing in the future which will require solutions from chemists. Difficulties in obtaining sufficient material for analysis and the culturing of responsible microorganisms have been the major obstacles in marine toxin research. Nevertheless, tremendous progress has been made in the structural elucidation of toxins, especially polyether types. Progress has also been made in identifying toxigenic sources, as seen in the case of ciguatoxin and tetrodotoxin. Moreover, the contributions that toxins have made to life science as biochemical or pharmacological tools have been invaluable. Thus, concerted effort by chemists, biochemists, and biologists is expected to open a new era in marine toxin studies.

X. References and Notes

(1) *Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel*; Kao, C. Y., Levinson, S. R., Ed.; The New York Academy of Sciences: New York, 1986.
(2) (a) Bialojan, C.; Takai, A. *Biochem. J.* **1988**, *256*, 283–290. (b) Haystead, T. A. J.; Sim, A. T. R.; Carling, D.; Honnor, R. C.; Tsukitani, Y.; Cohen, P.; Hardie, D. G. *Nature* **1989**, *337*, 78.
(3) Hashimoto, K. *Marine Toxins and Other Bioactive Marine Metabolites*; Japan Scientific Societies Press: Tokyo, 1979.
(4) *Seafood Toxins*, ACS Symposium Series 262; Ragelis, E. P., Ed.; American Chemical Society: Washington, DC, 1984.
(5) *Marine Toxins*, ACS Symposium Series 418; Hall, S., Strichartz, G., Eds.; American Chemical Society: Washington, DC, 1990.
(6) Yasumoto, T.; Yotsu, M.; Endo, A.; Murata, M.; Naoki, H. *Pure Appl. Chem.* **1989**, *61*, 505.
(7) Yasumoto, T.; Yasumura, D.; Yotsu, M.; Michishita, T.; Endo, A.; Kotaki, J. *Agric. Biol. Chem.* **1986**, *50*, 793.
(8) Simidu, U.; Kita-Tsukamoto, K.; Yasumoto, T.; Yotsu, M. *Int. J. System. Bacteriol.* **1990**, *40*, 331.
(9) Noguchi, T.; Jeon, J. K.; Arakawa, O.; Sugita, H.; Deguchi, Y.; Shida, Y.; Hashimoto, K. *J. Biochem.* **1986**, *99*, 311.
(10) Simidu, U.; Noguchi, T.; Hwang, D. F.; Shida, Y.; Hashimoto, K. *Appl. Env. Microbiol.* **1987**, *53*, 714.
(11) Matsui, T.; Taketsugu, S.; Kodama, K.; Ishii, A.; Yamamori, K.; Shimizu, C. *Nippon Suisan Gakkaishi* **1989**, *55*, 2199.
(12) Yasumoto, T.; Michishita, T. *Agric. Biol. Chem.* **1985**, *49*, 893.
(13) Yotsu, M.; Endo, A.; Yasumoto, T. *Agric. Biol. Chem.* **1989**, *53*, 893.
(14) Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H. *J. Am. Chem. Soc.* **1989**, *110*, 2344.
(15) Endo, A.; Khora, S. S.; Murata, M.; Naoki, H.; Yasumoto, T. *Tetrahedron Lett.* **1988**, *29*, 4127.
(16) Khora, S. S.; Yasumoto, T. *Tetrahedron Lett.* **1989**, *30*, 4393.
(17) Yotsu, M.; Yasumoto, T.; Kim, Y.-H.; Naoki, H.; Kao, C. Y. *Tetrahedron Lett.* **1990**, *31*, 3187.
(18) Yotsu, M.; Hayashi, Y.; Khora, S. S.; Sato, S.; Yasumoto, T. *Biosci. Biotech. Biochem.* **1992**, *56*, 370.
(19) Shimizu, Y.; Kobayashi, M. *Chem. Pharm. Bull.* **1983**, *31*, 3625.
(20) Wu, B. Q.; Yang, L.; Kao, C. Y.; Yotsu, M.; Yasumoto, T. *Biophys. J.* **1991**, *59*, 261a (Abstract).
(21) Yang, L.; Kao, C. Y. *J. Gen. Physiol.* **1992**, *100*, 609.
(22) Yang, L.; Kao, C. Y.; Yasumoto, T. *Toxicon* **1992**, *30*, 635.
(23) *Toxic Marine Phytoplankton*; Graneli, E., Sundström, B., Edler, L., Anderson, D. M., Eds.; Elsevier: New York, 1989.
(24) Jachin, E.; Gentile, J. *Scince* **1968**, *162*, 915.
(25) Alam, M.; Shimizu, Y.; Ikawa, M.; Sasner, J. J., Jr. *J. Environ. Sci. Health* **1978**, *A13*, 493.
(26) Ikawa, M.; Wegner, K.; Faxall, T. L.; Sasner, J. J., Jr. *Toxicon* **1982**, *20*, 747.
(27) Kodama, M.; Ogata, T.; Sato, S. *Agric. Biol. Chem.* **1988**, *52*, 1075.
(28) Kotaki, Y.; Tajiri, M.; Oshima, Y.; Yasumoto, T. *Nippon Suisan Gakkaishi* **1983**, *49*, 283.
(29) Sullivan, J. J.; Iwaoka, W. T. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 297.
(30) Oshima, Y.; Sugino, K.; Yasumoto, T. *Mycotoxins and Phycotoxins '88*; Natori, S., Hashimoto, K., Ueno, Y., Eds.; Elsevier Science Publishers: Amsterdam, 1989; p 319.
(31) Shimizu, Y. *Progress in the Chemistry of Organic Natural Products*; Herz, W., Griesbach, H., Kirby, G. W., Eds.; Springer: New York, 1984; p 235.
(32) Harada, T.; Oshima, Y.; Yasumoto, T. *Agric. Biol. Chem.* **1983**, *47*, 191.
(33) Sullivan, J. J.; Iwaoka, W. T.; Liston, J. *J. Biochem. Biophys. Res. Commun.* **1983**, *114*, 465.
(34) Oshima, Y.; Sugino, K.; Itakura, H.; Hirota, M.; Yasumoto, T. *Toxic Phytoplankton*; Graneli, E., Sundström, B., Edler, L., Anderson, E. M., Eds.; Elsevier: New York, 1989; p 391.
(35) Oshima, Y.; Itakura, H.; Lee, K.; Yasumoto, T.; Blackburn, S.; Hallegraeff, G. *Abstracts of Papers*, 5th International Conference on Toxic Marine Phytoplankton, Newport, RI, 1991; p 92.
(36) Shimizu, Y. *Chem. Rev.* In this issue.
(37) Yasumoto, T.; Bagnis, R.; Vernoux, J. P. *Bull. Jpn. Soc. Sci. Fish.* **1976**, *42*, 359–365.
(38) Yasumoto, T.; Nakajima, I.; Ohshima, Y.; Bagnis, R. *Toxic Dinoflagellate Blooms*; Taylor, D. L., Seligner, H., Eds.; Elsevier: North-Holland, 1979; p 65.
(39) Bagnis, R. *Hawaii Med. J.* **1965**, *28*, 25.
(40) (a) Scheuer, P. J.; Takahashi, W.; Tsutsumi, J.; Yoshida, T. *Science* **1976**, *155*, 1267. (b) Tachibana, K. Ph.D. Thesis, University of Hawaii, 1980. (c) Nukina, M.; Koyanagi, L. M.; Scheuer, P. J. *Toxicon* **1984**, *22*, 169.
(41) (a) Murata, M.; Legrand, A.-M.; Ishibashi, Y.; Yasumoto, T. *J. Am. Chem. Soc.* **1989**, *111*, 8927–8931. (b) Murata, M.; Legrand, A. M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1990**, *112*, 4380–4386.
(42) Legrand, A.-M.; Litaudon, M.; Genthon, J. N.; Bagnis, R.; Yasumoto, T. *J. Appl. Phycol.* **1989**, *1*, 183.
(43) (a) Lewis, R. J.; Sellin, M.; Poli, M. A.; Norton, R. S.; MacLeod, J. K.; Sheil, M. M. *Toxicon* **1991**, *29*, 1115. (b) Satake, M.; Murata, M.; Yasumoto, T. *Symposium papers*, 34th Symposium on the Chemistry of Natural Products, Tokyo; Organizing Committe of the symposium: Tokyo, 1992; p 87.
(44) Satake, M.; Murata, M.; Yasumoto, T. *Tetrahedron Lett.* **1993**, *34*, 1975.
(45) Legrand, A.-M.; Cruchet, P.; Bagnis, R.; Murata, M.; Ishibashi, Y.; Yasumoto, T. *Toxic Marine Phytoplankton*; Graneli, E., Sundström, B., Edler, L., Anderson, D. M., Eds.; Elsevier: New York, 1989; p 374.
(46) Yokoyama, A.; Murata, M.; Oshima, Y.; Iwashita, T.; Yasumoto, T. *J. Biochem.* **1988**, *104*, 184–187.
(47) (a) Murata, M.; Naoki, H.; Iwashita, T.; Matsunaga, S.; Sasaki, M.; Yokoyama, A.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 2060. (b) Murata, M.; Iwashita, T.; Yokoyama, A.; Sasaki, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1992**, *114*, 6594.
(48) Takahashi, M.; Ohizumi, Y.; Yasumoto, T. *J. Biol. Chem.* **1982**, *257*, 7287.
(49) For a review, see: Gusovsky, F.; Daly, J. W. *Biochem. Pharmacol.* **1990**, *39*, 1633.

(50) Kobayashi, M.; Ochi, R.; Ohizumi, Y. *Br. J. Pharmacol.* **1987**, *92*, 665.
(51) Soergel, D. G.; Yasumoto, T.; Daly, J. W.; Gusovsky, F. *Mol. Pharmacol.* **1992**, *41*, 487.
(52) Satake, M.; Murata, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 361.
(53) Nagai, H.; Torigoe, K.; Satake, M.; Murata, M.; Yasumoto, T.; Hirota, H. *J. Am. Chem. Soc.* **1992**, *114*, 1102.
(54) Nagai, H.; Murata, M.; Torigoe, K.; Satake, M.; Yasumoto, T. *J. Org. Chem.* **1992**, *57*, 5448.
(55) Halstead, B. W. *Poisonous and Venomous Marine Animals of the World*; U.S. Government Printing Office: Washington, DC, 1967; Vol. 2, p 124.
(56) Davis, C. C. *Bot. Gaz.* **1947**, *109*, 358–360.
(57) Lin, Y.-Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775.
(58) (a) Shimizu, Y.; Gupta, S.; Norte, A.; Hori, A.; Genenah, A.; Kobayashi, M. *Proc. Int. Conf. on Toxic Dinoflagellates* **1985**, *3*, 271. (b) Nakanishi, K. *Toxicon* **1985**, *23*, 473–479.
(59) Shimizu, Y.; Chou, H.-N.; Bando, H.; Duyne, G. V.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 514–515.
(60) Pawlak, J.; Tempesta, M. S.; Golik, J.; Zagorski, M. G.; Lee, M. S.; Nakanishi, K.; Iwashita, T.; Gross, M. L.; Tomer, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 1144.
(61) Van Duyne, G. D. *Marine Toxins*, ACS Symposium Series 418; Hall, S., Strichartz, G., Ed.; American Chemical Society: Washington, DC, 1990; pp 144–165.
(62) Catterall, W. A.; Risk, M. *Mol. Pharmacol.* **1981**, *19*, 345.
(63) Bidard, J. N.; Vijverberg, H. P. M.; Frelin, C.; Chunge, E.; Legrand, A. M.; Bagnis, R.; Lazdunski, M. *J. Biol. Chem.* **1986**, *479*, 204–220.
(64) Lombet, A.; Bidard, J. N.; Lazdunski, M. *FEBS Lett.* **1987**, *219*, 355–359.
(65) Li, K. M. *Science* **1965**, *147*, 1580.
(66) Rayner, M. D.; Kosaki, T. I.; Fellmeth, E. L. *Science* **1968**, *160*, 70.
(67) (a) Murata, M.; Legrand, A.-M.; Scheuer, P. J.; Yasumoto, T. *Tetrahedron Lett.* **1992**, *33*, 525–526. (b) Baden, D. G.; Rein, K. S.; Kinoshita, M.; Gawley, R. E. *Ciguatera*; Tosteson, T. R., Ed.; Polyscience Publication: Quebec, 1992; pp 103–114.
(68) Yasumoto, T.; Oshima, Y.; Yamaguchi, M. *Nippon Suisan Gakkaishi* **1978**, *44*, 1249.
(69) Yasumoto, T.; Oshima, Y.; Sugawara, W.; Fukuyo, Y.; Oguri, H.; Igarashi, T.; Fujita, N. *Nippon Suisan Gakkaishi* **1980**, *46*, 1405–1411.
(70) Lee, J.-S.; Igarashi, T.; Fraga, S.; Dahl, E.; Hovgaard, P.; Yasumoto, T. *J. Appl. Phycol.* **1989**, *1*, 147.
(71) (a) Kumagai, M.; Yanagi, T.; Murata, M.; Yasumoto, T.; Kat, M.; Lassus, R.; Rodoriguez-Vazquez, J. A. *Agric. Biol. Chem.* **1986**, *50*, 2853–2857. (b) Kat, M. *Toxic Dinoflagellate Blooms*; Taylor, D. L., Seliger, H. H., Eds.; Elsevior: North-Holland, 1979; p 215.
(72) Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, H.; Engen, D. V.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469.
(73) Murakami, Y.; Oshima, Y.; Yasumoto, T. *Nippon Suisan Gakkaishi* **1982**, *48*, 69.
(74) Murata, M.; Shimatani, M.; Sugitani, H.; Oshima, Y.; Yasumoto, T. *Nippon Suisan Gakkaishi* **1982**, *48*, 549–552.
(75) In the previous report,[74] C35 was incorrectly assigned to *S* while the configuration of C35 was consistent with the present structure.
(76) Yasumoto, T.; Murata, M.; Oshima, Y.; Matsumoto, G. K.; Clardy, J. *Tetrahedron* **1985**, *41*, 1019–1025.
(77) Hu, T.; Doyle, J.; Jackson, D.; Marr, J.; Nixon, E.; Pleasance, S.; Quilliam, M. A.; Walter, J. A.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1992**, 39.
(78) Isobe, M.; Ichikawa, Y.; Goto, T. *Tetrahedron Lett.* **1986**, *27*, 963.
(79) Takai, A.; Bialojan, C.; Troschka, M.; Ruegg, J. C. *FEBS Lett.* **1988**, *217*, 81.
(80) Suganuma, M.; Fujiki, H.; Suguri, H.; Yoshizawa, S.; Hirota, M.; Nakayasu, M.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Sugimura, T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1768.
(81) Sassa, T.; Richter, W. W.; Uda, N.; Suganuma, M.; Suguri, H.; Yoshizawa, S.; Hirota, M.; Fujiki, H. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 939.
(82) For example, see: Nishiwaki, S.; Fujiki, H.; Suganuma, M.; Furuya-Suguri, J.; Matsushima, R.; Iida, Y.; Ojika, M.; Yamada, K.; Uemura, D.; Yasumoto, T.; Schmitz, F. J.; Sugimura, T. *Carcinogenesis* **1990**, *11*, 1837.
(83) Murata, M.; Sano, M.; Iwashita, T.; Naoki, H.; Yasumoto, T. *Agric. Biol. Chem.* **1986**, *50*, 2693.
(84) Terao, K.; Ito, E.; Oarada, M.; Murata, M.; Yasumoto, T. *Toxicon* **1990**, *28*, 1095.
(85) Murata, M.; Kumagai, M.; Lee, J. S.; Yasumoto, T. *Tetrahedron Lett.* **1987**, *28*, 5869.
(86) Naoki, H.; Murata, M.; Yasumoto, T. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 179.
(87) Moore, R. E.; Bartolini, G. *J. Am. Chem. Soc.* **1981**, *103*, 2491.
(88) Uemura, D.; Ueda, K.; Hirata, Y. *Tetrahedron Lett.* **1981**, *22*, 2781.
(89) Armstrong, R. W.; Beau, J.-M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W.-H.; Hawkins, L. D.; Jin, H.; Kang, S. H.; Kishi, Y.; Martinelli, M. J.; McWhorter, W. W.; Mizuno, M.; Nakata, M.; Stutz, A. E.; Talamas, F. X.; Taniguchi, M.; Tino, J. A.; Ueda, K.; Uenishi, J.; White, J. B.; Yonaga, M. *J. Am. Chem. Soc.* **1989**, *111*, 7530.
(90) Moore, R. E.; Scheuer, P. J. *Science* **1971**, *172*, 495.
(91) Maeda, M.; Kodama, R.; Tanaka, T.; Yoshizumi, H.; Nomoto, K.; Takemoto, T.; Fujita, M. *Symposium Papers*, 27th Symposium on the Chemistry of Natural Products, Hiroshima; Organizing Committee of the symposium: Hiroshima, 1985; p 616.
(92) (a) Yasumoto, T.; Yasumura, D.; Ohizumi, Y.; Takahashi, M.; Alcala, A. C.; Alcala, L. C. *Agric. Biol. Chem.* **1986**, *50*, 163. (b) Alcala, A. C.; Alcala, L. C.; Garth, J. S.; Yasumura, D.; Yasumoto, T. *Toxicon* **1988**, *26*, 105–107.
(93) Fukui, M.; Murata, M.; Inoue, A.; Gawel, M.; Yasumoto, T. *Toxicon* **1987**, *25*, 1121–1124.
(94) For a review, see: Haberman, E. *Toxicon* **1989**, *27*, 1171.
(95) Kobayashi, J.; Ishibashi, M.; Wälchili, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Am. Chem. Soc.* **1988**, *110*, 490.
(96) Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861.
(97) Nakajima, I.; Oshima, Y.; Yasumoto, T. *Nippon Suisan Gakkaishi* **1981**, *47*, 1029–1033.
(98) Torigoe, K.; Murata, M.; Yasumoto, T.; Iwashita, T. *J. Am. Chem. Soc.* **1988**, *110*, 7876–7877.
(99) Murakami, M.; Makabe, K.; Yamaguchi, K.; Konosu, S.; Wälchli, M. R. *Tetrahedron Lett.* **1988**, *29*, 1149.
(100) Kosuge, T.; Tsuji, K.; Hirai, K. *Chem. Pharm. Bull.* **1982**, *30*, 3255.
(101) Kosuge, T.; Tsuji, K.; Fukuyama, T.; Nukaya, H.; Ishida, H. *Chem. Pharm. Bull.* **1985**, *33*, 2890.
(102) Kosuge, T.; Tsuji, K.; Hirai, K.; Fukuyama, T. *Chem. Pharm. Bull.* **1985**, *33*, 3059.
(103) Inoue, S.; Okada, K.; Tanino, H.; Kakoi, H. *Tetrahedron Lett.* **1986**, *27*, 5225.
(104) Yotsu-Yamashita, M.; Haddock, R. L.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 1147.
(105) Kato, Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 2245.
(106) Myndrese, J. S.; Moore, R. E.; Kashiwagi, M.; Norton, T. R. *Science* **1977**, *196*, 538.
(107) Aikawa, S.; Suzuki, M.; Ono, K. *Rep Yamagata Prefect. Inst. Public Health* **1981**, *13*, 81.
(108) Sonoda, T. *U. Food Hyg. Jpn.* **1983**, *24*, 507.
(109) Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K.; Fujita, T. *Tetrahedron Lett.* **1987**, *28*, 633.
(110) Bates, S. S.; Bird, C. J.; deFreitas, A. S. W.; Foxall, R.; Quilliam, M. A.; Sim, P. G.; Smith, J. C.; Subba Rao, D. V.; Todd, E. C. D.; Walter, J. A.; Wright, J. L. C. *Can. J. Fish. Aquatic Sci.* **1989**, *46*, 1203.
(111) Fritz, L.; Quilliam, M. A.; Wright, J. L. C. *J. Phycol.* **1992**, *28*, 439.
(112) Murakami, S.; Takemoto, T.; Shimizu, Z.; Daigo, K. *Jpn. J. Pharm. Chem.* **1953**, *25*, 571.
(113) Laycock, M. V.; deFreitas, A. S. W.; Wright, J. L. C. *J. Appl. Phycol.* **1989**, *1*, 113.
(114) Moore, R. E. *Marine Natural Products*; Scheuer, P. J., Ed.; Academic Press: New York, 1981; Vol. IV, p 1.
(115) Fujiki, H.; Suganuma, M.; Nakayasu, M.; Hoshino, H.; Morre, R. E.; Sugimura, T. *Gann* **1982**, *73*, 495.