Review

Chemistry, etiology, and food chain dynamics of marine toxins

By Takeshi YASUMOTO*),**)

Emeritus Professor, Tohoku University
(Communicated by Saburo TAMURA, M. J. A.)

Abstract: Highly complex chemical structures of marine natural toxins involved in seafood poisoning were determined using small samples. The biogenetic process by which the toxins accumulate in fish and shellfish was explained by the food chain. The primary sources of the toxins were identified as dinoflagellates, bacteria, and blue-green algae. The topics related to ciguatera, puffer fish, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, azaspiracid poisoning, red tides, and *Gracilaria* red alga poisoning are described.

Key words: Ciguatera; dinoflagellate; puffer fish toxin; shellfish toxin; red tide toxin; blue-green algae.

Introduction. Fish and shellfish, though delicious and considered good for one's health, occasionally accumulate natural toxins and thereby cause food poisoning. However, relevant studies have been hampered by the very sporadic and unpredictable occurrence of such poisoning. My research group (we, hereafter) has focused on elucidating toxin structures and discovering biogenetic origins of the toxins. The toxin molecules are large and available in very limited quantities, making them a most challenging target for study. The goals were accomplished by extensive use of NMR spectroscopy, collision induced dissociation fast-atom bombardment tandem mass spectrometry (CID FAB MS/MS), anisotropic NMR reagents, and fluorescent chiral HPLC reagents combined with chemical degradation and partial synthesis. The etiological study was a similarly challenging task to perform, because the pursuing food chain involved perspectives knowledge in both marine ecology and analytical chemistry. To confirm the toxigenicity in a candidate organism, mostly unicellular alga referred to as dinoflagellate, we had to culture the organism but some species were difficult or unable to culture. Adding to the problem, not all clones produced toxins in enough quantities. In the case of unculturable species, we prepared a homogeneous sample for analysis by picking up more than ten thousands of target cells with a capillary under microscope. With all the efforts made, we could identify the toxin sources for the seafood poisoning known so far. This paper gives details of studies on "ciguatera fish poisoning", because these studies encompass all of the above elements characteristic to marine toxin studies.

Historical aspects of ciguatera. In warm temperate, subtropical, and tropical regions many species of fish may cause upon ingestion neurological and gastrointestinal syndromes generally referred to as "ciguatera", a Caribbean word originally used for poisoning due to ingestion of small marine snails called "cigua". Although fatal cases are rare, the mortality rate is high, estimated annual number of patients being 20,000~60,000 worldwide. Ciguatera involves a broad phylogenetic spectrum of fish of widely ranging biology: more than 400 species listed in the literature. Since fish toxicity markedly fluctuates individually, regionally, seasonally, and annually, the exogenous origin of the toxin is easily conceived. In the late 1960, a research group in Hawaii proposed that the causative toxin was accumulated in fish by the food chain. This group also isolated from moray eels a lipophilic toxin named ciguatoxin (CTX).[1] However, the toxin structure remained

*) Collaboration of Regional Entities for the Advancement of Technological Excellence in Okinawa, Japan Science and Technology Agency, 12-75 Suzaki, Gushikawa, Okinawa 904-2234.
**) Recipient of the Imperial Prize and the Japan Academy Prize in 2004.

unknown.

Discovery of the primary source of ciguatoxin. To pursue the food chain down to the origin, we chose a small surgeonfish, *Ctenochaetus striatus*, as a probable first link for the following three reasons. First, the fish accounted for 65% of ciguatera outbreaks in Tahiti. Second, having small and loosely attached teeth, the fish feed on food of limited composition, microalgae and other microorganisms. Third, the fish was frequently found in the stomach of carnivores, implying that the fish was actually one of the links with large fish. The first breakthrough in toxin etiology was made in 1977, when we observed that the abundance of a disk-shaped dinoflagellate in the digestive contents of *C. striatus* showed a good correlation with the toxicity of the viscera of the fish.[2] The disk-shaped dinoflagellate was initially assigned as *Diplopsalis* sp. but in a later study a new genus and a new species was created to name it *Gambierdiscus toxicus*.[3] *G. toxicus* grew densely on the surface of a calcareous alga, *Jania* sp. in the Gambier islands, where construction on surrounding reefs had caused massive death of corals and subsequent proliferation of *Jania* sp. on dead corals. The collected cells of *G. toxicus* contained two types of toxins markedly different in polarity. The less polar toxin resembled CTX, while the polar toxins was found to correspond maitotoxin (MTX), which was previously detected in the gut contents of *C. striatus* and named after the Tahitian name of the fish. A clone culture of *G. toxicus* was successfully prepared. The culture produced MTX but no CTX, despite our effort to induce CTX production by applying various culture conditions. Thus argument was raised whether CTX detected in the wild sample of *G. toxicus* was a genuine product of this dinoflagellate or a product of other contaminant organisms. Six years later, we made another expedition to the Gambier Islands for collection. Of 12 clone cultures newly prepared, only one clone produced CTX congeners in a sufficient quantity for chemical characterization, indicating that toxin profiles varied widely among clones. From the new clone couture we could isolate precursor toxins of CTX, finally putting an end to the years of arguments about the origin of ciguatera toxins. The epiphytic nature of *G. toxicus* explained why ciguatera poisoning often flared up following destructions of coral reefs. When coral dies, calcareous algae proliferate on dead corals and thereby provide *G. toxicus* with the substrata to grow.

Structures of ciguatoxins. The first breakthrough in chemical studies was made in a bilateral collaboration for over ten years between a research group in Tahiti and us. We collected 4 tons of moray eels from ciguatera-endemic areas in French Polynesia, extracted 124 kg of the viscera, and obtained 0.35 mg of pure CTX (**1**). From the sample of wild *G. toxicus* collected in the Gambier Islands, was obtained 0.75 mg of a CTX analog coded CTX4B (**2**). Working extensively on these small samples by NMR spectroscopy, we successfully determined the structures of the two toxins as shown in Fig. 1.[4] The cultured *G. toxicus* produced, in addition to CTX4B, two new poly-cyclic-ether toxins, CTX3C (**3**) and gambierol (**4**), and a potent antifungal compound, gambieric acid (**5**) respectively (Fig. 1).[5)-8)] Additionally, four CTX congeners and three congeners of gambieric acid were determined by NMR analysis (structures not shown to save space).

Although we could elucidate planar structures and their relative stereochemistry with small samples, the absolute configurations in the toxin molecules could not be determined by the conventional NMR methods. In addition, the quantities available for stereochemical studies were even smaller, because the pure toxins had to be used for other studies as well: e.g. developing analytical methods and a toxicological study to evaluate the health risks. The goals were successfully accomplished by combining new chiral fluorescent HPLC reagents or new anisotropic NMR reagents with chemical degradation and synthesis of partial structures. A typical example is seen in our experiment to determine the absolute configuration at C2 in CTX (**1**). As shown in Scheme 1, hydroxyl groups of **1** (5 µg) were protected as (benzyloxy)methyl (BOM) ether. The resultant ether was cleaved at the C3/C4-double bond with $OsO_4/NaIO_4$ to produce an aldehyde, which was immediately reduced with $NaBH_4$ to yield a glycerol derivative. The alcohol was esterified with a fluorescent chiral reagent, [(*S*)-TBMB-carboxylic acid], and the resultant ester was compared by HPLC with synthetically prepared (2*S*)- and (2*R*)-TBMB esters. The retention times of the derivative CTX (**1**) on two different chromatographic columns agreed well with those of the reference TBMB ester from 2*S*-standard. The C2 configuration in CTX (**1**) was thus determined to be *S* using only 5 µg of the toxin.[9]

Chromatographic analysis of extracts prepared from three clones of *G. toxicus* and three species of fish revealed presence of many CTX congeners, but they were too small to be studied by NMR experiments. The spectra produced by conventional FAB MS/MS methods were dominated by ions resulting from sequential loss of water, providing little structural information. However,

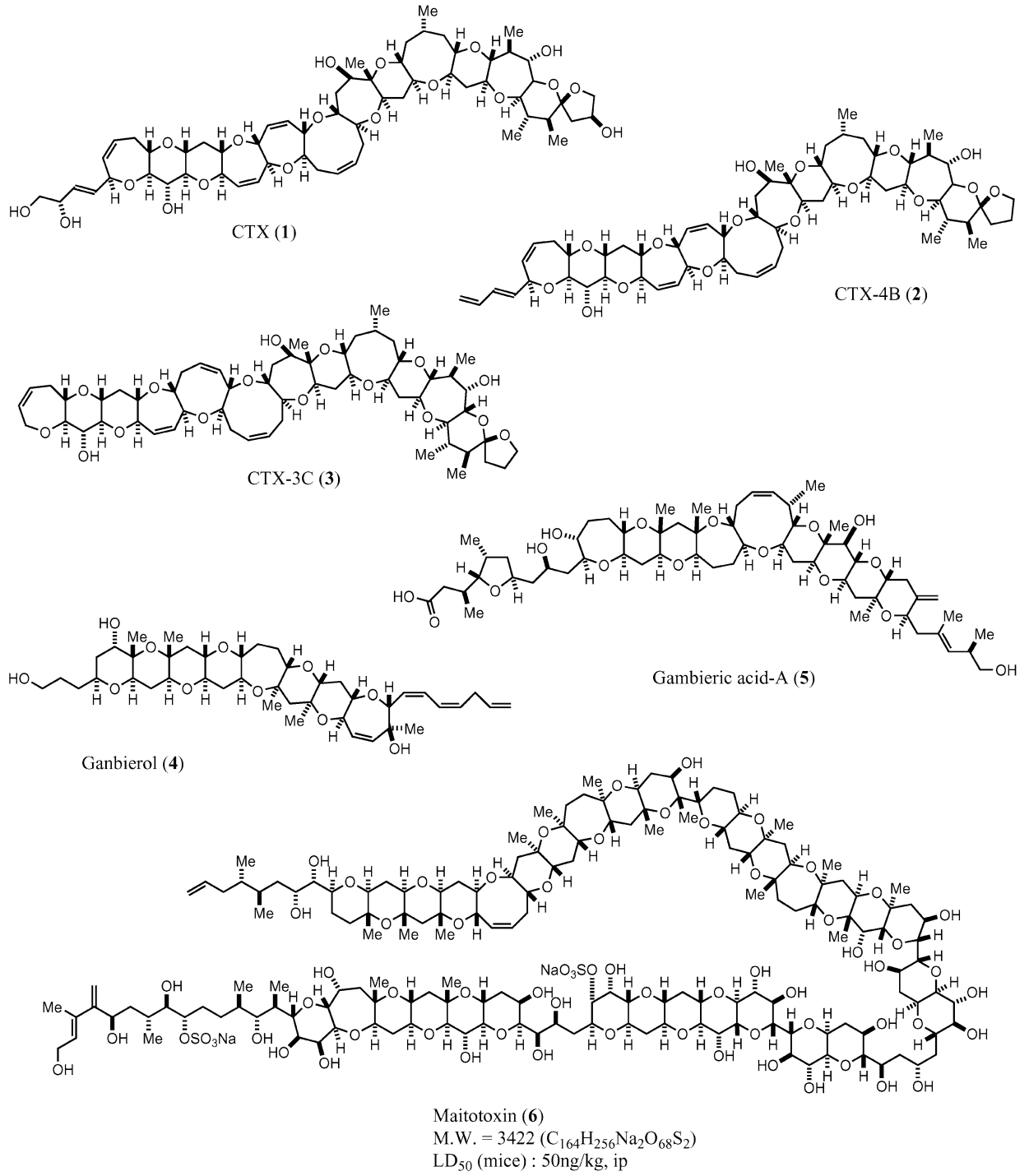
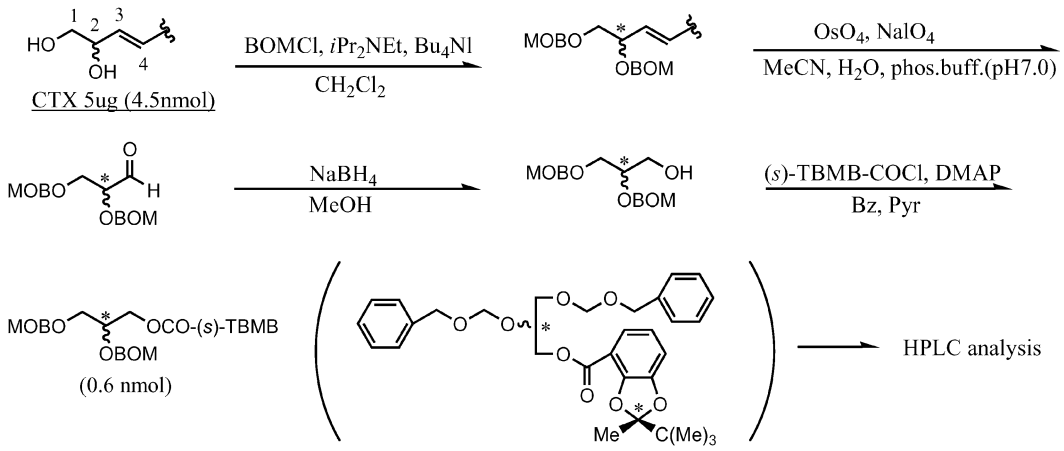


Fig. 1. Structures of representative toxins produced by *Gambierdiscus toxicus*.

we found that addition of sodium chloride to matrix markedly enhanced the intensity of sodiated ions and that collision induced fragment ions of the sodiated ions mimicked the fragmentation patterns typical for charge-remote CID FAB MS/MS. Obviously, the sodium ion was localized on two proximal oxygen atoms residing



Scheme 1

at a terminus of the molecules and thereby acted as a charge site to produce the charge-remote fragmentation pattern. The spectra thus obtained enabled straightforward interpretation of the ring alignment and the type and position of substitutions. In some instances where the above experiments did not clarify the structures, we introduced a strong negative charge into the molecules in question by preparing 2-sulfo-benzoates and conducted negative FAB MS/MS experiments. The resultant negative ion spectra provided more detailed information, than the positive ion spectra did, and enabled us to clarify the type and position of a functional group. Structures of as many as 23 CTX congeners could be thus elucidated using less than 10 µg of samples.[10] The 23 congeners are not shown for the limited space, but it was evident from their structures that CTXs produced by *G. toxicus* underwent metabolic changes in fish. The structural modifications took place at the terminal parts of the molecules. The type of the modification was mostly oxidation but included in a few examples opening of an ether ring and epimerization of spiro rings. Interestingly, oxidation of CTX4B to CTX in fish increased the mouse lethality by ten times. The lethality of CTX (0.35~0.40 µg/kg) determined in mice by intraperitoneal (i.p.) injection implies that CTX is 25 times more toxic than tetrodotoxin (10 µg/kg) of puffer fish. The minimum dose to cause illness in human by oral intake was estimated to be 70 ng. The structural variety and the extremely low levels of CTXs in fish lay a serious obstacle in developing appropriate detection methods for the toxins.

Structure of maitotoxin (MTX). Having the molecular weight of 3422, MTX (**6**) is the largest natural product hitherto elucidated, except for biopolymers. The structural elucidation by NMR was facilitated by the use of ^{13}C-enriched MTX prepared by adding $NaH^{13}CO_3$ to the culture medium of *G. toxicus*. The presence of two sulfate groups in the molecule facilitated charge-remote fragmentation experiments by FAB MS/MS and effectively provided data to support the structure deduced by NMR.[11] Confirmation of the stereochemistry was achieved by a new NMR methodology and partial synthesis.[12] Notably, MTX is the most toxic compound except for a few proteinous bacterial toxins. Its lethality (50 ng/kg, mice, i.p.) is 200 times more potent than tetrodotoxin. MTX caused Ca^{2+} ion influx into cells in all cell lines tested and thus was suggested to serve as an excellent tool to probe ubiquitous but hitherto uncharacterized channels. Though potent, MTX may play little role in ciguatera poisoning because of its low concentration in fish and a poor absorption rate from digestive tracts.

Etiology of tetrodotoxin. Tetrodotoxin (TTX) in puffer fish is famous for its unique structure and specific action to block the voltage-dependent sodium channel. The toxin is also known for its frequent implication in fatal food poisoning. Despite the generally accepted notion that the toxin was a genuine product of puffer fish, we came to infer its exogenous origin when we detected in our survey on ciguatera low levels of the toxin in the viscera of an angelfish and two parrotfish, all unrelated to puffer fish.[13] In support for the hypothesis, we confirmed that TTX concentrations in puffer fish fluctuated markedly both individually and regionally, analogous with CTX in ciguatera fish. Such fluctuations in tox-

icity should have contributed to the ignorance in some people of the potential danger of eating livers. Otherwise, nobody would dare eat the notorious organ. As a candidate for the first link in the hypothetic food chain of TTX, we chose three crabs, *Zosimus aeneus*, *Atergatis floridus*, and *A. integerrimus*. These small crabs inhabiting coral reefs accumulated TTX at high concentrations and their stomachs were full of a calcareous alga, *Jania* sp. The calcareous alga was collected in Okinawa Island and shown to contain TTX. Subsequent survey revealed TTX contents in the alga to fluctuate widely depending on the site and season of collection, suggesting attaching organisms being responsible for the TTX production. Subsequently two species of bacteria were isolated from the algal surface and one species was confirmed to produce TTX in culture.[14] The bacterium was first classified to *Pseudomonas* sp. but, because it was a new species, renamed *Shewanella alga*.[15] From the bacterium culture, TTX (200 µg) and 4,9-anhydroTTX (55 µg) were isolated and unambiguously identified by mouse toxicity, liquid chromatography, mass spectrometry, and chemical degradation to 2-amino-6-hydroxymethyl-8-hydroxyquinazoline.[14] Later, we detected TTX in two other species, *Vibrio pelagius* biovar II and *Alteromonas tetraodonis*.[16] These results demonstrated for the first time that bacteria produce TTX and the toxin is transmitted to marine animals through the food chain. In addition to the algae and crabs, we proved occasional occurrence of TTX in the annelid *Pseudopotamia occelata* and the ivory shell *Babylonia japonica*, and deduced that they also were a part of the food chain links.[14] To facilitate the survey on TTX, we constructed a fluorescence-detection HPLC analyzer,[17] which enabled us to detect, isolate, and determine structures of seven new analogs of TTX from puffer fish, newts, and a frog (structure not shown).[18)-20)]

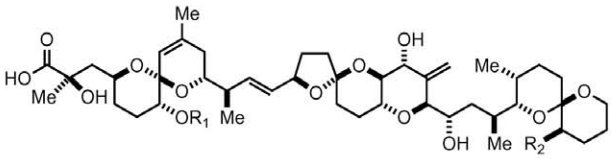
Diarrhetic shellfish poisoning (DSP). In 1976 and 1977 a number of people suffered from diarrhea after eating blue mussels *Mytylus edulis* produced in the northeastern region of Japan.[21] We named this previously unreported enteritis "diarrhetic shellfish poisoning". Two causative toxins isolated were new derivatives of okadaic acid (OA, **7**), a cyclic-ether containing carboxylic acid first isolated from sponges.[22] The new toxins were named dinophysistoxin-1 (DTX1, **8**) and dinopnysistoxin-3 (DTX3, **9**) after the generic name of the dinoflagellate that produced the toxins.[23),24)] Okadaic acid (**7**) itself was isolated from blue mussels implicated in poisoning outbreaks in Europe.[25] Additionally, a new macrolide toxin, pectenotoxin (**10**), and a new ladder-shape cyclic polyether toxin, yessotoxin (**11**), were isolated from the Japanese scallop, *Patinopecten yessoensis*.[24),26),27)] We identified 8 species of dinoflagellates belonging to *Dinophysis* as the sources of OA (**7**) and DTX1 (**8**), and *D. fortii* of pectenotoxin (**10**).[28),29)] Another dinoflagellate *Protoceratium reticulatum* produced **11**.[30] In shellfish, these toxins underwent metabolic change in structures to produce DTX3 (**9**) and several other congeners (not shown). The major toxin structures are shown in Fig. 2, together with photos of *D. fortii*, and *P. reticulatum*.

Azaspiracid poisoning. In 1995 and thereafter incidents of new shellfish poisoning took place in Europe. We isolated the causative toxin and four analogs and named the main toxin azaspiracid, based on a unique azaspiro structure in the molecule.[31)-33)] The proposed structure was recently amended to **12** (Fig. 1) by total synthesis conducted by the K. C. Nicolaou's group.[34] The origin of **12** was identified as *Protoperidinium crassipes* (Fig. 2) that we first reported as *Protoperidinium* sp. because of ambiguity in taxonomy.[35]

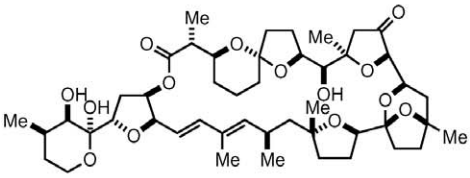
Neurotoxic shellfish poisoning. When the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*) blooms in the sea, shellfish in nearby areas cause upon ingestion peculiar syndromes named neurotoxic shellfish poisoning. The dinoflagellate was known to produce ladder-shape neurotoxins named brevetoxin A and brevetoxin B,[36),37)] but the structures of the toxins accumulated in shellfish were unknown. We isolated for the first time four new toxins from shellfish associated with poisoning outbreaks in New Zealand and demonstrated that they were not the original toxins produced by *K. brevis* but were the metabolites of brevetoxin B. The four toxins were code-named BTXB1 through BTXB4.[37)-41)] The structures for BTX1 (**13**), BTXB2 (**14**), and BTX4 (**15**) are shown in Fig. 2 together with the causative dinoflagellate, *K. brevis*. The structures of BTXB3 indicated that an ether ring in BTXB was cleaved to expose a hydroxyl group which in turn was esterified with a varying kinds of fatty acid (structure not shown).

Red tide toxins. Worldwide, red tides of various unicellular phytoplankton pose serious threats to the aquatic ecosystem and aquaculture by killing wide range of fauna and flora. Involvement of toxins in the devastation was easily perceived but the chemical entities of the toxins were unknown, except for those of *K. brevis*. We investigated *Prymnesium parvum*, one of the most notorious red tide species to occur in brackish

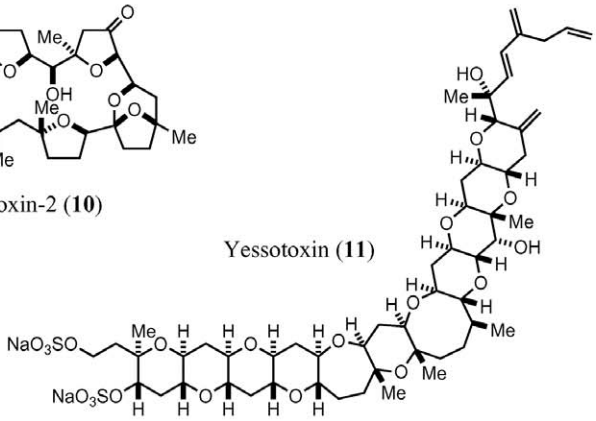
Dinophysis fortii



	R1	R2
(**7**) OA	H	H
(**8**) DTX-1	H	Me
(**9**) DTX-3	acyl	Me



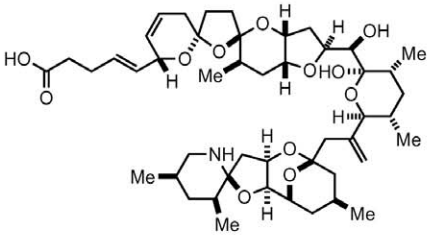
Pectenotoxin-2 (**10**)



Yessotoxin (**11**)

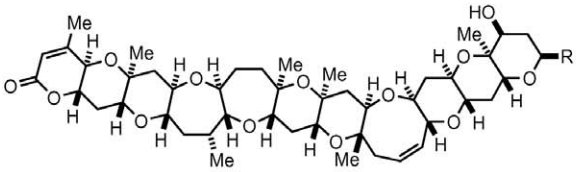
Protoceratium reticulatum

Azaspiracid (**12**)



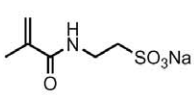
Protoperidinium crassipes

Brevetoxin

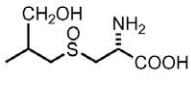


Karenia brevis

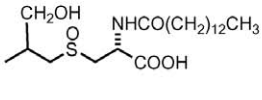
R=



BTXB1 (**13**)



BTXB2 (**14**)



BTXB4 (**15**)

Fig. 2. Toxins involved in shellfish poisoning and the causative dinoflagellates.

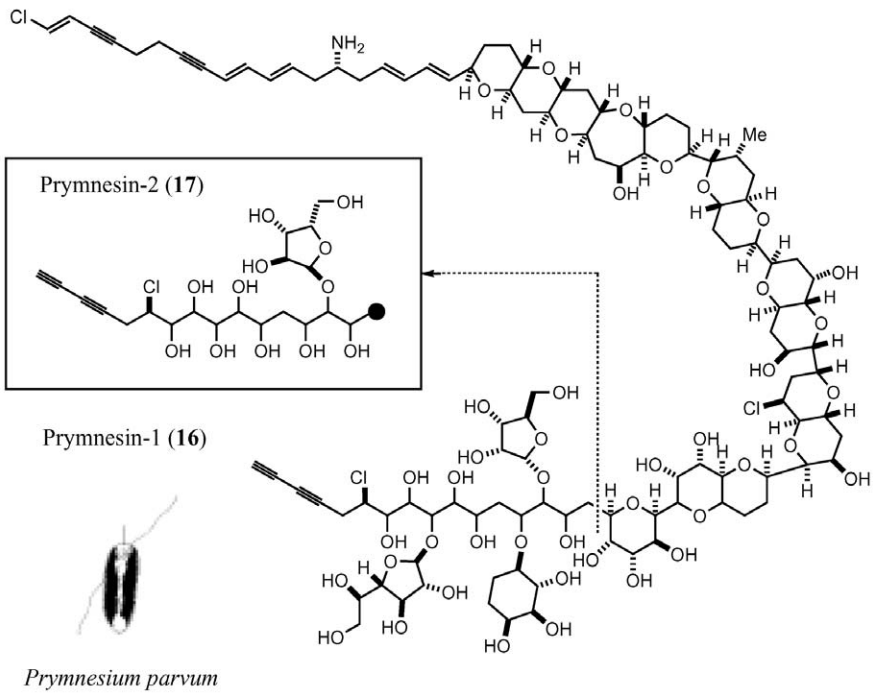


Fig. 3. Structures of red tide toxins, prymnesin-1 and prymnesin-2.

water, and elucidated the complex structures of two major toxins, prymnesin-1 (**16**) and prymnesin-2 (**17**) (Fig. 3).[42),43)] From *K. mikimotoi*, a similarly notorious marine species to occur in Japan, Asia, and Europe, we isolated and determined the structure of a new ladder-shape polyether toxin, gymnocin-A (not shown). Gymnocin-A resembles brevetoxin-B in structure but is larger than the latter.[44)] Another closely related red tide species, *Karenia* sp., collected in New Zealand, a cyclic-imine-macrolide named gymnodimine was determined (not shown).[45)]

Toxins of marine blue-green algae. Red algae of genus *Gracilaria* and some related species are eaten in many parts of the world but occasionally cause severe, or often fatal, food poisoning. From *Polycavernosa tudai* (syn. *Gracilaria edulis*) respectively involved in fatal incidents in Guam and the Philippines, we isolated new macrolide glycosides.[46)-48)] The structure for the major toxin named polycavernoside-A (**18**) is shown in Fig. 4. The macrolide skeleton in polycavernoside-A (**18**) resembles that in aplysiatoxin, a product of the blue-green alga, *Lyngbya majuscula*.[49)] Furthermore, toxicity could be detected in a filamentous blue-green alga attaching *P. tsudai*. It was highly probable, therefore, that **18** had the origin in the blue-green alga rather than *P. tsudai*. In a study on another red alga implicated in poisoning in Hawaii, aplysiatoxin (**19**) was isolated.[50)] From meat of the marine turtle that caused fatal poisoning in Madagascar, we detected lyngbyatoxin-A (**20**),[51)] another toxic product of *L. majuscula*.[52)] Because this blue-green alga often grows attached to turtle grass, the favorite food of turtles, it was inferred that lyngbyatoxin-A found in the turtle meat was derived from the blue-green alga.

Discussion. We determined chemical structures of many toxins implicated in seafood poisoning. Only representative structures are shown in this paper but further information is available in our two review papers.[53),54)] The characteristic features of the toxins are summarized as follows: 1) Many toxins possess multiple ether rings, often aligned in a ladder-shape. 2) Toxins are large, maitotoxin (**6**) being the largest (Mw 3422). 3) The toxicity is extremely potent, maitotoxin (**6**) being the most toxic. Its LD_{50} (50 ng/kg, mice by intraperitoneal injection) indicates that it is 200 times more toxic than tetrodotoxin and is only exceeded by a few proteinous toxins of bacteria. Also prominent feature of CTX (**1**) is that oral intake of as small as 70 ng can cause ill-

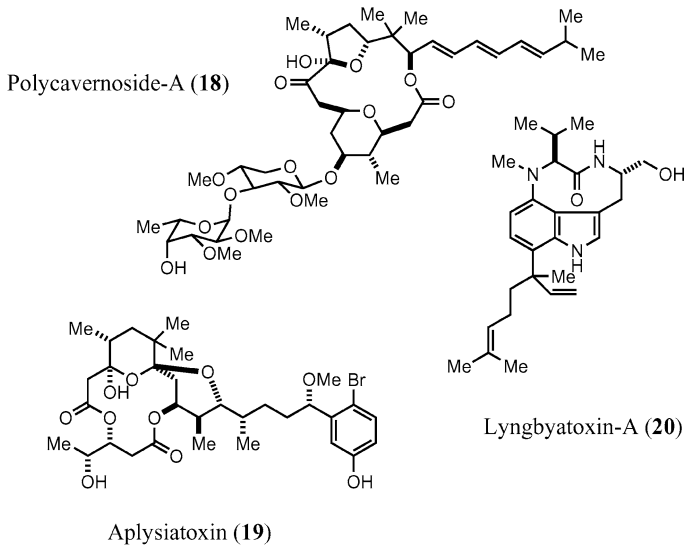


Fig. 4. Toxins of blue-green algae involved in seafood poisoning.

ness in an adult. It should be noted that in natural product chemistry isolation of target substances comprises a significant part of the time and labor involved. My strategy to combine NMR with FAB MS/MS for elucidating planar structures and relative stereochemistry and to combine chiral reagents with synthesis of partial structures proved to be highly efficient. The stereochemical information obtained in our studies should assist chemists who are endeavoring to determine the total synthesis of marine toxins.

From the etiological point of view, the importance of the food chain was clearly demonstrated. The majority of the toxins are produced by dinoflagellates, with a few exceptions of bacteria and blue-green algae. Phylogenetically, dinoflagellates hold a unique position between prokaryotes and eukaryotes and are often referred to as mesokaryotes, but their biological and ecological importance was obscure. Our disclosure of toxicity in many dinoflagellate species, especially in benthic species, sheds new light on the significance of these intriguing organisms in marine ecosystems.

Acknowledgements. The author thanks his former colleagues in Tohoku University and those in oversea institutions for their excellent contribution. The FAB MS/MS studies were conducted in collaboration with Dr. H. Naoki of the Suntory Institute for Bioorganic Research. Drs. A. M. Legrand and R. Bagnis were most helpful in the ciguatera studies.

References

1) Scheuer, P. J., Takahashi, W., Tsutsumi, J., and Yoshida, T. (1967) Science **155**, 1267-1268.
2) Yasumoto, T., Nakajima, I., Bagnis, R., and Adachi, R. (1977) Bull. Japan. Soc. Sci. Fish. **43**, 1021-1026.
3) Adachi, R., and Fukuyo, Y. (1978) Bull. Japan. Soc. Sci. Fish. **45**, 67-71.
4) Murata, M., Legrand, A. M., Ishibashi, Y., and Yasumoto, T. (1989) J. Am. Chem. Soc. **111**, 8929-8931.
5) Nagai, H., Torigoe, K., Satake, M., Murata, M., Yasumoto, T., and Hirota, H. (1992) J. Am. Chem. Soc. **114**, 1102-1103.
6) Satake, M., Murata, M., and Yasumoto, T. (1993) Tetrahedron Lett. **34**, 1975-1978.
7) Satake, M., Murata, M., and Yasumoto, T. (1993) J. Am. Chem. Soc. **115**, 361-362.
8) Satake, M., Ishibashi, Y., Legrand, A. M., and Yasumoto, T. (1997) Biosci. Biotech. Biochem. **60**, 2103-2105.
9) Satake, M., Morohashi, M., Oguri, H., Oishi, T., Hirama, M., Harada, N., and Yasumoto, T. (1997) J. Am. Chem. Soc. **119**, 11325-11326.
10) Yasumoto, T., Igarashi, T., Legrand, A. M., Cruchet, P., Chinain, M., Fujita, T., and Naoki, H. (2000) J. Am. Chem. Soc. **122**, 4988-4989.
11) Murata, M., Naoki, H., Iwashita, T., Matsunaga, S., Sasaki, M., Yokoyama, A., and Yasumoto, T. (1993) J. Am. Chem. Soc. **115**, 2060-2062.
12) Nonomura, T., Sasaki, M., Matsumori, N., Murata, M., Tachibana, K., and Yasumoto, T. (1996) Angew. Chem. Int. Ed. Engl. **35**, 1675-1678.
13) Yasumoto, T., Nagai, H., Yasumura, D., Michishita, T., Endo, A., Yotsu, M., and Kotaki, Y. (1986) Ann. N. Y. Acad. Sci. **479**,

44-51.
14) Yasumoto, T., Yasumura, D., Yotsu, M., Michishita, T., Endo, A., and Kotaki, Y. (1986) Agri. Biol. Chem. **50**, 793-795.
15) Shimizu, U., Kita-Tsukamoto, K., Yasumoto, T., and Yotsu, M. (1990) Internat. J. System. Bacteriol. **40**, 331-336.
16) Yotsu, M., Yamazaki, T., Meguro, Y., Endo, A., Murata, M., Naoki, H., and Yasumoto, T. (1987) Toxicon **25**, 225-228.
17) Yotsu, M., Endo, A., and Yasumoto, T. (1989) Agric. Biol. Chem. **53**, 893-895.
18) Endo, A., Khora, S. S., Murata, M., Naoki, H., and Yasumoto, T. (1988) Tetrahedron Lett. **29**, 4127-4128.
19) Yasumoto, T., Yotsu, M., Murata, M., Naoki, H. (1988) J. Am. Chem. Soc. **110**, 2344-2345.
20) Yotsu, M., Yasumoto, T., Kim, Y. H., Naoki, H., and Kao, C. Y. (1990) Tetrahedron Lett. **31**, 3187-3190.
21) Yasumoto, T., Oshima, Y., and Yamaguchi, M. (1978) Bull. Japan. Soc. Sci. Fish. **44**, 1249-1255.
22) Tachibana, K., Scheuer, P. J., Tsukitani, Y., Kikuchi, H., Engen, D. V., Clardy, J., Gopichand, Y., and Schmitz, F. J. (1981) J. Am. Chem. Soc. **103**, 2469-2471.
23) Murata, M., Shimatani, M., Sugitani, H., Oshima, Y., and Yasumoto, T. (1982) Bull. Japan. Soc. Sci. Fish. **48**, 525-530.
24) Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G. K., and Clardy, J. (1985) Tetrahedron **41**, 1019-1025.
25) Kumagai, M., Yanagi, T., Murata, M., Yasumoto, T., Kat, M., Lassus, P., and Rodriguez-Vazquez, J. A. (1986) Agric. Biol. Chem. **50**, 2853-2857.
26) Sasaki, K., Wright, J. L. C., and Yasumoto, T. (1998) J. Org. Chem. **63**, 2475-2480.
27) Murata, M., Kumagai, M., Lee, J. S., and Yasumoto, T. (1987) Tetrahedron Lett. **28**, 5869-5872.
28) Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., and Fujita, N. (1980) Bull. Japan. Soc. Sci. Fish. **46**, 327-331.
29) Lee, J. S., Igarashi, T., Fraga, S., Dahl, E., Hovgaad, P., and Yasumoto, T. (1989) J. Appl. Phycol. **1**, 147-152.
30) Satake, M., MacKenzie, L., and Yasumoto, T. (1997) Nat. Toxins **5**, 164-167.
31) Satake, M., Ofuji, K., Naoki, H., James, K. J., Furey, A., McMahon, T., Silke, J., and Yasumoto, T. (1998) J. Am. Chem. Soc. **120**, 9967-9968.
32) Ofuji, K., Satake, M., McMahon, T., Silke, J., James, K. J., Naoki, H., Oshima, Y., and Yasumoto, T. (1999) Nat. Toxins **7**, 99-102.
33) Ofuji, K., Satake, M., McMahon, T., James, K. J., Naoki, H., Oshima, Y., and Yasumoto, T. (2001) Biosci. Biotechnol. Biochem. **65**, 740-742.
34) Nicolaou, K. C., Koftis, T. V., Vyskocil, S., Petrovic, G., Ling, T., Yamada, Y. M. A., Tang, W., and Frederick, M. O. (2004) Angew. Chem. Int. Ed. **43**, 4318-4324.
35) Yasumoto, T. (2001) Chem. Record **1**, 228-242.
36) Shimizu, Y., Chou, H. N., Bando, H., Duyne, G. V., and Clardy, J. (1986) J. Am. Chem. Soc. **108**, 514-515.
37) Lin, Y. Y., Risk, M., Ray, S. M., Van Engen, D., Clardy, J., Golik, J., James, J. C., and Nakanishi, K. (1981) J. Am. Chem. Soc. **103**, 6773-6775.
38) Ishida, H., Nozawa, A., Totoribe, K., Muramatsu, N., Nukaya, H., Tsuji, K., Yamaguchi, K., Yasumoto, T., Kaspar, H., Berkett, N., and Kosuge, T. (1995) Tetrahedron Lett. **36**, 725-728.
39) Morohashi, A., Satake, M., Murata, K., Naoki, H., Kaspar, H. F., and Yasumoto, T. (1995) Tetrahedron Lett. **36**, 8995-8998.
40) Murata, K., Satake, M., Naoki, H., Kaspar, H. F., and Yasumoto, T. (1998) Tetrahedron **54**, 735-742.
41) Morohashi, A., Satake, M., Naoki, H., Kaspar, H. F., Oshima, Y., and Yasumoto, T. (1999) Nat. Toxins **7**, 45-48.
42) Igarashi, T., Satake, M., and Yasumoto, T. (1996) J. Am. Chem. Soc. **118**, 479-480.
43) Igarashi, T., Satake, M., and Yasumoto, T. (1999) J. Am. Chem. Soc. **121**, 8499-8511.
44) Satake, M., Shoji, M., Oshima, Y., Naoki, H., Fujita, T., and Yasumoto, T. (2002) Tetrahedron Lett. **43**, 5829-5832.
45) Seki, T., Satake, M., Mackenzie, L., Kaspar, H. F., and Yasumoto, T. (1995) Tetrahedron Lett. **36**, 7093-7096.
46) Yotsu-Yamashita, M., Haddock, R. L., and Yasumoto, T. (1993) J. Am. Chem. Soc. **115**, 1147-1148.
47) Yotsu-Yamashita, M., Seki, T., Paul, V. J., Naoki, H., and Yasumoto, T. (1995) Tetrahedron Lett. **36**, 5563-5566.
48) Yotsu-Yamashita, M., Yasumoto, T., Yamada, S., Bajarias, F. F. A., Formeloza, M. A., Romero, M. L., and Fukuyo, Y. (2004) Chem. Res. Toxicol. **17**, 1265-1271.
49) Kato, Y., and Scheuer, P. J. (1974) J. Am. Chem. Soc. **96**, 2245-2246.
50) Nagai, H., Yasumoto, T., and Hokama, Y. (1996) Toxicon **34**, 753-761.
51) Yasumoto, T. (1998) Toxicon **36**, 1515-1518.
52) Cardellina, II, J. H., Marner, F.-J., and Moore, R. E. (1979) Science **204**, 193-195.
53) Yasumoto, T., and Murata, M. (1993) Chem. Rev. **93**, 1897-1909.
54) Murata, M., and Yasumoto, T. (2000) Nat. Prod. Rep. **17**, 293-314.

(Received Jan. 13, 2005; accepted Feb. 14, 2005)