Marine Toxins and Nonmarine Toxins: Convergence or Symbiotic Organisms?†

John W. Daly*

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, Maryland 20892-0820

Received January 28, 2004

Bioactive marine natural products occur only rarely in nonmarine sources. The converse also is true. Divergent evolutionary pathways for the biosynthesis of bioactive secondary metabolites seem to be the rule. Marine biosynthetic pathways lead to a wide variety of different structural classes, among which polyethers, macrolides, terpenes, unusual amino acids/peptides, and alkaloids are notable. Nonmarine biosynthetic pathways also lead to a similar wide variety of structural classes. However, the structures are usually quite different from the marine analogues. The alkaloids of plants are notable, but again there appears little convergence between the marine and nonmarine alkaloids. However, tetrodotoxin, a remarkable, highly polar, marine alkaloid, does occur in various amphibians. The occurrence and possible origin of tetrodotoxin and congeners, including chiriquitoxin, and of the saxitoxin analogue zetekitoxin in amphibians are reviewed.

The impact of marine and nonmarine natural products on biomedical research and therapeutics has been formidable. Without the extensive array of such secondary metabolites as research probes and lead structures, it is hard to imagine where biomedical science would be today. The marine natural products that have had the greatest impact typically occur only in the marine environment, where they arise from algae, bacteria, bryozoans, dinoflagellates, molluscs, sponges, tunicates, fish, etc.[1−7] Such products include polyethers, such as brevetoxin, ciguatoxin, okadaic acid, maitotoxin, palytoxin, and yessotoxin, macrolides, such as bryostatin and lophotoxin, terpenes, such as manoalide, and alkaloids, such as calyculin, eudistomin, neosurugatoxin, saxitoxin, and tetrodotoxin. Unique marine peptides, such as the conotoxins, and unique marine amino acids, such as domoic and kainic acid, also have had major biomedical impact. None of these important marine natural products with the exception of tetrodotoxin and a saxitoxin analogue have been found in a nonmarine organism structure. Sources, pharmacological activity, and potential therapeutic targets of marine natural products are to be found in various reviews.[2−4,6,7]

Conversely, the nonmarine natural products that have had the greatest impact on biomedical research seem not to occur in marine organisms. They have come primarily from bacteria/fungi that have yielded antibacterials and antifungals and from plants that have been a rich source of alkaloids. Only a few alkaloids from animal sources have had a significant impact. These include batrachotoxin, histrionicotoxin, and epibatidine, all isolated from poison frog skin, but all appearing to be obtained directly from dietary arthropods.[8] Peptides, such as the antibiotic magainins from frog skin,[9] have opened up new areas of research. Alkaloids from plants that have played critical roles in biomedical research include atropine, caffeine, camptothecin, cocaine, colchicine, ephedrine, galanthamine, quinine, morphine, muscarine, nicotine, paclitaxel, physostigmine, reserpine, and vincristine. Plant steroids and terpenes, such as artemisinin, digitalis, forskolin, and thapsigargin, have markedly advanced biomedical research. Such alkaloids, steroids, and terpenes have not been forthcoming from marine sources. The divergence of biosynthetic pathways that lead to bioactive compounds in marine and nonmarine sources has been discussed.[10] Structures and pharmacological activities of the above nonmarine natural products are covered in standard reference works.[11] The present minireview focuses on "marine alkaloids" now found in nonmarine organisms, namely, amphibians. These are the tetrodotoxins and a novel saxitoxin analogue. A few classes of relatively simple alkaloids found in both marine and nonmarine sources appear to represent examples of convergent biosynthetic pathways.

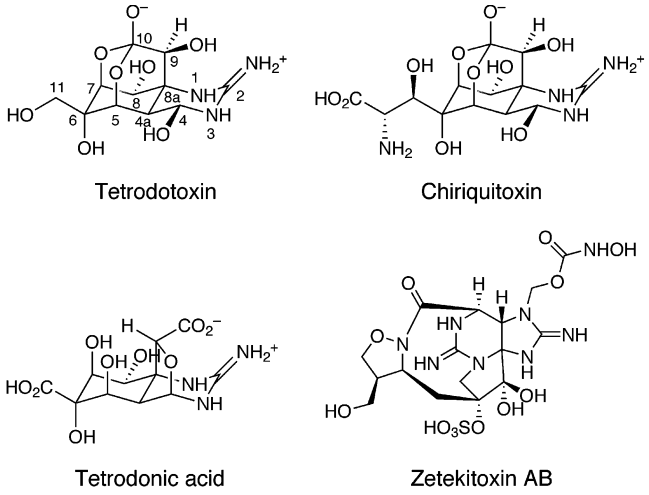

Figure 1. Structures of guanidinium toxins from atelopid frogs: tetrodotoxin, chiriquitoxin, tetrodonic acid, and zetekitoxin AB.

Tetrodotoxins and Saxitoxins

The principle toxin from the puffer fish *Spheroides rubripes* was named tetrodotoxin in 1910.[12] After isolation from ovaries of the puffer fish[13] and from eggs of the California newt, *Taricha torosa*,[14] the structure (Figure 1) was elucidated and reported in 1964 at the IUPAC Natural Product Symposium in Kyoto, Japan, by four groups,

† Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

* To whom correspondence should be addressed. Tel: (301) 496-4024. Fax: (301) 402-0008. E-mail: jdaly@nih.gov.

10.1021/np040016t CCC: $27.50
Published on Web 06/29/2004

namely, those of K. Tsuda, T. Goto, R. B. Woodward, and H. S. Mosher.[15]

Subsequently, tetrodotoxin and congeners have been reported from a variety of marine organisms, as reviewed by Miyazawa and Noguchi:[16] (1) puffer fish from several genera, including species from brackish waters; in the freshwater puffer *Takifuga fungi* of Thailand, saxitoxin was the major toxin;[17] (2) the marine goby fish *Yongeichthys criniger*, where, unlike the puffer fish that have high levels in liver and ovaries, tetrodotoxin levels were highest in skin;[18] the levels varied considerably for gobies collected in Japanese versus Taiwanese waters; (3) various marine worms of different phylla, namely, flatworms (Platyhelminthes), ribbon worms (Nemertea), arrow worms (Chaetognatha), and seqmented worms (Annelida);[16] (4) the blue-ringed octopus, *Hapalochloena maculosus*, where the toxin from venom glands was initially named maculotoxin and then identified as tetrodotoxin;[19] (5) various gastropods, first from the trumpet shell-fish *Charonica sauliae*[20] and then from seven other genera;[16] (6) the horseshoe crab, *Carcinoscorpius rotundicauda*;[16] (7) xanthid crabs from five different genera;[16] paralytic shellfish toxins, namely, saxitoxins and gonyautoxins, were also found; (8) starfish of the genus *Astropectan*.[16]

The origin of tetrodotoxin found in the wide range of marine organisms still needs to be definitively established. It has been argued rather convincingly that symbiotic bacteria are the putative source. A variety of marine bacteria, in some cases isolated from tetrodotoxin-containing hosts, have been shown to produce tetrodotoxin.[16] Most studies have implicated bacteria of the genus *Vibrio* as the putative source. *Pseudomonas* species also have been proposed,[21,22] as have *Actinomycetes*.[23] A red algae of the genus *Jamia*[24] and a dinoflagellate[25] appear to contain tetrodotoxin, although for the algae a symbiotic bacteria may be involved. In 1995, the possibility of false positives for tetrodotoxin in the standard HPLC assays was raised.[26]

Indirect evidence for a symbiont comes from studies on hatchery-raised puffer fish, which are not toxic, but become so when fed tetrodotoxin-containing liver of wild-caught puffer fish[27] and after being fed tetrodotoxin-producing bacteria.[28] Furthermore, although atelopid frogs in the wild contained significant levels of tetrodotoxin in skin, the same species when raised from eggs in captivity had no detectable tetrodotoxin in skin extracts.[29] A tetrodotoxin-binding protein has been reported from toxic puffer fish.[30] Results, based on nerve electrophysiology with the Atlantic puffer fish *Spheroides maculates*,[31] confirm the well-known resistance of puffer fish to the toxic effects of tetrodotoxin.

Tetrodotoxin was first discovered in an amphibian in 1964, where it was isolated from eggs of the Californian newt *Taricha torosa*.[14] Since that time tetrodotoxin and congeners have been found in a wide range of amphibians (see Table 1). Tailed amphibians (Caudata) from five genera of the family Salamandridae have been reported to have tetrodotoxin or tetrodotoxin-like toxins in extracts. Only one of the species of this family that have been studied did not contain tetrodotoxin. That was the European fire salamander, *Salamandra salamandra*, known to contain the highly toxic, steroidal alkaloid samandarine.[51] In one study,[40] tetrodotoxin was reported from the newt *Ambystoma tigrinium* of the family Ambystomatidae (Table 1). However, in another study on a variety of newts/salamanders from eight families, tetrodotoxin-like activity was not detected in *Ambystoma tigrinium*.[34] Tetrodotoxin-like activity also was not detected in newts/salamanders of the following genera: *Ambystoma*, *Amphiuma*, *Aneides*, *Batrachoseps*, *Crytobranchus*, *Ensatina*, *Necturus*, *Salamandra*, and *Siren*, nor was tetrodotoxin-like activity detected in frogs/toads of the genera *Bufo*, *Hyla*, *Rana*, and *Xenopus*.[34]

The newts of the genus *Taricha* proved to be by far the most toxic (Table 1). The toxicity[34] reported for one collection of *Taricha granulosus* would equate to nearly 6 mg of tetrodotoxin per newt, a rather remarkable amount. Levels of tetrodotoxin-equivalents have been reported over a wide range for different collections of the same species and even among individuals of the same collection. An early paper suggested that female newts might be more toxic than male newts,[52] but apparently this possibility has not been investigated in detail.[37] Levels appear to be maintained or even increased in newts in captivity.[33,36,37] However, putative radioactive precursors were not incorporated into the tetrodotoxin.[35,36] The small, brightly colored, terrestrial efts of *Notophthalmus* were reported to be severalfold more toxic than the dull-colored, aquatic adults.[34] In contrast, efts of *Cynops ensicauda* had only trace amounts of toxins.[41] Larvae of *Taricha* newts declined in toxicity as they matured.[52] Tetrodotoxin was the predominant alkaloid identified from all the newts except for two instances, one with *Cynops* where 6-epiTTX was a major alkaloid, and one with *Triturus alpestris* where 11-deoxyTTX was a major alkaloid (Table 1).

Tailless amphibians (Anura) appear to be much less toxic than the *Taricha* newts (Table 1). Only three had toxicities approaching those of the *Taricha* newts. These were *Atelopus oxyrhyncus*,[48,49] one collection of *Atelopus zeteki*,[46] and a few specimens of a *Polypedates* species.[50] Another collection of *A. zeteki* was much less toxic.[46] It would appear that most anurans of Table 1 contain less than 20 μg of tetrodotoxin-equivalents per frog/toad. The predominant alkaloid in all anuran extracts was tetrodotoxin, with but two exceptions. Small amounts of 4-epitetrodotoxin and 4,9-anhydrotetrodotoxin were detected in some cases. One exception was *Atelopus chiriquensis*, where about 70% of the toxins proved to be a remarkable alkaloid, chiriquitoxin, while the remainder was tetrodotoxin.[46] Unlike the many tetrodotoxin congeners, chiriquitoxin has an altered carbon skeleton in which the 11-CH_2OH of tetrodotoxins is replaced by a 11-$CHOHCHNH_2COOH$ moiety (Figure 1).[53] Chiriquitoxin has not been detected from a marine source, nor from any other amphibian. It is at least as toxic as tetrodotoxin.[47] The second exception was the Panamanian golden frog, *Atelopus zeteki*, which had only small amounts (<5%) of tetrodotoxin with the main toxins being two (or three) closely related guanidinium toxins, originally termed atelopid toxin[54,55] and later zetekitoxins AB and C.[46,56] The spectral data indicated that the zetekitoxins were not of the tetrodotoxin class. The structure of zetekitoxin AB ($C_{16}H_{25}N_8O_{12}S$) has now been elucidated through detailed analysis of mass spectral and NMR spectral data.[57] It proved to be an unprecedented analogue of saxitoxin (Figure 1), manyfold more potent than saxitoxin as a sodium channel blocker.

Tetrodotoxin has been detected in almost all bufonid species of *Atelopus* that have been examined (Table 1). There have been two exceptions. Extracts of *Atelopus cruciger* were found to be nontoxic.[48] In addition, the preserving alcohol of one museum specimen of *Atelopus spumarius* was nontoxic.[49] Tetrodotoxin has not been found in other bufonids (see above). Tetrodotoxin also has been detected in anurans from three other families. The dendrobatid *Colostethus inguinalis*, a riparian frog of Central America, had very low levels of tetrodotoxin in skin

Table 1. Occurrence of Tetrodotoxin (TTX) and Congeners, Chiriquitoxin (CTX) and Zetekitoxin (ZTX) in Amphibians

order family genus species	source	major alkaloids	TTX equivalents,[a] M.U.	other alkaloids	reference
Caudata					
Salamandridae					
Cynops ensicauda	Japan	TTX	60−120		14, 34
	Japan	TTX	1400	4-epiTTX, 6-epiTTX, 4,9-anhydroTTX[b]	32
	Japan	TTX, 6-epiTTX	4400−7000	11-deoxyTTX	39
Cynops pyrrhogaster	Japan	TTX	60−100		14, 34
	Japan	TTX	50		33
	Japan	TTX, 6-epiTTX	1800−2800	11-deoxyTTX	39
	Japan	TTX, 6-epiTTX	400	4,9-anhydroTTX, 4,9-anhydro-6-epiTTX	40
Paramesotriton hongkongensis	China	TTX	50		33
	China	TTX	90		39
Notopthalmus viridescens	USA (Northeast)	TTX	60		14, 34
	USA (Northeast)	TTX	180		40
	USA (Northeast)	TTX	130−350	4-epiTTX, 6-epiTTX, 4,9-anhydroTTX	33, 34, 39
	USA (Northeast)	TTX	1000	6-epiTTX, 11-deoxyTTX	39
	USA (Northeast)	TTX	200−540	6-epiTTX	38
Taricha granulosa	USA (West)	TTX	26 000		33
	USA (West)	TTX	14 000		36
	USA (West)	TTX	6200		39
	USA (West)	TTX	13 000	1-hydroxy-5,11-dehydroTTX	35
Taricha rivularis	USA (West)	TTX	600		34
	USA (West)	TTX	2500		33
Taricha torosa	USA (West)	TTX	1200		33
	USA (West)	TTX	800−1200		34
	USA (West)	TTX	3800−6000		36
Triturus alpestris	Europe	TTX	<1		34
	Europe	TTX, 11-deoxyTTX	190	6-epiTTX	39
Triturus cristatus	Europe	TTX	<1		34
Triturus marmoratus	Europe	TTX	1−3		14, 34
Triturus vulgaris	Europe	TTX	1		34
	Europe	TTX	50	6-epiTTX, 11-deoxyTTX	39
Ambystomatidae					
Ambystoma tigrinium[c]	North America	TTX	80	6-epiTTX, 11-deoxyTTX	39
Anura					
Brachycephalidae					
Brachycephalus ephippium	Brazil	TTX	140	4-epiTTX, 6-epiTTX, 4,9-anhydroTTX, 11-oxoTTX tetrodonic acid, 11-nor-6-hydroxyTTX	42−44
Dendrobatidae					
Colostethus inquinalis	Panamá	TTX	0.5−6		45
Bufonidae					
Atelopus chiriquiensis	Costa Rica	CTX>TTX	350		46
Atelopus ignescens	Colombia	TTX	1−3	4-epiTTX	45
Atelopus oxyrhynchus[d]	Venezuela	TTX	200 900	4-epiTTX, 4,9-anhydroTTX	47
" "	Venezuela	TTX	900		48
Atelopus peruensis[d]	Peru	TTX	20		49
Atelopus spumarius	Brazil	TTX	10−16	4-epiTTX, 4,9-anhydroTTX	45
Atelopus spurelli	Colombia	TTX	3−5		45
Atelopus subornatus[d]	Colombia	TTX	20−80	4-epiTTX, 4,9-anhydroTTX	49
Atelopus varius varius	Costa Rica	TTX	100		46
Atelopus varius ambulatorius	Costa Rica	TTX	100		46
Atelopus varius	Panamá	TTX	10−13	4-epiTTX, 4,9-anhydroTTX	29, 45
Atelopus zeteki	El Valle, Panamá	ZTX	1200	TTX	46
" "	Cope, Panamá	ZTX	23		45
Rhacophoridae					
Polypedates sp.[e]	Bangladesh	TTX	30−900		50

[a] A mouse unit (M.U.) corresponds to 0.16−0.22 μg of tetrodotoxin. Values are per individual. In some cases, a tentative value was estimated from the cited literature data. [b] Trace amounts of 11-deoxyTTX, 11-deoxy-4-epiTTX, and 11-deoxy-4,9-anhydroTTX also were isolated. [c] Another study on *Ambystoma*[34] reported no TTX. [d] Analyses were conducted on alcohol used to preserve specimen(s). [e] Frogs from another location had no TTX.[49]

extracts.[45] Tetrodotoxin was not detected in eight other species of *Colostethus*. The tiny, golden, brachycephalid frog *Brachycephalus epihippium* had much higher levels, with tetrodotoxin being high in both skin and liver.[43] Finally, in a rhacophorid frog of the genus *Polypedates*, the highest levels of tetrodotoxin approached those of the *Taricha* newts, but there was great variation in levels for individual frogs.[50]

There are many problems with the mouse unit (M.U.) estimates of Table 1. Each M.U. probably is equivalent to 0.16−0.22 μg of tetrodotoxin. In particular, tetrodotoxin does not extract well into alcohol alone, but needs alcohol−acetic acid or aqueous acetic acid to extract well (see ref 45). In some cases, the alcohol used to preserve a museum specimen of the atelopid species was analyzed.[49] Stability of tetrodotoxin at room temperatures in alcohol over years

could be a factor. Finally, not all tetrodotoxin congeners have the same activity in mouse or binding assays, with some, like 11-oxotetrodotoxin, being very toxic.[44]

A range of anurans from the following genera of eight families have been reported to lack tetrodotoxin-like alkaloids.[35,45,49] Bufonidae: *Bufo*, *Dendrophryniscus*, *Melanophryniscus*, *Oreophrynella*. Dendrobatidae: *Aromabates*, *Dendrobates*, *Phyllobates*. Hylidae: *Cyclorana*, *Hemiphractus*, *Hyla*, *Litoria*, *Nyctimystes*, *Osteocephalus*, *Phrynohyas*. Leptodactylidae: *Eleutherodactylus*. Microhylidae: *Otophryne*, *Phrynomerus*, *Scaphiophryne*. Mantellidae: *Mantella*. Myobatrachidae: *Heleioporus*, *Notaden*, *Pseudophryne*. Pipidae: *Xenopus*. Ranidae: *Rana*. All but the *Hyla*, *Oreophrynella*, and *Xenopus* results are based wholly or in part on lack of inhibition of [^{3}H]saxitoxin binding.[45] Such an assay also would detect saxitoxins.

The presence of tetrodotoxins, chiriquitoxins, and now the saxitoxin analogue zetekitoxin in certain lineages of amphibians raises questions as to their origin. As in marine organisms, a symbiotic microorganism, presumably a bacteria, is a possible source. However, it is difficult to rationalize how three species of Central American atelopid frogs found in similar montane, riparian habitats would contain mainly tetrodotoxin for *Atelopus varius*, a mixture of chiriquitoxin and tetrodotoxin for *Atelopus chiriquiensis*, and mainly the saxitoxin analogue zetekitoxin for *Atelopus zeteki*. Of course, it is possible that species in different watersheds might contain different symbiotic organisms. With regard to saxitoxins, marine dinoflagellates[58] and certain freshwater algae[59−61] produce such alkaloids. Such algae could be considered a possible source of zetekitoxin. It, however, is remarkable that zetekitoxin, rather than saxitoxin itself, was the alkaloid found in that montane atelopid frog of Panamá.

When *Atelopus varius* was raised from eggs at the National Aquarium in Baltimore, the frogs at adulthood 2−3 years later had no detectable tetrodotoxin in skin extracts.[29] Certainly, this result is consonant with the necessity of a dietary source or a symbiotic microorganism. In the wild, eggs of *Atelopus chiriquiensis* contain both tetrodotoxin and chiriquitoxin,[62] as might be expected, since the egg-laying parent contains such alkaloids and the eggs may merely have been sequestered from ovarian sources.

As well-known for the puffer fish, the tetrodotoxin-containing newts of the genera *Taricha* and *Notophthalmus* are resistant to tetrodotoxin,[14,31,41,63] but remarkably not to saxitoxin.[31] Newts and a predator garter snake, *Thamnophis sirtalis*, seem in an arms-race, with snakes being highly resistant to tetrodotoxin at sites where the newts are highly toxic.[63,64]

The extent to which tetrodotoxin and perhaps zetekitoxin occur in other toxic amphibians requires further study. A facile method to detect other species with tetrodotoxin-equivalents would be based on an [^{3}H]saxitoxin assay, much as ouabain-equivalents, determined with [^{3}H]ouabain, were used to assay a wide range of frogs/toads for bufadienolide-like steroids.[65]

Other Alkaloids

There may well be other bioactive marine secondary metabolites that also occur in nonmarine organisms. Certainly, ring systems often have evolved in both spheres, probably reflecting similar biosynthetic pathways or precursor amino acids. Simple pyrrolidines and piperidines are known from plants, with hygrine, solenopsin, coniine, arecoline, and lobeline being notable examples. Such alkaloids find analogues in the pyrrolidine villatamines from a marine flatworm[66] and the piperidine pseudodistomins from a marine tunicate.[67] Decahydroquinolines occur in many poison frogs, probably arising from dietary arthropods.[8] Such decahydroquinolines find a parallel in the decahydroquinoline lepadine from a marine flatworm.[68] Simple izidines are common in plants, with swainsonine, castanospermine, sparteine, cytosine, and lupinine being notable. Marine parallels include the quinolizidine pictamine from a marine tunicate.[69] Similar parallels exist for simple pyridines, where in plants nicotine and cytosine and in frogs epibatidine are most notable. The pyridine alkaloid anabaseine occurs in both a myrmicine ant[70] and a marine nemertine worm.[71] Other relatively simple pyridines occur in marine organisms, as in ikimines from sponges[72] and louludinium chloride from a marine alga.[73] Many β-carbolines and analogous tetrahydroquinolines are known from plants, notably emetine. Manzamine from a sponge represents an example of a marine β-carboline alkaloid.[74] The eudistomins from marine tunicates are another example.[75] The indolic plant alkaloid physostigmine and the pseudophrynamines of Australian *Pseudophryne* frogs[76] find a parallel in the flustramines from a marine bryozoan.[77] The imidazole alkaloid spinacine occurs in marine sharks/fish,[78] while a close analogue, spinceamine, occurs in a leptodactylid frog.[79] Staurosporine was originally isolated from the bacteria *Streptomyces stauroporeus*,[80] and such indolocarbazoles also are known from fungi and blue-green algae. In 1992, a hydroxystaurosporine was described from a marine tunicate.[81] The polyether mycalamide A from a marine sponge[82] has nearly the same complex structure as pederin from a blister beetle,[83] differing only in the nature of the oxy substitution. Finally, macrocyclic peptides occur from both marine[5,6] and nonmarine[84] sources, but a comparison is beyond the scope of the present overview.

Undoubtedly, many other parallels exist, but identity of marine and nonmarine natural products is not common. The macrolides, terpenes, and polyethers of marine organisms appear to have few if any counterparts from nonmarine sources, while the diversity of tyrosine- and tryptophan-derived alkaloids found in plants finds few parallels in the marine world. Both spheres have yielded a rich harvest of unique bioactive natural products, and perhaps fortunately the divergence of biosynthetic strategies has increased the harvest and made for few redundant discoveries of structures relevant to biomedical research.

References and Notes

(1) Scheuer, P. J. *Med. Chem. Res.* **1989**, *9*, 535−545.
(2) Scheuer, P. J. *Science* **1990**, *248*, 173−177.
(3) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49.
(4) Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *93*, 1897−1909.
(5) Kobayashi, J.; Ishibashi, M. In *The Alkaloids*; Brossi, A., Cordell, G. A., Eds.; Academic Press: NewYork, 1992; Vol. 41, pp 42−124.
(6) König, C. M.; Wright, A. D. *Planta Med.* **1996**, *62*, 193−211.
(7) Carté, B. K. *Bioscience* **1996**, *46*, 271−286.
(8) Daly, J. W. *J. Med. Chem.* **2003**, *46*, 445−452.
(9) Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449−5453.
(10) Fenical, W. *Science* **1982**, *215*, 923−928.
(11) *The Merck Index*, 13th ed.; Merck & Co.: Whitehouse Station, NJ, 2001.
(12) Tahara, Y. *Biochem. Z.* **1910**, *10*, 255−275.
(13) Tsuda, K.; Kawamura, M. *Chem. Pharm. Bull. Jpn.* **1953**, *1*, 112−113.
(14) Mosher, H. S.; Fuhrman, F. A.; Buchwald, M. D.; Fisher, H. G. *Science* **1964**, *144*, 1100−1110.
(15) Mosher, H. S.; Fuhrman, F. A. In *ACS Symposium Series, No. 262 Seafood Toxins*; Ragelis, E. P., Ed.; American Chemical Society: Washington D.C., 1984; Chapter 28, pp 333−344.
(16) Miyazawa, K.; Noguchi, T. *J. Toxicol. Toxin Rev.* **2001**, *20*, 11−33.
(17) Sato, S.; Kodama, M.; Ogata, T.; Saitanu, K.; Furuya, M.; Hirayama, K.; Kakinuma, K. *Toxicon* **1997**, *35*, 137−140.
(18) Noguchi, T.; Hashimoto, Y. *Toxicon* **1973**, *11*, 305−307.
(19) Schemack, D. D.; Howden, M. E. H. *Science* **1978**, *199*, 188−189.
(20) Narita, H.; Noguchi, T.; Maruyama, J.; Ueda, Y.; Hashimoto, K.; Watanabe, V.; Hida, K. *Bull. Jpn. Soc. Sci. Fish.* **1981**, *47*, 935−941.

(21) Yasumoto, T.; Yasumura, D.; Yotsu, M.; Michishita, T.; Endo, A.; Kotaki, Y. *Agric. Biol. Chem*. **1986**, *50*, 793−795.
(22) Yotsu, M.; Yamazaki, T.; Meguro, Y.; Endo, A.; Murata, M.; Naoki, H.; Yasumoto, T. *Toxicon* **1987**, *25*, 225−228.
(23) Do, H. K.; Kogure, K.; Imada, C.; Noguchi, T.; Ohwada, K.; Simidu, U. *J. Appl. Bacteriol*. **1991**, *70*, 464−468.
(24) Yasumoto, T.; Yotsu, M.; Endo, A.; Murata, M.; Naoki, H. *Pure Appl. Chem*. **1989**, *61*, 505−508.
(25) Kodama, M.; Sato, S.; Sakamoto, S.; Ogata, J. *Toxicon* **1996**, *34*, 1101−1105.
(26) Matsumara, K. *Appl. Environ. Microbiol*. **1995**, *61*, 3468−3470.
(27) Matsui, T.; Hamada, S.; Kanosu, S. *Bull. Jpn. Soc. Sci. Fish*. **1981**, *47*, 535−537.
(28) Matsui, T.; Taketsugu, S.; Sato, H.; Yamamori, K.; Kodama, K.; Ishii, A.; Hirose, H.; Shimizu, C. *Nippon Suisan Gakkaishi* **1990**, *56*, 705.
(29) Daly, J. W.; Padgett, W. L.; Saunders: R. L.; Cover, J. F. *Toxicon* **1997**, *35*, 705−709.
(30) Matsui, T.; Yamamori, K.; Furukawa, K.; Kono, M. *Toxicon* **2000**, *38*, 463−468.
(31) Kao, C. Y.; Fuhrman, F. A. *Toxicon* **1967**, *5*, 25−32.
(32) Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H. *J. Am. Chem. Soc.* **1988**, *110*, 2344−2345.
(33) Brodie, E. D., Jr.; Hensel, J. L., Jr.; Johnson, J. A. *Copeia* **1974**, 506−511.
(34) Wakely, J. F.; Fuhrman, G. J.; Fuhrman, F. A.; Fischer, H. G.; Mosher, H. S. *Toxicon* **1966**, *3*, 195−203.
(35) Kotaki, Y.; Shimizu, Y. *J. Am. Chem. Soc.* **1993**, *115*, 827−830.
(36) Shimizu, Y.; Kobayashi, M. *Chem. Pharm. Bull*. **1983**, *31*, 3625−3631.
(37) Hanifin, C. T.; Brodie, E. D., III; Brodie, E. D., Jr. *Toxicon* **2002**, *40*, 1149−1153.
(38) Yotsu-Yamashita, M.; Mebs, D. *Toxicon* **2001**, *39*, 1261−1263.
(39) Yotsu, M.; Forizzi, M.; Yasumoto, T. *Toxicon* **1990**, *28*, 238−241.
(40) Tsuruda, K.; Arakawa, O.; Noguchi, T. *J. Nat. Toxins* **2001**, *10*, 79−89.
(41) Levenson, C. H.; Woodhull, A. M. *Toxicon* **1979**, *17*, 184−187.
(42) Sebben, A.; Schwartz, C. A.; Valente, D.; Mendes, E. G. *Toxicon* **1986**, *24*, 799−806.
(43) Pires, O. R., Jr.; Sebben, A.; Schwartz, E. F.; Largura, S. W. R.; Bloch, C., Jr.; Morales, R. A. V.; Schwartz, C. A. *Toxicon* **2002**, *40*, 761−766.
(44) Pires, O. R., Jr.; Sebben, A.; Schwartz, E. F.; Bloch, C., Jr.; Morales, R. A. V.; Schwartz, C. A. *Toxicon* **2003**, *42*, 563−566.
(45) Daly, J. W.; Gusovsky, F.; Myers, C. W.; Yotsu-Yamashita, M.; Yasumoto, T. *Toxicon* **1994**, 279−285.
(46) Kim, Y. H.; Brown, G. B.; Mosher, H. S.; Fuhrman, F. A. *Science* **1975**, *189*, 151−152.
(47) Yotsu-Yamashita, M.; Mebs, D.; Yasumoto, T. *Toxicon* **1992**, *30*, 1489−1492.
(48) Mebs, D.; Schmidt, K. *Toxicon* **1989**, *22*, 819−825.
(49) Mebs, D.; Yotsu-Yamashita, Yasumoto, T.; Lötters, S.; Schlüter, A. *Toxicon* **1995**, *33*, 246−249.
(50) Tanu, M. B.; Mahmud, Y.; Tsuruda, K.; Arakawa, O.; Noguchi, T. *Toxicon* **2001**, *39*, 937−941.
(51) Schöpf, Cl. *Experientia* **1961**, *17*, 285−328.
(52) Twitty, V. C. *J. Exp. Zool*. **1937**, *76*, 67−104.
(53) Yotsu, M.; Yasumoto, T.; Kim, Y. H.; Naoki, H.; Kao, C. Y. *Tetrahedron Lett*. **1990**, *31*, 3187−3190.
(54) Fuhrman, F. A.; Fuhrman, G. J.; Mosher, H. S. *Science* **1969**, *165*, 1376−1377.
(55) Shindelman, J.; Mosher, H. S.; Fuhrman, F. A. *Toxicon* **1969**, *7*, 315−319.
(56) Brown, G. B.; Kim, Y. H.; Küntzel, H.; Mosher, H. S.; Fuhrman, G. J.; Fuhrman, F. A. *Toxicon* **1977**, *15*, 115−128.
(57) Yotsu-Yamashita, M.; Kim, Y. H.; Dudley, S. C., Jr.; Choudhary, G.; Pfahal, A.; Oshima, Y.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4346−4351.
(58) Shimizu, Y.; Hsu, C.-P.; Fallon, W. E.; Oshima, Y.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6791−6793.
(59) Jackim, E.; Gentile, J. *Science* **1968**, *162*, 915−916.
(60) Sevcik, C.; Noriega, J.; Dśuze, G. *Toxicon* **2003**, *42*, 359−366.
(61) Mahmood, N. A.; Carmichael, W. W. *Toxicon* **1986**, *24*, 175−186.
(62) Pavelka, L. A.; Kim, Y. H.; Mosher, H. S. *Toxicon* **1977**, *15*, 135−139.
(63) Brodie, E. D., III; Brodie, E. D., Jr. *Evolution* **1991**, *45*, 221−224.
(64) Brodie, E. D., III; Brodie, E. D., Jr. *Evolution* **1990**, *44*, 651−659.
(65) Flier, J.; Edwards, M. W.; Daly, J. W.; Myers, C. W. *Science* **1980**, *208*, 503−505.
(66) Kubanek, J.; Williams, D. E.; Dilipde Silva, E.; Allen, T.; Anderson, R. J. *Tetrahedron Lett*. **1993**, *36*, 6189−6192.
(67) Ishibashi, M.; Ohizumi, Y.; Sasaki, T.; Nakamura, N.; Hirata, Y.; Kobayashi, J. *J. Org. Chem*. **1987**, *52*, 450−453.
(68) McCoy, M. C.; Faulkner, D. J. *J. Nat. Prod*. **2001**, *64*, 1087−1089.
(69) Kong, F.; Faulkner, D. J. *Tetrahedron Lett*. **1991**, *32*, 3667−3668.
(70) Wheeler, J. W., Olubajo, O.; Storm, C. B.; Duffield, R. M. *Science* **1981**, *211*, 1051−1052.
(71) Kem, W. R., Scott, K. N.; Duncan, J. H. *Experientia* **1976**, *32*, 684−686.
(72) Carroll, A. R.; Scheuer, P. J. *Tetrahedron* **1990**, *46*, 6637−6644.
(73) Yoshida, W. Y.; Scheuer, P. J. *Heterocycles* **1996**, *47*, 1023−1027.
(74) Ichiba, T.; Corgiat, J. M.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod*. **1994**, *57*, 168−170.
(75) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. B., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc*. **1984**, *106*, 1524−1526.
(76) Spande, T. F.; Edwards, M. W.; Pannell, L. K.; Daly, J. W. *J. Org. Chem*. **1988**, *53*, 1222−1226.
(77) Carlé, J. S.; Christophersen, C. *J. Org. Chem*. **1981**, *46*, 3440−3443.
(78) Ackermann, D. *Hoppe-Seyler's Z. Physiol. Chem*. **1962**, *328*, 275.
(79) Erspamer, V.; Vitali, T.; Roseghini, M.; Cei, J. M. *Experientia* **1963**, 346.
(80) Furasaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. *J. Chem. Soc., Chem. Commun*. **1978**, 800−801.
(81) Kinnel, R.; Scheuer, P. J. *J. Org. Chem*. **1992**, *57*, 6327−6329.
(82) Perry, M. D.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *J. Am. Chem. Soc*. **1988**, *110*, 4850−4851.
(83) Furnsaki, A.; Watanabe, T. *Tetrahedron Lett*. **1968**, 6301−6304.
(84) Itokawa, H.; Takeya, K.; Hitotsuyanagi, Y.; Morita, H. In *The Alkaloids*, *Chemistry and Pharmacology*; Cordell, G. A., Ed.; Academic Press: New York, 1997; Vol. 49, pp 301−387.

NP040016T