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Marine Toxins

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He who oversees everything also created very many poisonous fish, and in this way he punishes those who seek them.

This quotation, which is credited[1] to the sixteenth century French poet and physician Jacques Grévin, serves as a dual reminder that man's concern with toxic marine organisms has a long history and that those who pursue research of marine toxins do at times find the experience painful, not to say punishing. But I hope to show in this Account that here, as elsewhere, punishment can have its own rewards. Most of our chemical knowledge of marine toxins is quite recent and has resulted from a study of but few organisms. Judging, therefore, from an admittedly narrow sample it appears nonetheless that future research in this field may reveal rich and diverse chemistry and will provide important clues for a better understanding of marine ecosystems. This Account will attempt to illustrate these points with a discussion of some recent research findings. For various other aspects of marine toxin research the reader should consult the ample review literature.[2-12]

Terminology, as it often does, tends to get in the way, and a brief section on definitions is worthwhile. The dictionary[13] defines *toxin* more narrowly than *poison*, but in line with common practice we will use the terms interchangeably.

In its broadest sense then, a toxin or poison is an entity, natural or manmade, that has an adverse physiological effect on a living organism. Historically, man used his own organism as the yardstick by which he measured harmful physiological activity. In recent years, though, our outlook has become less anthropocentric, as we have become increasingly aware of the interdependence of all the earth's biota. Still, much of the existing literature deals with substances that are harmful to man and other warm-blooded (principally domesticated) animals.

Toxicology is the science that deals with the effects of poisons on living organisms. A distinction that separates natural (toxinology) and man-made (toxicology) poisons could be very useful (particularly in *Chemical Abstracts*!), but has been adopted only sparingly.[14,15]

One group of naturally occurring poisons forms a ready subdivision. *Venoms* are animal secretions that are produced by discrete glands and are delivered by specific mechanisms for defensive or offensive purposes. Venoms, which have been isolated from some species of sea snakes, scorpion fishes, coelenterates, and others, have proved to be proteinaceous macromolecules; they are being studied by biochemical methods and are not considered in this Account. Poisons (or toxins), although concentrated in specific organs (e.g., tetrodotoxin in pufferfish ovaries[16]), most frequently are of dietary origin (saxitoxin) or are produced by the host organism (pahutoxin[17]). Chemically, these compounds have proved to be small molecules that lack common functionality or unifying structural features.

While poisons that constitute a public health hazard (red tide toxins, ciguatoxin) continue to be important research targets, it is well to include in our purview

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(1) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World", Vol. 2, U.S. Government Printing Office, Washington, D.C., 1967, p 162.
(2) G. Habermehl, *Bull. Inst. Pasteur, Paris*, **74**, 107–111 (1976).
(3) M. Alam, *Tex. Rep. Biol. Med.*, **33**, 183–199 (1975).
(4) G. Habermehl, *Naturwissenschaften*, **62**, 15–21 (1975).
(5) P. J. Scheuer, *Lloydia*, **38**, 1–7 (1975).
(6) E. Karlsson, *Experientia*, **29**, 1319–1327 (1973).
(7) D. Mebs, *Experientia*, **29**, 1328–1334 (1973).
(8) E. Zlotkin, *Experientia*, **29**, 1453–1466 (1973).
(9) E. J. Schantz in "Microbial Safety of Fishery Products", C. O. Chichester & H. D. Graham, Ed., Academic Press, New York, N.Y., 1973, pp 151–162.
(10) P. J. Scheuer in "Toxins of Animal and Plant Origin", Vol. 2, A. De Vries and E. Kochva, Ed., Gordon and Breach, London, 1972, pp 560–572.
(11) F. E. Russell in "Pharmacology and Toxicology of Naturally Occurring Toxins", Vol. 2, H. Raskova, Ed., Pergamon Press, New York, N.Y., 1971, pp 3–114.
(12) P. J. Scheuer, *Naturwissenschaften*, **58**, 549–554 (1971).
(13) "Webster's Third New International Dictionary", G. & C. Merriam, Springfield, Mass., 1971.
(14) T. A. Freyvogel and B. A. Perret, *Experientia*, **29**, 1317–1319 (1973).
(15) *Toxicon* (Pergamon Press, Oxford) is the official journal of the International Society on Toxinology and is "devoted to the exchange of knowledge on the poisons derived from animals, plants and microorganisms".
(16) K. Tsuda, *Naturwissenschaften*, **53**, 171–176 (1966).
(17) D. B. Boylan and P. J. Scheuer, *Science*, **155**, 52–56 (1967).

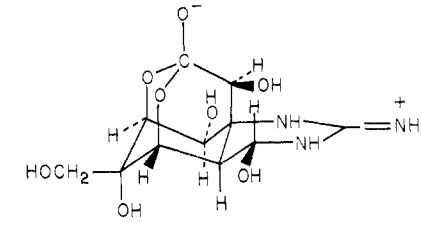
metabolites with known harmful physiological effects on any other organism. Such an approach provides vital data for our understanding of the marine environment. Nor does such a broad-based conception overlook man's needs and interests. Through pharmacological research on tetrodotoxin and on the amphibian toxins, for instance, we have learned that bioactive molecules can become valuable and highly specific tools for the study of drug action, particularly in neurophysiology.[18]

Because of man's traditional and vital concern with those marine toxins that are hazardous to his health and safety, we will begin this *Account* with a consideration of tetrodotoxin, saxitoxin, and ciguatoxin. In a subsequent section we will look at aplysiatoxin and palytoxin, neither of which has been isolated from organisms that are part of man's diet or has been considered a public health problem.

Tetrodotoxin

Poisonous pufferfishes (family Tetraodontidae, hence the name), which are the best known source of tetrodotoxin, were recognized in early records from China and perhaps even Egypt and are therefore among the earliest described toxic marine organisms. Halstead's comprehensive treatment[19] should be consulted for fascinating historical details as well as for biological and medical aspects of human intoxication from consumption of puffers. Japan is the only major country where puffers (*Fugu*) are a culinary delicacy. Invariably, the ovaries contain the highest concentration of toxin, followed by the liver and the intestines, but even in season not each individual of the most notorious species (*Fugu rubripes* or *torafugu*[20]) has toxic ovaries. These circumstances make it difficult to conceive of a rational biogenesis of the toxin. But perhaps even more puzzling than the incidence of toxic individuals among the puffers is the discovery of the indentical toxin in several unrelated animal species. Hashimoto and co-workers[21] isolated tetrodotoxin from a phyletically unrelated fish, *Gobius criniger*, which had been implicated in sporadic outbreaks of poisonings in the Philippines and in Taiwan. Toxic gobies from the Ryukyu and Amami islands and from Taiwan exhibited the greatest toxin concentration in the skin and in the testes, but not the ovaries. In further contrast to the puffer toxicity pattern, the gobies showed virtually no seasonal but pronounced and narrow geographical variation in toxicity. Phyletically even more remote are the other two recorded occurrences of tetrodotoxin, both in amphibians. Mosher and co-workers[22] followed up the reported toxicity of the eggs and embryos (and occasionally the blood of adult females) of the western American salamander *Taricha torosa*. Characterization of the toxin established its identity with tetrodotoxin. More recently, Mosher's group[23] discovered and confirmed the presence of tetrodotoxin in the skin (but not the viscera) of male and female Costa Rican frogs of the genus *Atelopus*. One of the frogs, *A. chiriquiensis*, contained in addition to tetrodotoxin a structurally related toxin, designated chiriquitoxin.

The structure of tetrodotoxin (1) proved to be as



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fascinating as the biological aspects of the problem. Elucidation was completed in four laboratories simultaneously in 1964. Beside Mosher's group that worked with California newts,[22] Tsuda,[16] Goto and co-workers,[24] and Woodward[25] carried out their work with toxin isolated from puffer ovaries. The structure has been confirmed by an x-ray diffraction of crystalline tetrodotoxin hydrobromide.[26] The intriguing chemistry of this molecule, which has been synthesized by the Nagoya group,[27] is described in the primary literature and has been reviewed.[28] The pharmacology of tetrodotoxin, which has become a valuable tool in neurophysiology research, has been studied and summarized by Narahashi.[29]

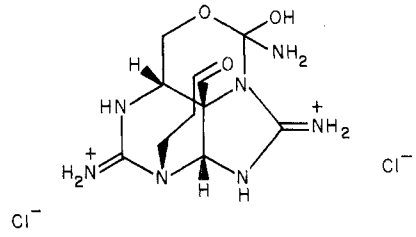
Saxitoxin

"Red tides", the sporadic and unpredictable appearance of red colored organisms over large stretches of ocean in the temperate zones, have been known and feared since antiquity. The earliest reference is generally believed to be the passage in Exodus 7, 20–21: ". . . and all the water changed into blood. The fish died and the river stank. . .". Massive fish kills and shellfish toxicity, which generally last a few weeks and result in incidents or outbreaks of paralytic shellfish poisoning, have been linked to the red tides, but the first clear indication that the bloom of the dinoflagellate *Gonyaulax catenella* was the cause of mussel and clam toxicity off the Pacific coast of North America dates back only 40 years.[30,31] And it was only 10 years ago that Schantz, Rapoport, and co-workers[32] proved the identity of the causative agent, saxitoxin, which they isolated as a metabolite of an axenic culture of *G. catenella* and as a dietary constituent from the hepatopancreas of the mussel *Mytilus californianus*. In spite of the unpredictability of its occurrence, the etiology of paralytic shellfish poisoning is now well

(18) G. Brown and B. Witkop, *Israel J. Chem.*, **12**, 697–709 (1974).
(19) B. W. Halstead, ref 1, pp 679–901.
(20) *Tora* is the Japanese word for tiger, a word which has become familiar through the motion picture of the Pearl Harbor attack, "Tora, Tora, Tora".
(21) T. Noguchi and Y. Hashimoto, *Toxicon* **11**, 305–307 (1973).
(22) H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, and H. G. Fischer, *Science*, **144**, 1100–1110 (1964).
(23) Y. H. Kim, G. B. Brown, H. S. Mosher, and F. A. Fuhrman, *Science*, **189**, 151–152 (1975).
(24) T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron*, **21**, 2059–2088 (1965).
(25) R. B. Woodward, *Pure Appl. Chem.*, **9**, 49–74 (1964).
(26) A. Furusaki, Y. Tomiie, and I. Nitta, *Bull. Chem. Soc. Jpn.*, **43**, 3332–3341 (1970).
(27) Y. Kishi, T. Fukuyama, M. Aratani, F. Nakatsubo, T. Goto, S. Inoue, H. Tanino, S. Sugiura, and H. Kakoi, *J. Am. Chem. Soc.*, **94**, 9219–9221 (1972).
(28) P. J. Scheuer, "Chemistry of Marine Natural Produts", Academic Press, New York, N.Y., 1973, pp 149–157.
(29) T. Narahashi, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **31**, 1115–1132 (1972).
(30) H. Sommer and K. F. Meyer, *Arch. Pathol.*, **24**, 560–598 (1937).
(31) H. Sommer, W. F. Whedon, C. A. Kofoid, and R. Stohler, *Arch. Pathol.*, **24**, 537–559 (1937).
(32) E. J. Schantz, J. M. Lynch, G. Vayvada, K. Matsumoto, and H. Rapoport, *Biochemistry*, **5**, 1191–1195 (1966).

understood and is in sharp contrast to the puzzling genesis of tetrodotoxin (vide supra). However, the incidence of saxitoxin in the Alaskan butter clam, *Saxidomus giganteus*, which stores the posion in its siphon for long periods of time and which also has lent its name to the toxin, has not been linked to *G. catenella* or any other elaborating organism.[33]

The structural determination of saxitoxin proved to be as challenging as that of tetrodotoxin. Both are low molecular weight (about 300 daltons) compounds, with more of the weight contributed by nitrogen and oxygen than by carbon. Both molecules contain unprecedented functionalities and exhibit pK values that are difficult to interpret. Neither toxin yielded a crystalline derivative readily. Virtually all structural work on saxitoxin was carried out in Rapoport's laboratory,[34,35] resulting in 1971 in a proposal of the saxitoxin structure (**2**),[36] which was based entirely on oxidative degradation



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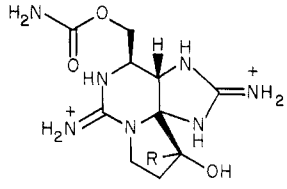
and spectral (largely proton NMR) data. This structure had to be revised to **3a** in 1975, when a group at

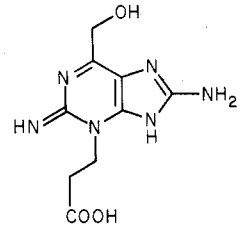


3a, R = OH
b, R = OEt
c, R = H

Wisconsin led by Schantz[37] succeeded in preparing a crystalline bis(*p*-bromobenzenesulfonate); this was soon followed by Rapoport's[38] crystalline ethyl hemiketal dihydrochloride (**3b**).

The x-ray data also shed some light on a rational interpretation of the pK_a data. The high pK_a value of saxitoxin, 11.60, had confidently been assigned to one of the guanidines; the low value, 8.24, was attributed to the hydroxy proton in **2**, since this value appeared to be far too low to arise from the second guanidine unit; moreover, it increases to 9.05 in 50% aqueous ethanol, a value characteristic of proton dissociation from oxygen but not from nitrogen. The low pK_a value cannot be ascribed to the carbonyl hydrate in the revised structure **3a**, since the corresponding secondary alcohol (**3c**) has pK_a values that are identical to those of **3a**. Rapoport[37] concludes that both pK_a's must be due to the two guanidine moieties. The proximity of the two guanidine nitrogens, which are separated by only 3.48 Å, makes the low value plausible, but still leaves the pK_a change when going from a solvent of high to a solvent of low dielectric constant unexplained for the time being.

Oxidation of saxitoxin with alkaline hydrogen peroxide[35] had led to a purine derivative (**4**), which Bates



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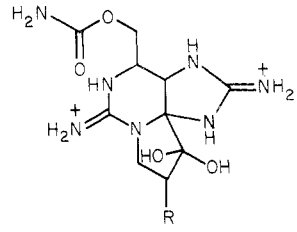


5a, R = α-OH
b, R = β-OH

and Rapoport[39] have utilized for a chemical assay, based on the electronic absorption properties of the purine and far more sensitive than the bioassay for the toxin.

The North Atlantic coasts of America and Great Britain have also experienced sporadic red tides with concomitant shellfish infestation. Early observations indicated that the causative organism was not *G. catenella* and that the toxin was not identical with saxitoxin. Massive red tides along the coast of New England in 1972 and 1974 have recently led to the isolation of identical toxins from the soft shell clam *Mya arenaria* and from cultures of *G. tamarensis*. Shimizu and co-workers[40] have shown that the Atlantic shellfish poison is a mixture of saxitoxin and three new compounds. Two of the new toxins (**5a**,**b**) are interrelated and undergo spontaneous interconversion.

The four toxins proved to be difficult to separate. Purification was hampered by the instability of at least some of the toxins, which would tolerate no solvents except aqueous ethanol and very dilute acetic acid. By spectral analysis it has been demonstrated that two of the new toxins, gonyautoxins II (**5a**) and III (**5b**), are epimeric hydroxysaxitoxins,[41] while the structure of the fourth toxin is still under investigation. A sensitive TLC–fluorometric assay for the gonyautoxins, no doubt related to the spectral assay for saxitoxin,[39] has been described by Ikawa and co-workers.[42]

Beside the common occurrence of saxitoxin in mollusks as a result of a dinoflagellate bloom, saxitoxin has been isolated from a crustacean. Interestingly, the exoskeleton of the crab *Zosimus aeneus* proved to be the most highly toxic part of the animal when Hashimoto and co-workers[43] conducted an epidemiological survey of intoxication of humans and domestic animals

(33) E. J. Schantz and H. W. Magnusson, *J. Protozool.*, **11**, 239–242 (1964).
(34) W. Schuett and H. Rapoport, *J. Am. Chem. Soc.*, **84**, 2266–2267 (1962).
(35) J. L. Wong, M. S. Brown, K. Matsumoto, R. Oesterlin, and H. Rapoport, *J. Am. Chem. Soc.*, **93**, 4633–4634 (1971).
(36) J. L. Wong, R. Oesterlin, and H. Rapoport, *J. Am. Chem. Soc.*, **93**, 7344–7345 (1971).
(37) E. J. Schantz, V. E. Ghazarossian, H. K. Schnoes, F. M. Strong, J. P. Springer, J. O. Pezzanite, and J. Clardy, *J. Am. Chem. Soc.*, **97**, 1238–1239 (1975).
(38) J. Bordner, W. E. Thiessen, H. A. Bates, and H. Rapoport, *J. Am. Chem. Soc.*, **97**, 6008–6012 (1975).
(39) H. A. Bates and H. Rapoport, *J. Agric. Food Chem.*, **23**, 237–239 (1975).
(40) Y. Shimizu, M. Alam, Y. Oshima, and W. E. Fallon, *Biochem. Biophys. Res. Commun.*, **66**, 731–737 (1975).
(41) Y. Shimizu, L. S. Buckley, M. Alam, Y. Oshima, W. E. Fallon, H. Kasai, I. Miura, V. P. Gullo, and K. Nakanishi, *J. Am. Chem. Soc.* **98**, 4514–4516 (1976).
(42) L. J. Buckley, M. Ikawa, and J. J. Sasner, Jr., *J. Agric. Food Chem.*, **24**, 107–111 (1976).
(43) T. Noguchi, S. Konosu, and Y. Hashimoto, *Toxicon*, **7**, 325–326 (1969); Y. Hashimoto, S. Konosu, T. Yasumoto, A. Inoue, and T. Noguchi, *ibid.*, **5**, 85–90 (1967).

in the Ryukyu and Amami islands. As far as is known, the crab toxicity has not been linked to a known algal bloom, nor have other invertebrates been implicated so far.

Ciguatoxin

Among the intoxications that are caused by marine organisms and that constitute a real or potential public health hazard, an affliction called ciguatera remains one with many unanswered questions. Two recent reviews[44,45] should be consulted for comprehensive background and literature citations. The word ciguatera is of Spanish origin and perhaps was first used by the Spanish conquistadores in Cuba to describe the illness that was apparently caused by eating a marine mollusk, *Turbo pica* or *cigua*. Current use of the word ciguatera refers to a human disorder that ensues following ingestion of a number of tropical and subtropical coral reef fishes, but rarely if ever of pelagic fishes from the tropics or of coral reef invertebrates. Although the name ciguatera was coined in the Caribbean, similar or identical fish poisonings were also reported from the Pacific, where at present ciguatera is of widespread occurrence. The crew of the Spanish explorer Pedro Fernandez Quiros came down with an illness that was ascribed to eating infected fish, probably snapper, off the New Hebrides in 1606. A carefully documented early account of ciguatera intoxication also occurred in the New Hebrides in 1774: members of Captain Cook's H.M.S. *Resolution* became ill after eating what was probably red snapper. The fish viscera were sufficiently toxic to kill a pig that ate the fish entrails aboard the vessel.

Clinical ciguatera symptoms are neurological and gastrointestinal and include tingling of the lips, mouth, and fingertips, itching of the skin, reversal of temperature sensation, loss of motor ability, vomiting, and diarrhea. The disease is rarely fatal, but is prolonged and disabling and tends to recur upon ingestion of seemingly nontoxic fish.

Over the years many species of fish have been reported to cause ciguatera.[45] Bagnis, who has studied ciguatera in French Polynesia, found that 96% of some 2800 clinical cases could be traced to ten families of fishes, most prominent among them the surgeon fishes.[46]

Reports of ciguatera outbreaks from Pacific archipelagoes have often been characterized by narrow geographic limits. A species of fish, but rarely all individuals of a given species, would be toxic at one reef, and not at another a short distance away. These repots, plus the sporadic, unpredictable, and sudden incidence of outbreaks that were apparently not linked to seasonal events, have made ecological hypotheses tenuous at best. We do know[44] that, initially, herbivorous, then carniverous, fishes become ciguateric and that the causative toxin will accumulate and remain stored in the large carnivores, principally in the liver and viscera, for many years.[47] Recent observations by Yasumoto[48] in the Society and Gambier islands have for the first time provided a meaningful clue to the origin of the toxin. Yasumoto compared the microflora of toxic and nontoxic reefs and was able to implicate an organism of as yet unconfirmed identity, perhaps a dinoflagellate. This discovery if substantiated raises an immediate question. Why should the etiology and ecology of tropical ciguatera and of temperate zone shellfish poisoning differ so radically if both toxins originate in a phytoflagellate? The answer must lie in the fundamental differences between a highly compartmentalized tropical reef ecosystem with its unique food web and the ecosystems of temperate zone continental shelves. The long persistence of ciguatoxin in the large carnivores has a parallel in the prolonged storage of saxitoxin in the siphon of the Alaskan butter clam.

One of the many difficulties in the isolation and characterization of the causative toxin, ciguatoxin, lies in the fact that only two assay animals have been found that will suffer observable and characteristically ciguateric symptoms after a meal of toxic fish: cats and mongooses. These animals, which must be fed raw fish at 10 to 15% of body weight, are relatively large, thus prohibiting the screening of small fish for possible toxin isolation. While French Polynesia has been for some years the richest known area for ciguatoxic fish, the most abundant of the affected species are small herbivorous surgeon fishes (principally *Ctenochaetus striatus*, "maito") that cannot be individually screened. In our work we have therefore concentrated on the large carnivores from accessible areas, first red snappers (*Lutjanus bohar*) from Palmyra Island, and more recently moray eels (*Gymnothorax javanicus*) from Johnston Island,[49] 1100 km southwest of Hawaii and under U. S. jurisdiction. The eels have to be speared individually, frozen, and returned to Hawaii, where each fish is screened for toxicity by mongoose feeding. On the average, 20% of the specimens will be toxic. One kilogram of highly toxic fish muscle (killing the assay mongoose) will yield about 1 mg of TLC-homogeneous ciguatoxin. Since the skin, fat, and bones of the eel are nontoxic and constitute about 60% of the weight, 2.5 kg of toxic eel or 12.5 kg of eel (two fish) from a toxic reef have to be speared in order to produce 1 mg of toxin. The purification is monitored by mouse bioassay,[50] which again consumes relatively large amounts of toxin. Ciguatoxin is a viscous, UV-transparent oil of approximate molecular weight 1500. Its most prominent function is the hydroxy group, which has allowed us to prepare a stable, UV-active, but noncrystalline 3,5-dinitrobenzoate. We have been unable to obtain mass spectral signals either from the pure toxin or its derivative, even by field desorption techniques.[51] Proton NMR spectra of the toxin indicate that ciguatoxin is a hydroxylated lipid molecule with little olefinic character and without observable proton signals below δ 6.[51]

If Yasumoto's recent observation[48] is confirmed, an axenic culture of the ciguatoxin-producing organism can be attempted. Sufficient toxin production will then permit structural elucidation, design of a simple

(44) P. J. Scheuer, *Adv. Food Res.*, **18**, 141–161 (1970).
(45) A. H. Banner in "Biology and Geology of Coral Reefs", Vol. III, O. A. Jones and R. Endean, Ed., Academic Press, New York, N.Y., 1975, pp 177–213.
(46) R. Bagnis, *Rev. Hyg. Med. Soc.*, **15**, 619–646 (1967).
(47) T. Yasumoto and P. J. Scheuer, *Toxicon*, 7, 273–276 (1969).
(48) T. Yasumoto, private communication, March 1976.
(49) P. J. Scheuer, W. Takahashi, J. Tsutsumi, and T. Yoshida, *Science*, **155**, 1267–1268 (1967).
(50) A. H. Banner, P. J. Scheuer, S. Sasaki, P. Helfrich, and C. B. Alender, *Ann. N.Y. Acad. Sci.*, **90**, 770–787 (1960); A. H. Banner, S. Sasaki, P. Helfrich, C. B. Alender, and P. J. Scheuer, *Nature (London)*, **189**, 229–230 (1961).
(51) D. B. Boylan and P. J. Scheuer, unpublished data.

screening test for toxic fish, and perhaps even an attempt at a rational therapy for ciguatera patients.

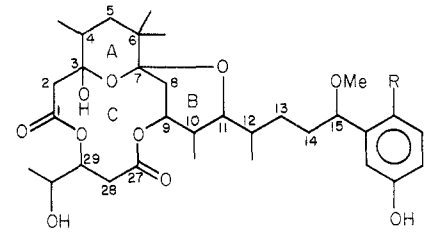
All three toxins, tetrodotoxin, saxitoxin, and ciguatoxin, have been studied because each is accumulated by marine organisms that are eaten by man. There is no record that the toxins to be discussed, the aplysiatoxins and palytoxin, have ever been a dietary human threat, which is not to say that their existence in marine biota has remained unobserved. In fact, both toxins have acquired a certain historical notoriety.

Aplysiatoxins

These compounds have been isolated from members of the gastropod molluscan family Aplysiidae or sea hares. Unlike many mollusks which possess spectacular shells, these invertebrates are without external shell and are generally inconspicuous. They are best known, at least among biologists, for their usefulness in physiological research. Their reputed toxicity had received little scientific attention, although it appears to have been known at least since Roman times. Halstead[52] quotes Jacques Grevin thus: "So deadly is the force of this poison that it poisons not only those who took it in by mouth, but also those who touched or looked at it, as Pliny reports, and if a pregnant woman sees it or even comes near it, especially if this happens to be a young woman, she immediately feels pain in the belly and nausea, and then she has an abortion".

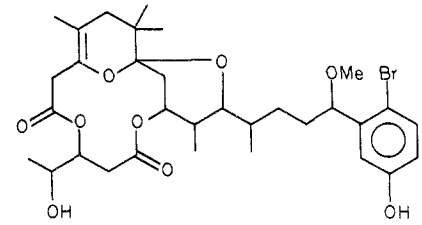
Flury[53] was the first modern investigator who attempted to define the toxic principle. More recently, Watson,[54] who studied several species of sea hares common to Hawaiian reefs, clearly linked the toxic properties of the animals to constituents of their midgut or digestive gland. Watson[54] further showed that not all species of sea hares are toxic. Early suspicion that digestive gland constituents are not true sea hare metabolites but are a reflection of the animals' algal diet was strengthened in recent years by isolation of a broad spectrum of organic compounds from sea hares[55-64] and was conclusively proven by Faulkner's isolation of identical metabolites from *Aplysia californica* and from their red algal diet.[65] The demonstration that sea hares will accumulate in their digestive glands a wide variety of dietary constituents enhances the credibility of unusual bioactivity which the Romans had ascribed to Mediterranean sea hares.[52]

Watson[54] had shown that the midgut toxin of several species of sea hares from Hawaiian reefs could be fractionated into water-soluble and ether-soluble entities that differed in pharmacological properties. In our work,[66-69] we isolated from the ether-soluble toxic fraction of *Stylocheilus longicauda* a mixture of two compounds, aplysiatoxin (**6a**) and debromoaplysiatoxin (**6b**), which were separable only after acetylation of the



6a, R = Br
b, R = H

phenol and secondary alcohol functions. These diacetates are nontoxic, as are the alcoholic monoacetates. Once we had determined reproducible UV spectral and TLC properties which allowed us to monitor the purification spectrophotometrically instead of by bioassay, we discovered that the alcoholic monoacetates occur naturally as sea hare metabolites. This lucky observation approximately doubled our supply of starting material for structural work. However, every compound in the series, the toxic hydroxyphenols and the nontoxic mono- and diacetates, possesses a tertiary hydroxyl at C-3, which is lost with great ease and leads to a parallel series of compounds, of which anhydroaplysiatoxin (**7**)



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is a representative. This spontaneous dehydration reaction takes place to varying degrees during chromatography, thereby doubling the number of components. The resulting pairs appeared to have identical composition since during electron impact mass spectrometry the dehydration reaction occurred in the inlet and was quantitative. Only during chemical ionization mass spectrometry and only with ammonia as the carrier gas did the C-3 hydroxyl survive.

Chemically, the aplysiatoxins are bislactones of 3,4-dihydroxyvaleric acid and a pentamethylpentahydroxy-ω-phenylpentadecanoic acid, constructed into three oxa rings, a trioxa 12-ring (C) and two tetrahydropyrans (A and B). The toxins have unique structures and do not fall into a simple or obvious biogenetic pattern.

In an attempt to uncover clues to the aplysiatoxin biogenesis we searched among the *Stylocheilus* lipids for nontoxic companion substances. Rose[70] succeeded

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(55) S. Yamamura and Y. Hirata, *Tetrahedron*, 19, 1485–1496 (1963).
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(59) D. J. Faulkner, M. O. Stallard, J. Fayos, and J. Clardy, *J. Am. Chem. Soc.*, 95, 3413–3414 (1973).
(60) D. J. Faulkner and M. O. Stallard, *Tetrahedron Lett.*, 1171–1174 (1973).
(61) F. J. Schmitz and F. J. McDonald *Tetrahedron Lett.*, 2541 (1974).
(62) D. J. Faulkner, M. O. Stallard, and C. Ireland, *Tetrahedron Lett.*, 3571–3574 (1974).
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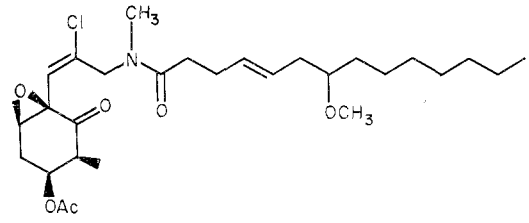
(64) L. Minale and R. Riccio, *Tetrahedron Lett.*, 2711–2714 (1976).
(65) M. O. Stallard and D. J. Faulkner, *Comp. Biochem. Physiol. B*, 49, 25–35, (1974); 49, 37–41 (1974).
(66) Y. Kato, "Toxic Constituents of the Marine Mollusk *Stylocheilus longicauda*", Ph.D. Dissertation, University of Hawaii, 1973.
(67) Y. Kato and P. J. Scheuer, *J. Am. Chem. Soc.*, 96, 2245–2246 (1974).
(68) Y. Kato and P. J. Scheuer, *Pure Appl. Chem.*, 41, 1–14 (1975).
(69) Y. Kato and P. J. Scheuer, *Pure Appl. Chem.*, in press.
(70) A. F. Rose, "Nontoxic Constituents of the Marine Mollusk *Stylocheilus longicauda*", Ph.D. Dissertation, University of Hawaii, 1975.

in elucidating the structures of two unique constituents, stylocheilamide (**8**) and its deacetyl analog (**9**), albeit



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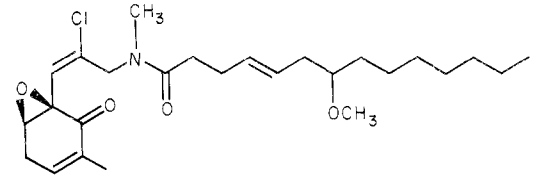

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of no apparent structural relationship to the aplysiatoxins (**6**).

Despite the unsavory reputation of toxic sea hares, we observed no ill effects from handling the animals. Manipulating the toxic extracts, however, produced reddening and swelling of mucous membranes of eyes and nose. Accidental skin contact with a toxic extract generated slow-healing sores.

As was mentioned earlier, there now exists compelling evidence[65] that the midgut constituents of sea hares are of dietary origin. We had our own demonstration of this phenomenon when we discovered that large colonies of *Stylocheilus longicauda*, which grew up in the eel tanks at Oceanic Institute of Makapuu, Oahu, were nontoxic.[71] Although Makapuu is only a few kilometers from one of our customary collection sites in Kaneohe Bay, *Stylocheilus* eggs or larvae were introduced into the eel tanks with the sea water intake, but in contrast to the natural habitat in Kaneohe Bay, the commercial eel diet contained no aplysiatoxin-harboring component.

Our own occasional search for an aplysiatoxin-producing macroalga at our collecting sites was fruitless. Recently, however, Moore and his collaborators[72] isolated debromoaplysiatoxin (**6b**) from several species of bluegreen algae collected at Enewetak atoll in the Marshall islands. These workers also showed that debromoaplysiatoxin (**6b**) is one of the algal metabolites with antileukemia activity. Interestingly, one of these bluegreen algae, *Lyngbia majuscula*, has long been associated in Hawaii with occasional outbreaks of a skin rash known as "swimmers' itch".[73] Our own observations of bioactivity during toxin isolation from *Stylocheilus* (vide supra) now finds an explanation in the established link between debromoaplysiatoxin and a dermatitis-producing blue-green alga.

Palytoxin

Somehow, it had never occurred to us to implicate blue-green algae as the aplysiatoxin-producing organism because of the implied[74] and later confirmed[65] connection between red algae and sea hare constituents, in California[65] and in Japan.[74] Ciguatoxin, on the other hand, had, according to an often cited hypothesis,[75] long been believed to be elaborated by a blue-green alga. Our interest in ciguatoxin and its potential algal precursor prompted us[76] some years ago to follow a lead provided by an entry in the Hawaiian–English Dictionary,[77] which lists under *limu* (seaweed), *limu-make-o-Hana*, the deadly seaweed of Hana. (Hana is a town on the island of Maui.) Further information on this intersting *limu* may be found in a book by David Malo,[78] the first chronicler of Hawaiian customs ". . . in Muolea, in the district of Hana, grew a poisonous moss in a certain pool or pond close to the ocean. It was used to smear on the spear points to make them fatal . . . The moss is said to be of a reddish color and is still to be found. It grows nowhere else than at that one spot." We soon learned that only few Maui islanders knew the location of "that one spot" and that even fewer would admit to knowing it, let alone show it to strangers. We further learned that a reason for this secretiveness is an ancient Hawaiian *kapu* (taboo), which holds that diaster will befall anyone who collects the toxic *limu*. The *kapu* in turn is traced to an Hawaiian legend,[79] according to which the *limu* in the pool became toxic after the body of a man was thrown into it when the people of Muolea discovered that the suspiciously acting man was part man and part shark.

Our first field collection on December 31, 1961, was an emphatic reminder of the old *kapu*, as it coincided with a fire of unknown origin that destroyed the main laboratory building of the Hawaii Institute of Marine Biology on Coconut Island. Cursory examination of the *limu* revealed that the organism was not an alga, but a coelenterate, probably *Palythoa* sp.,[80] and that the toxin was so potent and so fast acting that it could have no possible connection with ciguatera poisoning. We isolated and characterized the toxic principle, which we named palytoxin.[81]

In a fascinating parallel investigation, Hashimoto, who has also had a long interst in ciguatera, isolated a water-soluble toxin from a filefish, *Alutera scripta*, following field reports from the Ryukyu islands that the viscera of this fish when eaten by pigs would be lethal.[82] Hashimoto and co-workers[82] found that the gut contents of the toxic fish consisted mainly of a crushed zoanthid identified as *Palythoa tuberculosa*. Examination of the implicated zoanthid revealed an identical toxin, later shown to be palytoxin.[83]

A third independent discovery of a toxic Caribbean zoanthid[84] by the University of Oklahoma group led subsequently to isolation of palytoxin.[85] Observations

(71) We are indebted to Ross Nigrelli who told us of these *Stylocheilus* populations.

(72) J. S. Mynderse, R. E. Moore, M. Kashiwagi, and T. R. Norton, *Science*, in press.

(73) A. H. Banner, *Hawaii Med. J.*, **19**, 35–36 (1959).

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(75) J. E. Randall, *Bull. Mar. Sci. Gulf Carib.*, **8**, 236–267 (1958).

(76) I am much indebted to Professor A. H. Banner for the original suggestion and to Dr. P. Helfrich for making the first field collection possible.

(77) M. K. Pukui and S. H. Elbert, "Hawaiian-English Dictionary", 3rd ed, University of Hawaii Press, Honolulu, Hawaii, 1965, p 191.

(78) D. Malo, "Hawaiian Antiquities", B. P. Bishop Museum Special Publication 2, 2d ed., Honolulu, Hawaii, 1951, p 201.

(79) Manuscript notes by Katherine Livermore on file at B. P. Bishop Museum, Honolulu.

(80) The organism has since been designated *Palythoa toxica* [G. E. Walsh and R. L. Bowers, *Zool. L. Linnean Soc.*, **50**, 161–180 (1971).]

(81) R. E. Moore and P. J. Scheuer, *Science*, **172**, 495–498 (1971).

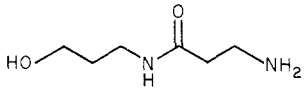
(82) Y. Hashimoto, N. Fusetani, and S. Kimura, *Nippon Suisan Gakkaishi*, **35**, 1086–1093 (1969).

(83) S. Kimura and Y. Hashimoto, *Publ. Seto Mar. Biol. Lab.*, **20**, 713–718 (1973).

(84) L. S. Ciereszko and D. H. Attaway, Annual Report, Petroleum Research Fund, Vol. 6, American Chemical Society, Washington, D.C., 1961, pp 107–108.

by the Oklahoma group indicated a seasonal toxicity cycle of the Caribbean zoanthids, unlike the Maui *P. toxica* which seems to be of uniform toxicity throughout the year. Hashimoto et al.[82] in their initial report had noted that toxicity of *P. tuberculosa* not only differed widely from colony to colony but even within a single colony of animals. In a careful follow-up study of this phenomenon the Japanese workers[86] found that toxicity of *P. tuberculosa* is confined to the female polyps, where it is greatest in the mature eggs, which are present from May through September. This link between observed toxicity and the reproductive cycle of an organism is yet another example of an intersting phenomenon. It will be recalled that saxitoxin results from the bloom of a dinoflagellate; that tetrodotoxin, at least in the salamander, is found principally in the eggs and embryos; and that recent observations point to a blooming phytoflagellate as the elaborator of ciguatoxin.

Palytoxin is unique in many respects. Its intravenous lethality exceeds that of tetrodotoxin or saxitoxin by at least an order of magnitude;[81] when the toxin is injected into dogs rather than mice, its lethality is increased about tenfold.[87] Its molecular size, about 3000 daltons, is much larger than conventional "small" molecules, as steroids, alkaloids, or antibiotics. Yet its lack of obvious repetitive units, amino acids, or common sugars sets the molecule apart from small peptides or oligosaccharides. As a consequence, no existing methodology is quite suitable for the structural elucidation of palytoxin. Current instrumentation offers only limited resolution for the intact molecule. Hydrolytic and oxidative reactions have tended to be destructive, while attempted enzymatic or reductive degradations have left the toxin either unaltered or modified only slightly. Mild acid hydrolysis of hydrogenated palytoxin has led to the identification of *N*-(3-hydroxypropyl)-3-aminopropionamide (**10**).[88]



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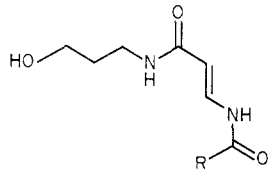

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(85) D. Attaway, "Isolation and Partial Characterization of Caribbean Palytoxin", Ph.D. Dissertation, University of Oklahoma, 1968.

(86) S. Kimura, Y. Hashimoto, and K. Yamazato, *Toxicon*, **10**, 611–617 (1972).

(87) J. S. Whiles, J. A. Vick, and M. K. Christensen, *Toxicon*, **12**, 427–433 (1974).

Spectral data of intact palytoxin and of model compounds have further shown that the UV maximum of the toxin at 263 nm arises from the corresponding, though unsaturated, structural moiety (**11**), an *N*-(3-hydroxypropyl)-*trans*-3-amidoacrylamide. This is a small but significant part of this large molecule. It contains two of an estimated four nitrogen atoms, and loss of the 263-nm chromophore in acid parallels loss of toxicity.

Few details of the pharmacology of the toxin have been published. Walsh and Bowers[80] describe its effect on humans: "A student collector who accidentally touched a colony to an open lesion suffered malaise, muscle pain, and abdominal cramps, and required hospitalization for two days". Beyond that, Quinn et al.[89] have reported antitumor properties of the toxin, while Kaul and co-workers[90] and Rayner et al.[91] have described its cardiac activity.

I began this selective Account of recent research on marine toxins by quoting a rather unredeeming sixteenth century viewpoint, which sees only punishment for those who pursue this research. A much more hopeful outlook is expressed by Shakespeare in *The Tempest*, Act I, Scene II:[92]

> *Full fadom five thy father lies;*
> *Of his bones are coral made;*
> *Those are pearls that were his eyes;*
> *Nothing of him that doth fade*
> *But doth suffer a sea-change*
> *Into something rich and strange.*

Shakespeare's view comes to mind as one hikes from the road near Hana over a black lava flow to the shore, to a single tidepool, perhaps 2 m × 60 cm, and only 50 cm deep at low tide, sparkling in the sun, which is the habitat of *limu-make-o-Hana.*

I am grateful to the Public Health Service, the National Science Foundation, NOAA, Office of Sea Grant (04-6-158-44026), and Hoffmann-La Roche for financial support of our marine toxins research.

(88) R. E. Moore, R. F. Dietrich, B. Hatton, T. Higa, and P. J. Scheuer, *J. Org. Chem.*, **40**, 540–542 (1975).

(89) R. J. Quinn, M. Kashiwagi, R. E. Moore, and T. R. Norton, *J. Pharm. Sci.*, **63**, 257–260 (1974).

(90) P. N. Kaul, M. R. Farmer, and L. S. Ciereszko, *Proc. West. Pharmacol. Soc.*, **17**, 294–301 (1974).

(91) M. D. Rayner, B. J. Sanders, S. M. Harris, Y. C. Lin, and B. E. Morton, *Res. Commun. Chem. Pathol. Pharmacol.*, **11**, 55–64 (1975).

(92) I am grateful to Alice Scheuer for calling this passage to my attention.