TOXINS OCCURRING IN COMMERCIALLY IMPORTANT MARINE ORGANISMS

by

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Abstract

Thousands of aquatic animal species, some of which affect our lives directly and/or indirectly, have been listed as poisonous or venomous. Marine animals which are important as food sources in the Indo-Pacific region often cause poisoning. Puffer fish poisoning, ciguatera poisoning and paralytic shellfish poisoning present the most serious problems for the fishing industry, as well as for the food hygienist and have been studied extensively for the past twenty years. Poisonings from fish roes and wax-rich fish are also worthy of note. Marine toxins are known to be synthesized by the causative organisms or to be accumulated through food chains; they are not bacterial in origin. On the other hand, some marine organisms are known to cause environmental damage such as mass mortalities of commercially important fish and shellfish. In this review, the current state of our biological and chemical knowledge of these toxins is presented.

INTRODUCTION

Marine organisms have been a source of food for human beings from the dawn of history. In recent years, the rapid growth of the world's population has caused us to pay more attention to marine fauna and flora as food and to utilize hitherto unexploited species.

Thousands of marine organisms have been listed as poisonous or venomous, some of which cause discomfort, illness or even death in man (Halstead, 1965, 1967, 1970). More and more toxic species will be found as fishing activities increase and it is therefore very important to accumulate a knowledge of toxic species and their toxic factors, in order to utilize marine food sources more efficiently and to safeguard human health. We must also pay attention to the increasing number of red-tide outbreaks which have caused losses of commercially important fish and shellfish. However, little is known of the red-tide organisms and their toxins (Fusetani, 1976).

In this review, the current state of our knowledge of toxins in marine organisms will be discussed. Emphasis will be given to toxins isolated from fish and shellfish which are valuable food sources.

TOXINS FROM FISH

Tetrodotoxin

Poisoning from puffer fish is perhaps the best documented marine intoxicant. Historically, human poisoning was reported in the Chinese medical literature 2 000 years ago (Tsuda, 1966). Puffer fish poisoning has been a public health problem in Japan because 'fugu' (puffer fish) in a culinary delicacy. Many incidents of puffer poisoning have occurred and even now, although only licensed cooks may prepare fugu dishes in restaurants, around 100 people are poisoned every year (Halstead, 1967).

The toxic principle, tetrodotoxin, appears to be concentrated mainly in the ovaries and liver of some species belonging to the Tetraodontidae (Halstead, 1967). It is noteworthy that some tropical puffers (e.g., *Lagocephalus lunaris lunaris*) possess a high concentration of the toxin in the muscle (Berry and Hassan, 1973). The pure tetrodotoxin was first isolated in 1948 by a Japanese chemist. After extensive studies the structure of tetrodotoxin (Appendix 1) was elucidated in four laboratories simultaneously in 1964 (Tsuda, 1966; Goto *et al.*, 1965; Woodward, 1964; Mosher *et al.*, 1944) Tetrodotoxin is a weakly basic (pKa 8.5) compound with a composition $C_{11}H_{17}N_3O_8$. It shows novel structural features; only one carbocyclic ring, a guanidine moiety, six hydroxyl groups and an unprecedented hemilactal function. A total synthesis of tetrodotoxin has been accomplished by Kishi *et al.*, 1972.

Tetrodotoxin has been isolated from several unrelated animal species. Moguchi and Hashimoto (1973) isolated the toxin from the goby, *Gobius criniger*, which has caused human poisoning in the Philippines and in Taiwan. Mosher's group (1964) reported that the toxic principle, originally named taricha-toxin, found in the eggs of the California newt, *Taricha torosa* was identical to tetrodotoxin. More recently, they have detected tetrodotoxin in the skin of Costa Rican frogs of the genus *Atelopus* (Kim *et al.*, 1975). The wide distribution of tetrodotoxin in phyletically unrelated animals is puzzling when the biogenesis of the toxin is considered.

Symptoms of puffer fish poisoning are numbness and tingling of the lips, tongue and mouth, nausea, vomiting, diarrhoea and in severe cases, respiratory paralysis followed by death. The mortality rate has been estimated to be greater than 60 percent. The pure tetrodotoxin shows a toxicity of LD_{50} 9 μg/kg in mice. It appears to block nerve conduction by specifically interfering with the initial passive increases in sodium permeability of the membrane (Narahashi, 1972). Tetrodotoxin has become an indispensable pharmacological reagent in understanding the mechanism of membrane permeability. No specific antidote or recommended specific treatment for the poisoning is known as yet.

Ciguatera toxins

Ciguatera food poisoning is caused by ingestion of a large variety of sub-tropical and tropical fish associated with coral reefs, but rarely of coral reef invertebrates (Halstead, 1967). Outbreaks of ciguatera have been reported from various areas in the Caribbean, Pacific and Indian oceans where many ciguateric fish are commonly eaten. Therefore, the problem of ciguatera is of paramount importance in public health terms.

Symptoms of ciguatera have neurological and gastrointestinal phases which include tingling of the lips, mouth and fingertips, itching of the skin, reversal of temperature sensation, loss of motor ability, vomiting and diarrhoea. The disease is rarely fatal but is prolonged; recovery may take months (Bagnis, 1968). It has also been observed that a person who has suffered from ciguatera becomes somehow sensitized so that he may exhibit symptoms even if he eats non-toxic fish.

A large number of fish have been listed as causing ciguatera. Halstead (1967) listed well over 400 species but Bagnis (1967), who surveyed ciguatera in French Polynesia, considered 32 species in 15 families to have produced the disease. In general, ciguateric species are limited to those fish that feed on algae or detritus on coral reefs and the larger reef carnivores that prey on these herbivores. They mostly belong to the Acanthuridae, Scaridae, Carcharhinidae, Muraenidae, Carangidae, Labridae, Lutjanidae, Lethrinidae, Serranidae and Sphyraenidae. At present, it is generally accepted that the principal toxin in these ciguateric fish is ciguatoxin which was isolated and named by Scheuer *et al.*, (1967). The species of fish in which the presence of ciguatoxin has been confirmed experimentally are few but include such important species as the red snapper, *Lutjanus bohar;* moray eel, *Gymnothorix javanicus;* amberjack, *Seriola aureovittata;* grouper, *Epinephelus microdon;* parrotfish, *Scarus gibbus;* shark, *Carcharhinus menisorrah* and surgeonfish, *Ctenochaetus striatus* (Scheuer *et al.*, 1967; Hashimoto and Fusetani, 1968; Yasumoto *et al.*, 1971; Chanteau *et al.*, 1976; Chungue *et al.*, 1977).

Ciguatoxin has not yet been obtained in pure form but the most purified preparation showed a toxicity of 8 μg/kg in mice (Chanteau *et al.*, 1976). Little is known of the structural features; a hydroxylated lipid molecule of approximate molecular weight 1 500 (Scheuer, 1977). The toxin is positive to Dragendorff reagent. Ciguatoxin has a rather widespread action on excitable membranes involving an initial increase in excitability followed by conduction block; this might be explained by an increase of sodium ion permeability. This action is antagonized by either tetrodotoxin or calcium ions (Banner, 1976).

Numerous difficulties prevent development of anti-ciguatera measures. The major difficulties are:

(a) No simple and reliable bioassay method has been developed (currently, feeding tests on cats or mongooses are adopted)

(b) Variability of toxicity in ciguateric fish is extremely high

(c) Isolated geographic distribution of toxic fish (fish can be toxic at one reef and not at another a short distance away)

(d) Fish become toxic sporadically, unpredictably and suddenly (Banner, 1976)

The phenomena listed above support the food chain hypothesis originally advanced by Randall (1958). Over the years, many organisms have been suggested as the original elaborator of the toxin. Quite recently, Yasumoto and collaborators (1977) reported that the dinoflagellate *Diplopsalis* sp. *nov*, collected from detritus on the coral reefs of French Polynesian islands produced ciguatoxin and maitotoxin. It would seem reasonable to assume that this organism is picked up concomitantly by herbivorous animals and the accumulated toxin is then transmitted to carnivores.

The complexity of clinical symptoms of ciguatera poisoning indicates that toxins other than ciguatoxin are involved in ciguateric organisms (Hashimoto, 1976). Recently, a fat-soluble secondary toxin, named scaritoxin, has been isolated along with ciguatoxin from the muscle of the parrotfish, *S. gibbus,* which frequently causes human poisoning in French Polynesia (Chungue *et al.,* 1977). Scaritoxin occurs as the major toxin in the muscle of *Scarus* spp. and shows a close similarity to ciguatoxin in biological and chemical properties. Yasumoto and co-workers (1971, 1976) isolated three toxins, ciguatoxin, maitotoxin and an unknown toxin, from the viscera and gut contents of the herbivorous surgeonfish, *C. striatus,* the most well-known ciguateric species in Tahiti. Maitotoxin reveals a lipoproteinic nature and gives rise to fatty acids, sugars and 15 amino acids on hydrolysis. It is toxic to guppies as well as to mice (MLD 15-20 mg/kg). Ciguatoxin, scaritoxin and maitotoxin were also found in the viscera of the turban shell, *Turbo argyrostoma,* which provoked ciguatera-like poisoning in 1968 in Marcus Island (Yasumoto and Kanno, 1976). Palytoxin has been detected in the gut contents and viscera of the filefish, *Alutera scripta,* that was once labelled as a ciguateric fish (Hashimoto *et al.,* 1969; Kimura and Hashimoto, 1973). The toxin was shown to be accumulated by the fish that fed on the toxic zoanthid *Palythoa tuberculosa.*

Dinogunellin

Poisoning caused by eating fish eggs other than the roe of puffer fish is also known. The symptoms are characterized by gastrointestinal disorders including vomiting, diarrhoea and abdominal pain. Halstead (1967) listed over 50 species which cause this disease and suggested the term 'ichthyootoxins' for the causative agents.

In northern Japan, the roe of the northern blenny, *Stichaeus grigorjewi,* has long been known to be toxic and caused a number of human poisonings. The toxic factor, dinogunellin, has been obtained pure and identified as a lysophospholipid (Appendix 1), with a unique structure (Hatano and Hashimoto, 1974; Hatano *et al.,* 1976). Dinogunellin is present in the roe as a lipoprotein. Recently, the toxin in the roe of the cabezon, *Scorpaenichthys marmoratus,* a well known ichthyootoxic species on the Pacific coast of North America, was found to be identical to dinogunellin (Hashimoto *et al.,* 1976). It would be interesting to know whether dinogunellin is common in other ichthyootoxic fish.

Wax esters and diacyl glyceryl ethers

Recent fisheries activities in the deep seas have evoked a new type of poisoning from the consumption of fish flesh, in which non-glyceridic lipids are predominant. These fish, known as 'diving fish' undergo vertical migrations of some hundreds of metres. The castor oil fish, *Lepidocybium flavobrunneum* and *Ruvettus pretiosus,* and lantern fish (family Myctophidae) were shown to have a high oil content in the muscle. The oil consisted predominantly of wax esters (Appendix 1) and produced diarrhoea and seborrhoea in assay animals (Mori *et al.,* 1966; Nevenzel *et al.,* 1969).

On the other hand, stromatid fish such as *Stromateus maculatus, Cubiceps gracilis* and *Centrolophus* sp. have been reported to contain high percentages of diacyl glyceryl ethers (Appendix 1); approximately 90 percent of unsaponifiable matters (Mori *et al.*, 1972). Iida (1971) suggested a possible toxicity of diacyl glyceryl ethers. Great care must be taken with the muscle lipids of fish caught by deep sea trawlers.

TOXINS FROM SHELLFISH

Paralytic shellfish poisons

Sporadic outbreaks of poisoning from the ingestion of mussels and clams along the Pacific coast of North America have long been known. From the symptoms, which consist of numbness of lips, tongue and fingertips, muscular incoordination, respiratory distress and death, the intoxication has been called 'paralytic shellfish poisoning' (Halstead, 1970). It was not established until 1939 that molluscs become toxic from accumulation of the toxic red-tide organism *Gonyaulac catenella* (Sommer *et al.*, 1937). The shellfish become too toxic for humans to consume when the dinoflagellates reach a count of 100-200 cells/ml.

The toxic principle, saxitoxin, was first isolated from the Alaskan butter clam, *Saxidomus giganteus* (Schuett and Rapoport, 1962). It was later found to be identical to the toxin obtained from axenic cultures of *G. catenella* and to the toxin from the mussel, *Mytilus californianus* (Schantz *et al.*, 1966). Chemical studies have been made using saxitoxin obtained from cultured *G. catenella.* Saxitoxin is a diacidic base (pKa 8.1, 11.5) of low molecular weight ($C_{10}H_{17}N_7O_4$, M. W 299). The structure (Appendix 1), was first presented by Rapoport's group (Wong *et al.*, 1971), but was later revised by Schantz *et al.*, (1975), who applied x-ray diffraction method to a crystalline bis (*p*-bromobenzenesulfonate)

Saxitoxin has approximately the same lethality as tetrodotoxin (LD_{50} 9 μg/kg in mice). It acts as a neuromuscular blocking agent and inhibits conductance of sodium ions as does tetrodotoxin (Narahashi, 1972).

Along the North Atlantic coasts of America and Great Britain, blooms caused by the toxic dinoflagellate, *G. tamarensis,* have also presented public health and economic problems. Shimizu and co-workers (1975) recently isolated saxitoxin and three new toxins, gonyautoxins I-III, from cultures of *G. tamarensis* and the soft shell clam. *Mya arenaria,* infested with the dinoflagellate. The structures of gonyautoxins, II and III have been elucidated mainly by spectral data (Shimizu *et al.*, 1976) (Appendix 1). It was quite recently reported that three more toxins, gonyautoxin IV, gonyautoxin V and neosaxitoxin, were detected in *G. tamarensis* (Oshima *et al.*, 1977).

Red tides caused by *Gonyaulax* sp. have been observed recently along the Japanese coast. It was found that the Japanese shellfish poison is a mixture of saxitoxin and five new compounds including gonyautoxins I-III as is the Atlantic poison (Oshima *et al.*, 1976).

Saxitoxin has also been detected in such warm-water organisms as xanthid crab, *Zosimus aeneus,* in the Amami Islands, green turban shell, *Turbo marmorata,* in the Ryulyus and Palauan bivalves (Noguchi *et al.*, 1969; Yasumoto and Kotaki, 1977; Kamiya, personal communication). This may indicate that marine organisms other than *Gonyaulax* spp. produce saxitoxin. It should be noted that paralytic shellfish poisoning occurred in Papua New Guinea, where shellfish were considered to be infested by the dinoflagellate *Pyrodinium bahamense* (Worth *et al.*, 1975).

Surugatoxin

The carnivorous gastropod, *Babylonia japonica,* commonly eaten in Japan, sometimes causes human intoxication, which may be fatal. Symptoms are largely gastrointestinal and neurological. Hashimoto (1968) and Shibota and Hashimoto (1970), found that the toxic extract produced dramatic mydriasis in cats and mice. On purification, a light yellow powder was obtained which evoked mydriasis in mice at a minimum dose of 20 μg/kg.

Independently of Hashimoto's group, Kosuge and co-workers (1972) succeeded in crystallizing the toxic factor, surugatoxin, as a heptahydrate and determined its structure by x-ray diffraction (Appendix 1). Surugatoxin consists of 6-bromoxyindole, pteridine and myoinositol. It has a strong anti-nicotinic activity on ganglia.

Recently, a gram-positive bacterium (tentatively named F-2), isolated from the bottom mud of the toxic area, was reported to produce a surugatoxin-like substance (Yamamoto *et al.*, 1976). This may suggest that the gastropod becomes toxic by accumulation of the toxic bacterium.

OTHER MARINE TOXINS

Many red-tide organisms are known to produce toxic substance which cause mass mortality of fish or shellfish and shellfish infestations. Most of them belong to the dinoflagellates, among which *Gonyaulax* spp. are most well known.

Another well-known species, *Gymnodinium breve,* which frequently causes massive fish kills along the coast of the Gulf of Mexico, was found to contain a variety of toxic substances (Alam, 1975). Shimizu and co-workers (1976) isolated a pure toxin with an LD_{50} 0-5 mg/kg (mice) which was considered to be a steroid attached to a large polar moiety. The related species *G. nagasaki,* was reported to cause mass mortality of cultured yellowtail *Seriola quinqueradiata* in Japan (Fusetani, 1976).

The dinoflagellate *Noctiluca miliaris* was found to secrete a large amount of ammonia which causes massive fish kills in Japan (Okaichi and Nishio, 1976). Such toxic dinoflagellates as *Rhodomonas baltica, Exuviaella* sp. and *Hornellia* sp. are known to cause mass mortality of fish along the coast of Japan, though their toxic principles are poorly characterized (Fusetani, 1976).

CONCLUSION

Although our awareness of the sea has been rapidly growing, our knowledge of toxic factors in marine organisms lags far behind our knowledge of toxins elaborated by terrestrial organisms. Less than 1 percent of toxic marine organisms has been examined for their chemical properties and pharmacological activities. We need to expand our research effort in this area and increase our basic knowledge of toxic marine flora and fauna in order to utilize marine resources more efficiently.

Recently, another approach has been made toward toxic marine organisms. Exploitation and investigation to develop drugs from the hitherto 'untapped' ocean have been attempted (der Marderosian, 1969). It is natural to consider the vast aquatic environment, in which approximately 80 percent of the earth's animal life lives, as a potential source of drugs. Indeed, many marine toxins isolated so far have remarkable biological activities and chemical properties; some valuable drugs have been developed from them. A new type of insecticide (Cartap) has been derived from the toxin principle (nereistoxin) isolated from the marine worm, *Lumbriconereis heteropoda* (Hashimoto *et al.*, 1973). Tetrodotoxin and saxitoxin are valuable tools in neurophysiology research as pharmacological reagents. (Narahashi, 1972).

In conclusion, we need to accumulate more knowledge of toxic marine creatures.

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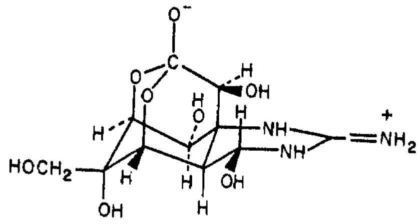
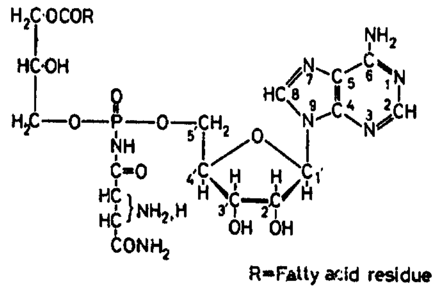
Appendix 1
Structural Formulae



Tetrodotoxin



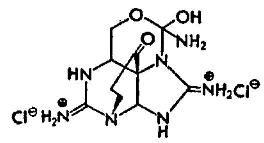
I Dinogunellin

R_1OCOR_2

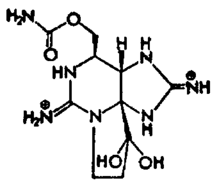
H_2COR_1
$HCOCOR_2$
H_2COCOR_3

Wax ester

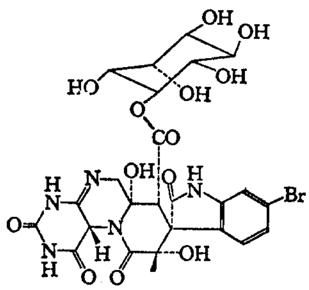
Diacyl glyceryl ether



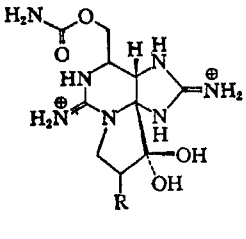
Saxitoxin
(according to Wong *et al.*, 1971)



Saxitoxin
(According to Schantz *et al.*, 1975)



Surugatoxin



Gonyautoxin
R= a–OH, I
R= B–OH, II