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PHYCOTOXINS FROM BLUE-GREEN ALGAE

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Abstract - Unpredictable poisonings of livestock and wildlife by waterblooms or scums of freshwater cyanophytes (cyanobacteria) occur when toxic strains of the common species dominate composition sufficiently to provide above-threshold levels of phycotoxins by susceptible animals. Known phycotoxins from freshwater cyanophytes are alkaloids, polypeptides or pteridines. New toxins produced by new strains have been described recently. Freshwater cyanophyte waterblooms can cause allergic reactions and, in water supplies, are suspected of causing outbreaks of human gastro-enteritis. The structures of dermatitis-producing and non-dermatitis-producing phycotoxins from species of marine cyanophytes have been reported recently.

INTRODUCTION

Species of marine algae have been gathered and used for food in Japan and China for centuries. To a lesser extent they have been gathered and used in Europe, North America, and other parts of the world as well. In recent times much research has been done to develop methods for culturing, harvesting and drying both seaweeds and freshwater micro-algae. These methods are being utilized more and more in different countries to supply food for man or for livestock. The chief micro-algae that are being grown for human or animal consumption are eukaryotic chlorophytes, such as Chlorella and Scenedesmus. The coccoid bloom-forming cyanophyte Microcystis is the principal species which has long been grown on human and animal wastes as a food source for fish in manmade ponds and lagoons in India, Pakistan and other countries in south-east Asia. Another cyanophyte, Spirulina, traditionally eaten by people in central Africa and Mexico is now being cultured, or being considered for culture, as a food in Belgium, Mexico, Peru, California, Israel, South Africa and Thailand (16). Freshwater cyanophytes are potentially excellent sources of food because they have a high protein content and good digestibility. They also grow quite rapidly and can be cultured relatively easily on by-product or waste sources of nutrients. However, one of the serious drawbacks to using bloom-forming cyanophytes as food for livestock or man is the fact that toxic strains of several species of cyanophytes are now known to exist which are usually morphologically indistinguishable from non-toxic strains.

Poisonings of livestock and other animals attributed to toxic blooms of freshwater cyanophytes occur with considerable frequency but unpredictably in western Canada and mid-western United States, and in similar climatic and geographic regions of South America, Europe, Asia, South Africa and Australia. These poisonings can cause considerable economic loss and hence are of concern to veterinarians. They are also of some concern to wildlife biologists, environmentalists and public health officials. More than 12 species belonging to 9 genera of freshwater cyanophytes have been implicated in case histories of animal poisonings, but toxic strains of the three most common bloom-forming species, Microcystis aeruginosa Kütz. emend Elenkin, Anabaena flos-aquae (Lyngb.) de Bréb. and Aphanizomenon flos-aquae (L.) Ralfs. have been responsible for most of the episodes. There are fewer reports of toxic marine than freshwater cyanophytes. Collections of Lyngbya majuscula Gomont and a mixture of Oscillatoria nigroviridis Thwaites and Schizothrix calcicola (Ag.) Gom. from certain sites have been found which produce toxins that kill mice and are responsible for sporadic outbreaks of a contact dermititis in Hawaii, Okinawa and other Pacific islands.

The literature on toxic cyanophytes has been reviewed by Ingram and Prescott (43), Olson (60), Vinberg (78), Gorham (29), Schwimmer and Schwimmer (69, 70), Gentile (25), Shilo (71), Moore (56), Kirpenko et al. (48), Collins (19), Gorham and Carmichael (30) and Carmichael and Gorham (16). Chemical identities of the toxins and their toxicological and pharmacological properties are still very incomplete. So, too, is our knowledge of the many ecophysiological factors which must interact to produce a toxic episode. It requires the growth and concentration of an algal population that is sufficiently dominated by toxic strains(s) for a threshold or larger dose to be acquired by a susceptible animal. In what follows we shall

survey what is currently known about the phycotoxins of blue-green algae or cyanophytes and their properties, starting with the freshwater species and ending with the marine species.

MICROCYSTIS TOXINS

Louw (52) was one of the first to isolate and attempt to identify a phycotoxin from a bloom dominated by a species of Microcystis, called M. toxica Stephens, that developed in the Vaal Dam reservoir in South Africa in 1942-43. He concluded that it was an alkaloid with acute and chronic hepatotoxic properties of undetermined structure. Hughes et al. (42) were the first to obtain a toxic colony isolate of Microcystis aeruginosa, designated NRC-1, from a non-toxic bloom dominated by another species, which was collected from Little Rideau Lake, Ontario, Can. in 1954. It produced a Fast Death Factor (FDF), later called microcystin (mcyst) (50) which, with large differences in species tolerances, killed mice, rabbits, guinea pigs, lambs, calves, and chickens by both the oral and intraperitoneal routes. Enlargement and congestion of the liver with necrosis of the hepatic cells and punctate haemorrhages were constant and pathognomic. For mice, a minimal lethal dose (LD_{min}) caused a latent period of about 30 min, followed by signs of convulsions, pallor and death in about 60 min. Freeze-dried NRC-1 cells were sent to So. Africa and Dr. D. G. Steyn reported that they produced signs of poisoning in laboratory animals that were indistinguishable from those produced by the M. toxica bloom and toxin that he and Louw had studied previously. Bishop et al. (9) found that mcyst was not an alkaloid. It was a dialyzable, stable, acidic, probably cyclic peptide containing 7 amino acids (Table 1) with an estimated molecular weight of 1300-2600. The LD_{min} (i.p. mouse) was 0.47 mg kg^{-1}. Murthy and Capindale (57) later found that mcyst, isolated from a culture of NRC-1 by a different procedure had an LD_{min} (i.p. mouse) of 0.1 mg kg^{-1}, produced the same toxic signs, but had different electrophoretic properties and had 7 other amino acids in addition to the original 7 (Table 1).

TABLE 1. Amino acid composition of Microcystis toxins from Canada, Australia and U.S.S.R.

Canada[1] (mcyst)			Australia[2]		U.S.S.R.[3] (16.6% component)	
moles	%	%	moles	%	%	%
Asp (1)	14	Thr 7	β CH_3-Asp (1)	Asp 5	Thr 16	Lys 2
Glu (2)	14	Pro 2	D-Glu (1)	Glu 12	Pro tr	Cys 7
D-Ser (1)	4	Gly 6	D-Ala (1)	Ser 5	Gly 8	His 3
Val (1)	5	iLeu 4	Met (1)	Val + Met 4	- -	
Orn (1)	4	Tyr 2	Tyr (1)	- -	Tyr 5	
Ala (2)	15	Phe 1	+	Ala 9	Phe 4	
Leu (2)	15	Arg 7	CH_3NH_2 (1)	Leu 16	Arg 5	

[1] From Bishop et al. (9) in moles; from Murthy and Capindale (57) in per cent

[2] From (23)

[3] From (47)

Rabin and Darbre (64) using a more rapid adaptation of the Murthy and Capindale extraction procedure isolated a single toxic peptide with a molecular weight of 1750 ± 450 from freeze-dried cells of NRC-1 that gave most of the common protein amino acids and ornithine upon hydrolysis. NRC-1 was meanwhile found (27) to be a mixture of two strains and not clonal, as originally assumed. This may have been a contributing factor to the differences in structural composition of the toxins obtained from it at various times.

Elleman et al. (23) used an altered procedure of Murthy and Capindale and obtained a high recovery of toxin from a potent bloom of M. aeruginosa that occurred in Malpas Dam Reservoir, New South Wales, Australia in 1973. They found a single peptide with an LD_{100} (i.p. mouse) = 0.07 mg kg^{-1} which produced the same toxic signs as mcyst including extensive liver haemorrhage, and death within 1 to 3 h. The preparation had no free amino group, and was composed of equimolar quantitites of five amino acids and methylamine (Table 1). On the basis of mass spectrometry and the amino acid analysis the minimum molecular weight was calculated as 654. It was noted that a cyclic structure or a dimeric molecule could not be excluded.

Kirpenko et al. (47) have reported on the structure of a toxin isolated by ion exchange resins from an M. aeruginosa bloom collected from the Dnieper basin. It killed rats, had a molecular weight of 19,400 ± 1,400, and consisted of acetomethylene, isothiocyanate and carbohydrate groups, hydrogen and disulphide bonds and 16.6% peptide. The latter contained the same 12 amino acids reported by Murthy and Capindale plus four others (Table 1).

Toerien et al. (76) have reinvestigated the nature of Microcystis toxin found in South Africa. Using a modified Murthy and Capindale procedure they have isolated two toxic peptides from a bloom collected from the Hartbeesport Dam in 1974 which consisted of 90% M. aeruginosa forma aeruginosa (that Komáreck (49) believes is identical to M. toxica). The toxic signs produced in rats and mice were similar to those produced by mcyst. Work is continuing on the structure of the peptides that are described as similar to the ones found in Canada. They have concluded that the earlier suggestion of Louw (52) that Microcystis toxin is an alkaloid was erroneous.

Carmichael and Gorham (16) have recently isolated toxic clones of M. aeruginosa from lakes in Alberta and Saskatchewan, Can. during the summers of 1975-77 which produce signs of poisoning that differ from those of mcyst. They produce signs of general weakness followed in 1 - 2 h by cardiovascular collapse. Pronounced liver damage is, however, observed. Since the signs of poisoning resemble those produced by type-c Anabaena toxin (antx-c) they have been designated type-c Microcystis clones. Carmichael (unpublished) has separated Microcystis type-c toxins into two low-molecular-weight toxic fractions. One is a peptide with hepatotoxic properties like mcyst. The other causes death in mice by respiratory arrest which indicates a neurotoxin.

It would appear that there are a number of peptide or peptide-containing toxins of undefined structure produced by strains and blooms of Microcystis. These differ greatly in molecular weight, physical properties and amino acid composition. Most have cytotoxic properties which produce characteristic liver damage. However, because of the survival times involved, it is doubtful whether liver damage is the primary cause of death. Instead, recent work has indicated that neurotoxic activity located in the same or a different fraction as the hepatotoxic activity is the cause of death.

ANABAENA TOXINS

Olson (60), Firkins (24) and Rose (65) were among the first to investigate toxic blooms of Anabaena flos-aquae (An. Lemmermanii) associated with cases of animal poisonings in Minnesota and Iowa. Gorham et al. (32) were the first to report toxic and non-toxic colony isolates of An. flos-aquae from toxic blooms collected from Burton Lake, Saskatchewan in 1960 and 1961. Lethal i.p. or oral doses of toxic strain or bloom killed mice in 1-4 min preceded by signs of paralysis, tremors and mild convulsions. The signs of poisoning were indistinguishable from those observed in Minnesota and Iowa, and they called the toxin Very Fast Death Factor (VFDF). Devlin et al. (21) have determined the structure of VFDF, renamed anatoxin-a (antx-a), obtained from non-axenic clone NRC-44h (also axenic NRC-44-1) derived from one of the original colony isolates. It is an alkaloid, 2-acetyl-9-azabicyclo [4.2.1] non-2-ene (Fig. 1) with a molecular weight of 165. The structure has been confirmed by x-ray crystallography (41) and synthesis (11). The LD_{min} (i.p. mouse) = 0.3 mg kg^{-1}, 4-5 min survival.

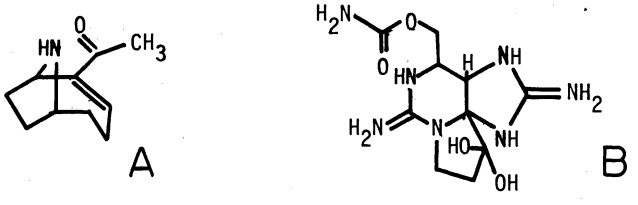

Fig. 1. A. Anatoxin-a. B. Saxitoxin

Antx-a is a powerful, post-synaptic, depolarizing neuromuscular blocking agent which causes death by respiratory arrest (12). The oral lethal dose for ducks and calves is much less than for mice and rats (14). The effects of antx-a are unusually long-lasting. Detoxification under artificial respiration, which was maintained for 8 h in rats and 30 h in calves (17), is too slow to be a practical recovery treatment. Biggs and Dryden (8) have found that antx-a also exhibits presynaptic action which reduces the frequency of miniature end plate potentials and the quantal content of end plate potentials. The combination of pre- and post-synaptic effects may account for the long duration of action. Carmichael et al. (13) have compared the pharmacology of natural and synthetic antx-a and found that they have

similar properties. It exhibits both muscarinic and nicotinic activity, behaves qualitatively like acetylcholine, carbachol and decomethonium, and is not affected by neostigmine or tetrodotoxin. Studies, so far, have not indicated any practical antidote.

Antx-a produces a characteristic post-synaptic, depolarizing sign of opisthotonous and muscle rigidity in avian species such as duck, pheasant and chick (12). This has been used (15) in conjunction with differences in survival times, salivation and lachrymation in mice, rats and chicks and chromodacryorrhea in rats to identify three, and possibly five, additional Anabaena toxins, called antx-b, -c, and d, produced by clones of An. flos-aquae isolated from blooms or scums collected from several lakes in Western Canada during the periods of 1972-75. Clones which produce salivation as well have been designated antx-a(S) and antx-b(S) to indicate that they may produce a salivation factor that is separate from the toxin(s). Work is continuing on these additional toxin types. It is possible that there may be structural similarities with known toxins or that some or all may be mixtures. Short survival times suggest that types -b and -d are likely to contain low-molecular-weight alkaloids. Carmichael (unpublished) has recently found that antx-c, like Microcystis type-c, is a mixture of two low-molecular-weight toxins. One is a peptide with hepatotoxic properties like mcyst. The other causes death in mice by respiratory arrest and this indicates a neurotoxin.

APHANIZOMENON TOXINS

Blooms of Aphanizomenon flos-aquae have been implicated in animal poisonings in Iowa, Minnesota and Oregon, U.S.A. and Manitoba, Can. for many years and several investigations have been carried out (10, 43, 53, 61, 62, 63). However, numerous blooms and cultures of this species that we have tested from Alberta, Saskatchewan and Ontario, Can. over a long period have been non-toxic (27, 31). Highly toxic blooms from Klamath Lake, Oregon in 1952 and 1960 that consisted of 50:50 Aphanizomenon:Microcystis produced signs and survival times with mice that were indistinguishable from those produced by mcyst and it was concluded that the Aphanizomenon component was either non-toxic or it, too, was producing an mcyst-like toxin (27).

Toxic blooms appeared in Lake Winnisquam in 1964 and in Kezar Lake, New Hampshire, U.S.A. in 1966 and 1967. Sawyer et al. (67) and Gentile and Maloney (26) reported the isolation of an atypical non-colony-forming non-axenic clone of Aph. flos-aquae from the Kezar Lake bloom which, with different doses, killed fish, mice and Daphnia catawba but was non-toxic to copepods; ostracods and cladocerans. Jackim and Gentile (44), Gentile (25) and Alam et al. (1) have reported that the toxin produced by the clone, called aphantoxin (abbrev. aphtx), has chemical and pharmacological properties which resemble some, but not all, of those possessed by saxitoxin (the paralytic shellfish poison produced by the marine dinoflagellate, Gonyaulax catenella). More recent work indicates that aphtx is a mixture of several toxins, one of which is saxitoxin (2, 72). The structure of saxitoxin has been established by Schantz et al. (68) as a tetrahydropurine alkaloid (Fig. 1). Aphtx mixture has a fast-acting neuromuscular action and inhibits nerve conduction of an action potential without affecting the transmembrane resting potential, i.e. without depolarization (25, 67). Saxitoxin has a similar action, has minimal effects on the cardiovascular system (72) and causes death by respiratory failure without significant depression of the medullary respiratory center (25).

Gentile (25) has reported the isolation and mass culture of a non-fasciculate Aph. flos-aquae culture from a typical bloom of fasciculate colonies from Klamath Lake which was toxic to fish and to mice (LD_{min} = 10 mg kg^{-1}, 5 min survival). He later induced fasciculate growth in a non-fasciculate culture from Klamath Lake and reported that both forms were toxic.

FRESHWATER CYANOPHYTE TOXINS

Table 2 provides a summary of the toxins which have, in recent years, been isolated or identified from cultures or blooms of freshwater cyanophytes collected from lakes or reservoirs in various countries around the world. In addition to those from Microcystis, Anabaena and Aphanizomenon, an unstable ichthyotoxin called TX-06, has recently been identified from Synechococcus sp. ATCC 18800 and its structure has been investigated by Amman (3). It appears to be a mixture of a pteridine, that closely resembles but is not identical to biopterin, and a peptide containing 7 amino acids (serine, glycine, glutamic acid, proline, alanine, lysine, and aspartic acid) in different proportions from those of any other phycotoxin. Because there was no stoichiometric relation between the pteridine and the peptide it was concluded that the peptide was not an integral part of TX-06 but what roles the two components play in determining the toxicity of the mixture was not made clear. The study used guppy mortality and survival times as the assay but did not provide any other toxicological data.

TABLE 2. Toxins which have been isolated or identified from cultures or blooms of freshwater cyanophytes from various sites.

Cyanophyte	Collection Site	Toxin[1]	Structure	References
Microcystis aeruginosa				
NRC-1 (mixed)	Little Rideau L., Ont., Can.	mcyst	peptides[2] (7, 14, 20±)	(9, 57, 64)
bloom	Malpas Dam, N.S. Wales, Australia	mcyst-like	peptide (5)+ methylamine	(23)
bloom	Dnieper Basin, Ukraine, U.S.S.R.	complex	16.6% peptide (16); aceto-methylene, isothiocyanate, carbohydrate groups	(47)
bloom	Hartbeesport Dam, S. Africa	mcyst-like mixture	2 peptides	(76)
type-c clones e.g. A-143-a-[3]	Bendig's Pond, Sask.; Hastings L., Alta., Can.	type-c, mixture	peptide + unknown	(16)
Anabaena flos-aquae				
NRC-44-h-	Burton L., Sask., Can.	antx-a	alkaloid $C_{10}H_{15}NO$	(21)
NRC-525-17-, NRC-525-26-	Buffalo Pound L. Sask., Can.	antx-a (S)[4]	unknown	(15, 16)
A-52-2-	Disney L., Alta., Can.	antx-b	unknown	(15, 16)
S-UTH-1-	Echo L., Sask., Can.	antx-b (S)	unknown	(15, 16)
A-113, A-113-9-	Beaverhill L., Alta., Can.	antx-c mixture	peptide + unknown	(15, 16)
S-23-g, S-29-a	Bendig's Pond, Sask., Can.	antx-d	unknown	(15, 16)
Aphanizomenon flos-aquae				
bloom (50% *Microcystis*	Klamath L., Oregon, U.S.A.	mcyst-like	unknown	(27)
clone	Kezar L., N.H., U.S.A.	aphtx, mixture	alkaloid $C_{10}H_{19}N_7O_4$ + unknowns	(1, 2, 44, 72)
clone	Klamath L., Oregon, U.S.A.	aphtx-like	unknown	(25)
Synechococcus sp.				
ATCC-18800	-	TX-06, mixture	pteridine + peptide (7)	(3)

[1] See text for abbreviations
[2] Figures in parentheses denote number of different amino acids reported in hydrolysates
[3] A-143-a- indicates clone "a" from colony isolate A-143. A dash following a clone letter (or number) indicates one or more subsequent reclonings, usually to establish axenic conditions
[4] (S) = salivation factor present

The review to this stage serves to emphasize the statement made at the outset that there is a great deal to be learned about the chemical, toxicological and pharmacological properties of the toxins from the freshwater cyanophytes. It has now become clear that different strains and not just different species are capable of producing distinctly different toxins. Some of these are low-molecular-weight, very-fast acting alkaloids with neurotoxic activity while others are slower acting peptides or peptide mixtures with hepatotoxic as well as neurotoxic activity. It has also become clear that some strains are capable of producing mixtures of more than one kind of toxin and some of the structure vs function anomalies which have been reported may have been caused by such mixtures. One surprising discovery with interesting evolutionary and biosystematic implications is the ability of a strain of a blue-green alga to synthesize the same alkaloid as a phylogenetically distant dinoflagellate. How many other types of toxins and toxin mixtures the bloom-forming freshwater cyanophytes are capable of producing and the full range of their effects are open questions which await answers.

FRESHWATER CYANOPHYTES AND MAN

There is much evidence that freshwater cyanophytes can cause a variety of adverse effects in man (69, 70). Cohen and Reif (18) have documented a case of repeated episodes of erythrematous papulo-vesicular dermatitis from swimmers who have made contact with Anabaena in Lake Carey, Pa., U.S.A. They demonstrated special sensitivity to the phycocyanin pigment derived from Anabaena and also concluded that blue-green algae were responsible for itching, swelling and redness of conjunctivae experienced by many swimmers. Heise (38, 39) has also documented repeated episodes of hay fever involving itching of eyes, nasal discharge and blockage, asthma and generalized urticaria in two patients who came in contact with species of Oscillatoriaceae while swimming in three lakes in Wisconsin, U.S.A.

Outbreaks of human gastroenteritis which occurred in Charleston, West Virginia and the area served by the Anacostia reservoir near Washington, D.C. in the drought years of 1930 and 1931 were attributed to unusual growths of cyanophytes in the water supplies (74, 75, 77). The usual bacterial causes of gastroenteritis could be excluded but direct effects of the algae (or bacteria associated with the algae which can also be toxic (27)), were not established. Better evidence for gastroenteritis caused by cyanophyte blooms in lakes in Saskatchewan, Can. has been reported by Dillenberg and Dehnel (22). A man developed such severe headache, nausea and gastrointestinal upset a few hours after swimming in Lake Katepwa, that he was hospitalized for 24 hr. The clinical diagnosis was enteritis or amoebic dysentery. No Salmonella or Entamoeba were found in the stool but there were many cells with the size and morphology of Microcystis. A physician accidentally fell into an algal bloom in Echo Lake. The surprise caused him to swallow about a half pint (250 ml) of the water. Three to five hours later he experienced stomach pains, vomiting and painful diarrhea. Still later he had a fever, severe headache, pain in muscles and joints and weakness. Examination of the slimy green stool revealed innumerable Microcystis cells and a number of well-preserved chains of Anabaena circinalis but no other pathogens. Additional cases of gastroenteritis and one case of coma with labored breathing associated with involuntary ingestion of blooms of Aphanizomenon, Microcystis and Anabaena have been noted by Dillenberg (69, 70). Aziz (4) has recently described a diarrhea toxin that causes fluid accumulation in ligated loops of small intestine of guinea pigs. The non-dialyzable toxin was obtained from a clone of Microcystis aeruginosa isolated from a city pond in Dacca, Bangladesh. The dialyzable fraction produced no diarrhea but an i.p. dose of 0.5 ml killed rats. The diarrhea toxin was, therefore, different from the animal poison.

Concern aroused in Saskatchewan by the cases of human and animal poisoning by cyanophytes led to toxicity tests of the blooms in two impoundments used as water supplies for three cities. While the raw waters gave indications of toxicity the treated waters did not (22). Wheeler et al. (79) found that toxins produced by a Microcystis aeruginosa bloom were not inactivated by the laboratory equivalent of water purification processes including alum coagulation, filtration, chlorination and activated carbon treatment. Massive amounts of activated carbon were needed to render the effluent non-toxic (60, 79). Gorham (28) considered that mcyst and antx-a would normally be diluted or inactivated by the usual water treatment practices to a level that would likely be too low to cause human poisoning. A recent investigation of a sudden waterborne outbreak of gastroenteritis which affected 62% of the population served by a water utility in Sewickley, Pa., U.S.A. has been described by Lippy and Erb (51). Bacteriological monitoring at the onset and during the outbreak did not indicate a grossly contaminated water which would be suspected of causing widespread illness. An uncovered finished water reservoir which had piles of algae around the edge, the bottom and the surface, consisting predominantly of the cyanophyte, Schizothrix calcicola, was suspected of being the source of entry of the unidentified contaminant into the system. Further studies on the potential toxicity of a number of non-axenic cultures of S. calcicola from different sources are being carried out (73). To date, some LPS-"endotoxin"-like activity has been observed in strain 1937 from the R. C. Starr collection, University of Texas, Austin, Texas. An outbreak of pyrogenic reactions has been traced to an increase in "endotoxin" contamination of tap water, possibly caused by an algal bloom involving cyano-

phytes (40).

Different species and strains of freshwater cyanophytes are capable of producing toxins or mixtures of toxins that can cause sickness or death in man. Many are new, have unknown structures and incompletely defined properties. Some can cause problems in recreational use of water bodies, others in the provision and delivery of safe drinking water. There may well be other toxins still to be found and more than one may occur simultaneously. Until more exact information about toxic freshwater cyanophytes becomes available, there needs to be systematic monitoring as well as detailed studies of the potential hazards of these algae to man.

MARINE CYANOPHYTE TOXINS

The marine cyanophytes have not been implicated in wildlife or public health problems to the same degree as the freshwater forms. Sams (66) described a "seabathers eruption" which occurred sporadically along the east coast of Florida. It was an acute erythrematous, papulo-vesicular contact dermititis, which could be prevented by immediate showering after swimming. He was unable to determine the cause. A similar severe "swimmers itch" which occurred occasionally during summer months on the windward side of Oahu, Hawaii, U.S.A. and at the Gushikawa beach on Okinawa Island, Japan has been investigated and is caused by direct contact with particular populations of the cyanophyte Lyngbya majuscula Gomont (= Microcoleus lyngbyaceous (Kütz.) Crouan (37)) (5, 6, 33, 34, 35, 36, 37, 54, 55, 59). The toxic principle was identified as a lipid-soluble phenolic compound (36,55). An i.v. dose of 12 mg caused death of a rat in 6 min from respiratory failure preceded by electrocardiogram and electroencephalogram irregularities (54). It also lysed protozoan and red blood cells and possessed antibacterial activity (54). In the tropical and subtropical Pacific, fish intoxication poses a public health problem. The most common is ciguatera, a neurological and gastrointestinal disorder that occurs sporadically following the ingestion of toxic fish. The toxins from Lyngbya majuscula and Shizothrix calcicola and other toxic cyanophytes from various atolls and islands in the Pacific have been shown not to be ciguatoxin (6, 7, 35). A relationship may exist, however, between poisonous rabbitfish (Siganus fuscescens) and toxic L. majuscula, a species which the fish have been observed to eat (37). Sea-hare, Stylocheilus longicauda, a gastropod mollusk having little or no shell, accumulates two toxins (LD_{min}, i.p. mice, 0.3 mg kg^{-1} for both) called aplysiatoxin (abbrev. aplytx) and debromoaplysiatoxin (abbrev. deBr-aplytx) in its digestive tract. The structures of these toxins have been elucidated by Kato and Scheuer (45, 46) (Fig. 2).

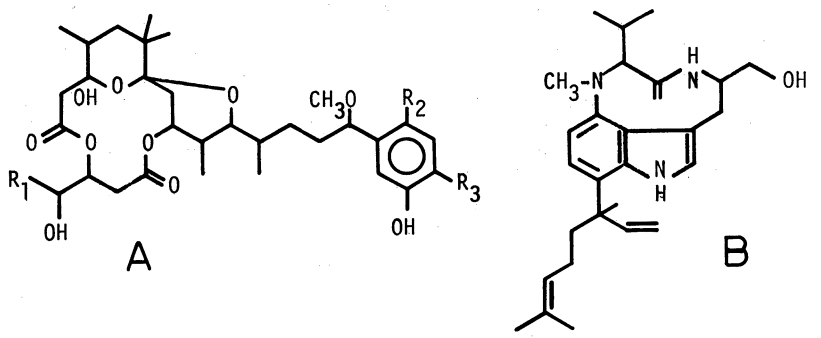

Fig. 2. A. Debromoaplysiatoxin and related toxins:

1. R_1=CH_3, R_2=R_3=H deBr-aplytx
2. R_1=CH_3, R_2=Br, R_3=H aplytx
3. R_1=CH_3, R_2=R_3=Br 19 Br-aplytx
4. R_1=R_2=R_3=H oscilltx A
5. R_1=R_3=H, R_2=Br 21 Br-oscilltx A
6. R_1=H, R_2=R_3=Br 19, 21 Br-oscilltx A

B. Lyngbyatoxin A

The sea-hare grazes on Lyngbya majuscula. From deep-water L. majuscula, collected from Einewetak atoll, Marshall Islands and Laie Bay, Oahu, Hawaii, U.S.A., Mynderse et al. (59) have isolated crystalline deBr-aplytx and showed that it produces erythrematous dermititis. They have isolated deBr-aplytx from a mixture of Oscillatoria nigroviridis and Schizothrix calcicola from Einewetak, and have found that this compound also exhibits fair activity against P-388 lymphocytic mouse leukemia. Mynderse and Moore (58) subsequently isolated and identified a second major component (nor-deBr-aplytx) from the same mixture of O. nigroviridis and S. calcicola and named it oscillatoxin A (abbrev. oscilltx A). Two minor bromine-containing oscilltx's and 19-Br-aplytx were isolated and identified from the same source. Cordellina II et al. (20) have isolated a highly inflammatory and vesicatory substance from the

lipid extract of L. majuscula collected from Kahala Beach, Oahu. It is an indole alkaloid called lyngbyatoxin A (abbrev. lyngtx A) (Fig. 2), with a structure that is closely related to that of teleocidin B from Streptomyces (which causes severe irritation and eruptive vesiculations on human skin). The LD_{min} (i.p. mouse) for both of these toxins is 0.3 mg kg^{-1}, the same as for the aplysiatoxins. From L. majuscula, Moore (56) has also identified four non-toxic lipopeptides.

TABLE 3. Toxins which have been isolated or identified from collections of marine cyanophytes from various sites.

Cyanophyte	Collection Site	Toxin*	Structure	References
Lyngbya majuscula collections (deep water)	Enawetak atoll, Marshall Is., Laie Bay, Oahu Hawaii, U.S.A. Gushikawa Beach, Okinawa I., Japan	deBr-aplytx	phenol $C_{32}H_{48}O_{10}$	(20, 36, 37, 59)
collection (shallow water)	Kahala Beach, Oahu, Hawaii, U.S.A.	lyngtx A	indole alkaloid $C_{27}H_{39}N_3O_2$	(20)
Oscillatoria nigroviridis & Schizothrix calcicola collection	Enawetak atoll, Marshall Is., U.S.A.	Mixture: deBr-aplytx, oscilltx A	phenols $C_{32}H_{48}O_{10}$ $C_{31}H_{46}O_{10}$	(59)
	19, 21	Br-oscilltx A	$C_{31}H_{45}BrO_{10}$	
	19, 21	Br-oscilltx A	$C_{31}H_{44}Br_2O_{10}$	
	19	Br-aplytx	$C_{32}H_{46}Br_2O_{10}$	

* See text for abbreviations

Table 3 provides a summary of the toxins which have been isolated or identified to date from marine cyanophytes collected from various sites in the Pacific islands. There are two kinds of toxins, phenolic and alkaloid, and both have about the same toxicity towards mice. Their structures and toxicology bear little, if any, relation to those of the peptide and alkaloid toxins that are so far known from freshwater species. The known marine toxigenic species are closely related members of the family Oscillatoriaceae which grow and form tufts on rocks in both shallow and deep water. Spirulina, the cyanophyte being grown for human food, is a member of the same family. As with the freshwater cyanophytes, there appear to be toxic and non-toxic strains as judged by toxic and non-toxic collections from different sites and different depths. Whether the differences in toxicity represent genetic or ecophysiological differences, or a combination of both, needs further study making use, if possible, of unialgal and/or axenic cultures.

The lipid-soluble phenolic toxins are chemically related derivatives of brominated or debrominated aplytx. While they occur naturally as mixtures, each of them is toxic to mice, is dermatitis-producing, and exhibits anti-leukemic properties. Further studies of their toxicology and pharmacology are needed to learn more about specific structure in relation to these three different toxic properties. The discovery that a major component of the toxic mixture from Lyngbya majuscula, lyngtx A, is structurally related and has a similar toxicity to teleocidin B from the fungus Streptomyces poses another important question of phylogeny, just as the saxitoxin component of aphtx does. Lyngbya majuscula and Schizothrix calcicola are cyanophytes which grow in freshwater as well as marine environments. When and if toxic marine strains of these species can be cultured it would be valuable to see if they can be grown in freshwater media and whether they would produce the same or different toxin(s). Similarly, if freshwater strains of Schizothrix calcicola prove to be toxic to animals or man, their ability to grow and produce toxins should be checked in marine media.

Research on marine cyanophyte toxins has, historically, been motivated by their potential role in ciguatera poisoning. While it is now established that ciguatoxin does not originate with cyanophyte toxins, there is evidence that the latter may be transmitted via the food chain to other kinds of fish. This, together with the knowledge we have about paralytic shellfish poison (that originates with toxic dinoflagellates) emphasizes the need for constant concern about the potential hazards of cyanophyte toxins in the food chain. Great caution should be exercised in exploiting any of the cyanophytes as sources of food for man and animals until a great deal more is known about toxins and toxin production by the many different species and strains.

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