Toxicon 56 (2010) 813–828

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Review

Neurotoxic cyanobacterial toxins

Rómulo Aráoz [a,*], Jordi Molgó [a], Nicole Tandeau de Marsac [b,**]

[a] CNRS, Institut de Neurobiologie Alfred Fessard, FRC2118, Laboratoire de Neurobiologie Cellulaire et Moléculaire, UPR9040, 1 avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France
[b] Unité des Cyanobactéries (CNRS, URA 2172), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

ARTICLE INFO

Article history:
Received 25 February 2009
Received in revised form 27 July 2009
Accepted 30 July 2009
Available online 4 August 2009

Keywords:
Cyanobacteria
Neurotoxins
Cyanotoxins

ABSTRACT

Worldwide development of cyanobacterial blooms has significantly increased in marine and continental waters in the last century due to water eutrophication. This phenomenon is favoured by the ability of planktonic cyanobacteria to synthesize gas vesicles that allow them to float in the water column. Besides, benthic cyanobacteria that proliferate at the bottom of lakes, rivers and costal waters form dense mats near the shore. Cyanobacterial massive proliferation is of public concern regarding the capacity of certain cyanobacterial strains to produce hepatotoxic and neurotoxic compounds that can affect public health, human activities and wild and stock animals. The cholinergic synapses and voltage-gated sodium channels constitute the targets of choice of cyanobacterial neurotoxins. Anatoxin-a and homoanatoxin-a are agonists of nicotinic acetylcholine receptors. Anatoxin-a(s) is an irreversible inhibitor of acetylcholinesterase. Saxitoxin, kalkitoxin and jamaicamide are blockers of voltage-gated sodium channels, whereas antillatoxin is an activator of such channels. Moreover the neurotoxic amino acid L-beta-*N*-methylamino-L-alanine was shown to be produced by diverse cyanobacterial taxa. Although controversial, increasing *in vivo* and *in vitro* evidence suggest a link between the ingestion of L-beta-*N*-methylamino-L-alanine and the development of amyotrophic lateral sclerosis/Parkinsonism–dementia complex, a neurodegenerative disease. This paper reviews the occurrence of cyanobacterial neurotoxins, their chemical properties, mode of action and biosynthetic pathways.

1. Introduction

Cyanobacteria, the oldest known fossils (2.15 billions years) (Rasmussen et al., 2008), are photosynthetic ubiquitous micro-organisms that played a key role in the oxygenation of earth's atmosphere. Cyanobacteria constitute the most important nitrogen fixing organisms and possess the ability to harvest solar irradiance at wavelengths that most of photosynthetic organisms are unable. Cyanobacteria can occupy any terrestrial ecosystem including freshwater, marine and soil environments, as well as extreme habitats such as desserts, hot spring waters, and Artic and Antarctic environments (Sompong et al., 2005; Taton et al., 2006). Cyanobacterial massive proliferation, when dominated by toxic cyanobacteria, is of serious concern for public health, human activities, and animal life. Indeed, certain species of cyanobacteria are able to synthesize hepatotoxins, cytotoxins and neurotoxins (Carmichael, 1994; Sivonen and Jones, 1999; Briand et al., 2003). The lethal effects of cyanobacterial blooms upon stock animals were first described by Francis (1878) in a lake of the estuary of the Murray (Australia).

Cyanobacteria produce important hepatotoxins e.g., microcystins, nodularins and cylindrospermopsins. Microcystins are cyclic heptapeptides that possess the

* Corresponding author. Tel.: +33 (0) 1 69 82 36 42; fax: +33 (0) 1 69 82 41 41.
** Corresponding author. Tel.: +33 (0) 1 45 68 84 15; fax: +33 (0) 1 40 61 30 42.
E-mail addresses: araoz@nbcm.cnrs-gif.fr (R. Aráoz), ntmarsac@pasteur.fr (N. Tandeau de Marsac).

0041-0101/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2009.07.036

general structure (-D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha-), where X and Z are variable L-amino acids, D-MeAsp is D-erythro-β-methyl aspartic acid, Mdha is *N*-methyldehydroalanine, and Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Sivonen and Jones, 1999). There exists more than 80 identified microcystin variants with different degrees of toxicity (i.p. mouse LD_{50} ranging from 50 to >1200 μg kg^{-1}) (Hotto et al., 2007). Microcystins are almost exclusively produced by planktonic cyanobacterial members of the genera *Microcystis*, *Anabaena*, *Planktothrix*, and in a small proportion by members of the genera *Anabaenopsis*, *Hapalosiphon* and *Nostoc* (Sivonen and Jones, 1999; Oksanen et al., 2004). Microcystins are powerful liver tumor promoters and potent inhibitors of the serine/threonine protein phosphatase-1 and -2A (Sivonen and Jones, 1999; Maynes et al., 2006). Nodularin is a cyclic pentapeptide hepatotoxin that differs from microcystins by lacking the amino acids D-Ala and X and by having L-Arg and *N*-methyldehydrobutyrine (Mdhb) instead of the variable amino acid Y and Mdha, respectively (Rinehart et al., 1988). Nodularin is produced by the bloom forming strain *Nodularia spumigena*, a hepatotoxic cyanobacterium that occurs worldwide in brackish waters, and by *Nodularia harveyana* PCC 7804 (Sivonen and Jones, 1999; Koskenniemi et al., 2007; Saito et al., 2001). Nodularin is a potent tumor promoter and inhibits serine/threonine protein phosphatase-1 and -2A (i.p. mouse LD_{50}: 50–70 μg kg^{-1}) (Gulledge et al., 2003). Cylindrospermopsin is a cytotoxic alkaloid consisting of a tricyclic guanidine moiety combined with hydroxymethyluracil that inhibits the synthesis of protein and of glutathione leading to cell death (i.p. CH_3 mouse LD_{50}: 2.1 mg kg^{-1}) (Ohtani et al., 1992; Runnegar et al., 2002). Cylindrospermopsin is produced by *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Aphanizomenon flos-aquae*, *Umezakia natans*, *Raphidiopsis curvata* and *Anabaena bergii* (Preussel et al., 2006). Cyanobacterial hepatotoxins are a serious threat for domestic and wild animal life as well as for human health (Carmichael et al., 2001; Koskenniemi et al., 2007; Hawkins et al., 1985). The World Health Organization has established a provisional drinking-water guideline for microcystin-LR: 1 mg l^{-1} (World Health Organization, 2004).

Cyanobacterial neurotoxins target (i) cholinergic synapses: anatoxin-a and homoanatoxin-a are potent agonists of muscular and neuronal nicotinic acetylcholine receptor (nAChR) subtypes (Spivak et al., 1980; Thomas et al., 1993; Wonnacott et al., 1992), while anatoxin-a(s) is a potent irreversible inhibitor of acetylcholinesterase (Mahmood and Carmichael, 1986b) and (ii) sodium channels: saxitoxins are a group of structurally related molecules that block voltage-gated sodium channels (for review, see Llewellyn, 2006). The lipopeptides purified from marine cyanobacteria, kalkitoxin and jamaicamides A, B and C, also block voltage-gated sodium channels (LePage et al., 2005; Edwards et al., 2004), while antillatoxins A and B activate them (Li et al., 2001). Additionally, the non-protein neurotoxic amino acid L-beta-*N*-methylamino-L-alanine, produced by diverse taxa of cyanobacteria, may be associated with the development of the amyotrophic lateral sclerosis/Parkinsonism–dementia complex, a neurodegenerative disease (Cox et al., 2003). The purpose of this paper is to review the occurrence of cyanobacterial neurotoxins, their chemical properties, mode of action and biosynthetic pathways.

2. Neurotoxic alkaloids in freshwater cyanobacteria

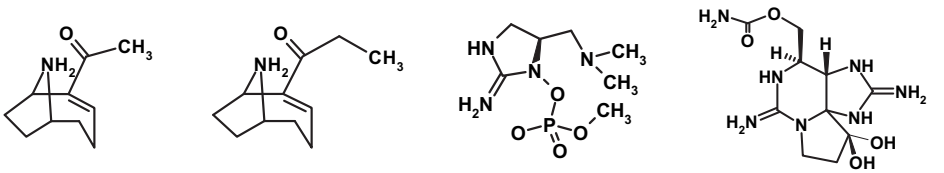
2.1. Anatoxin-a and homoanatoxin-a

The neurotoxins anatoxin-a (2-acetyl-9-azabicyclo[4.2.1]non-2-ene; UV λ_{max} at 227 nm; $\varepsilon = 12{,}000$; $C_{10}H_{15}NO$; EI-MS *m*/*z* $[M]^+$ 165; Fig. 1) and its methylene homologue homoanatoxin-a (2-(propan-1-oxo-1-yl)-9-azabicyclo[4.2.1]non-2-ene; UV λ_{max} at 230 nm; $C_{11}H_{18}NO$; EI-MS *m*/*z* $[M]^+$ 179; Fig. 1), are low molecular weight bicyclic secondary amines exclusively produced by cyanobacteria (Devlin et al., 1977; Skulberg et al., 1992). Anatoxin-a is synthesized by various members of the genera *Anabaena* (Harada et al., 1989), *Aphanizomenon* (Selwood et al., 2007), *Cylindrospermum* (Sivonen et al., 1989), *Microcystis* (Park et al., 1993), *Oscillatoria* (Sivonen et al., 1989), *Planktothrix* (Viaggiu et al., 2004) and *Raphidiopsis* (Namikoshi et al., 2003). Homoanatoxin-a is synthesized by some species corresponding to the genera *Oscillatoria* (Skulberg et al., 1992), *Anabaena* (Furey et al., 2003a), *Raphidiopsis* (Namikoshi et al., 2003) and *Phormidium* (Wood et al., 2007). Simultaneous synthesis of anatoxin-a and homoanatoxin-a was demonstrated for *Raphidiopsis mediterranea* Skuja (Namikoshi et al., 2003), and the axenic *Oscillatoria* PCC 9029 (Aráoz et al., 2005). The presence of anatoxin-a and homoanatoxin-a in several axenic cyanobacterial species – exempt of bacterial contaminants – of the genera *Anabaena* (Rouhiainen et al., 1995) and *Oscillatoria* (Aráoz et al., 2005; Cadel-Six et al., 2007) confirmed the cyanobacterial origin of these toxins.

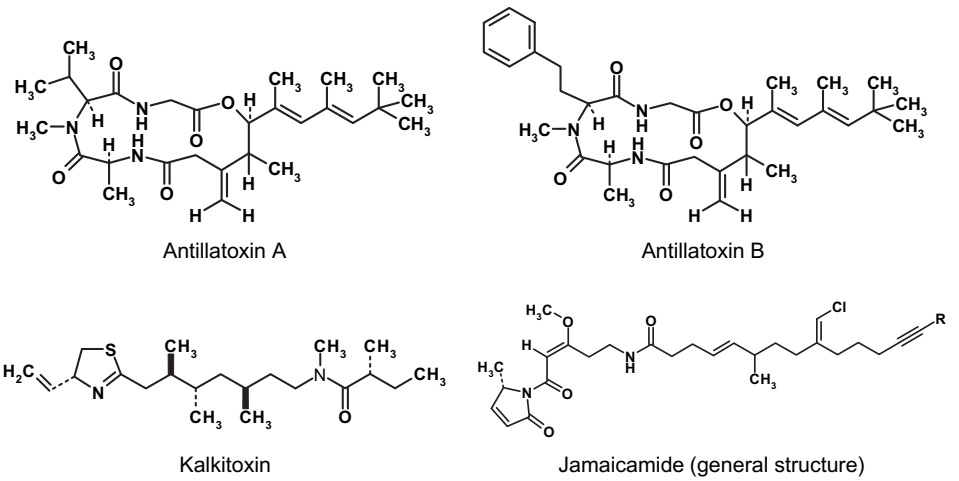
Massive proliferation of benthic neurotoxic cyanobacteria producing anatoxin-a and/or homoanatoxin-a near the shore of lakes and rivers has been shown to be fatal for wild and domestic animals, e.g., cows in Canada (Carmichael and Gorham, 1978), dogs in Scotland (Edwards et al., 1992), France (Gugger et al., 2005; Cadel-Six et al., 2007) and New Zealand (Wood et al., 2007), and Lesser flamingos in Kenya (Krienitz et al., 2003).

Electrophysiological experiments showed that anatoxin-a is a potent agonist of the muscle-type $\alpha 1_2\beta\gamma\delta$ nAChR. Thus, anatoxin-a induced neuromuscular blockade (of the depolanzing type), contracture of the frog's rectus abdominis muscle, depolarization of the frog sartorius muscle, desensitization, and alteration of the action potential (Spivak et al., 1980). Later, Thomas et al. (1993) working with chicken $\alpha 4\beta 2$ nAChR subunits expressed on mouse M 10 cells and chicken $\alpha 7$ nAChR expressed in oocytes from *Xenopus laevis*, showed that anatoxin-a is also a potent agonist of neuronal nAChR. Anatoxin-a, formerly known as "Very Fast Death Factor", kills mice 2–5 min after intraperitoneal injection preceded by twitching, muscle spasm, paralysis and respiratory arrest (i.p. mouse LD_{50}: 250 μg kg^{-1}; Devlin et al., 1977). Anatoxin-a is a depolarizing neuromuscular blocking agent. Neuromuscular blockade induced by anatoxin-a results from muscle membrane depolarization and desensitization. The binding of anatoxin-a to the nAChR induces the opening of the

Neurotoxic alkaloids in freshwater cyanobacteria



Neurotoxic lipopeptides in marine cyanobacteria



Cyanobacterial neurotoxic amino acid

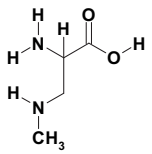


Fig. 1. Structure of the cyanobacterial neurotoxins.

receptor's channel allowing positively charged ions to move across it. This results in membrane depolarization. Prolonged exposure to anatoxin-a causes the desensitization of the nAChR that ultimately leads to the blockade of neuromuscular transmission (Spivak et al., 1980). Homoanatoxin-a was shown to be a potent analogue of anatoxin-a (Wonnacott et al., 1992). Additional mechanisms of toxicity were observed by systemic perfusion of sub-lethal and lethal doses of anatoxin-a to mice. Anatoxin-a seriously impaired blood pressure, heart rate and gas exchange (pO_2 and pCO_2) causing hypoxia and respiratory arrest, and severe acidosis that accompanied animal death (Adeymo and Sirén, 1992). Also, anatoxin-a induces the contraction of frog *rectus abdominis* muscle, as well as guinea pig ileum. In the latter case, the activation of nAChR from ganglionic interneurons by anatoxin-a, induced the release of acetylcholine that in cascade stimulated the muscarinic acetylcholine receptors leading to ileal contractions (Gordon et al., 1992).

The novel 9-azabicyclo[4.2.1]nonane semi rigid skeleton, and the potential pharmacological applications of anatoxin-a have led numerous organic chemists to develop diverse strategies for total synthesis of anatoxin-a (for reviews see Mansell, 1996; Parsons et al., 2000; Brenneman et al., 2004; Roe and Stockman, 2008). A series of eighteen anatoxin-a analogues were synthesized to use them as probes for characterizing the binding sites of anatoxin-a in muscle and neuronal types of nAChRs (Swanson et al., 1991; Wonnacott et al., 1991). These studies confirmed the important role that the side-chain stereochemistry of

anatoxin-a plays in the binding of this toxin to the nAChR. Thus, the series of anatoxin-a analogues showed varying degrees of specificity for neuronal acetylcholine receptor subtypes. Actually, the anatoxin-a side-chain chemistry led to the synthesis of the methylene homologue named homoanatoxin-a (Wonnacott et al., 1991), before being purified from an oscillatorian strain (Skulberg et al., 1992).

Mass spectrometry coupled to gas or liquid chromatography is currently used for anatoxin-a and homoanatoxin-a detection at nanogram and picogram levels from water samples and cyanobacterial extracts. Liquid chromatography–tandem mass spectrometry and nano-electrospray hybrid quadrupole time-of-flight mass spectrometry provide, reliability, sensitivity, selectivity, and structural and quantitative information for the analysis of anatoxin-a and homoanatoxin-a from cultured cyanobacteria and environmental samples (Himberg, 1989; Harada et al., 1993; Furey et al., 2003b; Maizels and Budde, 2004; James et al., 2005). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has also been applied to the detection of the low molecular mass anatoxin-a (165 Da) and homoanatoxin-a (179 Da) directly on cyanobacterial filaments of axenic strains of the genus *Oscillatoria* (Aráoz et al., 2008).

Feeding experiments with *Anabaena flos-aquae* and *Oscillatoria formosa* (producers of anatoxin-a and homoanatoxin-a, respectively) using ^{13}C-labeled precursors, showed that both, anatoxin-a and homoanatoxin-a biosynthesis proceed from acetate and glutamate (Hemscheidt et al., 1995). The source of carbons atoms is the same for anatoxin-a and homoanatoxin-a except for C-12. These results allowed Hemscheidt et al. (1995) to hypothesize the existence of a common precursor for both toxins which, following decarboxylation, may give rise to: i) anatoxin-a through enzymatic reduction of the common precursor, or to ii) homoanatoxin through enzymatic addition of a C_1 unit to the common precursor from the methyl donor *S*-adenosyl-L-methionine. Later, Namikoshi et al. (2004) identified L-methionine as the biosynthetic precursor of the methyl group at C-12 of homoanatoxin-a using L-[methyl-^{13}C]-methionine in the culture of the-cyanobacterium *Raphidiopsis mediterranea* (Fig. 2). Recently, Selwood et al. (2007) identified and chemically characterized for the first time the 11-carboxyl anatoxin-a derivative by high-resolution mass spectrometry in *Aphanizomenon issatschenkoi* (CAWBG02).

Note of the authors: as the present paper was under revision, a polyketide synthase coding sequence that is specific for strains producing anatoxin-a, or homoanatoxin-a was identified in *Oscillatoria* strain PCC 6506 (Cadel-Six et al., 2009). Partial genome sequencing of this cyanobacterium strain resulted in the identification of the putative gene cluster responsible for anatoxin-a and homoanatoxin-a production (Méjean et al., 2009). After gene annotation and deduction of their putative functions, Méjean et al. (2009) proposed a biosynthetic route for anatoxin-a and homoanatoxin-a. Moreover, feeding experiments using L-[U-^{13}C-^{15}N]Glu, L-[U-^{13}C-^{15}N]Arg, L-[U-^{13}C-^{15}N]Pro or L-[U-^{13}C]Glu precursors, allowed the same authors to propose l-proline as the starter unit for anatoxin-a and homoanatoxin-a biosynthesis.

However, proline could be readily produced from either glutamate or arginine in the primary metabolism of cyanobacteria (Méjean et al., 2009). Therefore, we believe that more work needs to be done to gain direct insight into these neurotoxins biosynthetic route.

2.2. Anatoxin-a(s)

Anatoxin-a(s) (UV λ_{max} at 220 nm; $C_7H_{17}N_4O_4P$; HRFABMS $[M+H]^+$ *m/z* 253.1066; Fig. 1) is an unusual natural organophosphate, structurally unrelated to anatoxin-a, that irreversibly inhibits acetylcholinesterase (Mahmood and Carmichael, 1986b). Its activity is similar to organophosphorous and carbamate insecticides such as paraoxon, physostigmine and pyridostigmine (Cook et al., 1988), and the chemical warfare agent sarin (Pita et al., 2003). Anatoxin-a(s) is produced by *Anabaena flos-aquae* strain NRC 525-17 (Mahmood et al., 1988) and *Anabaena lemmermannii* (Henriksen et al., 1997). Additionally,

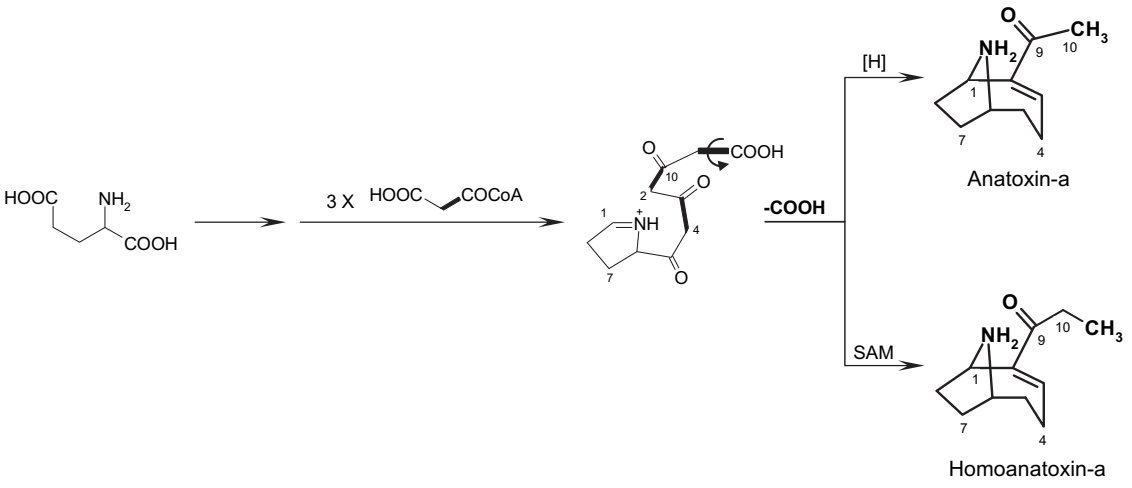


Fig. 2. Proposed biosynthetic route for homoanatoxin-a and anatoxin-a. Adapted, with permission, from Namikoshi et al. (2004). Copyright 2004 American Chemical Society. SAM: *S*-adenosyl-L-methionine.

acetylcholinesterase inhibition activity was found in extracts of *Anabaena spirolides*, isolated from environmental blooms of a water reservoir in Tapacurá, Brazil (Molica et al., 2005). When extracts of *A. flos-aquae* strain NRC 525-17 or purified anatoxin-a(s) were administered i.p. to mice, animal death was preceded by salivation, lacrimation, fasciculation, urinary incontinence and respiratory failure in 240 min (i.p. mouse LD_{50}: 20 μg kg^{-1}) (Mahmood and Carmichael, 1986b; Onodera et al., 1997a). Anatoxin-a(s) was involved in the poisoning of dogs, birds and swine in Canada (Mahmood et al., 1988), and wild birds in Denmark (Henriksen et al., 1997).

The Ellman test is currently used for the detection of anatoxin-a(s) (Mahmood and Carmichael, 1986b; Henriksen et al., 1997; Molica et al., 2005). Bachmann et al. (2000) adapted an electrochemical biosensor for the detection of anatoxin-a(s). The sensor system was composed of an Ag/AgCl reference electrode and a graphite working electrode in which acetylcholinesterase from *Drosophila* was immobilized. Acetylcholinesterase activity is monitored electrochemically from the formation of thiocholine following hydrolysis of acetylthiocholine chloride by the immobilized acetylcholinesterase. Devic et al. (2002) used a four-mutant set of *Drosophila* acetylcholinesterase to improve the specificity and the sensibility of the biosensor for anatoxin-a(s) detection.

Matsunaga et al. (1989) performed feeding experiments with *A. flos-aquae* NRC 525-17 using 50% ^{13}C and 90% ^{15}N to determine the chemical structure of this unique organophosphate. NMR analysis of the radioactive purified compound indicated that the *N*-CH_3 group was connected to the side chain of the imidazoline residue, and that the methylphosphate group was attached to one of the nitrogens through an ester bond. Anatoxin-a(s) slowly undergoes autolysis to give a mixture of a degradation product containing an imidazoline ring (High-Resolution Fast Atom Bombardment mass Spectrometry (HRFABS) $[M+H]^+$ *m/z* 159.1245) and monomethylphosphate that were separated by HPLC. The degradation product *m/z* 159.1245 was converted into *4S*-2-imino-4(dimethylaminomethyl)-imidazolidine (HRFABS $[M+H]^+$ *m/z* 143.1298) by catalytic hydrogenation. NMR analysis of both degradation products confirmed the stereochemistry of anatoxin-a(s) and the position of methylphosphate group at N_1. For detailed RMN data see Matsunaga et al. (1989) and Onodera et al. (1997a). The degradation compound *4S*-2-imino-4(dimethylaminomethyl)-imidazolidine was synthesized starting from D- and L-asparagine in four steps by Matsunaga et al. (1989). However, total synthesis of anatoxin-a(s) has not yet been reported. Shimizu (1996) proposed a biosynthetic route for this natural organophosphate compound: the biosynthesis of anatoxin-a(s) may proceed from the unusual catabolism of arginine by a retro-Claisen condensation mechanism which involves the loss of a glycine moiety from an arginine molecule (Fig. 3).

2.3. Saxitoxin

Saxitoxin ($C_{10}H_{17}N_7O_4$; $M_r = 299.286480$; Fig. 1), mostly related with marine dinoflagellates outbreaks, was first purified from the Alaskan butter clam, *Saxidomus giganteus* in 1957 (Schantz et al., 1957). Shellfish accumulate saxitoxins through filter-feeding on toxic dinoflagellates. Saxitoxins are produced by some species of the genera *Alexandrium* (*A. andersoni*, *A. catenella*, *A. excavatum*, *A. fundyense*, *A. minutum*, *A. ostenfeldii*, *A. tamarense* and *A. tamiyavanichi*), *Gymnodinium catenatum* and *Pyrodinium bahamense* (see Llewellyn, 2006 for references). Some members of the freshwater cyanobacteria *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya* and *Planktothrix* were also reported to produce saxitoxins (see Table 1 for references). Freshwater mussels were also seen to accumulate saxitoxin when fed with toxic cyanobacteria (Negri and Jones, 1995).

Saxitoxin structure was determined by X-ray crystallography twenty years after its discovery (Schantz et al., 1975; Bordner et al., 1975). Soon later, Shimizu et al. (1976) isolated several new paralytic shellfish toxins, named gonyautoxins from shellfish samples and from the causative organism *Gonyaulax tamarensis*. Saxitoxins comprise a series of 30 structurally related natural molecules with two guanidinium moieties often referred to as paralytic shellfish poisoning. Structurally, saxitoxins are divided into two categories: saxitoxin and neosaxitoxin series. In each series, the structural variations are created by the presence and differential stereochemistry of the sulphate groups at C-11, and the occurrence of *N*-sulphate carbamoyl groups at C-17 (Shimizu, 1986; Negri et al., 1995, Fig. 4; Table 1).

Saxitoxins block sodium permeability of excitable membranes by reversible binding to specific amino acid residues located in the outer pore loops of the voltage-dependent sodium channels at site 1, as tetrodoxin does (Cestèle and Catterall, 2000; Bricelj et al., 2005). The blockage of voltage-gated sodium channels prevents the generation of a proper action potential in nerves and muscle fibres leading to neuromuscular paralysis and death by respiratory arrest. Saxitoxins are among the most toxic compounds known (i.p. mouse LD_{50}: 5–10 μg kg^{-1}; Schantz et al., 1975). The rank order of sodium channel blockade is

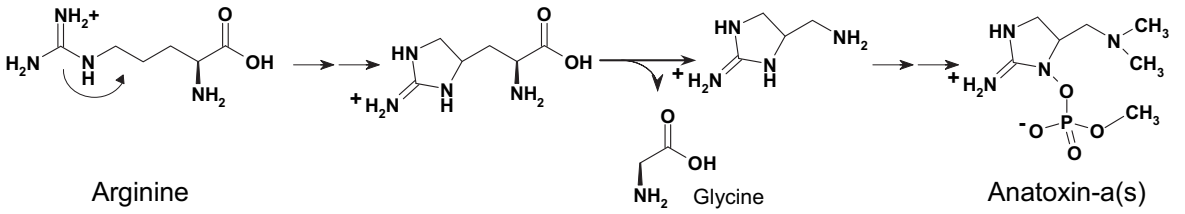


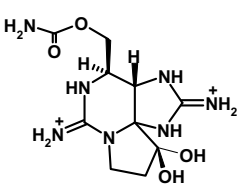
Fig. 3. Proposed anatoxin-a(s) biosynthetic pathway. Biosynthesis of anatoxin-a(s) involves the loss of glycine from arginine. Partially reprinted from Shimizu (1996), with permission from the *Annual Review of Microbiology*, Volume 50 © 1996 by Annual Reviews (www.annualreviews.org).

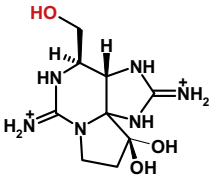
Table 1
Saxitoxin analogues produced by freshwater cyanobacteria.

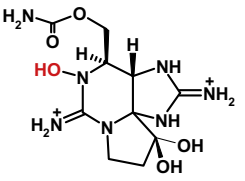
Toxin	Cyanobacterial strain	References
Saxitoxin (STX)	*Anabaena circinalis*	Negri et al., 1995; Teste et al., 2002
	Anabaena lemmermannii	Rapala et al., 2005
	Aphanizomenon flos-aquae	Ikawa et al., 1982; Mahmood and Carmichael, 1986a; Dias et al., 2002; Pereira et al., 2000
	Aphanizomenon gracile LMECYA40	Pereira et al., 2004
	Aphanizomenon issatschenkoi	Nogueira et al., 2004
	Cylindrospermopsis raciborskii	Lagos et al., 1999; Molica et al., 2002
	Cylindrospermopsis raciborskii C10	Castro et al., 2004
	Cylindrospermopsis raciborskii T3	Pomati et al., 2004
	Planktothrix sp.	Pomati et al., 2000
Decarbomoyl saxitoxin (dcSTX)	*Anabaena circinalis*	Negri et al., 1995
	Aphanizomenon DC-1	Liu et al., 2006
	Aphanizomenon flos-aquae	Pereira et al., 2000; Dias et al., 2002
	Aphanizomenon issatschenkoi	Nogueira et al., 2004
	Cylindrospermopsis raciborskii C10	Castro et al., 2004
	Lyngbya wollei	Carmichael et al., 1997
Neosaxitoxin (neoSTX)	*Aphanizomenon* DC-1	Liu et al., 2006
	Aphanizomenon flos-aquae	Ikawa et al., 1982; Mahmood and Carmichael, 1986a; Dias et al., 2002; Pereira et al., 2000
	Aphanizomenon gracile LMECYA40	Pereira et al., 2004
	Aphanizomenon issatschenkoi	Nogueira et al., 2004
	Cylindrospermopsis raciborskii	Lagos et al., 1999; Molica et al., 2002
Gonyautoxin 1 (GTX1)	*Aphanizomenon flos-aquae*	Ferreira et al., 2001
Gonyautoxin 2 (GTX2)	*Anabaena circinalis*	Negri et al., 1995; Jones and Negri, 1997: Teste et al., 2002;
	Cylindrospermopsis raciborskii	Lagos et al., 1999
	Cylindrospermopsis raciborskii C10	Castro et al., 2004
Gonyautoxin 3 (GTX3)	*Anabaena circinalis*	Negri et al., 1995; Jones and Negri, 1997; Teste et al., 2002
	Aphanizomenon flos-aquae	Ferreira et al., 2001
	Cylindrospermopsis raciborskii	Lagos et al., 1999
	Cylindrospermopsis raciborskii C10	Castro et al., 2004
Gonyautoxin 4 (GTX4)	*Aphanizomenon flos-aquae*	Ferreira et al., 2001
Gonyautoxin 5 (GTX5)	*Anabaena circinalis*	Negri et al., 1995
	Aphanizomenon flos-aquae	Pereira et al., 2000; Dias et al., 2002
	Aphanizomenon issatschenkoi	Nogueira et al., 2004
Gonyautoxin 6 (GTX6)	*Aphanizomenon flos-aquae*	Pereira et al., 2000
	Cylindrospermopsis raciborskii	Molica et al., 2002
Decarbomoyl gonyautoxin 2 (dcGTX2)	*Anabaena circinalis*	Negri et al., 1995
	Lyngbya wollei	Carmichael et al., 1997; Onodera et al., 1997b
Decarbomoyl gonyautoxin 3 (dcGTX3)	*Anabaena circinalis*	Negri et al., 1995; Teste et al., 2002
	Aphanizomenon DC-1	Liu et al., 2006
	Lyngbya wollei	Carmichael et al., 1997; Onodera et al., 1997b
C1	*Aphanizomenon flos-aquae*	Ferreira et al., 2001
	Anabaena circinalis	Negri et al., 1995; Jones and Negri, 1997; Teste et al., 2002
	Cylindrospermopsis raciborskii T3	Pomati et al., 2004
C2	*Anabaena circinalis*	Negri et al., 1995; Jones and Negri, 1997; Ferreira et al., 2001; Teste et al., 2002
	Cylindrospermopsis raciborskii T3	Pomati et al., 2004
Lyngbya wollei toxin 1	*Lyngbya wollei*	Onodera et al., 1997b
Lyngbya wollei toxin 2	*Lyngbya wollei*	Onodera et al., 1997b
Lyngbya wollei toxin 3	*Lyngbya wollei*	Onodera et al., 1997b
Lyngbya wollei toxin 4	*Lyngbya wollei*	Onodera et al., 1997b
Lyngbya wollei toxin 5	*Lyngbya wollei*	Onodera et al., 1997b
Lyngbya wollei toxin 6	*Lyngbya wollei*	Onodera et al., 1997b

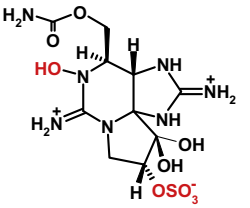
the following: carbamate saxitoxins > saxitoxin > neosaxi toxin > gonyautoxins > decarbamoyl saxitoxins > *N*-sulfocarbamoyl derivatives saxitoxins (Deeds et al., 2008; Indrasena and Gill, 1998).

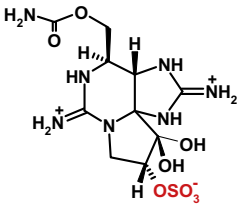
A cyanobacterial bloom dominated by *Anabaena circinalis* in a dam near Forbes in central New South Wales (Australia) was suspected to cause the death of 14 sheeps. C-toxins, gonyautoxins and saxitoxin were found in extracts of *A. circinalis* and in the intestine contents of dead sheep (Negri et al., 1995). Saxitoxin producing cyanobacterial strains were also found in the water reservoir of Tapacurá (Brazil) supplying drinking water to 1.3 million inhabitants (Molica et al., 2005). Saxitoxin was considered in the Schedule 1 of the Chemical Weapons Convention together with warfare agents such as mustard gas, sarin, ricin and many others (see review by Llewellyn, 2006 and references included). Several methods for saxitoxin detection have been developed which are highly sensitive and specific including fluorescence spectroscopy (Indrasena and Gill, 1998), HPLC with pre-column derivatization and fluorescence detection (Papageorgiou et al., 2005; Lawrence et al., 2005), radio-receptor binding assays (Ruberu et al., 2003), saxiphilin- (a saxitoxin binding protein) based methods (Llewellyn et al., 2001), immunodetection (Micheli et al., 2002) and cell-based assays (Humpage et al., 2007).

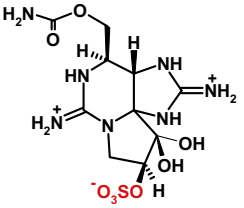

Saxitoxin
(STX)

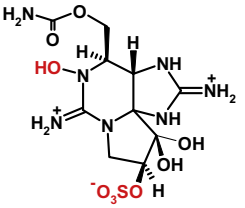

Decarbomoyl saxitoxin
(dcSTX)

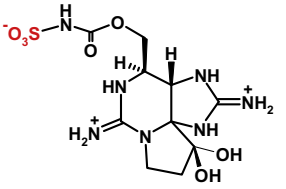

Neosaxitoxin
(neoSTX)

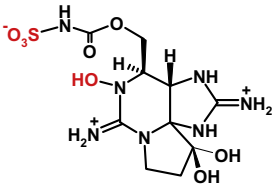

Gonyautoxin 1
(GTX1)

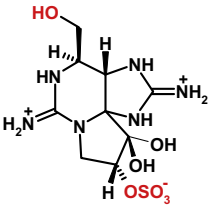

Gonyautoxin 2
(GTX2)

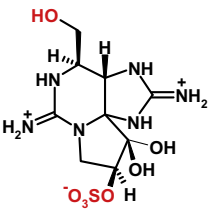

Gonyautoxin 3
(GTX3)

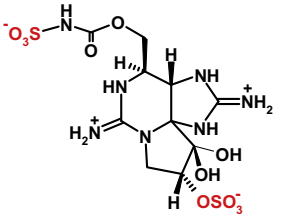

Gonyautoxin 4
(GTX4)

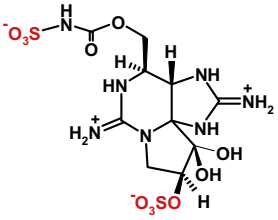

Gonyautoxin 5
(GTX5)

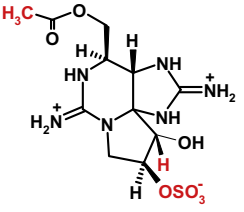

Gonyautoxin 6
(GTX6)

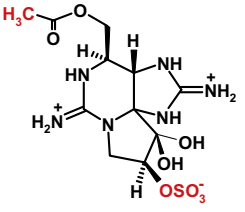

dcGonyautoxin 2
(dcGTX1)

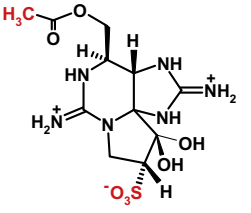

dcGonyautoxin 3
(dcGTX3)

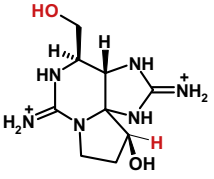

C1

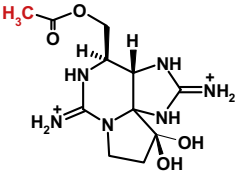

C2


Lyngbya wollei toxin 1
(Lw toxin 1)


Lyngbya wollei toxin 2
(Lw toxin 2)


Lyngbya wollei toxin 3
(Lw toxin 3)


Lyngbya wollei toxin 4
(Lw toxin 4)


Lyngbya wollei toxin 5
(Lw toxin 5)

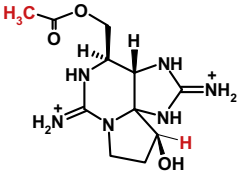

Lyngbya wollei toxin 6
(Lw toxin 6)

Fig. 4. Saxitoxin analogues produced by some members of different cyanobacteria genera. From Llewellyn (2006). Reproduced by permission of The Royal Society of Chemistry (http://dx.doi.org/10.1039/b501296c). See Table 1 for species names and references.

Saxitoxin shows nanomolar affinity for certain voltage-gated sodium channels types, and constitutes a suitable blueprint for developing new channel blockers for therapeutic purposes (Schantz and Johnson, 1992; Kohane et al., 2000; Garrido et al., 2007). Although the chemical synthesis of saxitoxin was achieved in the past (Tanino et al., 1977; Jacobi et al., 1984), novel synthetic pathways were designed to assemble the tricyclic skeleton that defines saxitoxin (Fleming and Du Bois, 2006; Fleming et al., 2007). Recently, the biosynthetic gene cluster of saxitoxin was discovered from the cyanobacterium *C. raciborskii* T3 (Kellmann et al., 2008). Saxitoxin gene cluster comprises more than 35 kb encoding for 31 open reading frames. By comparative sequence analysis, 30 enzymatic activities were assigned to 26 proteins of the saxitoxin synthase complex. The biosynthetic origin of saxitoxin comprises arginine, *S*-adenosylmethionine and acetate as it was proposed by Shimizu (1993) based on feeding experiments performed with *Alexandrium tamarense* and *Aphanizomenon flos-aquae*. The *in-silico* comparative DNA sequence analysis complemented

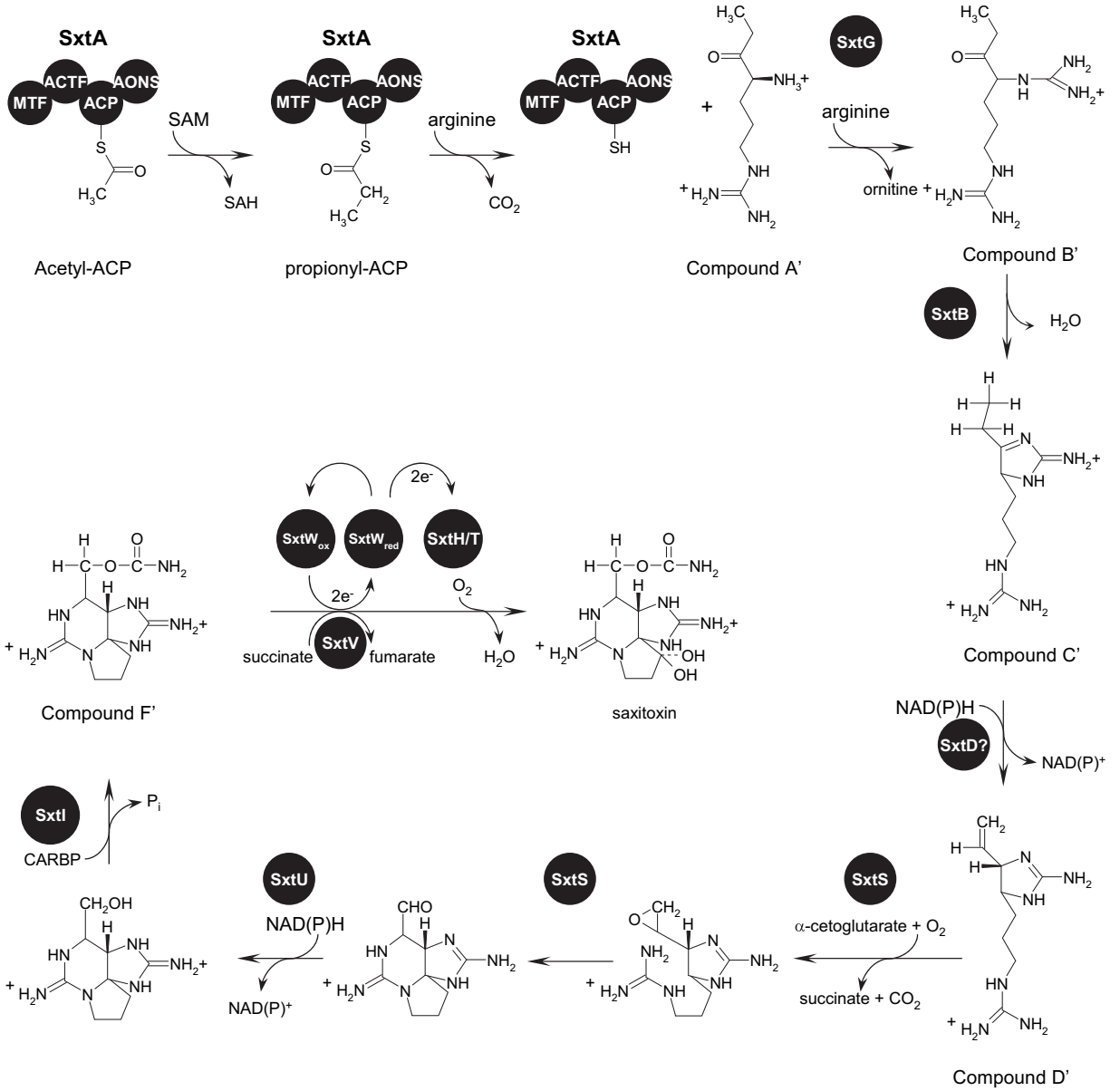


Fig. 5. Revised pathway for the biosynthesis of saxitoxin proposed by Neilan et al. From Kellmann et al. (*Appl. Environ. Microbiol.*, 2008, 74, 4044-4053 doi:10.1128/AEM.00353-08) and reproduced with permission from American Society for Microbiology. The *sxt* genes from *Cylindrospermopsis raciborskii* T3 and their predicted functions: *sxtA*: methyltransferase; *sxtG*: amidinotransferase; *sxtB*: cytidine deaminase; *sxtD*: sterole desaturase-like protein; *sxtS*: phytanoyl-CoA dioxygenase; *sxtU*: alcohol dehydrogenase; *sxtI*: carbamoyltransferase; *sxtW*: ferredoxin; *sxtV*: succinate dehydrogenase; *sxtH*: phenylpropionate dioxygenase; *sxtT*: phenylpropionate dioxygenase.

with liquid chromatography–tandem mass spectrometry experiments carried out with extracts of *Anabaena circinalis* to detect the presence of the intermediate metabolites A′, C′ and E′ allowed Neilan et al. to propose a revised pathway for saxitoxin biosynthesis (Fig. 5) (Kellmann et al., 2008).

3. Neurotoxic lipopeptides in marine cyanobacteria

3.1. Antillatoxin

Antillatoxin A ($C_{28}H_{45}N_3O_5$; UV λ_{max} at 230 nm; $\varepsilon = 12{,}000$; HRFABMS m/z $[M+H]^+$ 504.3436; Fig. 1) is a structurally novel lipopeptide with a high degree of methylation produced by the tropical marine cyanobacterium *Lyngbya majuscula* collected in Curaçao (Orjala et al., 1995). The cyclic lipopeptide antillatoxin A is composed of a tripeptide that forms both ester and amide linkages with a highly methylated lipid section. For complete ^1H and ^{13}C NMR data see Orjala et al. (1995), Yokokawa et al. (2000) and Li et al. (2004). In addition to antillatoxin A, specimens of *L. majuscula* collected from Collado Reef (Puerto Rico) and Bush Key, Dry Tortugas (Florida Keys, USA) were shown to produce an *N*-methyl homophenylalanine homologue called antillatoxin B ($C_{33}H_{48}N_3O_5$; UV λ_{max} at 209 nm, $\varepsilon = 50{,}119$ and at 240 nm, $\varepsilon = 114{,}824.06$; HRFABMS m/z $[M+Na]^+$ 566.3596; Fig. 1). For complete ^1H and ^{13}C NMR data see Nogle et al. (2001).

The structure of antillatoxin A, initially formulated from spectroscopic information, was revised at one stereocenter (C-4) after its chemical synthesis by Yokokawa et al. (1999, 2000). The preferred stereochemistry of antillatoxin A was further confirmed as a result of synthesis of four different stereoisomers of antillatoxin A (all possible C-4 and C-5 diastereomers) (Li et al., 2004). Consequently, natural antillatoxin A has a (*4R*,*5R*)-configuration (Yokokawa et al., 2000; Li et al., 2004). Total synthesis of antillatoxin A and its stereoisomers was achieved by Yokokawa and Shioiri (1998), Yokokawa et al. (1999, 2000) and recently by Lee and Loh (2006).

Antillatoxin A was first characterized as one of the most ichthyotoxic compounds (Lethal Concentration 50 (LC_{50}) = 0.1 μM) exceeded only by brevetoxins (Orjala et al., 1995). Later, it was shown that antillatoxin A induced a rapid neuronal death in cerebellar granule cell cultures ($LC_{50} = 0.18$ μM). The cytotoxicity of antillatoxin A was prevented by dextrorphan and MK-801, two non-competitive *N*-methyl-D-aspartic acid (NMDA) receptor antagonists (Berman et al., 1999). The work of Li et al. (2001) showed for the first time that the molecular target of antillatoxin A was the voltage-gated sodium channels. Indeed, they showed that neurotoxicity, as well as Ca^{2+} influx into cerebellar granule cells induced by antillatoxin A, was antagonized by tetrodotoxin, a well known sodium channel blocker. Furthermore, antillatoxin A was found to enhance Na^+ influx in intact neurons, effect that was also antagonized by tetrodotoxin ($EC_{50} = 98.2$ nM) confirming that antillatoxin A is an activator of voltage-gated sodium channels (Li et al., 2001).

Antillatoxin A is a member of the lipid-soluble gating modifier toxins of voltage-gated sodium channels that include brevetoxins, batrachotoxin, veratridine and gambierol (Cao et al., 2008). This group of toxins produces a rapid and concentration-dependent increase of intracellular Na^+ in neocortical neurons that can be antagonized by tetrodoxin. Intracellular Na^+ has been shown to modulate NMDA receptor activity. The increase of intracellular Na^+ selectively up-regulates synaptic responses mediated by NMDA receptors but not by non-NMDA receptors (Yu and Salter, 1998; Yu, 2006). In the case of exposure of neocortical neurons to antillatoxin A (300 nM) the increase of intracellular Na^+ exceeded 40 mM (Cao et al., 2008). Further work needs to be done in order to determine the binding site of antillatoxin A in voltage-gated sodium channels.

Antillatoxin B showed reduced sodium channel-activation properties ($EC_{50} = 1.77$ μM) and exhibited less ichthyotoxic activity ($LC_{50} = 1$ μM) compared to antillatoxin A, suggesting that the substitution of an *N*-methyl homophenylalanine residue for an *N*-methyl valine residue in antillatoxin B is responsible for its decreased activity (Nogle et al., 2001, Fig. 1). Similarly, the (*4R*,*5R*)-antillatoxin A is 25-fold more potent than its other three stereoisomers [(*4S*,*5R*)-, (*4S*,*5S*)- and (*4R*,*5S*)-antillatoxin A], indicating that the overall molecular topology of antillatoxin A is affected by changes at the stereocenter (C-4) (Li et al., 2004).

3.2. Kalkitoxin

Kalkitoxin ($C_{21}H_{38}N_2OS$; UV (MeOH) λ_{max} 250 nm; $\varepsilon = 2600$; High-resolution electron impact mass spectrometry m/z $[M]^+$ 366.2696; Fig. 1), is a thiazoline-containing lipopeptide discovered and purified from organic extracts of *Lyngbya majuscula* collected in the coasts of Curaçao using bioassay guided fractionation (Wu et al., 2000). Later, Nogle and Gerwick (2003) showed that *L. majuscula* specimens collected in the shallow coasts of Puerto Rico also produced kalkitoxin. Natural kalkitoxin possesses a 2,4-disubstituted thiazoline, a lipophilic chain and an unsaturated $CH_2{=}CH_2$ unit. For complete ^1H NMR (benzene-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) see Wu et al. (2000). Based on NMR analysis, Wu et al. (2000) assigned four stereochemical possibilities to kalkitoxin. In order to determine the absolute stereochemistry of natural (*3R*, *7R*, *8S*, *10S*, *2′R*)-kalkitoxin, they synthesized all possible stereoisomers and compared their ^{13}C NMR spectra. Total synthesis of (+)-kalkitoxin was also achieved by White et al. (2004).

Initial studies have shown that kalkitoxin was ichthyotoxic to the goldfish *Carassius auratus* ($LC_{50} = 700$ nM) and toxic to the aquatic crustacean brine shrimp (*Artemia salina*) with an $LC_{50} = 170$ nM (Wu et al., 2000). Accordingly, cytotoxic studies performed with synthetic (+)-kalkitoxin and two synthetic precursors using the human colon cell line HCT-116 showed that the thiazoline moiety of kalkitoxin is required for cytotoxicity ($IC_{50} = 1.0 \times 10^{-3}$ μg ml^{-1}) (White et al., 2004). Kalkitoxin was also shown to induce delayed neurotoxicity in cerebellar granule neurons in a concentration-dependent manner ($LC_{50} = 3.86$ nM). The delayed kalkitoxin toxicity was prevented only when the NMDA receptor antagonists (dextrorphan and MK-801) were present during the 22 h post-exposure period

(Berman et al., 1999). Kalkitoxin interaction with voltage-gated sodium channels was demonstrated using cerebellar granule neurons in culture (LePage et al., 2005). Kalkitoxin blocks veratridine-induced intracellular elevation of Ca^{2+} and neurotoxicity in a concentration-dependent manner ($EC_{50} = 262.7$ nM) showing indirectly that kalkitoxin blocks voltage-gated sodium channels. Furthermore, ligand-binding assay data using cerebellar granule cells showed that kalkitoxin alone did not interfere with [^{3}H]-batrachotoxin binding to the sodium channels; however, in the presence of deltamethrin, a positive allosteric modulator of sodium channels, kalkitoxin inhibited [^{3}H]-batrachotoxin binding to the voltage-gated sodium channels ($IC_{50} = 11.9$ nM; LePage et al., 2005). Since batrachotoxin binds to voltage-gated sodium channels only when the channel is in its open conformation (Catterall et al., 1981), the latter results provide evidences that kalkitoxin acts as a blocking agent of voltage-gated sodium channels.

3.3. Jamaicamide

Jamaicamide A ($C_{27}H_{37}O_4N_2ClBr$; UV (MeOH) λ_{max} 272 nm; $\varepsilon = 7943$; HRFABMS $[M+H]^+$ at m/z 567.1625; Fig. 1) is a novel highly functionalized neurotoxic lipopeptide possessing an unusual alkynyl bromide, vinyl chloride, β-methoxy eneone system, and a pyrrolinone ring. Jamaicamide A and two other isomers, jamaicamide B ($C_{27}H_{37}O_4N_2Cl$) and jamaicamide C ($C_{27}H_{39}O_4N_2Cl$;) were purified and characterized from a dark green strain of the marine cyanobacterium *Lyngbya majuscula* (strain JHB) growing in low abundance in Hector's Bay, Jamaica. Jamaicamide B is a debromo analogue of jamaicamide A, while in jamaicamide C, which also lacks the bromine atom, a terminal olefin replaces the terminal alkyne of jamaicamide B. For ^{1}H and ^{13}C NMR data of jamaicamides see Edwards et al. (2004). The exceptional structure of jamaicamide B that includes a vinyl chloride was exploited to visualize and distinguish *L. majuscula* strain "3L" (curacin A producer) from *L. majuscula* strain JHB (jamaicamides A, B and C producer) by MALDI-TOF imaging at m/z 374 for curacyn and at m/z 511 for jamaicamide B (Simmons et al., 2008).

The chemical structure of jamaicamides A, B and C suggested that these molecules derive from a mixture of polyketide (9 acetate units), amino acid (L-Ala, β-Ala) and methionine-derived methyl groups. Based on feeding experiments that strongly supported a mixed polyketide synthase/nonribosomal peptide synthetase assembly for jamaicamides, a 58 kb gene cluster of 17 open reading frames was cloned and the biosynthetic pathway of jamaicamides deciphered. The *jam* gene cluster from *L. majuscula* JHB is organized in a remarkably co-linear arrangement with respect to its proposed biosynthesis (Fig. 6) (Edwards et al., 2004).

The jamaicamides A, B and C showed similar cytotoxicity to H-460 human lung and Neuro-2a mouse neuroblastoma cell lines ($LD_{50} = 15$ μM). Jamaicamides were tested for their capacity either to activate or block voltage-gated sodium channels using an antagonism cell bioassay (Manger et al., 1995) with cerebellar granule neurons, ouabaine, veratridine, brevetoxin and saxitoxin. All jamaicamide isomers showed channel-blocking activity at 5 μM. None of the jamaicamide isomers exhibited sodium channel-activating activity (Edwards et al., 2004).

4. Neurotoxic amino acid

4.1. L-Beta-N-methylamino-L-alanine

The non-protein amino acid L-beta-*N*-methylamino-L-alanine (L-BMAA; 2-amino-3-methylaminopropanoic acid; $C_4H_{10}N_2O_2$; MW: 118.1344; Fig. 1) was initially isolated from highly toxic seeds of the false sago palm, the gymnosperm *Cycas circinalis* L. (Vega and Bell, 1967; Vega et al., 1967). A possible link was established between L-BMAA and the high incidence of a progressive neurological disease displaying clinical symptoms and histological aspects similar to amyotrophic lateral sclerosis/Parkinsonism–dementia complex (ALS/PDC), among the Chamorro people who traditionally used cycad seeds as a source of food and medicine in the Mariana islands of Guam and Rota (Spencer et al., 1986; Spencer et al., 1987a). Previous studies showed a correlation between exposure to cycad seeds and motor neurone disease in West New Guinea in Indonesia (Spencer et al., 1987b), and in Kii peninsula of Honshu island in Japan (Spencer et al., 1987c). Neurological deficits observed following repeated oral administration of L-BMAA to macaques (*Macaca fascicularis*) supported the hypothesis that cycad seeds might play a significant role in the aetiology of ALS/PDC in Guam and elsewhere (Spencer et al., 1986, 1987a). However, other experiments with primates fed on cycad flour (with unknown concentrations of L-BMAA) did not produce any clinical signs of neurological diseases (Duncan et al., 1988; Garruto et al., 1988, 1991).

Recently, the possibility that L-BMAA could trigger neurodegenerative disease has been put again at the forefront following the publications by Cox et al. who proposed biomagnification of cyanobacterial L-BMAA as a mechanism to generate large amount of neurotoxins in the Guam ecosystem (Cox and Sacks, 2002). This hypothesis was corroborated by (i) the discovery that L-BMAA occurs as both, free and protein-bound forms throughout tissues of flying foxes (*Pteropus mariannus mariannus*). Flying foxes feed on cycads (*Cycas micronesica*) and constitute a component of the traditional Chamorro diet (Banack et al., 2006), and (ii) the demonstration that diverse taxa of cyanobacteria including *Nostoc* symbionts isolated from coralloid roots of *C. microsenica* synthesize L-BMAA (Cox et al., 2003, 2005). Moreover, protein-bound L-BMAA was found to remain in Chamorro foods prepared from cycad flour (Banack et al., 2006). In Peru, people seasonally collect in highland lakes globular colonies of *Nostoc commune*, called "llullucha". Such colonies purchased in different markets from Cuzco (South East of Peru) were analyzed by UPLC–MS and LC/MS/MS and were found to contain L-BMAA (Johnson et al., 2008). Recent reports also described the presence of L-BMAA in a marine cyanobacterium isolated from the island of Oahu in Hawaii (Banack et al., 2007) and in cyanobacterial cultures representing the taxonomic diversity and geographic distribution in Southern Africa (Esterhuizen and Downing, 2008). Co-occurrence of

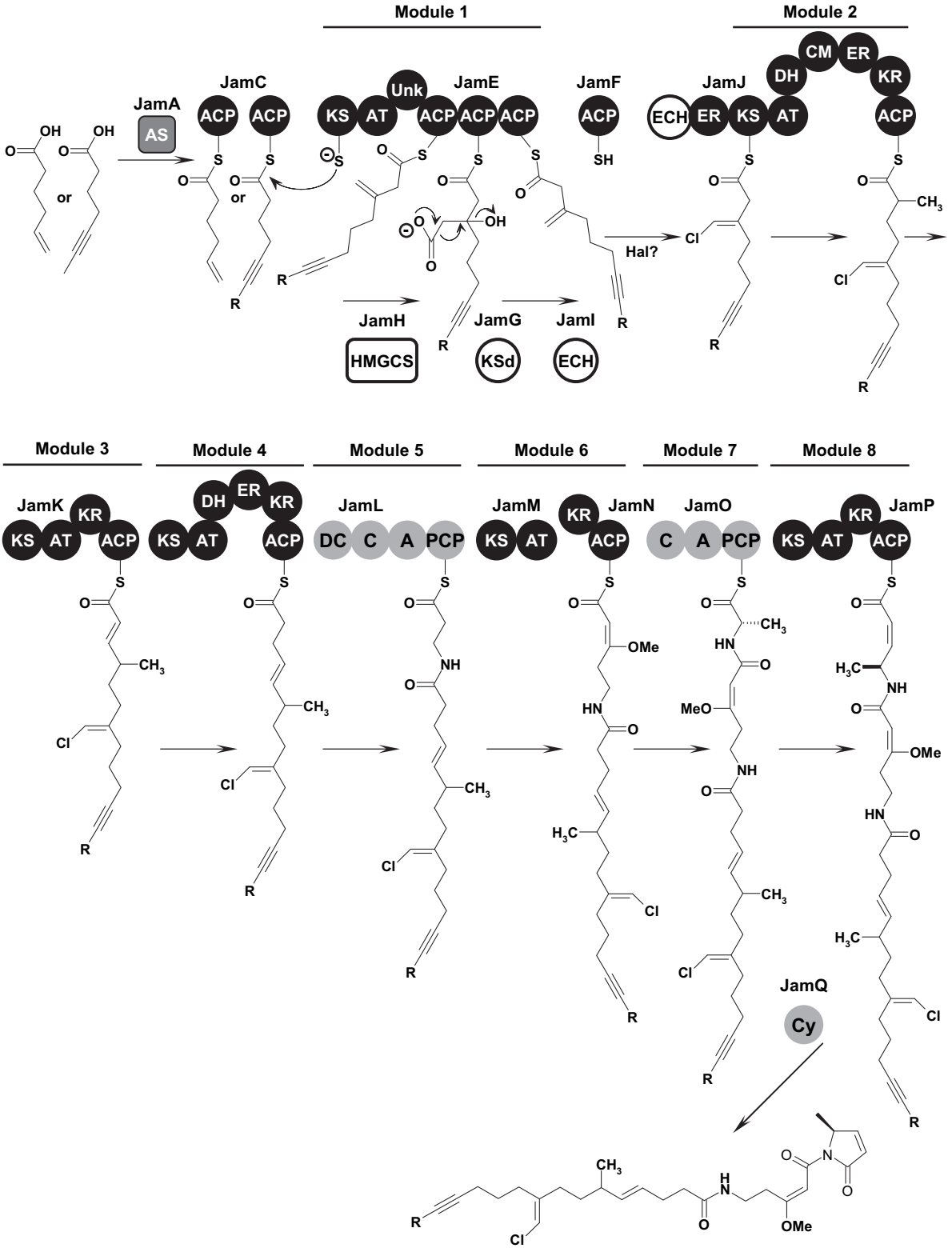


Fig. 6. Jamaicamide biosynthetic pathway. Reproduced, with permission, from Edwards et al. (2006). Copyright © 2004 Elsevier Ltd. The Jam proteins from *Lyngbya majuscula* JHB *jam* gene cluster and their predicted functions: JamA: hexanoyl-ACP synthetase; JamC: acyl carrier protein; JamE: polyketide chain extension; JamF: acyl carrier protein; JamG: ketosynthase/decarboxylase; JamH: 3-hydroxy-3-methylglutaryl-CoA synthase; JamI: enoyl hydratase/isomerase; JamJ: enoyl hydratase/isomerase/PKS protein; JamK: PKS protein; JamL: PKS/NRPS protein; JamM: PKS protein; JamN: PKS protein; JamO: NRPS protein; JamP: PKS/thioesterase; JamQ: cyclization.

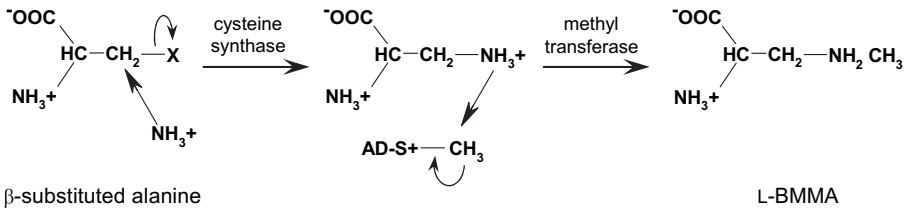


Fig. 7. Predicted two-step pathway for BMAA biosynthesis. From Brenner et al. (*Genome Biol*., 2003, 4, R78, doi:10.1186/gb-2003-4-12-r78). X = phosphoserine, cysteine, *O*-acetylserine or cyanioalanine. Ad-S^+ *S*-adenosyl-L-methionine.

L-BMAA (both, free and protein-bound forms; 8–287 μg g^{-1} cyanobacterial dry weight) and other known cyanobacterial toxins was found in bloom samples collected in British waterbodies between 1990 and 2004 (Metcalf et al., 2008).

Bioaccumulation of cyanobacterial L-BMAA could occur through different food chains in areas far from Guam and may explain the finding of L-BMAA in brain tissues of Alzheimer's patients from Canada (Cox et al., 2003; Murch et al., 2004a,b). Although there is mounting *in vivo* and *in vitro* evidence supporting a link between the presence of L-BMAA and ALS/PDC, the aetiology and pathogenesis of this neurodegenerative syndrome in the population of Western Pacific islands and in a small number of Caucasian and North American patients are still a matter of hot debate (Montine et al., 2005; Cox et al., 2006; Duncan and Marini, 2006; Garruto, 2006; Miller, 2006; Papapetropoulos, 2007; Steele and McGeer, 2008). Karymyan and Speth (2008) published a comprehensive review to which the reader is invited to refer for the description and critical evaluation of the animal models used for *in vivo* studies of L-BMAA neurotoxicity. Almost all these studies proved the neurotoxicity of this amino acid, which is generally associated with motor system disorder.

Based on analysis of expressed sequence tags, obtained from RNAs of young *Cycas rumphii* leaves, a two-step pathway has been proposed for the biosynthesis of L-BMAA in cycads (Brenner et al., 2003). In this pathway, L-BMAA synthesis begins with the transfer of NH_3 to a β-substituted alanine to form a metabolic intermediate. A cysteine synthase-like enzyme catalyzes this reaction. The second step is catalyzed by a methyl transferase that transfers a methyl group from *S*-adenosylmethionine to the new amine group of the intermediate compound (Fig. 7). Putative orthologous genes of both enzymes are present in cyanobacterial genomes, but gene inactivation is required to confirm that they are involved in the biosynthesis of L-BMAA in cyanobacteria (N. Tandeau de Marsac, unpublished data).

Considering the worldwide distribution of free-living and symbiotic cyanobacteria in most ecosystems, a search for the presence of L-BMAA in water supplies and human diets should be recommended. The ensemble of data collected, emphasizes the need for more research on L-BMAA and its relationships with neurodegenerative illnesses.

5. Conclusion

Cyanobacteria are ubiquitous primary producers that constitute up to 70% of the total phytoplankton biomass, they produce more than 30% of total free O_2 and account for more than 30% of total primary production (CO_2 fixation). Cyanobacterial excessive proliferation is of serious concern for public health as they produce hepatotoxins and neurotoxins. However, the toxigenicity of cyanobacteria is not uniform and even at the species level there is a wide variation of virulence. Cyanobacterial neurotoxins described to date target cholinergic synapses and voltage-gated sodium channels. The recent discovery of the lipopeptide neurotoxins antillatoxin, kalkitoxin and jamaicamide recalls the role of cyanobacteria as an important source for the discovery of emergent toxins. The complex structure of cyanobacterial neurotoxins represents a challenge for organic chemistry often leading to the development of novel synthetic pathways for their use as blueprints for the design of front-line drugs.

Acknowledgments

Supported in part by grant CESA 015 07 ARISTOCYA from the Agence Nationale de la Recherche, France (to J.M.), by the C.N.R.S. and the Institut Pasteur.

Conflict of interest

The authors have no conflict of interest.

References

Adeymo, O.M., Sirén, A.L., 1992. Cardio-respiratory changes and mortality in the conscious rat induced by (+)- and (±)-anatoxin-a. Toxicon 30 (8), 899–905.

Aráoz, R., Nghiêm, H.O., Rippka, R., Palibroda, N., Tandeau de Marsac, N., Herdman, M., 2005. Neurotoxins in axenic oscillatorian cyanobacteria: coexistence of anatoxin-a and homoanatoxin-a determined by ligand-binding assay and GC/MS. Microbiology 151 (4), 1263–1273.

Aráoz, R., Guérineau, V., Rippka, R., Palibroda, N., Herdman, M., Laprevote, O., von Döhren, H., Tandeau de Marsac, N., Erhard, M., 2008. MALDI-TOF-MS detection of the low molecular weight neurotoxins anatoxin-a and homoanatoxin-a on lyophilized and fresh filaments of axenic *Oscillatoria* strains. Toxicon 51 (7), 1308–1315.

Bachmann, T.T., Leca, B., Vilatte, F., Marty, J.L., Fournier, D., Schmid, R.D., 2000. Improved multianalyte detection of organophosphates and carbamates with disposable multielectrode biosensors using recombinant mutants of *Drosophila* acetylcholinesterase and artificial neural networks. Biosens. Bioelectron. 15 (3–4), 193–201.

Banack, S.A., Murch, S.J., Cox, P.A., 2006. Neurotoxic flying foxes as dietary items for the Chamorro people, Marianas Islands. J. Ethnopharmacol. 106 (1), 97–104.

Banack, S.A., Johnson, H.E., Cheng, R., Cox, P.A., 2007. Production of the neurotoxin BMAA by a marine cyanobacterium. Mar. Drugs 5 (4), 180–196.

Berman, F.W., Gerwick, W.H., Murray, T.F., 1999. Antillatoxin and kalkitoxin, ichthyotoxins from the tropical cyanobacterium *Lyngbya*

majuscula, induce distinct temporal patterns of NMDA receptor-mediated neurotoxicity. Toxicon 37 (11), 1645–1648.
Bordner, J., Thiessen, W.E., Bates, H.A., Rapoport, H., 1975. Structure of a crystalline derivative of saxitoxin. Structure of saxitoxin. J. Am. Chem. Soc. 97 (21), 6008–6012.
Brenneman, J.B., Machauer, R., Martin, S.F., 2004. Enantioselective synthesis of (+)-anatoxin-a via enyne metathesis. Tetrahedron 60 (34), 7301–7314.
Brenner, E.D., Stevenson, D.W., McCombie, R.W., Katari, M.S., Rudd, S.A., Mayer, K.F., Palenchar, P.M., Runko, S.J., Twigg, R.W., Dai, G., Martienssen, R.A., Benfey, P.N., Coruzzi, G.M., 2003. Expressed sequence tag analysis in *Cycas*, the most primitive living seed plant. Genome Biol. 4 (12), R78.
Briand, J.F., Jacquet, S., Bernard, C., Humbert, J.F., 2003. Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. Vet. Res. 34 (4), 361–377.
Bricelj, V.M., Connell, L., Konoki, K., MacQuarrie, S.P., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434 (7034), 763–767.
Cadel-Six, S., Peyraud-Thomas, C., Brient, L., Tandeau de Marsac, N., Rippka, R., Mejean, A., 2007. Different genotypes of anatoxin-producing cyanobacteria co-exist in the Tarn River, France. Appl. Environ. Microbiol. 73 (23), 7605–7614.
Cadel-Six, S., Iteman, I., Peyraud-Thomas, C., Mann, S., Ploux, O., Méjean, A., 2009. Identification of a polyketide synthase coding sequence specific for anatoxin-a-producing *Oscillatoria* cyanobacteria. Appl. Environ. Microbiol. 75 (14), 4909–4912.
Cao, Z., George, J., Gerwick, W.H., Baden, D.G., Rainier, J.D., Murray, T.F., 2008. Influence of lipid-soluble gating modifier toxins on sodium influx in neocortical neurons. J. Pharmacol. Exp. Ther. 326 (2), 604–613.
Carmichael, W.W., Gorham, P.R., 1978. Anatoxins from clones of *Anabaena flos-aquae* isolated from lakes of western Canada. Mitt. Int. Ver. Limmnol. 21, 285–295.
Carmichael, W.W., 1994. The toxins of cyanobacteria. Sci. Am. 270 (1), 78–86.
Carmichael, W.W., Evans, W.R., Yin, Q.Q., Bell, P., Moczydlowski, E., 1997. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. Appl. Environ. Microbiol. 63 (8), 3104–3110.
Carmichael, W.W., Azevedo, S.M., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109 (7), 663–668.
Castro, D., Vera, D., Lagos, N., Garcia, C., Vasquez, M., 2004. The effect of temperature on growth and production of paralytic shellfish poisoning toxins by the cyanobacterium *Cylindrospermopsis raciborskii* C10. Toxicon 44 (5), 483–489.
Catterall, W.A., Morrow, C.S., Daly, J.W., Brown, G.B., 1981. Binding of batrachotoxinin A 20-alpha-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. J. Biol. Chem. 256 (17), 8922–8927.
Cestèle, S., Catterall, W.A., 2000. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. Biochimie 82 (9–10), 883–892.
Cook, W.O., Beasley, V.R., Dahlem, A.M., Dellinger, J.A., Harlin, K.S., Carmichael, W.W., 1988. Comparison of effects of anatoxin-a(s) and paraoxon, physostigmine and pyridostigmine on mouse brain cholinesterase activity. Toxicon 26 (8), 750–753.
Cox, P.A., Sacks, O.W., 2002. Cycad neurotoxins, consumption of flying foxes, and ALS–PDC disease in Guam. Neurology 58 (6), 956–959.
Cox, P.A., Banack, S.A., Murch, S.J., 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc. Natl. Acad. Sci. U.S.A. 100 (23), 13380–13383.
Cox, P.A., Banack, S.A., Murch, S.J., Rasmussen, U., Tien, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., Bergman, B., 2005. Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. Proc. Natl. Acad. Sci. U.S.A. 102 (14), 5074–5078.
Cox, P.A., Banack, S., Murch, S., Sacks, O., 2006. Commentary on: return of the cycad hypothesis – does the amyotrophic lateral sclerosis/Parkinsonism dementia complex (ALS/PDC) of Guam have new implications for global health? Neuropathol. Appl. Neurobiol. 32 (6), 679–682.
Deeds, J.R., Landsberg, J.H., Etheridge, S.M., Pitcher, G.C., Longan, S.W., 2008. Non-traditional vectors for paralytic shellfish poisoning. Mar. Drugs 6 (2), 308–348.
Devic, E., Li, D., Dauta, A., Henriksen, P., Codd, G.A., Marty, J.L., Fournier, D., 2002. Detection of anatoxin-a(s) in environmental samples of cyanobacteria by using a biosensor with engineered acetylcholinesterases. Appl. Environ. Microbiol. 68 (8), 4102–4106.
Devlin, J.P., Edwards, O.E., Gorham, P.R., Hunter, N.R., Pike, R.K., Starvic, B., 1977. Anatoxin-a, a toxic alkaloid from *Anabaena flos-aquae* NRC-44h. Can. J. Chem. 55 (8), 1367–1371.
Dias, E., Pereira, P., Franca, S., 2002. Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA31 (cyanobacteria). J. Phycol. 38 (4), 705–712.
Duncan, M.W., Kopin, I.J., Garruto, R.M., Lavine, L., Markey, S.P., 1988. 2-Amino-3 (methylamino)-propionic acid in cycad-derived foods is an unlikely cause of amyotrophic lateral sclerosis/Parkinsonism. Lancet 2 (8611), 631–632.
Duncan, M.W., Marini, A.M., 2006. Debating the cause of a neurological disorder. Science 313 (5794), 1737.
Edwards, C., Beattie, K.A., Scrimgeour, C.M., Codd, G.A., 1992. Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30 (10), 1165–1175.
Edwards, D.J., Marquez, B.L., Nogle, L.M., McPhail, K., Goeger, D.E., Roberts, M.A., Gerwick, W.H., 2004. Structure and biosynthesis of the jamaicamides, new mixed polyketide–peptide neurotoxins from the marine cyanobacterium *Lyngbya majuscula*. Chem. Biol. 11 (6), 817–833.
Esterhuizen, M., Downing, T.G., 2008. Beta-N-methylamino-L-alanine (BMAA) in novel South African cyanobacterial isolates. Ecotoxicol. Environ. Saf. 71 (12), 309–313.
Ferreira, F.M.B., Franco Soler, J.M., Fidalgo, M.L., Fernandez-Vila, P., 2001. PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro river, northern Portugal). Toxicon 39 (6), 757–761.
Fleming, J.J., Du Bois, J., 2006. A synthesis of (+)-saxitoxin. J. Am. Chem. Soc. 128 (12), 3926–3927.
Fleming, J.J., McReynolds, M.D., Du Bois, J., 2007. (+)-Saxitoxin: a first and second generation stereoselective synthesis. J. Am. Chem. Soc. 129 (32), 9964–9975.
Francis, G., 1878. Poisonous Australian lake. Nature 18 (444), 11–12.
Furey, A., Crowley, J., Shuilleabhain, A.N., Skulberg, O.M., James, K.J., 2003a. The first identification of the rare cyanobacterial toxin, homoanatoxin-a, in Ireland. Toxicon 41 (3), 297–303.
Furey, A., Crowley, J., Lehane, M., James, K.J., 2003b. Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of anatoxins in cyanobacteria and drinking water. Rapid Commun. Mass Spectrom. 17 (6), 583–588.
Garrido, R., Lagos, N., Lagos, M., Rodríguez-Navarro, A.J., Garcia, C., Truan, D., Henriquez, A., 2007. Treatment of chronic anal fissure by gonyautoxin. Colorectal Dis. 9 (7), 619–624.
Garruto, R.M., Yanagihara, R., Gajdusek, D.C., 1988. Cycads and amyotrophic lateral sclerosis/Parkinsonism dementia. Lancet 2 (8619), 1079.
Garruto, R.M., Strong, M.J., Yanagihara, R., 1991. Experimental models of aluminum-induced motor neuron degeneration. Adv. Neurol. 56, 327–340.
Garruto, R.M., 2006. A commentary on neuronal degeneration and cell death in Guam ALS and PD: an evolutionary process of understanding. Curr. Alzheimer Res. 3 (4), 397–401.
Gordon, R.K., Gray, R.R., Reaves, C.B., Butler, D.L., Chiang, P.K., 1992. Induced release of acetylcholine from guinea pig ileum longitudinal muscle–myenteric plexus by anatoxin-a. J. Pharmacol. Exp. Ther. 263 (3), 997–1002.
Gugger, M., Lenoir, S., Berger, C., Ledreux, A., Druart, J.C., Humbert, J.F., Guette, C., Bernard, C., 2005. First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. Toxicon 45 (7), 919–928.
Gulledge, B.M., Aggen, J.B., Eng, H., Sweimeh, K., Chamberlin, A.R., 2003. Microcystin analogues comprised only of Adda and a single additional amino acid retain moderate activity as PP1/PP2A inhibitors. Bioorg. Med. Chem. Lett. 13 (17), 2907–2911.
Harada, K.-I., Kimura, Y., Ogawa, K., Suzuki, M., Dahlem, A.M., Beasley, V.R., Carmichael, W.W., 1989. A new procedure for the analysis and purification of naturally occurring anatoxin-a from the blue-green alga *Anabaena flos-aquae*. Toxicon 27 (12), 1289–1296.
Harada, K.I., Nagai, H., Kimura, Y., Suzuki, M., Park, H.D., Watanabe, M.F., Luukkainen, R., Sivonen, K., Carmichael, W.W., 1993. Liquid chromatography/mass spectrometric detection of anatoxin-a, a neurotoxin from cyanobacteria. Tetrahedron 49 (41), 9251–9260.
Hawkins, P.R., Runnegar, M.T., Jackson, A.R., Falconer, I.R., 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and

Subba Raju isolated from a domestic water supply reservoir. Appl. Environ. Microbiol. 50 (5), 1292–1295.
Hemscheidt, T., Rapala, J., Sivonen, K., 1995. Biosynthesis of anatoxin-a in *Anabaena flos-aquae* and homoanatoxin-a in *Oscillatoria formosa*. J. Chem. Soc. Commun. 13, 1361–1362.
Henriksen, P., Carmichael, W.W., An, J., Moestrup, Ø., 1997. Detection of an anatoxin-a(s)-like anticholinesterase in natural blooms and cultures of cyanobacteria/blue-green algae from Danish lakes and in the stomach contents of poisoned birds. Toxicon 35 (6), 901–913.
Himberg, K., 1989. Determination of anatoxin-a, the neurotoxin of *Anabaena flos-aquae* cyanobacterium, in algae and water by gas chromatography–mass spectrometry. J. Chromatogr. 481, 358–362.
Hotto, A.M., Satchwell, M.F., Boyer, G.L., 2007. Molecular characterization of potential microcystin-producing cyanobacteria in Lake Ontario embayments and nearshore waters. Appl. Environ. Microbiol. 73 (14), 4570–4578.
Humpage, A.R., Ledreux, A., Fanok, S., Bernard, C., Briand, J.F., Eaglesham, G., Papageorgiou, J., Nicholson, B., Steffensen, D., 2007. Application of the neuroblastoma assay for paralytic shellfish poisons to neurotoxic freshwater cyanobacteria: interlaboratory calibration and comparison with other methods of analysis. Environ. Toxicol. Chem. 26 (7), 1512–1519.
Ikawa, M., Wegener, K., Foxall, T.L., Sasner, J.J., 1982. Comparison of the toxins of the blue-green alga *Aphanizomenon flos-aquae* with the *Gonyaulax* toxins. Toxicon 20 (4), 747–752.
Indrasena, W.M., Gill, T.A., 1998. Fluorometric detection of paralytic shellfish poisoning toxins. Anal. Biochem. 264 (2), 230–236.
Jacobi, P.A., Martinelli, M.J., Polanc, S., 1984. Total synthesis of (±)-saxitoxin. J. Am. Chem. Soc. 106 (19), 5594–5598.
James, K.J., Crowley, J., Hamilton, B., Lehane, M., Skulberg, O., Furey, A., 2005. Anatoxins and degradation products, determined using hybrid quadrupole time-of-flight and quadrupole ion-trap mass spectrometry: forensic investigations of cyanobacterial neurotoxin poisoning. Rapid Commun. Mass Spectrom. 19 (9), 1167–1175.
Johnson, H.E., King, S.R., Banack, S.A., Webster, C., Callanaupa, W.J., Cox, P.A., 2008. Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. J. Ethnopharmacol. 118 (1), 159–165.
Jones, G.J., Negri, A.P., 1997. Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. Water Res. 31 (3), 525–533.
Karymyan, V.T., Speth, R.C., 2008. Animal models of BMAA neurotoxicity, a critical review. Life Sci. 82 (5–6), 233–246.
Kellmann, R., Mihali, T.K., Jeon, Y.J., Pickford, R., Pomati, F., Neilan, B.A., 2008. Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 74 (13), 4044–4053.
Kohane, D.S., Lu, N.T., Gökgöl-Kline, A.C., Shubina, M., Kuang, Y., Hall, S., Strichartz, G.R., Berde, C.B., 2000. The local anesthetic properties and toxicity of saxitonin homologues for rat sciatic nerve block in vivo. Reg. Anesth. Pain Med. 25 (1), 52–59.
Koskenniemi, K., Lyra, C., Rajaniemi-Wacklin, P., Jokela, J., Sivonen, K., 2007. Quantitative real-time PCR detection of toxic *Nodularia* cyanobacteria in the Baltic Sea. Appl. Environ. Microbiol. 73 (7), 2173–2179.
Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J.S., Codd, G. A., Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser flamingos at lake Bogoria, Kenya. FEMS Microbiol. Ecol. 43 (2), 141–148.
Lagos, N., Onodera, H., Zagatto, P.A., Andrinolo, D., Azevedo, S.M.F.Q., Oshima, Y., 1999. The first evidence of paralytic shellfish toxins in the fresh water cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. Toxicon 37 (10), 1359–1373.
Lawrence, J.F., Niedzwiadek, B., Menard, C., 2005. Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaborative study. J. AOAC Int. 88 (6), 1714–1732.
LePage, K.T., Goeger, D., Yokokawa, F., Asano, T., Shioiri, T., Gerwick, W.H., Murray, T.F., 2005. The neurotoxic lipopeptide kalkitoxin interacts with voltage-sensitive sodium channels in cerebellar granule neurons. Toxicol. Lett. 158 (2), 133–139.
Lee, K.C., Loh, T.P., 2006. Total synthesis of antillatoxin. Chem. Commun. 40, 4209–4211.
Llewellyn, L.E., Negri, A.P., Doyle, J., Baker, P.D., Beltran, E.C., Neilan, B.A., 2001. Radioreceptor assays for sensitive detection and quantitation of saxitoxin and its analogues from strains of the freshwater cyanobacterium, *Anabaena circinalis*. Environ. Sci. Technol. 35 (7), 1445–1451.
Llewellyn, L.E., 2006. Saxitoxin, a toxic marine natural product that targets a multitude of receptors. Nat. Prod. Rep. 23 (2), 200–222.
Li, W.I., Berman, F.W., Okino, T., Yokokawa, F., Shioiri, T., Gerwick, W.H., Murray, T.F., 2001. Antillatoxin is a marine cyanobacterial toxin that potently activates voltage-gated sodium channels. Proc. Natl. Acad. Sci. U.S.A. 98 (13), 7599–7604.
Li, W.I., Marquez, B.L., Okino, T., Yokokawa, F., Shioiri, T., Gerwick, W.H., Murray, T.F., 2004. Characterization of the preferred stereochemistry for the neuropharmacologic actions of antillatoxin. J. Nat. Prod. 67 (4), 559–568.
Liu, Y.M., Chen, W., Li, D.H., Shen, Y.W., Liu, Y.D., Song, L.R., 2006. Analysis of paralytic shellfish toxins in *Aphanizomenon* DC-1 from Lake Dianchi, China. Environ. Toxicol. 21 (3), 289–295.
Mahmood, N.A., Carmichael, W.W., 1986a. Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. Toxicon 24 (2), 175–186.
Mahmood, N.A., Carmichael, W.W., 1986b. The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium *Anabaena flos-aquae* NRC 525-17. Toxicon 24 (5), 425–434.
Mahmood, N.A., Carmichael, W.W., Pfahler, D., 1988. Anticholinesterase poisonings in dogs from a cyanobacterial (blue-green algae) bloom dominated by *Anabaena flos-aquae*. Am. J. Vet. Res. 49 (4), 500–503.
Maizels, M., Budde, W.L., 2004. A LC/MS method for the determination of cyanobacteria toxins in water. Anal. Chem. 76 (5), 1342–1351.
Manger, R.L., Leja, L.S., Lee, S.Y., Hungerford, J.M., Hokama, Y., Dickey, R.W., Granade, H.R., Lewis, R., Yasumoto, T., Wekell, M.M., 1995. Detection of sodium channel toxins: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxins, and seafood extracts. J. AOAC Int. 78 (2), 521–527.
Mansell, H.L., 1996. Synthetic approaches to anatoxin-a. Tetrahedron 52 (17), 6025–6061.
Matsunaga, S., Moore, R.E., Niemczura, W.P., Carmichael, W.W., 1989. Anatoxin-a(s), a potent anticholinesterase from *Anabaena flos-aquae*. J. Am. Chem. Soc. 111 (20), 8021–8023.
Maynes, J.T., Luu, H.A., Cherney, M.M., Andersen, R.J., Williams, D., Holmes, C.F.B., James, M.N.G., 2006. Crystal structures of protein phosphatase-1 bound to motuporin and dihydromicrocystin-LA: elucidation of the mechanism of enzyme inhibition by cyanobacterial toxins. J. Mol. Biol. 356 (1), 111–120.
Méjean, A., Mann, S., Maldiney, T., Vassiliadis, G., Lequin, O., Ploux, O. 2009. Evidence that biosynthesis of the neurotoxic alkaloids anatoxin-a and homoanatoxin-a in the cyanobacterium *Oscillatoria* PCC 6506 occurs on a modular polyketide synthase initiated by L-proline. J. Am. Chem. Soc. 131(22), 7512–7513.
Metcalf, J.S., Banack, S.A., Lindsay, J., Morrison, L.F., Cox, P.A., Codd, G.A., 2008. Co-occurrence of beta-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990–2004. Environ. Microbiol. 10 (3), 702–708.
Micheli, L., Di Stefano, S., Moscone, D., Palleschi, G., Marini, S., Coletta, M., Draisci, R., delli Quadri, F., 2002. Production of antibodies and development of highly sensitive formats of enzyme immunoassay for saxitoxin analysis. Anal. Bioanal. Chem. 373 (8), 678–684.
Miller, G., 2006. Neurodegenerative disease: Guam's deadly stalker: on the loose worldwide? Science 313 (5786), 428–431.
Molica, R., Onodera, H., Garcia, C., Rivas, M., Andrinolo, D., Nascimento, S., Meguro, H., Oshima, Y., Azevedo, S., Lagos, N., 2002. Toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii* (Cyanophyceae) isolated from Tabocas reservoir in Caruaru, Brazil, including demonstration of a new saxitoxin analogue. Phycologia 41 (6), 606–611.
Molica, R.J.R., Oliveira, E.J.A., Carvalho, P.V.V.C., Costa, A.N.S.F., Cunha, M.C. C., Melo, G.L., Azevedo, S.M.F.O., 2005. Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. Harmful Algae 4 (4), 743–753.
Montine, T.J., Li, K., Perl, D.P., Galasko, D., 2005. Lack of beta-methylamino-L-alanine in brain from controls, AD, or Chamorros with PDC. Neurology 65 (5), 768–769.
Murch, S.J., Cox, P.A., Banack, S.A., 2004a. A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. Proc. Natl. Acad. Sci. U.S.A. 101 (33), 12228–12231.
Murch, S.J., Cox, P.A., Banack, S.A., Steele, J.C., Sacks, O.W., 2004b. Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurol. Scand. 110 (4), 267–269.
Namikoshi, M., Murakami, T., Watanabe, M.F., Oda, T., Yamada, J., Tsujimura, S., Nagai, H., Oishi, S., 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. Toxicon 42 (5), 533–538.

Namikoshi, M., Murakami, T., Fujiwara, T., Nagai, H., Niki, T., Harigaya, E., Watanabe, M.F., Oda, T., Yamada, J., Tsujimura, S., 2004. Biosynthesis and transformation of homoanatoxin-a in the cyanobacterium *Raphidiopsis mediterranea* Skuja and structures of three new homologues. Chem. Res. Toxicol. 17 (12), 1692–1696.

Negri, A.P., Jones, G.J., 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. Toxicon 33 (5), 667–678.

Negri, A.P., Jones, G.J., Hindmarsh, M., 1995. Sheep mortality associated with paralytic shellfish poisoning toxins from the cyanobacterium *Anabaena circinalis*. Toxicon 33 (10), 1321–1329.

Nogle, L.M., Okino, T., Gerwick, W.H., 2001. Antillatoxin B, a neurotoxic lipopeptide from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. 64 (7), 983–985.

Nogle, L.M., Gerwick, W.H., 2003. Diverse secondary metabolites from a Puerto Rican collection of *Lyngbya majuscula*. J. Nat. Prod. 66 (2), 217–220.

Nogueira, I.C., Pereira, P., Dias, E., Pflugmacher, S., Wiegand, C., Franca, S., Vasconcelos, V.M., 2004. Accumulation of paralytic shellfish toxins (PST) from the cyanobacterium *Aphanizomenon issatschenkoi* by the cladoceran *Daphnia magna*. Toxicon 44 (7), 773–780.

Ohtani, I., Moore, R.E., Runnegar, M.T.C., 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. J. Am. Chem. Soc. 114 (20), 7941–7942.

Oksanen, I., Jokela, J., Fewer, D.P., Wahlsten, M., Rikkinen, J., Sivonen, K., 2004. Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I. Appl. Environ. Microbiol. 70 (10), 5756–5763.

Onodera, H., Oshima, Y., Henriksen, P., Yasumoto, T., 1997a. Confirmation of anatoxin-a(s), in the cyanobacterium *Anabaena lemmermannii*, as the cause of bird kills in Danish lakes. Toxicon 35 (11), 1645–1648.

Onodera, H., Satake, M., Oshima, Y., Yasumoto, T., Carmichael, W.W., 1997b. New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. Nat. Toxins 5 (4), 146–151.

Orjala, J., Nagle, D.G., Hsu, V., Gerwick, W.H., 1995. Antillatoxin: an exceptionally ichthyotoxic cyclic lipopeptide from the tropical cyanobacterium *Lyngbya majuscula*. J. Am. Chem. Soc. 117 (31), 8281–8282.

Papageorgiou, J., Nicholson, B.C., Linke, T.A., Kapralos, C., 2005. Analysis of cyanobacterial-derived saxitoxins using high-performance ion exchange chromatography with chemical oxidation/fluorescence detection. Environ. Toxicol. 20 (6), 549–559.

Papapetropoulos, S., 2007. Is there a role for naturally occurring cyanobacterial toxins in neurodegeneration? The beta-N-methylamino-L-alanine (BMAA) paradigm. Neurochem. Int. 50 (7–8), 998–1003.

Park, H.D., Watanabe, M.F., Harda, K., Nagai, H., Suzuki, M., Watanabe, M., Hayashi, H., 1993. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. Nat. Toxins 1 (6), 353–360.

Parsons, P.J., Camp, N.P., Edwards, N., Sumoreeah, L.R., 2000. Synthesis of (±)-anatoxin-a and analogues. Tetrahedron 56 (2), 309–315.

Pereira, P., Onodera, H., Andrinolo, D., Franca, S., Araujo, F., Lagos, N., Oshima, Y., 2000. Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal. Toxicon 38 (12), 1689–1702.

Pereira, P., Li, R.H., Carmichael, W.W., Dias, E., Franca, S., 2004. Taxonomy and production of paralytic shellfish toxins by the freshwater cyanobacterium *Aphanizomenon gracile* LMECYA40. Eur. J. Phycol. 39 (4), 361–368.

Pita, R., Anadón, A., Martínez-Larrañaga, M.R., 2003. Neurotoxins with anticholinesterase activity and their possible use as warfare agents. Med. Clin. (Barc.) 121 (13), 511–517.

Pomati, F., Sacchi, S., Rossetti, C., Giovannardi, S., Onodera, H., Oshima, Y., Neilan, B.A., 2000. The freshwater cyanobacterium *Planktothrix* sp. FP1: molecular identification and detection of paralytic shellfish poisoning toxins. J. Phycol. 36 (3), 553–562.

Pomati, F., Moffitt, M.C., Cavaliere, R., Neilan, B.A., 2004. Evidence for differences in the metabolism of saxitoxin and C1 + 2 toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii* T3. Biochim. Biophys. Acta 1674 (1), 60–67.

Preussel, K., Stüken, A., Wiedner, C., Chorus, I., Fastner, J., 2006. First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47 (2), 156–162.

Rapala, J., Robertson, A., Negri, A.P., Berg, K.A., Tuomi, P., Lyra, C., Erkomaa, K., Lahti, K., Hoppu, K., Lepistö, L., 2005. First report of saxitoxin in Finnish lakes and possible associated effects on human health. Environ. Toxicol. 20 (3), 331–340.

Rasmussen, B., Fletcher, I.R., Brocks, J.J., Kilburn, M.R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. Nature 455 (7216), 1101–1104.

Rinehart, K.L., Harada, K., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M. H.G., Blunt, J.W., Mulligan, P.E., Beasley, V.R., Dahlem, A.M., Carmichael, W.W., 1988. Nodularin, microcystin, and the configuration of Adda. J. Am. Chem. Soc. 110 (25), 8557–8558.

Roe, S.J., Stockman, R.A., 2008. A two-directional approach to the anatoxin alkaloids: second synthesis of homoanatoxin and efficient synthesis of anatoxin-a. Chem. Commun. 29, 3432–3434.

Rouhiainen, L., Sivonen, K., Buikema, W.J., Haselkorn, R., 1995. Characterization of toxin-producing cyanobacteria by using an oligonucleotide probe containing a tandemly repeated heptamer. J. Bacteriol. 177 (20), 6021–6026.

Ruberu, S.R., Liu, Y.G., Wong, C.T., Perera, S.K., Langlois, G.W., Doucette, G.J., Powell, C.L., 2003. Receptor binding assay for paralytic shellfish poisoning toxins: optimization and interlaboratory comparison. J. AOAC. Int. 86 (4), 737–745.

Runnegar, M.T., Xie, C., Snider, B.B., Wallace, G.A., Weinreb, S.M., Kuhlenkamp, J., 2002. In vitro hepatotoxicity of the cyanobacterial alkaloid cylindrospermopsin and related synthetic analogues. Toxicol. Sci. 67 (1), 81–87.

Saito, K., Konno, A., Ishii, H., Saito, H., Nishida, F., Abe, T., Chen, C., 2001. Nodularin-Har: a new nodularin from *Nodularia*. J. Nat. Prod. 64 (1), 139–141.

Schantz, E.J., Mold, J.D., Stanger, D.W., Shavel, J., Riel, F.J., Bowden, J.P., Lynch, J.M., Wyler, R.S., Riegel, B., Sommer, H., 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. J. Am. Chem. Soc. 79 (19), 5230–5235.

Schantz, E.J., Ghazarossian, V.E., Schnoes, H.K., Strong, F.M., Springer, J.P., Pezzanite, J.O., Clardy, J., 1975. The structure of saxitoxin. J. Am. Chem. Soc. 97 (5), 1238–1239.

Schantz, E.J., Johnson, E.A., 1992. Properties and use of botulinum toxin and other microbial neurotoxins in medicine. Microbiol. Rev. 56 (1), 80–99.

Selwood, A.I., Holland, P.T., Wood, S.A., Smith, K.F., McNabb, P.S., 2007. Production of anatoxin-a and a novel biosynthetic precursor by the cyanobacterium *Aphanizomenon issatschenkoi*. Environ. Sci. Technol. 41 (2), 506–510.

Shimizu, Y., Buckley, L.J., Alam, M., Oshima, Y., Fallon, W.E., Kasai, H., Miura, I., Gullo, V.P., Nakanishi, K., 1976. Structures of gonyautoxin II and III from the East Coast toxic dinoflagellate *Gonyaulax tamarensis*. J. Am. Chem. Soc. 98 (17), 5414–5416.

Shimizu, Y., 1986. Toxigenesis and biosynthesis of saxitoxin analogues. Pure Appl. Chem. 58 (2), 257–262.

Shimizu, Y., 1993. Microalgal metabolites. Chem. Rev. 93 (5), 1685–1698.

Shimizu, Y., 1996. Microalgal metabolites: a new perspective. Annu. Rev. Microbiol. 50, 431–465.

Simmons, T.L., Coates, R.C., Clark, B.R., Engene, N., Gonzalez, D., Esquenazi, E., Dorrestein, P.C., Gerwick, W.H., 2008. Biosynthetic origin of natural products isolated from marine microorganism–invertebrate assemblages. Proc. Natl. Acad. Sci. U.S.A. 105 (12), 4587–4594.

Sivonen, K., Himberg, K., Luukkainen, R., Niemelä, S.I., Poon, G.K., Codd, G. A., 1989. Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. Toxic. Assess. 4 (3), 339–352.

Sivonen, K., Jones, G., 1999. Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management. E & FN Spon, London, pp. 41–111.

Skulberg, O.M., Carmichael, W.W., Andersen, R.A., Matsunaga, S., Moore, R. E., Skulberg, R., 1992. Investigations of a neurotoxic oscillatorialean strain (Cyanophyceae) and its toxin. Isolation and characterization of homoanatoxin-a. Environ. Toxicol. Chem. 11 (3), 321–329.

Sompong, U., Hawkins, P.R., Besley, C., Peerapornpisal, Y., 2005. The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand. FEMS Microbiol. Ecol. 52 (3), 365–376.

Spencer, P.S., Nunn, P.B., Hugon, J., Ludolph, A., Roy, D.N., 1986. Motor-neurone disease on Guam: possible role of a food neurotoxin. Lancet 1 (8487), 965.

Spencer, P.S., Nunn, P.B., Hugon, J., Ludolph, A.C., Ross, S.M., Roy, D.N., Robertson, R.C., 1987a. Guam amyotrophic lateral sclerosis–Parkinsonism–dementia linked to a plant excitant neurotoxin. Science 237 (4814), 517–522.

Spencer, P.S., Palmer, V.S., Herman, A., Asmedi, A., 1987b. Cycad use and motor neurone disease in Irian Jaya. Lancet 2 (8570), 1273–1274.

Spencer, P.S., Ohta, M., Palmer, V.S., 1987c. Cycad use and motor neurone disease in Kii peninsula of Japan. Lancet 2 (8573), 1462–1463.

Spivak, C.E., Witkopf, B., Albuquerque, E.X., 1980. Anatoxin-a: a novel, potent agonist at the nicotinic receptor. Mol. Pharmacol. 18 (3), 384–394.

Steele, J.C., McGeer, P.L., 2008. The ALS/PDC syndrome of Guam and the cycad hypothesis. Neurology 70 (21), 1984–1990.

Swanson, K.L., Aronstam, R.S., Wonnacott, S., Rapoport, H., Albuquerque, E.X., 1991. Nicotinic pharmacology of anatoxin analogs. I. Side chain structure–activity relationships at peripherical agonist and noncompetitive antagonist sites. J. Pharmacol. Exp. Ther. 259 (1), 377–386.

Tanino, H., Nakata, T., Kaneko, T., Kishi, Y., 1977. A stereospecific total synthesis of D,L-saxitoxin. J. Am. Chem. Soc. 99 (8), 2818–2819.

Taton, A., Grubisic, S., Balthasart, P., Hodgson, D.A., Laybourn-Parry, J., Wilmotte, A., 2006. Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol. Ecol. 57 (2), 272–289.

Teste, V., Briand, J.F., Nicholson, B.C., Puiseux-Dao, S., 2002. Comparison of changes in toxicity during growth of *Anabaena circinalis* (cyanobacteria) determined by mouse neuroblastoma bioassay and HPLC. J. Appl. Phycol. 14 (5), 399–407.

Thomas, P., Stephens, M., Wilkie, G., Amar, M., Lunt, G.G., Whiting, P., Gallagher, T., Pereira, E., Alkondon, M., Albuquerque, E.X., Wonnacott, S., 1993. (+)-Anatoxin-a is a potent agonist at neuronal nicotinic acetylcholine receptors. J. Neurochem. 60 (6), 2308–2311.

Vega, A., Bell, E.A., 1967. α-Amino-β-methyl aminoproprionic acid, a new amino acid from seeds of *Cycas circinalis*. Phytochemistry 6 (5), 759–762.

Vega, A., Bell, E.A., Nunn, P.B., 1967. The preparation of L- and D-α-amino-β-methylaminopropionic acids and the identification of the compound isolated from *Cycas circinalis* as the L-isomer. Phytochemistry 7 (10), 1885–1887.

Viaggiu, E., Melchiorre, S., Volpi, F., Di Corcia, A., Mancini, R., Garibaldi, L., Crichigno, G., Bruno, M., 2004. Anatoxin-a toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. Environ. Toxicol. 19 (3), 191–197.

White, J.D., Xu, Q., Lee, C.S., Valeriote, F.A., 2004. Total synthesis and biological evaluation of +-kalkitoxin, a cytotoxic metabolite of the cyanobacterium *Lyngbya majuscula*. Org. Biomol. Chem. 2 (14), 2092–2102.

Wonnacott, S., Jackman, S., Swanson, K.L., Rapoport, H., Albuquerque, E.X., 1991. Nicotinic pharmacology of anatoxin analogs. II. Side chain structure–activity relationships at neuronal nicotinic ligand binding sites. J. Pharmacol. Exp. Ther. 259 (1), 387–391.

Wonnacott, S., Swanson, K.L., Albuquerque, E.X., Huby, N.J., Thompson, P., Gallagher, T., 1992. Homoanatoxin: a potent analogue of anatoxin-a. Biochem. Pharmacol. 43 (3), 419–423.

Wood, S.A., Selwood, A.I., Rueckert, A., Holland, P.T., Milne, J.R., Smith, K.F., Smits, B., Watts, L.F., Cary, C.S., 2007. First report of homoanatoxin-a and associated dog neurotoxicosis in New Zealand. Toxicon 50 (2), 292–301.

World Health Organization, 2004. Algae and cyanobacteria in fresh water. In: Guidelines for Drinking-water Quality, third ed., vol. 1. World Health Organization, Geneva, Switzerland. 407–408.

Wu, M., Okino, T., Nogle, L.M., Marquez, B.L., Williamson, R.T., Sitachitta, N., Berman, F.W., Murray, T.F., McGough, K., Jacobs, R., Colsen, K., Asano, T., Yokokawa, F., Shioiri, T., Gerwick, W.H., 2000. Structure, synthesis, and biological properties of kalkitoxin, a novel neurotoxin from the marine cyanobacterium *Lyngbya majuscula*. J. Am. Chem. Soc. 122 (48), 12041–12042.

Yokokawa, F., Shioiri, T., 1998. Total synthesis of antillatoxin, an ichthyotoxic cyclic lipopeptide, having the proposed structure. What is the real structure of antillatoxin? J. Org. Chem. 63 (24), 8638–8639.

Yokokawa, F., Fujiwara, H., Shioiri, T., 1999. Total synthesis and revision of absolute configuration of antillatoxin, an ichthyotoxic cyclic lipopeptide of marine origin. Tetrahedron Lett. 40 (10), 1915–1916.

Yokokawa, F., Fujiwara, H., Shioiri, T., 2000. Total synthesis and revision of absolute stereochemistry of antillatoxin, an ichthyotoxic cyclic lipopeptide from marine cyanobacterium *Lyngbya majuscula*. Tetrahedron 56 (12), 1759–1775.

Yu, X.M., Salter, M.W., 1998. Gain control of NMDA-receptor currents by intracellular sodium. Nature 396 (6710), 469–474.

Yu, X.M., 2006. The role of intracellular sodium in the regulation of NMDA-receptor-mediated channel activity and toxicity. Mol. Neurobiol. 33 (1), 63–80.