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Cyclic peptides and depsipeptides from cyanobacteria: a review

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An elaborate array of structurally-novel and biologically-active cyclic peptides and depsipeptides are found in blue-green algae (cyanobacteria). Several of these compounds possess structures that are similar to those of natural products from marine invertebrates. Most of these cyclic peptides and depsipeptides, such as the microcystins and the lyngbyatoxins, will probably only be useful as biochemical research tools. A few, however, have the potential for development into useful commercial products. For example, cryptophycin-1, a novel inhibitor of microtubule assembly from *Nostoc* sp GSV 224, shows impressive activity against a broad spectrum of solid tumors implanted in mice, including multidrug-resistant ones, and majusculamide C, a microfilament-depolymerizing agent from *Lyngbya majuscula*, shows potent fungicidal activity and may have use in the treatment of resistant fungal-induced diseases of domestic plants and agricultural crops.

Keywords: cyanobacteria; natural products; hepatotoxins; fungicides; antitumor agents; enzyme inhibitors

Early research on toxins

Chemical investigations to date indicate that cyclic peptides and depsipeptides are common constituents of blue-green algae (cyanobacteria). In fact, most of the cyanobacterial secondary metabolites that have been isolated and identified fall into these two classes. The most familiar ones are the microcystins, cyclic heptapeptides associated with many poisonous cyanobacterial blooms found in eutrophic freshwater lakes [12,13]. In 1959 Bishop *et al* [5] isolated a microcystin for the first time from a Canadian strain of *Microcystis aeruginosa*. This toxin was eventually designated microcystin-LR [14] and has been found to be the major hepatotoxin in most strains of *M. aeruginosa* from the Northern Hemisphere. Botes *et al* [6,7,76] established its gross structure in 1985 and Rinehart *et al* [73] reported its total structure three years later. Approximately 50 microcystins have now been isolated and identified [74]. The structures of the compounds referred to in this review are shown in Figure 1. Although the first blue-green alga to be implicated in hepatotoxic animal poisonings was *Nodularia spumigena* in 1878 [20], it was not until over a century later that a microcystin-related toxin, nodularin, albeit a cyclic pentapeptide, was isolated from this alga and its total structure determined [73].

Although reports abound in the literature describing animal kills from ingestion of microcystin-containing blue-green algae in drinking water [12], human deaths from cyanobacterial poisoning are undocumented. Nevertheless, cases of human liver injury from drinking microcystin-contaminated water have been reported [19] and the consequences of long term exposure are just now being realized. In China, for example, chronic cyanobacterial poisoning plays a significant role in the markedly higher incidence of human liver cancer in areas that are heavily dependent on surface drinking-water [88]. Fujiki has found that microcystin-LR is a potent liver tumor promoter [59] and nodularin is a liver carcinogen [60].

Almost all of the hepatotoxins that have been isolated from blue-green algae belong to the microcystin/nodularin class. Microcystins and nodularins appear to be widely distributed in aquatic and terrestrial cyanophytes [35,72,74], and recently have also been found in marine organisms. Microcystin-LR is one of the major toxins associated with mussels from Gillam Island, British Columbia [2], and reared (in the province of British Columbia or the state of Washington) Atlantic salmon suffering from 'netpen liver disease' (NLD) [3]. Recently a nodularin-related compound, motuporin [16], which possesses an L-valine unit in lieu of the L-arginine unit, has been isolated from *Theonella swinhoei*, a sponge which is known to harbor symbiotic blue-green algae [85].

Lyngbyatoxin A [8] is a modified cyclic dipeptide found in the seaweed *Lyngbya majuscula* that grows on leeward Oahu, Hawaii. This inflammatory agent is identical with teleocidin A-1 [75] from *Streptomyces mediocidicus*, a bacterium which sometimes contaminates antibiotic-producing *Streptomyces* and poses a hazard to workers in the drug industry. Although *L. majuscula* is the causative agent of an acute dermatitis inflicted on ocean swimmers and bathers who come into contact with the seaweed during the summer months in Hawaii (mostly on windward Oahu) and Okinawa [41], lyngbyatoxin A and related compounds [1] have never been implicated as the active agents in seaweed dermatitis. Structurally different (not amino acid-derived) but pharmacologically identical [55] toxins account for the inflammatory activity of this cyanophyte. Nevertheless, lyngbyatoxin A appears to have been the causative agent in the *L. majuscula* responsible for a severe oral and gastrointestinal inflammation suffered by a person who accidentally ingested the alga [79]. Fujiki showed that lyngbyatoxin A is a potent phorbol-ester-type tumor promoter [23], but no evidence has been found to implicate its involvement in human cancer. The gastroenteritis induced by lyngbyatoxin A is similar to that induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), a purgative still in use in some

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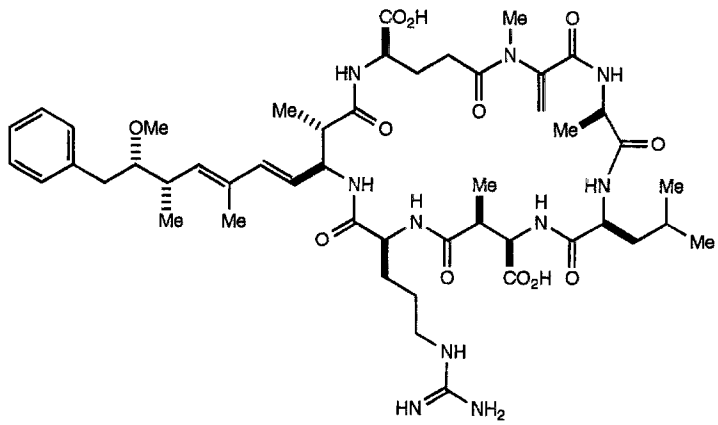
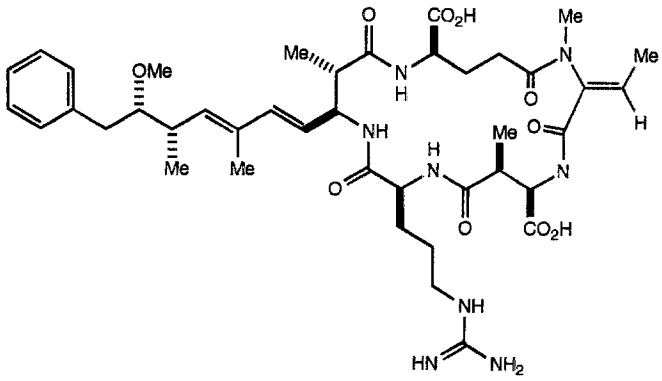
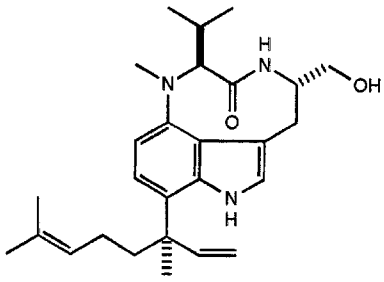


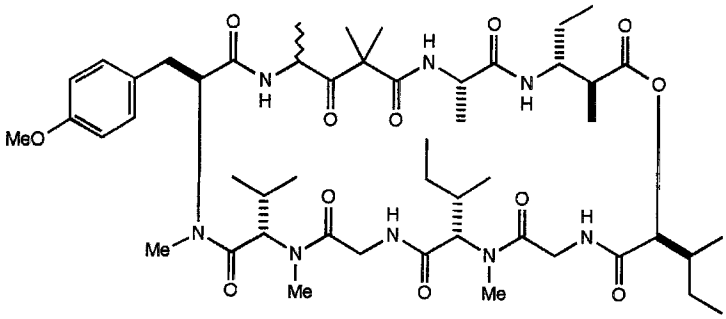
microcystin-LR



nodularin



lyngbyatoxin A

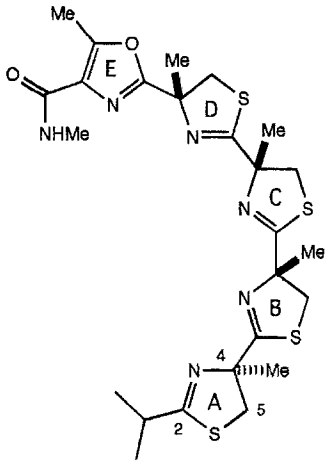


majusculamide C

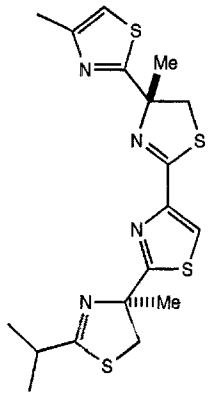
Figure 1 The structures of compounds referred to in the text. (Continued)

Third World countries and a well-known tumor promoter [54].

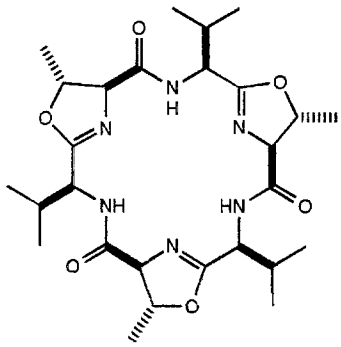
None of the peptidal toxins appear to have any useful activity for the treatment of animal or plant diseases; however, microcystin-LR, nodularin, and lyngbyatoxin A, which are commercially available, are serving as important research tools for probing certain biological processes and phenomena. All three toxins affect protein phosphorylation/dephosphorylation cycles that are paramount in controlling intracellular events as diverse as metabolism, contractility, membrane transport, cell division and gene transcription. Whereas lyngbyatoxin A stimulates the phosphorylation of serine and threonine residues in proteins, microcystin-LR and nodularin inhibit the dephosphorylation of the phosphorylated units. The net result is the same, an increase in phosphorylated proteins and a cascade of subsequent events, some of which lead to tumor promotion. The biological activities of lyngbyatoxin A and



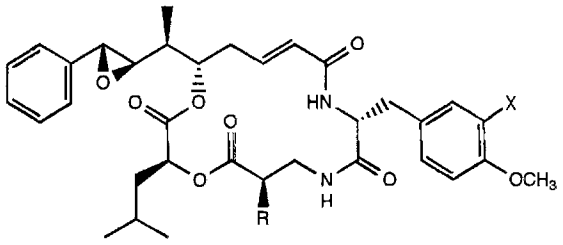
tantazole B



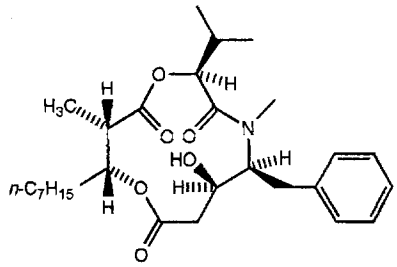
didehydromirabazole A



westiellamide



cryptophycin-1 X = Cl; R = CH_3
arenastatin A X = H; R = H



hapalosin

Figure 1 (Continued)

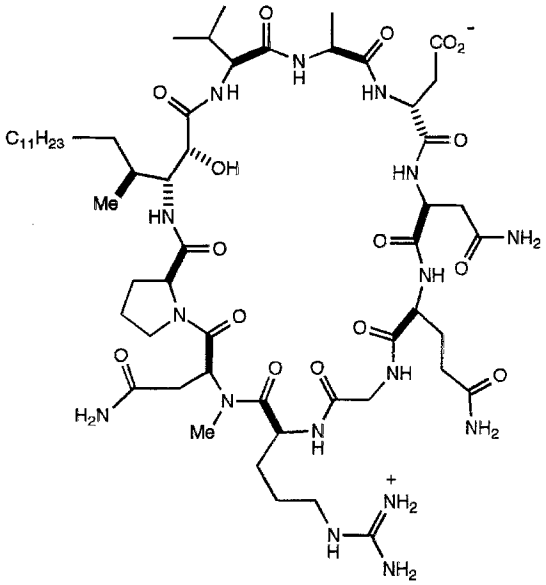
TPA are identical; both compounds are potent activators of protein kinase C. On the other hand, microcystin-LR [36,37,50,87] and nodularin [38,87] are highly effective inhibitors of protein phosphatases 1, 2A and 3, enzymes that are also strongly inhibited by okadaic acid. Under normal conditions microcystins are not cell-permeable, and therefore they are only cytotoxic to cells like hepatocytes that have transporters for these peptides. Consequently, hepatoenteritis is generally the only malady associated with the ingestion of microcystin-containing *M. aeruginosa*. Okadaic acid, however, is strongly cytotoxic since it can penetrate into all mammalian cells where it inhibits intracellular protein phosphatases, resulting in cell death. Gastroenteritis, similar to that induced by ingesting *L. majuscula* containing lyngbyatoxin A [79] or drinking tea containing TPA, results from the consumption of shellfish containing okadaic acid. A comparable gastroenteritis is sometimes observed with the ingestion of microcystin-containing *M. aeruginosa*, but it is not clear whether microcystin-LR is responsible. Other types of protein phosphatase inhibitors, however, appear to be present in blue-green algae, but none have been identified yet [35].

Screening programs

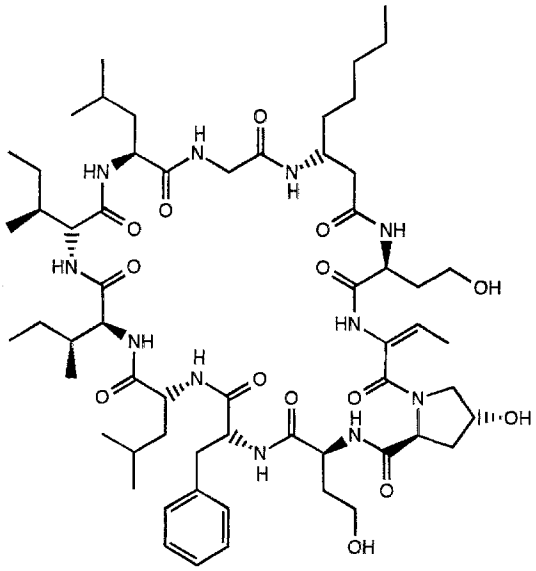
Stimulated by the interesting structures and bioactivities of cyanobacterial toxins, researchers at the University of Hawaii initiated a program in 1977 to screen extracts of field-collected blue-green algae for anticancer and antimicrobial activities using animal- and cell-based bioassays [53]. A high percentage of cyanophytes, mostly marine, showed interesting activities; however, only a small number of cyanophytes were found in sufficient quantities for follow-up isolation, identification, and biological evaluation of active compounds.

Early research on field-collected cyanophytes, however, led to the discovery of majusculamide C [15,86], a novel cyclic nonadepsipeptide from a deep-water variety of *L. majuscula* found in the Marshall Islands. Majusculamide C is strongly cytotoxic and has a cell cycle activity similar to the mitosis blocker cytochalasin B; however, it showed marginal to nil antitumor activity *in vivo*. Nevertheless, potent activity was observed against a broad-spectrum of fungal plant pathogens, including resistant strains, such as *Phytophthora infestans*, the causative organism of tomato late blight, and *Plasmopora viticola*, the causative organism of grape downy mildew [57]. It could become an important fungicide if the economics of its mass production were more favorable. Majusculamide C is closely related in structure to dolastatin 11, a potent cytotoxin that has been isolated from the sea hare *Dolabella auricularia*, and differs from majusculamide C in possessing an L-*N*-methylleucine unit in lieu of the L-*N*-methylisoleucine unit [70].

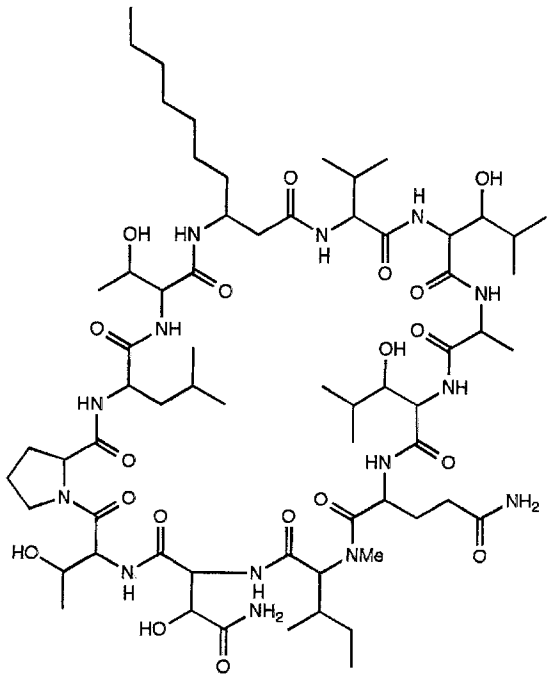
To circumvent problems associated with field-collec-



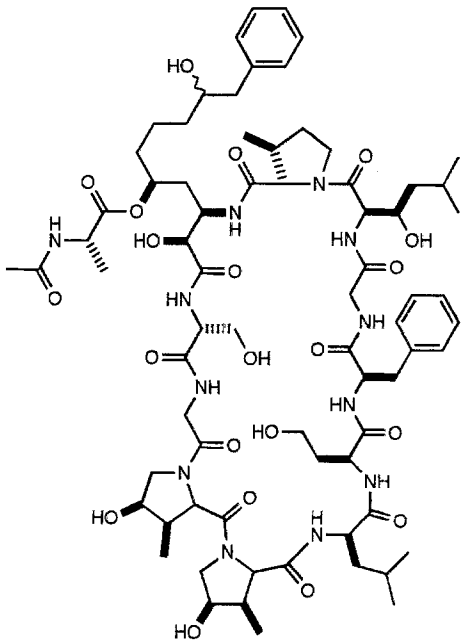
calophycin



laxaphycin A



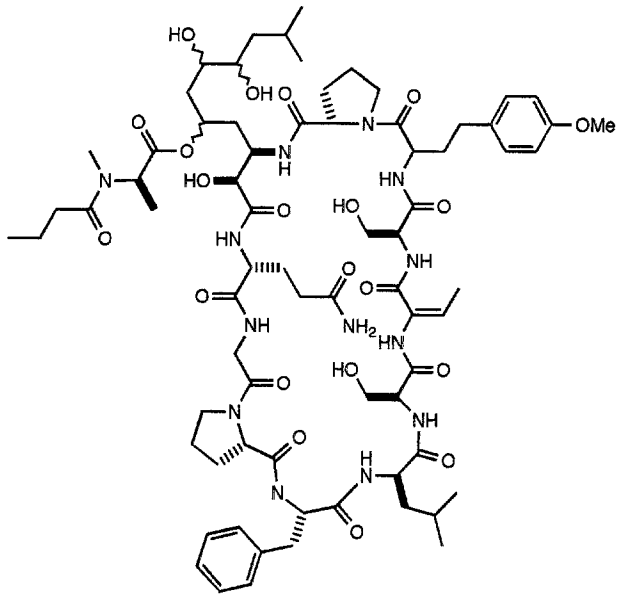
laxaphycin B



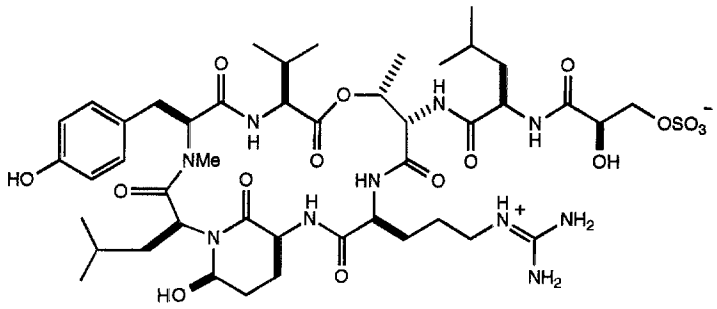
scytonemin A

Figure 1 (Continued)

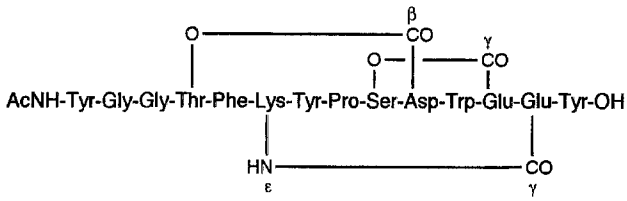
tions, the screening program at Hawaii was expanded in 1981 to include extracts of laboratory-cultured blue-green algae [58]. Extracts of more than 1500 strains representing some 400 species of blue-green algae were tested over the next 12 years [66], using mostly cell-based assays to discover new anticancer, antifungal, and antiviral agents. Six percent of the extracts were cytotoxic against human tumor cell lines at MICs $<20\ \mu g\ ml^{-1}$ [65]; however, less than 1% of the extracts were solid tumor-selective [84] and/or tumor-selective. Some of the non-cytotoxic extracts (<1%) showed multiple-drug-resistance (MDR)-reversing activity. Nine percent of the extracts were antifungal at 1 mg per disc against one or more test organisms, viz *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*. Approximately 10% of the cultures produced substances



schizotrin A



A90720A

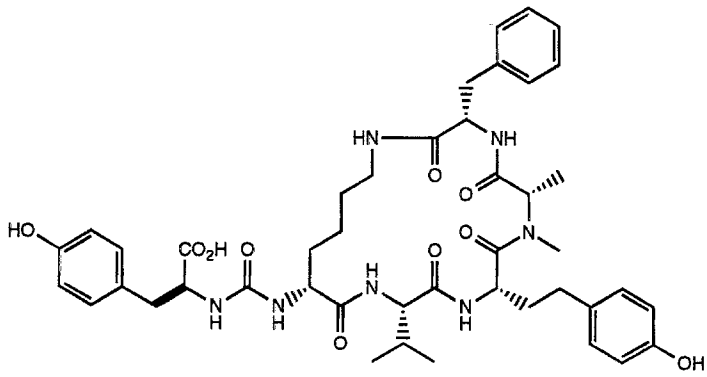


microviridin

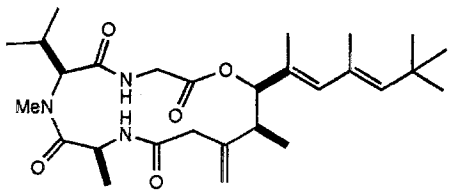
Figure 1 (Continued)

that caused significant reduction in cytopathic effects normally associated with viral infection [64] and 2% of the hydrophilic extracts showed inhibitory activity against the reverse transcriptases of avian myeloblastosis virus and human immunodeficiency virus, type 1 (HIV-1) [48].

Prior to the 1990s, the only significant research on secondary metabolites from blue-green algae outside of the Hawaii effort resulted from a modest screening program at Merck, Sharp & Dohme Research Laboratories, Rahway, NJ, USA [77]. Recently, however, workers at other laboratories have begun to screen extracts of blue-green algae, mostly strains of *Microcystis* and *Anabaena* spp, for various biological activities, using predominantly mechanism- and enzyme-based assays [28]. Interestingly, most of the active principles that have been isolated and identified from these latter cyanophytes to date are cyclic peptides and depsipeptides.



anabaenopeptin A



antillatoxin

Figure 1 (Continued)

Anticancer agents

Tantazoles [10,11,24] and mirabazoles [9,63] are modified heterocyclic peptides that comprise one of four classes of cytotoxins in terrestrial *Scytonema mirabile* BY-8-1. Both the tantazoles and mirabazoles contain a sequence of four contiguous cysteine-derived Δ^2-thiazoline rings attached 4,2′ to one another with an isopropyl group connected to C-2 of the first thiazoline ring (ring A). The tantazoles, however, differ from the mirabazoles in having a threonine-derived oxazole ring (ring E) attached to C-4 of the fourth thiazoline (ring D) via C-2. A glycine-derived appendage is linked to C-4 of the oxazole ring in the tantazoles. Most of the tantazoles and mirabazoles are tumor-selective cytotoxins, but tantazole B and didehydromirabazole A are also solid tumor-selective [84].

Westiellamide is a weakly cytotoxic, modified cyclic hexapeptide from *Westiellopsis prolifica* [71]. Its structure is identical with that of cycloxazoline from the ascidian *Lissoclinum bistratum* [30] and provides circumstantial evidence for algal symbionts (*Prochloron* spp) playing a role in the biosynthesis of closely-related cyclic peptides found in marine tunicates, eg bistratamides in *L. bistratum* [18] and lissoclinamides and patellamides in *L. patella* [17]. Similar cyclic peptides, eg dolastatin 3 [69], found in marine molluscs undoubtedly have a cyanobacterial origin.

The cryptophycins comprise the largest class of cyanobacterial depsipeptides to date (25 members) [25,82]. All of the cyclic cryptophycins consist of a δ-hydroxy acid unit (A), an α-amino acid unit (B), a β-amino acid unit (C), and an α-hydroxy acid unit (D), connected together in a cyclic ABCD sequence. Cryptophycin-1, the most important member, was first isolated from *Nostoc* sp ATCC 53787 as an antifungal agent [34,77] by researchers at Merck. In their hands, however, cryptophycin-1 appeared to be too toxic to be of practical use, at least as an antifungal agent. In a collaborative study at the University of Hawaii and Wayne State University, cryptophycin-1 was discovered to be a new microtubule depolymerizing agent [80] showing excellent activity against a broad spectrum of solid tumors implanted in mice, including drug-resistant and multiple drug-resistant ones [82]. The gross structure and relative and absolute stereochemistry of cryptophycin-1 were rigorously established using a combination of chemical and spectral techniques and total synthesis [4]. The Hawaii group had isolated cryptophycin-1 from *Nostoc* sp GSV 224 along with minor amounts of three other cyclic analogs and three acyclic artifacts. In the absence of methanol in the isolation scheme, the acyclic artifacts were not formed and 18 additional cyclic cryptophycins could be isolated as minor constituents [25]. One of the minor analogs was cryptophycin-24, which proved to be identical with arenastatin A from an Okinawan sponge identified as *Dysidea arenaria* [45–47].

Hapalosin, a novel cyclic depsipeptide from *Hapalosiphon welwitschii*, reverses P-glycoprotein-mediated multidrug-resistance (MDR) in tumor cells [81]. Its structure is (3*S*,4*R*,8*R*,9*S*,12*S*)-9-benzyl-4-heptyl-8-hydroxy-12-isopropyl-3,10-dimethyl-1,5-dioxa-10-azacyclododecane-2,6,11-trione as determined by a combination of spectroscopic and chemical methods.

Fungicides

Calophycin, a cyclic decapeptide containing a novel (2*R*,3*R*,4*S*)-3-amino-2-hydroxy-4-methylpalmitic acid unit

(Hamp), is the potent broad-spectrum fungicide in *Calothrix fusca* EU-10-1 [52]. The unusual Hamp unit has also been identified in puwainaphycin E, one of a family of non-fungicidal cyclic decapeptides from *Anabaena* sp BQ-16-1 [29,56]. Puwainaphycin E differs structurally from calophycin in possessing *O*-methyl-L-threonyl, L-threonyl, L-threonyl, and (*E*)-didehydrobutyrinyl units in lieu of the L-argininyl, L-asparaginyl, D-aspartyl, and L-alanyl units, respectively.

The laxaphycins are a large family of cyclic undeca- and dodecapeptides, the major representative of each class being laxaphycin A and laxaphycin B, respectively, that are responsible for the antifungal activity of the crude extract of *Anabaena laxa* FK-1-2 [21,22]. The antifungal effect exhibited by these peptides is unusual in that the peptides act synergistically with each other to inhibit growth. In order to achieve maximum biological potency, a member of each class of peptide must be present. The mode of action, however, is not novel and does not involve a specific receptor. Lysis of cells occurs in a non-specific manner. The laxaphycins closely resemble, both structurally and biologically, a group of cyclic peptides known as the hormothamnins that have been isolated from the marine cyanophyte *Hormothamnion enteromorphoides* [26,27]. Laxaphycin A differs from hormothamnin A in the geometry of the double bond in the didehydrobutyrinyl unit.

Although antifungal activity is commonly observed, significant antibacterial activity is not, at least in the extracts of blue-green algae grown in culture to date. A large percentage of the >1500 extracts screened at Hawaii showed weak to moderate activity against Gram-positive bacteria, but none was active against Gram-negative bacteria. Scytonemin A [33], a cyclic undecapeptide from *Scytonema* sp U-3-3, and schizotrin A [67], a cyclic undecapeptide from *Schizotrix* sp TAU IL-89-2, showed weak antibacterial activity and moderate activity against several fungi.

Enzyme inhibitors

Several biologically-active cyclic depsipeptides have been isolated recently from terrestrial blue-green algae which possess the unusual 3-amino-6-hydroxy-2-piperidone (Ahp) unit that was first described in dolastatin 13, one of the cytotoxins found in the sea hare *Dollabella auricularia* [68]. For example, A90720A, a serine proteinase (trypsin) inhibitor from *Microchaete loktakensis* IC-39-2, contains an Ahp unit. Its overall structure is closely related to that of dolastatin 13, suggesting that dolastatin 13 has a dietary and cyanobacterial origin. Interestingly, the total structure of A90720A was elucidated by an X-ray crystallographic study of the bovine trypsin-A90720A complex [49]. The same glyceric acid 3-*O*-sulfate unit is present in micropeptin-90 from *Microcystis aeruginosa* NIES-90 [39] and oscillapeptin from *Oscillatoria agardhii* NIES-204 [78]. Micropeptin-90, an inhibitor of plasmin and trypsin, but not of papain, chymotrypsin or elastase, differs from A90720A in having an L-phenylalanine unit instead of an L-leucine unit. In oscillapeptin, however, the *N*-methyltyrosine unit is *O*-methylated, homotyrosine units have replaced the D-leucine and L-arginine units, and L-isoleucine units have replaced the L-leucine and L-valine units. Oscillapeptin is an inhibitor of elastase and chymotrypsin, but not of trypsin, papain, thrombin, or plasmin. Micropeptins A and B are plasmin and trypsin inhibitors from *Microcystis aeruginosa* NIES-100 [61]. Each of these latter peptolides possesses L-glutamic acid (α-attached) and L-lysine units instead of D-leucine and L-arginine units, respectively, and the amino acid in the side chain is *N*-acylated by a hexanoyl or octanoyl group instead of a sulfated glyceric acid unit. Microcystilide A [83], a cell-differentiation-promoter from *M. aeruginosa* NO-15-1840, the aeruginopeptins [32] from *M. aeruginosa* TAC 95 and M228, and the cyanopeptolins [42,51] from *Microcystis* spp are related cyclic depsipeptides.

Microviridin, a tyrosinase inhibitor from *Microcystis viridis* NIES-102 [28,40], is both a novel tricyclic depsipeptide and a cyclic peptide. It consists of a tetradecapeptide backbone, viz NH_2-Tyr(I)-Gly(I)-Gly(II)-Thr-Phe-Lys-Tyr(II)-Pro-Ser-Asp-Trp-Glu(I)-Glu(II)-Tyr(III)-CO_2H where the NH_2 of Tyr(I) is acetylated, an ester bond connects the OH of Thr and the β-CO_2H of Asp, an ester bond connects the OH of Ser and the γ-CO_2H of Glu(I), and an amide bond connects the ε-NH_2 of Lys and the γ-CO_2H of Glu(II). All of the amino acid units have the L-configuration.

Other bioactive agents

Anabaenopeptins A and B [31] from *Anabaena flos-aquae* NRC 525-17 are unusual cyclic peptides that possess a ureido linkage between two of the amino acids, similar to the ones in keramamide A [43] and konbamide [44], two related cyclic peptides from Okinawan sponges belonging to the genus *Theonella*. Again the similarities of the structures suggest that cyanobacterial symbionts in the sponges may be involved in the biosyntheses of keramamide A and konbamide. The anabaenopeptins produce concentration-dependent relaxations of norepinephrine-induced contractions in rat aortic preparations.

Antillatoxin is an exceptionally ichthyotoxic cyclic depsipeptide from *Lyngbya majuscula* collected in Curacao [62]. It possesses an unusual δ-hydroxy acid unit, viz a (4*S*,5*R*,6*E*,8*E*)-5-hydroxy-4,6,8,10,10-pentamethyl-3-methyleneundeca-6,8-dienoic acid unit, which has a *t*-butyl group. The relative and absolute stereochemistry of the hydroxy acid unit has been proposed to be 4*S*,5*R* on the basis of a combination of molecular modeling, NMR and CD studies.

The future

To date relatively few blue-green algae have been examined for secondary metabolites. The very high incidence of novel, biologically-active cyclic peptides and depsipeptides, however, indicates that cyanobacteria are a rich resource of these potentially-useful natural products. Significant discoveries have already been made with cryptophycin-1 and majusculamide C, both of which appear to have potential commercial value because of their applicability to resistant systems. With the increased research in this area of natural products in the last few years, other important cyclic peptides and peptolides will undoubtedly be discovered in the near future.

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