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Bioactive compounds produced by cyanobacteria

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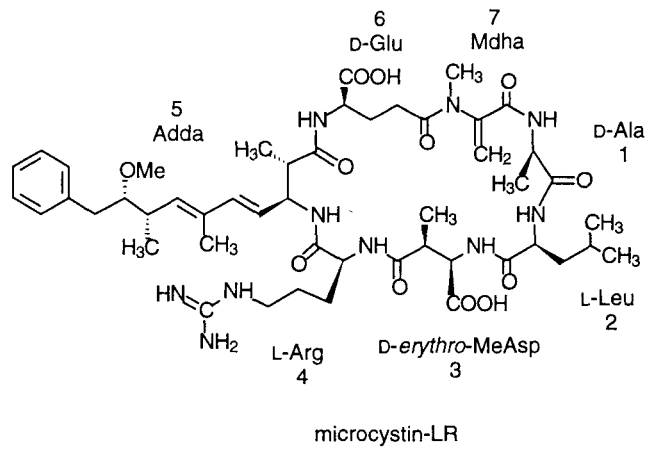
Cyanobacteria produce a large number of compounds with varying bioactivities. Prominent among these are toxins: hepatotoxins such as microcystins and nodularins and neurotoxins such as anatoxins and saxitoxins. Cytotoxicity to tumor cells has been demonstrated for other cyanobacterial products, including 9-deazaadenosine, dolastatin 13 and analogs. A number of compounds in cyanobacteria are inhibitors of proteases — micropeptins, cyanopeptolins, oscillapeptin, microviridin, aeruginosins — and other enzymes, while still other compounds have no recognized biological activities. In general cyclic peptides and depsipeptides are the most common structural types, but a wide variety of other types are also found: linear peptides, guanidines, phosphonates, purines and macrolides. The close similarity or identity in structures between cyanobacterial products and compounds isolated from sponges, tunicates and other marine invertebrates suggests the latter compounds may be derived from dietary or symbiotic blue-green algae.

Keywords: cyanobacteria; blue-green algae; toxins; enzyme inhibitors; peptides

Hepatotoxins from *Microcystis, Anabaena, Nostoc, Oscillatoria* and *Nodularia*

Toxic cyanobacterial (blue-green algal) waterblooms are found worldwide in eutrophic lakes, ponds, drinking water reservoirs and coastal waters, where they cause animal poisonings and pose risks to human health [8,9,11]. Several genera of cyanobacteria form these toxic waterblooms, with *Microcystis* being the most common [8,9,80]. Two types of toxins, hepatotoxins and neurotoxins, have been characterized from the toxic cyanobacteria, but hepatotoxicosis occurs more often than neurotoxicosis [8,9]; about 50% of *Microcystis* waterblooms show hepatotoxicity to mammals and other animals. The toxins responsible for the hepatotoxicity are the well known microcystins [10]. Microcystins are obtained not only from *Microcystis*, but also from *Anabaena, Nostoc* and *Oscillatoria* [74] which also form waterblooms.

The microcystins are cyclic heptapeptides like the representative microcystin-LR depicted in Figure 1 [72]. The unique structural feature in microcystins is the C_{20} amino acid, (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4*E*,6*E*-dienoic acid (Adda) [72], which plays an important role in their toxicity [21,54,55,59,74,82]. The suffix, LR, identifies the two variable L-amino acids at positions 2 and 4, ie, Leu and Arg [10]. More than 50 structural variations in microcystins have been found from the four genera of cyanobacteria [60,74]. The general structure of microcystins is cyclo(-D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha-), in which MeAsp is *erythro*-β-methylaspartic acid and Mdha indicates *N*-methyldehydroalanine [10,74]. X and Z are the commonly variable L-amino acids (positions 2 and 4), but structural modifications have been detected



microcystin-LR

Figure 1 Microcystin-LR.

in all seven amino acid units [60,74]. Some relationships between structural modifications and toxicity have been found for microcystins [21,54–57,59,60,74,82]. The structurally related toxins nodularins have been isolated from the brackish water-dwelling cyanobacterium *Nodularia spumigena* [59,72]. Nodularins are cyclic pentapeptides and have the Adda unit or its derivative (Figure 2).

Microcystins and nodularins show hepatotoxicity through inhibitory activity to protein phosphatases 1 and 2A [26,41,86] and are potent tumor promoters [15,61,62]. The cyclic structure of these toxins is essential for the toxicity, since linear compounds which have the same amino acid components as the cyclic peptides did not show apparent toxicity to mice [13,59,60].

Extensive efforts have been devoted to studies on cyanobacterial toxins resulting in understanding of the toxins and producing organisms. The results of studies on chemical structures, biosynthesis and structure-toxicity relationships of microcystins and nodularins have been summarized in our earlier review [74]. Microcystins are the most common causative agent of cyanobacterial waterbloom toxicosis,

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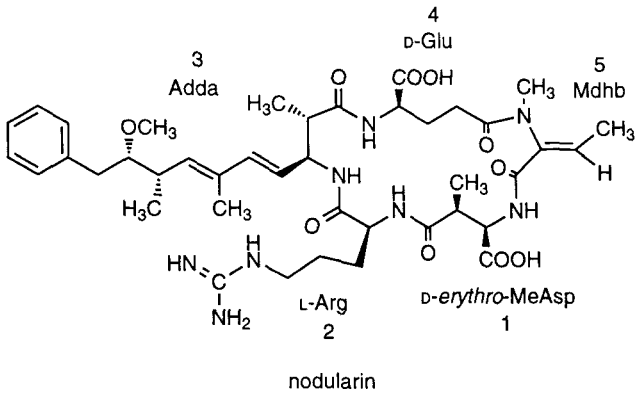


Figure 2 Nodularin isolated from the brackish water cyanobacterium *Nodularia spumigena*.

and show toxicity to mice at concentrations as low as 50 μg kg^{-1} (LD_{50}, intraperitoneal injection). The netpen liver disease of salmon observed on the Pacific Canadian coast is thought to be caused by microcystins [2]. A compound structurally related to nodularins, motuporin ([L-Val^2]nodularin), which has an L-Val unit instead of the L-Arg unit in nodularin, has been isolated from a marine sponge, *Theonella swinhoei* [14], but does not show cytotoxicity.

A structurally unrelated cyanobacterial hepatotoxin, cylindrospermopsin (Figure 3), has been isolated from *Cylindrospermopsis raciborskii* [63] and *Umezakia natans* [24] and has an acute (24 h) LD_{50} of 2.1 mg kg^{-1} (IP).

Neurotoxins from cyanobacteria

Neurotoxins (Figure 4) are the other group of cyanobacterial toxins (anatoxin-a from *Anabaena* and *Oscillatoria*, anatoxin-a(s) from *Anabaena flos-aquae*, homoanatoxin-a from *Oscillatoria rubescens*, saxitoxin and neosaxitoxin from *Aphanizomenon* and *Anabaena*) [8]. *Microcystis* species also produce anatoxin-a [67]. Saxitoxins are also produced by certain marine dinoflagellates [32,81].

During our studies on the metabolites of toxic cyanobacteria we isolated two nucleosides (Figure 5), 9-deazaadenosine and its 5′-α-D-glucopyranoside, from the freshwater cyanobacterium *Anabaena affinis* VS-1 [58]. These compounds showed cidal toxicity to a zooplankton, *Ceriodaphnia dubia* (LC_{50} = 0.1–0.5 μg ml^{-1}), and potent cytotoxicity to murine leukemia cells L1210 (IC_{50} = 0.002 and

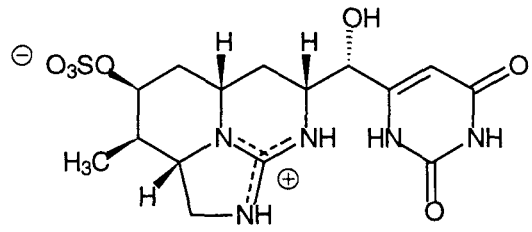


Figure 3 The structure of cylindrospermopsin.

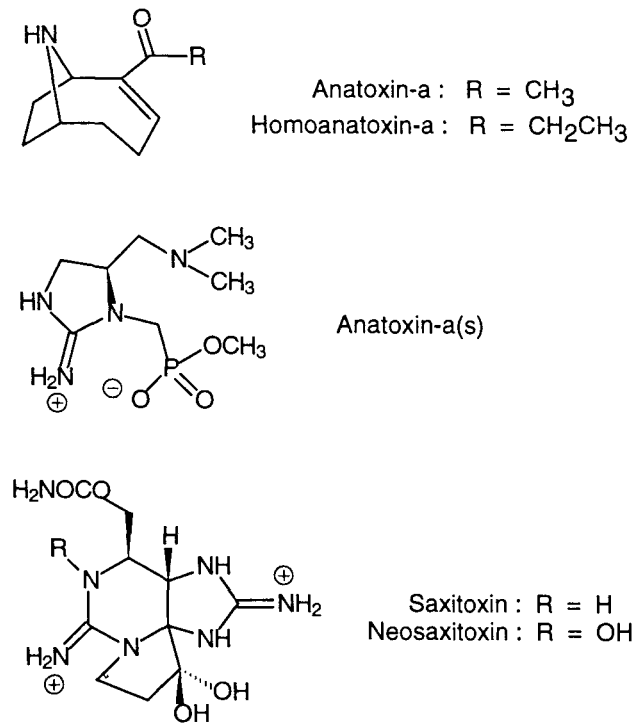


Figure 4 Anatoxin-a, homoanatoxin-a, anatoxin-a(s), saxitoxin and neosaxitoxin.

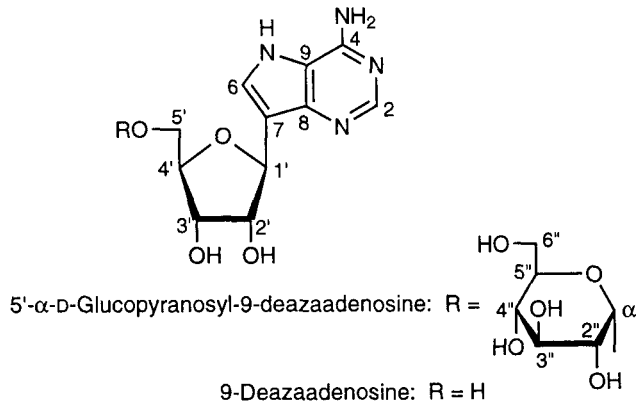


Figure 5 9-Deazaadenosine and its 5′-α-D-glucopyranoside isolated from the freshwater cyanobacterium *Anabaena affinis* strain VS-1.

0.02 μg ml^{-1}, respectively) [57,58]. The search for bioactive natural products, such as cytotoxins to tumor cells and antibacterial, antifungal and antiviral agents, from cyanobacteria has recently intensified [17,68]. The main effort for this purpose involved isolation of cyanobacteria strains from various environments and culturing of each strain to test for biological activities. Although toxins were the main subject of research on toxic cyanobacterial waterblooms, isolation of bioactive compounds other than biotoxins has recently been stressed.

Serine protease inhibitors from cyanobacteria

The production of bioactive components from *Microcystis* suggested a screening program for protease-inhibitory

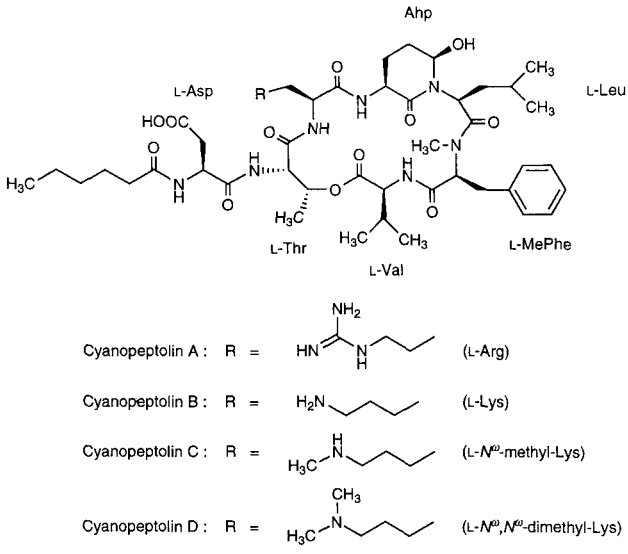


Figure 6 Cyanopeptolins A–D isolated from *Microcystis aeruginosa* PCC 7806.

activity of microalgal secondary metabolites [5]. The presence of non-toxic peptides in *Microcystis* in addition to microcystins was reported first by Weckesser and co-workers [4]. The structures of these peptides, termed cyanopeptolins A–D, isolated from microcystin-producing *Microcystis aeruginosa* PCC 7806, were assigned as shown in Figure 6 [42]. These compounds are cyclic depsipeptides (cyclic peptidolactones) and contain a unique amino acid unit, 3-amino-6-hydroxy-2-piperidone (Ahp). A number of closely related depsipeptides bearing the Ahp unit have been isolated since then from both toxic and non-toxic *Microcystis* species. The structures are summarized in Figure 7. Microcystilide A was isolated from *M. aeruginosa* NO-15-1840, which produced microcystins, and promoted cell differentiation of HL-60 cells at a concentration of 0.5 mg ml^{-1} [84]. Aeruginopeptins 95-A and B, and 228-A and B were isolated from *M. aeruginosa* strains TAC95 and M228, respectively [23]. Both strains produced microcystins, but no biological activity of these depsipeptides was noted. Five compounds belonging to this class were obtained as serine protease inhibitors. Micropeptins A and B, isolated from non-toxic *M. aeruginosa* NIES-100, showed inhibitory activities to plasmin and trypsin [65] and micropeptin 90 from non-toxic *M. aeruginosa* NIES-90 also inhibited plasmin and trypsin [28]. Cyanopeptolins S and SS were isolated together with microcystins from a waterbloom sample dominated by a *Microcystis* sp collected from a lake in Germany and showed inhibitory activity to trypsin, plasmin and thrombin [30,31]. The inhibitory activities to serine proteases by the depsipeptides isolated from *Microcystis* and other genera are summarized in Table 1 together with other serine protease inhibitors shown in Figures 8–10.

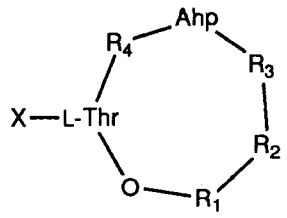
This class of depsipeptides has also been isolated from *Oscillatoria* spp and *Anabaena* spp. Oscillapeptin was obtained as a serine protease inhibitor from non-toxic *Oscillatoria agardhii* NIES-204 and inhibited elastase and chymotrypsin [78]. Oscillapeptin G was produced by *O. agardhii* NIES-610, together with microcystins, and showed inhibitory activity to tyrosinase at 1.0×10^{-4} M [77]. The isolation of anabaenopeptilides 90-A and B, and 202-A and B from *Anabaena circinalis* 90 and *Anabaena lemmermannii* 202 A2/41, respectively, was recently reported [16]. These two *Anabaena* spp were hepatotoxic, and microcystins have been characterized as the responsible toxins [79].

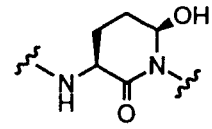
A closely related compound was isolated from a cyano-

Table 1 The inhibitory activity (IC_{50}) to serine proteases and tyrosinase by peptides isolated from cyanobacteria

	Trypsin	Plasmin	Thrombin	Elastase	Chymotrypsin	Tyrosinase	Ref
Micropeptin A	0.071[a]	0.026	n/i[b]	n/i	n/i		[65]
Micropeptin B	0.25	0.035	n/i	n/i	n/i		[65]
Micropeptin 90	2.0	0.1		n/i	n/i		[28]
Cyanopeptolin S	<0.2	<1	<5				[30]
Cyanopeptolin SS	<0.2	<1	<5				[31]
Oscillapeptin	n/i	n/i	n/i	0.3	2.2		[78]
Oscillapeptin G						1.0×10^{-4} M	[77]
A90720A	10 nM	30 nM	275 nM				[40]
Nostopeptin A	n/i	n/i	n/i	1.3	1.4		[44]
Microviridin A	n/i	n/i	n/i	n/i	n/i	3.3×10^{-4} M	[29]
Microviridin B	58	n/i	n/i	0.044	2.5		[66]
Microviridin C	32	n/i	n/i	0.084	4.9		[66]
Aeruginosin 298-A	1.0	n/i	0.3	n/i	n/i		[51]
Aeruginosin 98-A	0.6	6.0	7.0	n/i	n/i		[53]
Aeruginosin 98-B	0.6	7.0	10.0	n/i	n/i		[53]
Aeruginosin 98-C	3.9	5.0	3.3	n/i	n/i		[52]
Aeruginosin 102-A	0.2	0.3	0.04	n/i	n/i		[45]
Aeruginosin 102-B	1.1	0.8	0.1	n/i	n/i		[45]
Oscillamide Y					1.0×10^{-5} M		[76]

[a]The concentration is μg ml^{-1}, unless otherwise designated.
[b]Not inhibited (>10 or 100 μg ml^{-1}).





MePhe = *N*-methylphenylalanine
MeTyr = *N*-methyltyrosine
diMeTyr = *N,O*-dimethyltyrosine

Me(Cl)Tyr = *N*-methyl-3'-chlorotyrosine
MeLys = N^{ω}-methyllysine
diMeLys = N^{ω},N^{ω}-dimethyllysine
(H_4)Tyr = 1',2',3',4'-tetrahydrotyrosine
Hty = homotyrosine
Dhb = dehydrobutyrine

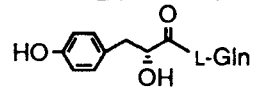
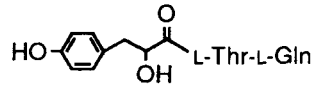
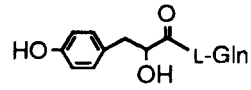
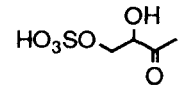
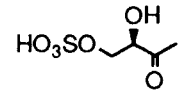
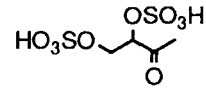
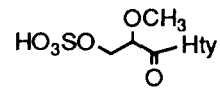
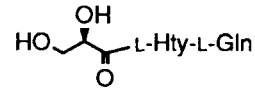
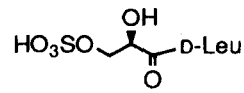
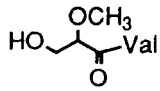
	R_1	R_2	R_3	R_4	X	Ref.
(from *Microcystis* spp.)						
Cyanopeptolin A	L-Val	L-MePhe	L-Leu	L-Arg	$CH_3(CH_2)_4CO$-L-Asp	[42]
Cyanopeptolin B	L-Val	L-MePhe	L-Leu	L-Lys	$CH_3(CH_2)_4CO$-L-Asp	[42]
Cyanopeptolin C	L-Val	L-MePhe	L-Leu	L-MeLys	$CH_3(CH_2)_4CO$-L-Asp	[42]
Cyanopeptolin D	L-Val	L-MePhe	L-Leu	L-diMeLys	$CH_3(CH_2)_4CO$-L-Asp	[42]
Microcystilide A	L-Ile	L-MeTyr	L-Leu	L-Tyr		[84]
Aeruginopeptin 95-A	L-Ile	L-MePhe	L-Thr	L-Tyr		[23]
Aeruginopeptin 95-B	L-Ile	L-MePhe	L-Thr	(H_4)Tyr		[23]
Aeruginopeptin 228-A	L-Ile	L-MePhe	L-Thr	L-Tyr		[23]
Aeruginopeptin 228-B	L-Ile	L-MePhe	L-Thr	(H_4)Tyr		[23]
Micropeptin A	L-Val	MeTyr	L-Leu	L-Lys	$CH_3(CH_2)_6CO$-L-Glu	[65]
Micropeptin B	L-Val	MeTyr	L-Leu	L-Lys	$CH_3(CH_2)_4CO$-L-Glu	[65]
Micropeptin 90	Val	MeTyr	L-Phe	Arg		[28]
Cyanopeptolin S	L-Ile	L-MePhe	L-Ile	L-Arg		[30]
Cyanopeptolin SS	L-Ile	L-MePhe	L-Ile	L-Arg		[31]
(from *Oscillatoria* spp.)						
Oscillapeptin	L-Ile	L-diMeTyr	L-Ile	Hty		[78]
Oscillapeptin G	L-Ile	L-MeTyr	L-Thr	L-Leu		[76]
(from *Anabaena* spp.)						
Anabaenopeptilide 90-A	L-Ile	L-diMeTyr	L-Thr	L-Hty	HCO-L-Gln	[16]
Anabaenopeptilide 90-B	L-Ile	L-Me(Cl)Tyr	L-Thr	L-Hty	HCO-L-Gln	[16]
Anabaenopeptilide 202-A	L-Ile	L-diMeTyr	L-Thr	L-Hty	HCO-L-Pro-L-Gln	[16]
Anabaenopeptilide 202-B	L-Ile	L-Me(Cl)Tyr	L-Thr	L-Hty	HCO-L-Pro-L-Gln	[16]
(from *Microchaete* sp.)						
A90720A	L-Val	L-MeTyr	L-Leu	L-Arg		[40]
(from *Dolabella auricularia*)						
Dolastatin 13	Val	MePhe	Phe	Dhb		[70]

Figure 7 19-Membered depsipeptides containing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit.

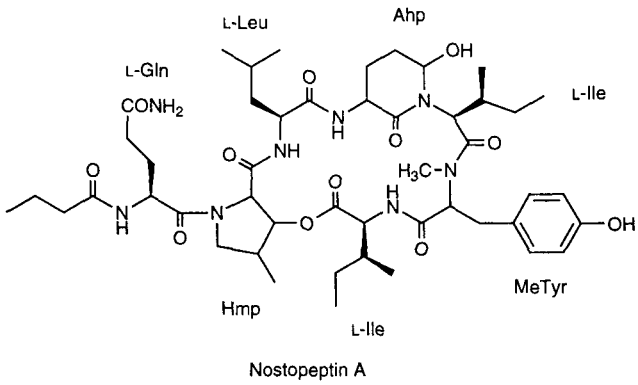


Figure 8 Nostopeptin A isolated from *Nostoc minutum* NIES-26.

bacterium other than the above species. *Microchaete loktakensis* produced A90720A, which showed inhibitory activity to trypsin, plasmin and thrombin similar to the other compounds.

A depsipeptide possessing the unique Ahp unit was first obtained from the Indian Ocean sea hare *Dolabella auricularia* and identified as a cytostatic agent [70]. This compound, dolastatin 13, has a very similar structure (Figure 7) to those of cyanobacterial metabolites, which would suggest that the primary producer of dolastatin 13 or its precursor may be a cyanobacterium on which the mollusc feeds.

These depsipeptides, listed in Figure 7, incorporated an L-Thr unit in a lactone linkage as another common structural feature, in addition to the Ahp unit. All compounds have an *N*-methylated amino acid unit as R_2. The R_4 amino

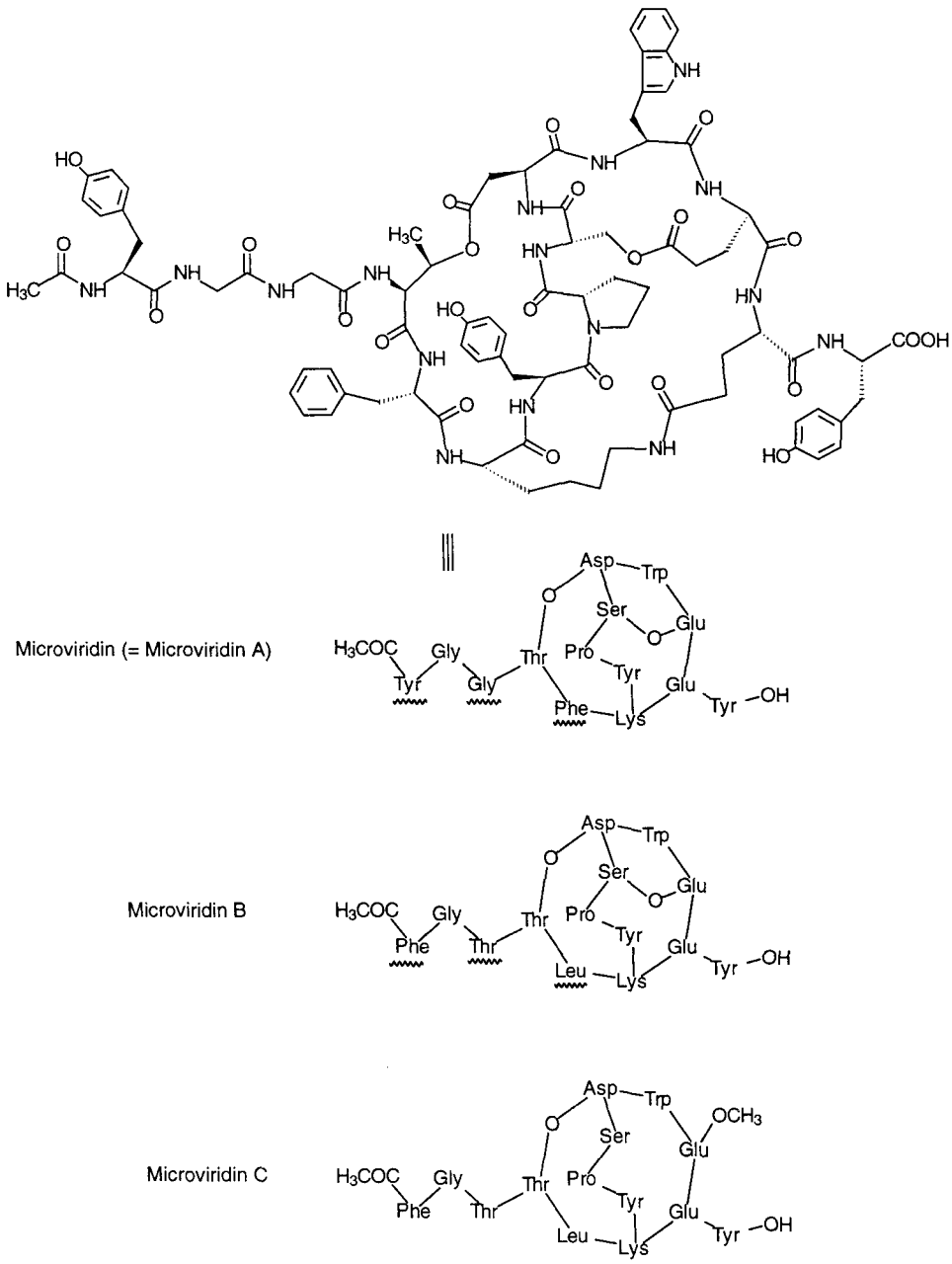
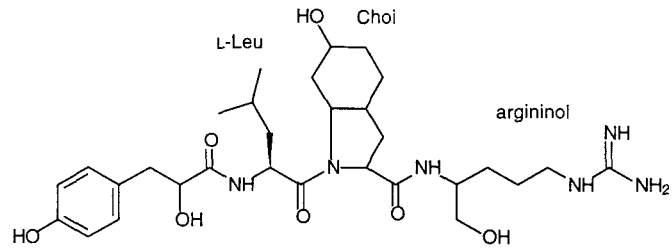
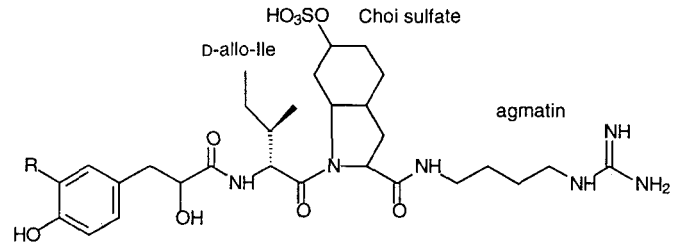


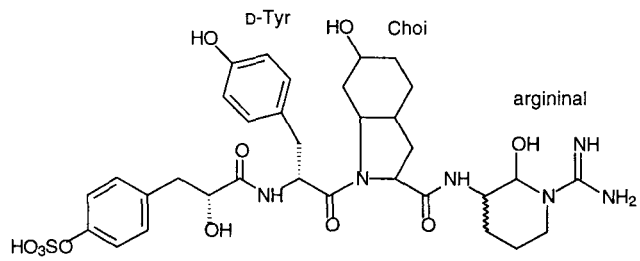
Figure 9 Tricyclic depsipeptides, microviridins A–C. (All amino acid components are the L-form.)



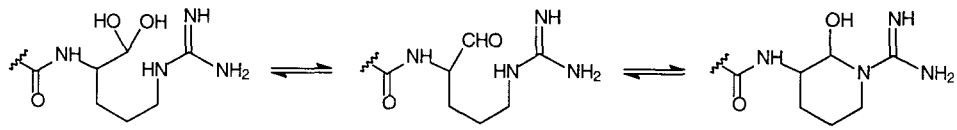
Aeruginosin 298-A



Aeruginosin 98-A R = Cl
Aeruginosin 98-B R = H
Aeruginosin 98-C R = Br



Aeruginosin 102-A : L-argininal
Aeruginosin 102-B : D-argininal



Tautomerism of argininal

Figure 10 Aeruginosins 298-A, 98-A, 98-B, 98-C, 102-A and 102-B isolated from *Microcystis* spp.

acid unit shows wide variety, while R_1 is either Val or Ile. The side chains attached to the amino group of L-Thr unit can be divided into four types, ie, the *N*-terminus is acylated by four types of carboxylic acids. One type is a hexanoyl or octanoyl acidic amino acid (Asp or Glu) residue. A second type has a *p*-hydroxyphenyllactyl unit attached to an amino acid or dipeptide unit. The third type of acid is a modified (sulfated, methylated) glyceric acid attached to the L-Thr unit directly or through an amino acid residue. The stereochemistry of glyceric acid in three compounds was determined to be D-. The fourth type is a formyl group attached to an amino acid or dipeptide.

The unusual amino acid (H_4)Tyr, 1′,2′,3′4′-tetrahydrotyrosine, was detected in aeruginopeptins 95-B and 228-B isolated from *Microcystis* spp. This amino acid was first found in microcystin-(H_4)YR obtained from a waterbloom of *Microcystis* spp [55,60]. Homotyrosine (Hty), found in the compounds from *Anabaena* spp and *Oscillatoria* spp, is also an amino acid component in microcystins isolated from *Anabaena* spp [22,56]. Dehydrobutyrine (Dhb), an amino acid component of dolastatin 13, is also found in nodularins as its *N*-methyl derivative (Figure 2).

The partial structures of the compounds in Figure 7 at the Thr unit, with a variable side chain attached to its amino group and its hydroxyl in a lactone bond in the ring, resemble those of didemnins isolated from the colonial tunicate *Tridemnum solidum* [73]. Didemnins are thought to be derived at least in part from symbiotic cyanobacterial metabolite(s).

A similar depsipeptide has been isolated from *Nostoc*

minutum NIES-26 as a serine protease inhibitor [44]. The compound, nostopeptin A, has the Ahp unit and a rare amino acid component, 3-hydroxy-4-methylproline (Hmp). The Hmp unit forms the lactone bond in the ring system, like the Thr unit in the above compounds. The 19-membered depsipeptides possessing the Ahp unit appear to be common metabolites in the freshwater cyanobacteria.

The second type of depsipeptides has the unique structural feature of a tricyclic ring system (Figure 9). The first example was isolated from the toxic *Microcystis viridis* NIES-102, which produced microcystins, and was named microviridin after the producing organism [29]. Although all amino acid components were L-, the structure assigned was quite unusual. Microviridin was reported to inhibit tyrosinase activity at a concentration of 3.3×10^{-14} M (Table 1). Two compounds closely related to microviridin have been reported recently from toxic *M. aeruginosa* NIES-298 [66] and named microviridins B and C. Another compound isolated from the same organism together with microviridins B and C was identified as microviridin and it has been suggested to rename microviridin as microviridin A. The structural differences between microviridins A and B are rather small: three amino acid components of Tyr, Gly and Phe in microviridin A are replaced by Phe, Thr and Leu, respectively, in microviridin B as indicated by underlining in Figure 9. The ester bond between the Ser hydroxyl group and the γ-carboxyl of the Glu unit found in microviridins A and B is missing in microviridin C, and the Glu γ-carboxyl is present as its methyl ester.

Microviridins B and C inhibited the activities of elastase, chymotrypsin and tyrosinase (Table 1), while microviridin A did not inhibit these serine proteases at 100 μg ml^{-1} [66]. The mechanism of action and structure-activity relationship of these compounds to serine proteases are interesting research targets.

The other structural type of serine protease inhibitors, aeruginosins, have been isolated from toxic and non-toxic *Microcystis* spp (Figure 10). They are linear peptides and have the unusual amino acid unit, 2-carboxy-6-hydroxyoctahydroindole (Choi), as the common amino acid component. Aeruginosin 298-A was isolated from the toxic strain of *M. aeruginosa* NIES-298 and inhibited thrombin and trypsin (Table 1) [51]. Microviridins A, B and C were also obtained from this strain (NIES-298), as noted above, together with microcystins. The non-toxic strain of *M. aeruginosa* NIES-98 produced aeruginosins 98-A, B and C as inhibitors of trypsin, thrombin and plasmin (Table 1) [52,53]. Aeruginosins 102-A and B were found in toxic *M. viridis* NIES-102 and inhibited thrombin, trypsin and plasmin (Table 1) [45]. This strain produced microcystins and microviridin A [29].

These compounds have *p*-hydroxyphenyllactic acid or its derivative at the *N*-terminus as the common structural unit. This acid unit is also found in five depsipeptides listed in Figure 7 as the masking group at the *N*-terminus. Aeruginosin 298-A has an L-Leu unit, while the other compounds have a D-amino acid unit at the same position.

The guanidine-containing units at the *C*-terminus are divided into three types, all derived from arginine. The argininal unit in aeruginosins 102-A and B is the aldehyde variant of Arg. These two compounds each showed three inseparable peaks on reversed-phase HPLC, which might be explained by the equilibria shown in Figure 10 [45]. The argininol unit in aeruginosin 298-A is a further reduced form of Arg containing a hydroxymethyl group. The third unit type, found in aeruginosins 98-A, B and C, is the decarboxyl variant of Arg, agmatin.

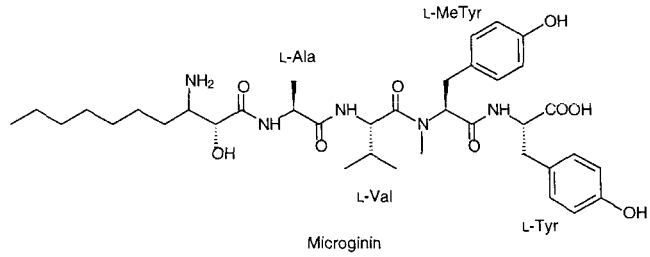
Cyanobacterial peptides with other bioactivities

Figure 11 shows another linear peptide isolated from the non-toxic strain of *M. aeruginosa* NIES-100. This strain also produced micropeptins A and B (see above). The linear peptide named microginin consisted of three usual and one *N*-methylated L-amino acids and a new β-amino acid at the *N*-terminus [64]. Microginin inhibited angiotensin-converting enzyme (LC_{50} = 7.0 μg ml^{-1}) but did not inbibit serine proteases.

Aeruginoguanidines 98-A, B and C (Figure 12) were obtained from *M. aeruginosa* NIES-98 during the isolation of serine protease inhibitors aeruginosins 98-A, B and C [52]. Aeruginoguanidines have unusual structures containing two *N*-methylated arginines with the *C*-terminus marked by an unusual highly sulfated aromatic amine for all three compounds, and two guanidine groups derivatized with prenyl and geranyl groups in aeruginoguanidine 98-A. The structures of aeruginoguanidines 98-B and C differ from that of 98-A in the isoprenoid units attached to the guanidino groups. These compounds were reported to show weak cytotoxicity to P388.

The 19-membered cyclic peptides shown in Figure 13 are not metabolites of *Microcystis* spp but were produced by toxic strains of *Anabaena* spp and an *Oscillatoria* sp. *Anabaena flos-aquae* NRC 525-17 gave anabaenopeptins A and B together with microcystins and anatoxin-a(s) [25]. These peptides showed weak relaxation activity to norepinephrine-induced constriction of rat aortic preparations. *A. circinalis* 90 produced anabaenopeptins A, B and C, and anabaenopeptins B and D were isolated from *A. lemmermannii* 202 A2/41 [16]. Both strains contained microcystins. Oscillamide Y was obtained as a serine protease inhibitor from the microcystin-producing strain of *O. agardhii* NIES-601 [76], which also produced oscillapeptin G. Oscillamide Y inhibited the activity of chymotrypsin (Table 1), which suggests that the other members of the class might also have this activity.

Anabaenopeptins have been detected from six *Anabaena* and two *Oscillatoria* strains of fresh-water cyanobacteria and a brackish-water cyanobacterium *Nodularia spumi-*



Microginin

Figure 11 Microginin isolated from *Microcystis aeruginosa*.

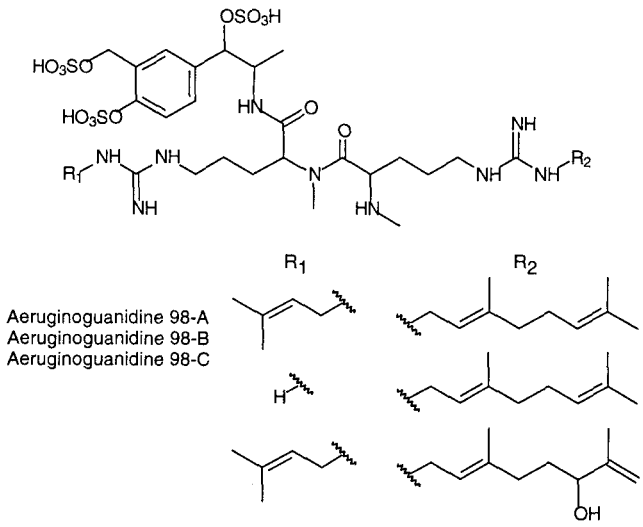
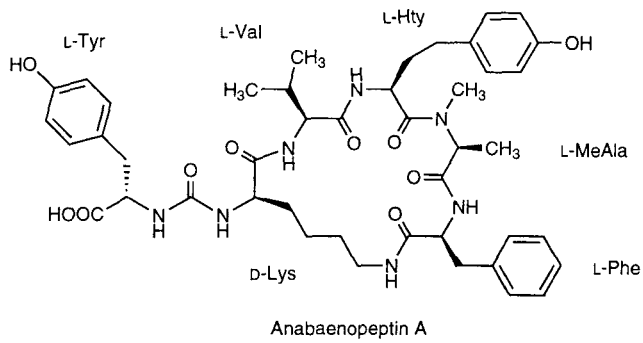
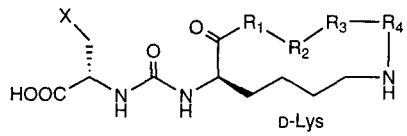


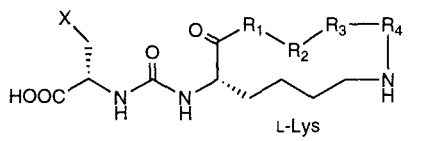
Figure 12 Aeruginoguanidines 98-A, 98-B and 98-C isolated from *Microcystis aeruginosa*.



Anabaenopeptin A



	R_1	R_2	R_3	R_4	X
Anabaenopeptin A	L-Val	L-Hty	L-MeAla	L-Phe	(L-Tyr)
Anabaenopeptin B	L-Val	L-Hty	L-MeAla	L-Phe	(L-Arg)
Anabaenopeptin C	L-Val	L-Hty	L-MeAla	L-Phe	(L-Lys)
Anabaenopeptin D	L-Val	L-Hty	L-MeAla	L-Phe	(L-Phe)
Oscillamide Y	L-Ile	Hty	MeAla	L-Phe	(L-Tyr)



	R_1	R_2	R_3	R_4	X
Konbamide	L-Ala	L-Leu	L-MeLeu	BhTrp	(L-Leu)
Keramamide A	L-Leu	L-Leu	MeCht	L-Phe	(L-Phe)

BhTrp = 2-bromo-5-hydroxytryptophan
MeCht = 6-chloro-5-hydroxy-*N*-methyltryptophan

Figure 13 19-Membered cyclic peptides isolated from *Anabaena* spp, *Oscillatoria* sp and marine sponges.

gena, together with hepatotoxins microcystins or nodularins (Table 2) [16].

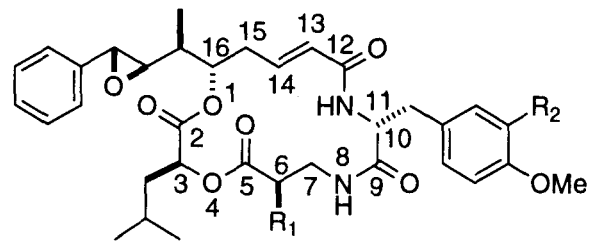
The unique structural features of these compounds are the ureido group and the ω-amido linkage of the D-Lys unit with the *C*-terminal amino acid unit (L-Phe). Interestingly, the stereochemistry of this Lys unit (D-) is different from the other amino acid units. The amino acid units R_2, R_3 and R_4 are the same in the five peptides and R_3 is *N*-methylated. The terminal amino acid (X) forming the ureido unit shows wide variety among these peptides, however.

Peptides structurally related to these compounds have been isolated from two species of marine sponges belonging to the *Theonella* genus. Konbamide [36] and keramamide A [37] have cyclic structures possessing the ureido unit and the ω-amide linkage similar to the above compounds (Figure 13). The position of the *N*-methylated amino acid unit is also the same as in the cyanobacterial metabolites. The difference is observed in the Lys unit: the compounds from marine sponges have an L-Lys unit instead of the D-form of the cyanobacterial metabolites. Konbamide and keramamide A each have one unique tryptophan derivative (BhTrp and MeCht) as shown in Figure 13.

The cyanobacterial genus *Microcystis* is a rich source of cyclic and linear peptides possessing a variety of biological activities, including the inhibition of certain enzyme activities. Structurally related peptides are also obtained from *Anabaena, Nostoc* and *Oscillatoria* spp. These peptides could be common secondary metabolites of the cyanobacteria which form toxic waterblooms, but since both toxic and non-toxic strains produce similar peptides, a relationship between these peptides and biotoxin production is still unclear.

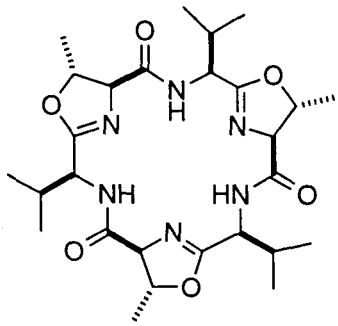
Relationship between cyanobacterial and invertebrate products

During studies on cyanobacterial secondary metabolites, structural relationships between the cyanobacterial secondary metabolites and compounds isolated from marine invertebrates have been observed. The structure of dolastatin 13, from the sea hare, resembles the structures of the cyanobacterial 19-membered depsipeptides in Figure 7, which are also similar to structures of didemnins from a colonial tunicate, as noted above. The 19-membered cyclic peptides shown in Figure 13 have structures related to those of compounds from marine sponges. The structural near-identity between nodularins from the cyanobacterium and



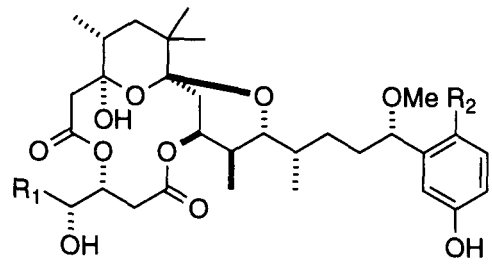
cryptophycin-1: $R_1 = CH_3$, $R_2 = Cl$
arenastatin A (cryptophycin-24): $R_1 = H$, $R_2 = H$

Figure 14 Cryptophycin A from a cyanobacterium and arenastatin from a sponge.



cycloxazoline (westiellamide)

Figure 15 Cycloxazoline, from a tunicate, the same as westiellamide, from a cyanobacterium.

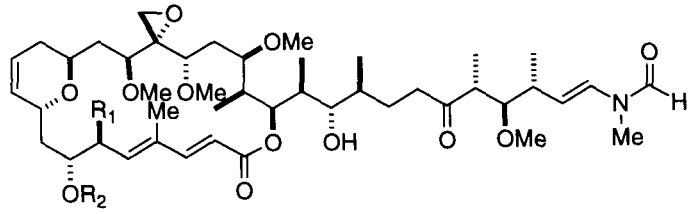


aplysiatoxin: $R_1 = CH_3$, $R_2 = Br$
oscillatoxin A: $R_1 = R_2 = H$

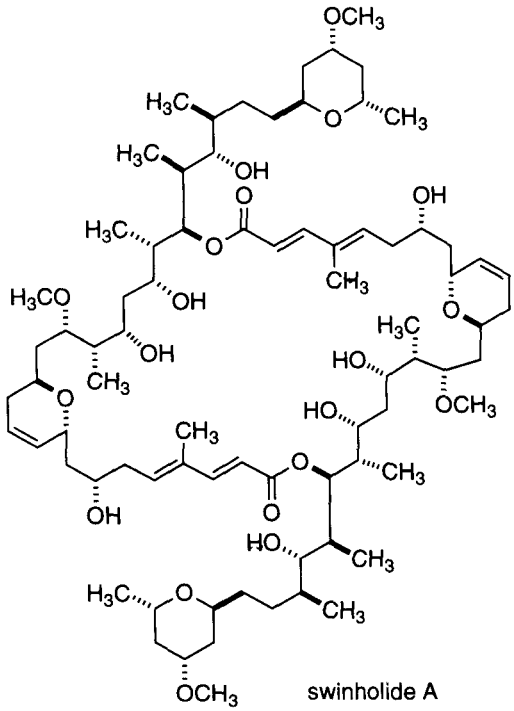
Figure 17 Aplysiatoxin, from a sea hare, and oscillatoxin A, from a cyanobacterium.

motuporin from a sponge was also noted above. Examples of these relationships are increasing as more structures of cyanobacterial secondary metabolites are assigned.

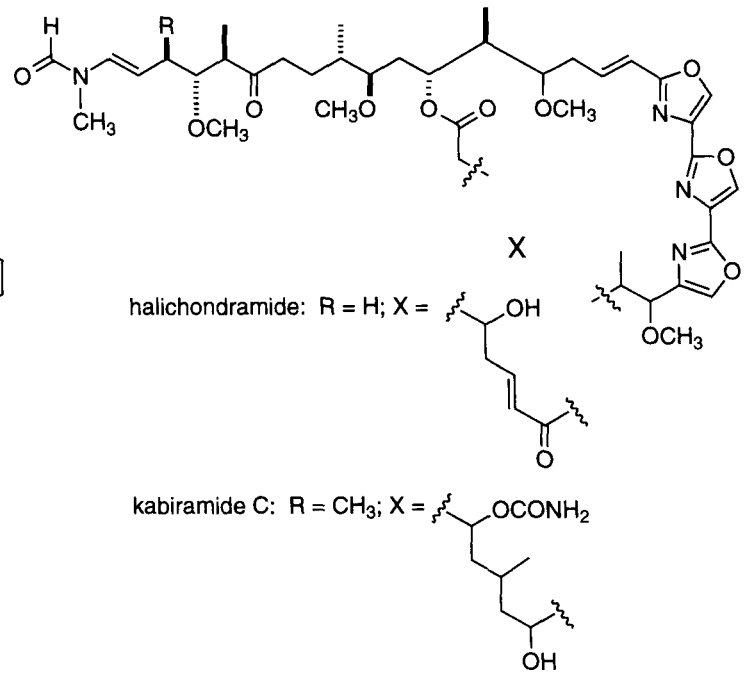
Several additional examples follow. Cryptophycins [18,83], isolated from terrestrial cyanobacteria (*Nostoc* spp) as antitumor agents, have structures very similar to that of arenastatin A [39], from the marine sponge *Dysidea arenaria* (Figure 14). A cytotoxic cyclic peptide cycloxazoline [20] from the colonial tunicate *Lissoclinum bistratum* is the same compound as westiellamide [71] obtained from the terrestrial cyanobacterium *Westiellopsis prolifica* (Figure 15). The terrestrial cyanobacteria *Scytonema* spp and *Tolypothrix* spp produce cytotoxic scytophycins and tolytoxins [6,27] whose structures are related to those of marine sponge metabolites swinholide A [7,35,38] from *Theonella*



tolytoxin: $R_1 = OH$, $R_2 = Me$
scytophycin B: $R_1 = R_2 = H$



swinholide A



halichondramide: R = H; X =

kabiramide C: R = CH_3; X =

Figure 16 Tolytoxin and scytophycin B, from cyanobacteria, swinholide A and halichondramide, from sponges, and kabiramide from nudibranch egg masses.

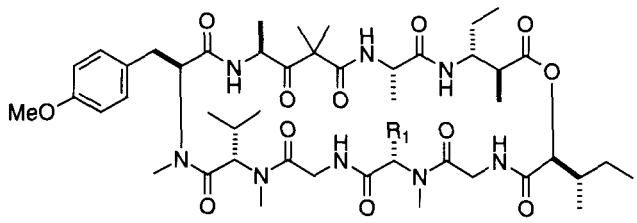


Figure 18 Majusculamide C, from a cyanobacterium, and dolastatin 11, from a sea hare.

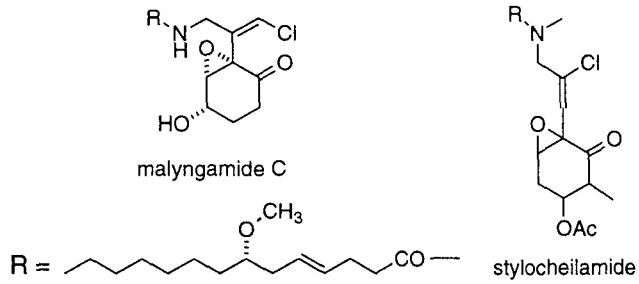


Figure 19 Malyngamide C, from a cyanobacterium, and stylocheilamide, from a sea hare.

Table 2 Detection of anabaenopeptins in hepatotoxic cyanobacteria [16]

Cyanobacteria	Hepatotoxins	Anabaenopeptins
Anabaena flos-aquae NRC 525-17	microcystins	A, B
Anabaena flos-aquae CYA 83/1	microcystins	B, D
Anabaena flos-aquae 202 A1	microcystins	B, D
Anabaena lemmermannii 66	microcystins	B, D
Anabaena lemmermannii 202 A2/41	microcystins	B, C
Anabaena circinalis 90	microcystins	A, B, C
Oscillatoria agardhii 97	microcystins	B, C
Oscillatoria agardhii CYA 128	microcystins	A, C
Nodularia spumigena BY1	nodularins	B

swinhoei and halichondramide [34] from a *Halichondria* sp, and to kabiramide C [46] from nudibranch eggmasses (Figure 16). Aplysiatoxins (Figure 17) were isolated from sea hares [33] and also from the marine cyanobacteria *Lyngbya* spp and *Oscillatoria* spp [49]. The marine cyanobacterial metabolites majusculamide C [12] and malyngamide C [1] from *L. majuscula* have structures similar to those of compounds from sea hares, dolastatin 11 [3,69] from *Dolabella auricularia* and stylocheilamide [75] from *Stylocheilus longicauda*, respectively (Figures 18 and 19). Majusculamide C was recently isolated from a sponge but was postulated to originate in a *Lyngbya majuscula* contaminant which provided the peptide through filter feeding by the sponge [85].

The sea hares apparently obtain these compounds or their precursors from their diets since certain marine cyanobacteria are favorite foods for these molluscs. The structural relationships between metabolites of terrestrial cyanobacteria and marine invertebrates, such as sponges and tunicates, are interesting. The primary producers of these compounds from sponges and tunicates may be symbiotic or associated cyanobacteria. In any case, it is quite interesting that marine and terrestrial cyanobacteria produce similar and sometimes the same secondary metabolites. Thus, it can be suggested that certain secondary metabolites isolated from marine invertebrates can be derived from certain microalgae.

General similarities in structural features are also observed between compounds from marine and terrestrial sources. Halogenated and sulfated compounds are characteristically found in marine organisms and have also been obtained from terrestrial cyanobacteria. Some examples were noted above in secondary metabolites from freshwater cyanobacteria. A number of sulfated metabolites have been obtained as bioactive metabolites (Figures 7, 10 and 12) and halogenated metabolites such as anabaenopeptilides 90-A and 202-B (Figure 7) and aeruginosins 98-A and C (Figure 10) are also isolated from freshwater cyanobacteria. Halogenated compounds are generally much more abundant in cyanobacteria than in other terrestrial organisms. Other examples of halogenated metabolites of terrestrial cyanobacteria are cyanobacterin from *Scytonema hofmanni* [43], hapalindole A from *Hapalosiphon fontinalis* [50], nostocyclophane D from *Nostoc linckia* [47] and puwainaphycins C and D from an *Anabaena* sp [19,48].

Both marine and terrestrial cyanobacteria produce structurally related secondary metabolites and it is of interest to speculate why the cyanobacteria produce such metabolites. One can imagine that ancient cyanobacteria evolved in the seas and lived together with certain marine invertebrates, providing metabolites produced by photosynthesis, and that some of the cyanobacteria left the seas and adapted to freshwater habitats and other terrestrial environments. The search for bioactive secondary metabolites from cyanobacteria is providing increasing numbers of useful and structurally characteristic compounds. Future research should yield more evidence for the biological relationships between marine invertebrates and cyanobacteria in marine and terrestrial habitats.

Acknowledgements

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