# *Microcystis aeruginosa:* Pharmacology and Phytochemistry

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**Summary**

*Microcystis aeruginosa* releases a variety of bioactive compounds during growth and can produce numerous potent toxins and represent an increasing environmental hazard. The literature on the chemical constituents and biological activity has been reviewed. Chemical studies show the presence of many compounds belonging mainly to the group of oligosaccharides, glycerolipids, enzymes, sulfur compounds, peptides such as microcystins, anabaenopeptins, microginins, aeruginosins, and cyanopeptolins. Biological studies reveal significant hepatotoxic, tumor promoters, cytotoxic, mutagenic, antialgal, antiviral activities. In this review the chemical constituents grouped according to structural classes and the biological activities are presented.

**Keywords:** Cyanobacteria, *Microcystis aeruginosa,* peptides, toxins, enzymes, neurotoxins, hepatotoxins, phosphatase activity, cytotoxicity, antiviral, environmental toxicology, protease and serine inhibitor

**Domains :** pharmaceutical sciences, therapeutic drug modeling

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## Introduction

Cyanobacteria are one of the earth´s most ancient life form (3.5 billion yerars) and are the dominant phytoplankton group in eutrophic freshwater bodies worldwide. *Microcystis aeruginosa* Kutzing is a potentially toxic cyanobacterium present in freshwater (lakes, ponds and rivers), where it creates abundant water blooms. This organism synthesizes hepatotoxins, which are microcystins that can kill fish and birds. These toxins also represent a risk for human health because they cause hepatic disorders and skin problems.

The study of the *Microcystis aeruginosa* genome is essential for understanding the mechanisms responsible for its proliferation and for developing means of fighting against this threat to health and the environment. Cyanobacteria produced two main group of toxin namely neurotoxins and peptide hepatotoxins [1]. The effects of environmental factors are associated with the amount of toxins found in cyanobacterial blooms. The chemical constituents grouped according to structural classes and the biological activities are presented in this review, are shown in Tables.

## Chemical Composition

### Carbohydrates

Carbohydrates of the gelatinous sheath and intracellular carbohydrates were separated by column chromatography on DEAE-Sephadex A-25. Polysaccharides of the gelatinous sheath produced by colonies showed marked heterogeneity in their sugar residues, while those of single cells were mainly composed of glucose. In the gelatinous sheath of colonies, xylose or glucose was abundant in two strains of *M. aeruginosa*, while in *M. wesenbergii* glucose, galactose, and xylose were the main constituents and in *M. viridis* mannose has been the characteristic major constituent. The relative amounts of the various sugar constituents of gelatinous sheath carbohydrates differed markedly among the three species of *Microcystis* cultured under the same conditions [2].

**Elemental components**

Mean elemental concentrations (mmol/kg dry wt.) from *M. aeruginosa* were: magnesium 125; silicon 1864; phosphorus 341; sulfur 122; chlorine 88; potassium 282; calcium 63 [3].

**Enzymes**

Ribulose diphosphate carboxylase has been purified from the unicellular blue-green alga, *M. aeruginosa*. This had a molecular weight of 518,000 and contained 2 types of subunits (large, 50,000 and small, 14,000) as shown by Na dodecyl sulfate-polyacrylamide gel electrophoresis. Ribulose diphosphate carboxylase from this photosynthetic prokaryote thus appears to resemble closely at the of eukaryotic microalgal chloroplasts in quaternary structure [4].

*Microcystis* strains contained endodeoxyribonucleases ApcTR183I (5'-TGCGCA-3') and Msp199I (5'-CCGG-3'). Principal component analysed showed that the most common cyanobacterial endonuclease types were AvaII, AvaI and AsuII. The cyanobacteria studied contained restriction endonucleases. The defined restriction endonucleases were isoschizomers of known enzymes [5].

The superoxide dismutase (SOD) enzymes of surface blooms, early degenerate and completely degenerate cultures were assayed by staining for SOD activity, immunoblotting and immunogold labeling. During surface bloom formation, Fe-SOD activity increased five-fold compared with that in control cells; no variation was detected in Mn-SOD activity. However, in early degenerate cultures, Fe-SOD activity decreased to that seen in control cultures, while activity disappeared in completely degenerate cultures. Immunogold labeling showed that Fe-SOD has been localized in the cytoplasmic and thylakoid membranes of *Microcystis*. The extent of labeling paralleled the course of Fe-SOD activity with an increase in particles in surface blooming cells. The results suggest Fe-SOD increased due to photooxidative stress. However, under prolonged photooxidative stress, high concentratios of active oxygen species could directly, or indirectly, inactivate and degrade Fe-SOD [6].

β-Carotene oxygenase is membrane bound, and is sensitive to SH reagents, antioxidants,

and chelating agents. Fe is apparently essential for enzyme activity [7].

A pyridine nucleotide dehydrogenase has been isolated and purified from extracts of *M. aeruginosa*. The enzyme is a globular flavoprotein located in the solution fraction after high-speed centrifugation of cell extracts. It exists as a noncovalently bound aggregate of approximate 8 identical subunits, the molecular weight of the octamer being 41,000 and that of the basic subunit 5100. It has been possible to demonstrate transfer of electrons from NAD(P)H to membrane particles. Oxidation of NADPH was unaffected by light, whereas $O_2$ uptake was impaired when membrane particles were energized by light. NADPH has been the superior electron donor and, based on studies with various electron acceptors, the enzyme has been classified as an NAD(P)H-quinone oxidoreductase [8]. Also β-carotene 7,8(7',8')-oxygenase has been isolated from *M. aeruginosa*. It cleaves β-carotene and zeaxanthin specifically at the positions 7-8 and 7'-8'; echinenone and myxoxanthophyll are not affected. The oxidative cleavage of β-carotene leads to the formation of β-cyclocitral and crocetindial and that of zeaxanthin to hydroxy-β-cyclocitral and crocetindial in nearly stoichiometric amounts [9].

A hydrogenase has been isolated from a unicellular and non-N2-fixing cyanobacterium, M. *aeruginosa* strain NIES 44. Hydrogenase was easily solubilized and has been capable of evolving $H_2$ in the presence of reduced Me-viologen and benzyl viologen. Hydrogenase was stimulated by divalent ions and showed a pH optimum at 6.8. Molecular weight of hydrogenase, estudied by gel filtration, was 50,000 [10]. Enzymes are shown in Table 1.

**Fatty acids**

The main glycerolipids (monogalactosyl-, digalactosyl-, sulfoquinovosyldiacylglycerol and phosphatidylglycerol) from 5 blue-green algae were presents in *Microcystis, Anabaena, Nostoc, Oscillatoria, Tolypothrix*. These plants showed occurrence of diglyceride species, and positional distribution of fatty acids between the sn-1- and sn-2-position of glycerol.

On the other hand, the positional distribution of fatty acids in all lipids has been controlled exclusively by chain length and not by the degree of unsaturation with C18-fatty acids at the sn-1- and C16-fatty acids at the sn-2-position.

**TABLE 1**
**Enzymes from *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
| Ribulose diphosphate carboxylase | Mol. wt. of 518,000, contained 2 types of subunits (large, 50,000 and small, 14,000), (4) |
| Endodeoxyribonuclease ApcTR183I (5'-TGCGCA-3'), | Contained restriction endonucleases (5) |
| Endodeoxyribonuclease Msp199I (5'-CCGG-3'), | Contained restriction endonucleases (5) |
| Superoxide dismutase | Induction and decay of a surface bloom (6) |
| β-Carotene oxygenase | Is sensitive to SH reagents, antioxidants, and chelating agents (7) |
| Pyridine nucleotide dehydrogenase | Is a globular flavoprotein, classified as an NAD(P)H-quinone oxidoreductase (8) |
| β-carotene 7,8(7',8')-oxygenase (9) | It cleaves β-carotene and zeaxanthin specifically at the positions 7-8 and 7'-8'; echinenone and myxoxanthophyll are not affected. |
| Hydrogenase | Molecular weight of hydrogenase, estudied by gel filtration, has been 50,000 (10) |

Thus, in prokaryotic organisms the diversity in diglyceride portions of lipids is reduced as compared to that in eukaryotic organisms [11].

The fatty acid profile of lipids in eight species of algae grown under controlled conditions has been studied, as part of a search for oil-producing algae. Fatty acids have been species specific, with changes occurring in the relative amounts of polyunsaturated fatty acids of cells cultivated under different conditions and during various growth phases. All green algae (*Chlorococcum oleofaciens, Chlorella ellipsoidea, Chlorella vulgaris, Stigeoclonium sp.* and *Scenedesums obliquus*) synthesized C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3; and C16:3 appeared in *C. vulgaris. Chrysophytacean* synthesized C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, C18:4, C20:3, C20:4 and C22:5. *Navicula pelliculosa,*

a *Chrysophytacean,* contains a great amount of C16:0 and C16:1, the remainder being C14:0, C18:1, C20:4 and C20:5. The fatty acid profile of the blue-green *Cyanophycean spirulina* sp. and *Microcystis aeruginosa* is the same as in the green algae [12].

In pure culture of *Microcystis aeruginosa* we detected 16:0, 18:2ω6, 18:3ω3, 18:3ω6, and 18:4ω3 acids as major fatty acids of *M. aeruginosa,* with trace amounts of C20 polyunsaturated fatty acids. In both pure culture and the field enclosure, the ratio of total fatty acid weight to dry weight decreased with decreasing availability of dissolved inorganic nitrogen. The ω3/ω6 ratios of C18 polyunsaturated fatty acids [(18:3ω3 + 18:4ω 3)/(18:2 ω6 + 18:3ω6)] varied greatly (range, 2-5) in response to the changes in physical and chemical conditions for *Microcystis* growth. The fatty acid compound is a useful indicator of the physiological state of *Microcystis* in freshwater lakes [13]. Fatty acids are shown in Table 2.

**TABLE 2**

**Fatty acids from *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
| Monogalactosylglycerol | Glycerolipid (11) |
| Digalactosylglycerol | Glycerolipid (11) |
| Sulfoquinovosyldiacylglycerol | Glycerolipid (11) |
| Phosphatidylglycerol | Glycerolipid (11) |
| 16:0 fatty acids | The fatty acid is a useful indicator of the physiological state of *Microcystis* in freshwater lakes (12) |
| 18:2ω6 fatty acids | The fatty acid is a useful indicator of the physiological state of *Microcystis* in freshwater lakes (13) |
| 18:3ω3 fatty acids | The fatty acid is a useful indicator of the physiological state of *Microcystis* in freshwater lakes (13) |
| 18:3ω6 fatty acids | The fatty acid is a useful indicator of the physiological state of *Microcystis* in freshwater lakes (13) |
| 18:4ω3 fatty acids | The fatty acid is a useful indicator of the physiological state of *Microcystis* in freshwater lakes (13) |

**Gas-vacuole membrane**

The gas-vacuole membrane from *M. aeruginosa*. is composed of only protein consisting of 10% basic, 18% acidic, and 52% nonpolar amino acids [14]. The hydrophobic protein comprising the gas vacuoles of a variety of procaryotic microorganisms is insoluble in detergents (e.g., SDS) and cannot be electrophoresed in detergent-containing systems. The gas vacuole protein from *Halobacterium halobium, H. Salinarium* strain 5, and the 2 species of cyanobacteria, *Anabaena flos-aquae* and *Microcystis aeruginosa* have been separated and identified. Mobility of the gas vacuole proteins in the 4 species are consistent with molecular weight of 16,800, 16,800, 14,700, and 15,700, respectively [15].

The primary structure and hydrophobicity of the gas vacuole membrane, containing only 1 protein and no lipid, typifies integral membrane proteins. Peptides obtained by trypsin digestion and N-bromosuccinimide treatment of the gas vacuole protein have been sequenced. Two aspects of the sequence analyses are of interest: a long stretch of 15 aliphatic residues and a thrice repeating octapeptide. The location of the aliphatic portion of the peptide between the N and C terminal of the gas vacuole protein sequence indicated that the protein is amphipathic, the protein has stretches of primarily either nonpolar residues or polar residues in its sequence. Based on this and a comparison of the relative polarities of gas vacuole protein with other integral membrane proteins, the gas vacuole protein is an integral membrane protein. The presence of the repeating octapeptide in the sequence suggested that it may serve as a structural building block for the membrane [16]

The complete amino acid sequence of the gas-vehicle protein (GVP) from *Anabaena flos-aquae* was of 70 residues long and has a molecular weight of 7388. Details of the sequence are related to the secondary β-sheet structure of the protein and its contrasting hydrophilic and hydrophobic surfaces. Extensive amino acid sequences have been also determined for GVPs from 2 other cyanobacteria, species of *Calothrix* and *Microcystis*; they are highly homologous with that of *Anabaena* GVP. Electrophoretic analyses indicates that GVPs of different cyanobacteria form a variety of stable oligomers [17].

The proteins present in gas vesicles of the cyanobacteria *Anabaena flos-aquae* and *Microcystis* sp. have been separated by SDS-polyacrylamide gel electrophoresis. The gas vesicles of the cyanobacterias contain, in addition to the principal Mr 7400 gas vesicle

protein GVPa, a second Mr 22,000 protein component, GVPc. GVPc can be removed from the gas vesicles, without their collapsing, by rinsing in solutions of SDS. It is concluded that GVPc is located on the outer surface of the hollow shell formed by GVPa. Removing GVPc causes a marked decrease in the critic collapse pressure of the gas vesicles. Apparently, the protein provides structural support and reduces pressures generated by surface tension. By measurement of the 35S-labeling of gas vesicles it has been demonstrated that GVPc, which contains 2 methionine residues per mol., accounts for 9% of the total gas vesicle protein, confirming the value suggested by amino acid analyses. 32P-labeling indicates that the phosphate content of gas vesicle protein is negligible [18].

The second protein of Mr 22K whose N-terminal amino acid sequences showed homology with the isolated from *Calothrix* sp. PCC 7601. The derived amino acid sequence for the gene product indicated a protein, GVPc, of 193 residues and Mr 21985 containing five highly conserved 33 amino acid repeats. The sequence was identical at the N-terminus to that of the Mr 22K protein present in gas vesicles and showed correspondence to seven tryptic peptides isolated from gas vesicles. This establishes that GVPc forms a second protein component of the gas vesicle, in addition to the main constituent, the 70 residue GVPa. Quantitative amino acid analysis of entire gas vesicles reveals that GVPc accounts for only 2.9% of the protein molecules and 8.2% of the mass present: this is insufficient to form the conical end caps of the gas vesicles. It is suggested that GVPc provides the hydrophilic outer surface of the gas vesicle wall; the 33 amino acid repeats may interact with the periodic structure provided by GVPa [19].

**Hydrocarbon, monoterpenes, sesquiterpenes and triterpenes**

A tobacco-like odorous compound isolated from a bloom of the blue-green alga *M. wesenbergii* and *M. aeruginosa* has been analyzed by gas-liquid chromatography and mass spectrometry. Its structure has been determined as β-cyclocitral. The hydrocarbon content was 0.10-0.18% of the dry weight *n*-hexadecane, *n*-heptadecane, 4-methylheptadecane, and *n*-octadecane constituted the main components [20, 21]. Also the triterpene Hop-22(29)-ene, is present in *Microcystis Aeruginosa* [22].

The volatile substances present in a eutrophic shallow lake have been determined

quality and quanty during the growing season, allowing a 1st-time analyses of the dynamics of these compounds in a water body. The major compounds have been β-cyclocitral, α-cyclocitral, β-ionone, 1,3,3-trimethylcyclohexene, 2,2,6-trimethylcyclohexanone, 2,6,6-trimethylcyclohex-2-en-1-one, eucalyptol, geosmin, 2 argosmin isomers, pent-1-en-3-ol, pent-1-en-3-one, heptadec-1-ene, heptadec-cis 5-ene, *cis*-1,8-heptadecadiene, decanal, and hexanal [23, 24]. Also compounds isolated from the cyanobacteria M. *aeruginosa* included 2-methylisoborneol, sesquiterpenes, 3-methyl-1-butanol, and 6-methyl-5-hepten-2-one [25]. Table 3 shows the hydrocarbon, monoterpenes, sesquiterpenes and triterpenes obtained from *M. aeruginosa*.

**Oligosaccharides**

Oligosaccharides have been extracted with phenol/water and purified, CsCl gradient ultracentrifugation from *M. aeruginosa*. The LPS contained significant amounts of 3-deoxy-D-manno-octulosonic acid, glucose, 3-doxy sugars, glucosamine, fatty acids, fatty acid esters, hexoses, and phosphate.

Oligosaccharides and lipid A hydrolyzate of LPS preparations were active in mouse lethality and Limulus lysate gelation. The lipid A moiety was slightly less active in toxicity and Limulus lysate gelatin assays than the intact LPS. The LPS and lipid A moiety of the 2 isolates of *M. aeruginosa* were less active in toxicity in mice [26].

Oligosaccharides were obtained from 3 strains (PCC 7806, PCC 7820 and UV-017) of the waterbloom-forming cyanobacterium *M. aeruginosa*. 3-Hydroxy fatty acids (3-OH-14:0, 3-OH-16:0, 3-OH-18:0) in addition to other fatty acids were identified in all 3 liposaccharides. Glucosamine, the only amino sugar found, presumably represents the backbone amino sugar of the phosphate-free lipid A moiety. Heptoses were absent and 2-keto-3-deoxyoctonate was not detected. Strains PCC 7806 and UV-017 were of the same chemotype, differing from that of strain PCC 7820. Polysaccharides with strain-specific chemical compounds different from those of the oligosaccharides have been obtained from each strain. The polysaccharides are likely to be in external cell envelope layers. Their sugar specificity was in agreement with the O-chain chemotypes of the oligosaccharides of the 3 strains [27].

**TABLE 3**
**Hydrocarbon , monoterpenes, sesquiterpenes and triterpenes from *Microcystis aeruginosa***

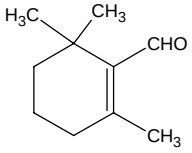
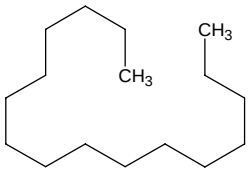
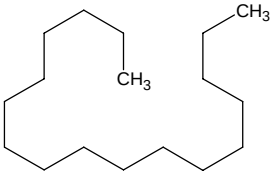
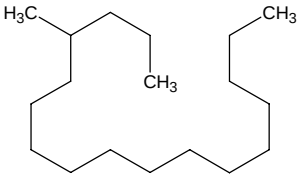
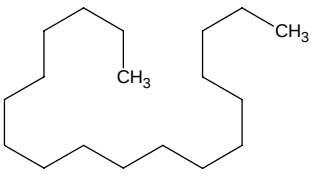
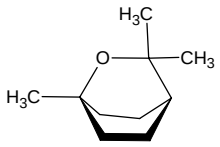
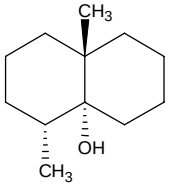
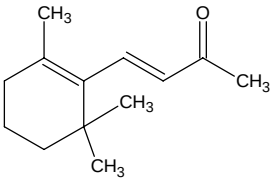
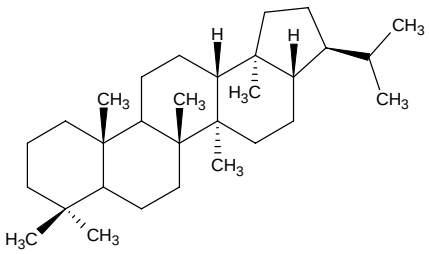
| Structure | Properties |
|---|---|
|  β-cyclocitral | Tobacco-like odorous compound (20-21) |
|  *n*-hexadecane | Main hydrocarbon content was 0.10-0.18% of the dry weight (20-21) |
|  *n*-heptadecane | Main hydrocarbon content was 0.10-0.18% of the dry weight (20-21) |
|  4-methylheptadecane | Main hydrocarbon content was 0.10-0.18% of the dry weight (20-21) |
|  *n*-octadecane | Main hydrocarbon content was 0.10-0.18% of the dry weight (20-21) |

**TABLE 3 (CONTINUED)**
**Hydrocarbon , monoterpenes, sesquiterpenes and triterpenes from *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
|  eucalyptol | Volatile substance (23,24) |
| 3-Methyl-1-butanol 1,3,3-trimethylcyclohexene 2,2,6-trimethyl cyclohexanone, 2,6,6-trimethyl cyclohex-2-en-1-one, pent-1-en-3-ol, pent-1-en-3-one, heptadec-1-ene, heptadec-cis 5-ene, *cis*-1,8-heptadecadiene, decanal, hexanal, 6-methyl-5-hepten-2-one and 2-methylisoborneol | Volatile substances (23-25) |
|  Geosmin | Volatile substances (23,24) |
|  β-ionone | Volatile substances (23,24) |
|  17β(H)-hopane | Appears to derive from bacterial reducen of hop-22(29)-ene, (22) |

**Microcystins and other peptides**

A high diversity of known cyanobacterial peptides such as microcystins, anabaenopeptins, microginins, aeruginosins, and cyanopeptolins, but also many unknown substances are present in the *Microcystis* colonies. Oligopeptide patterns were mostly related to specific *Microcystis* taxa.

Microcystin, a hepatotoxin known to be the cause of animal and human deaths, is produced by the bloom-forming cyanobacterium *Microcystis aeruginosa* in freshwater bodies worldwide. Microcystins are potent liver toxins and tumor promoters produced by several cyanobacteria genera.

Blooms of toxic cyanobacteria (blue-green algae) have become increasingly common in the surface waters of the world. Of the known toxins produced by cyanobacteria, the microcystins are the most significant threat to human and animal health. These cyclic peptides are potent inhibitors of eukaryotic protein phosphatases type 1 and 2A.

Synthesized nonribosomally, the microcystins contain a number unusual amino acid residues including the β-amino polyketide moiety Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid). The microcystin biosynthetic gene cluster spanning 55 kb, from *Microcystis aeruginosa* PCC7806 composed of 10 bidirectionally transcribed open reading frames arranged in two putative operons (mcyA-C and mcyD-J), has been correlated with microcystin formation by gene disruption and mutant analyses of the 48 sequential catalytic reactions involved in microcystin synthesis, 45 have been assigned to catalytic domains within six large multienzyme synthases/synthetases (McyA-E, G), which incorporate the precursors phenylacetate, malonyl-CoA, S-adenosyl-L-methionine, glutamate, serine, alanine, leucine, D-methyl-isoaspartate, and arginine. The additional four monofunctional proteins are putatively involved in O-methylation (McyJ), epimerization (McyF), dehydration (Mcyl), and localization (McyH). The unusual polyketide amino acid Adda is formed by transamination of a polyketide precursor as enzyme-bound intermediate, and not released during the process. The enzymic organization of the microcystin assembly represents an integrated polyketide-peptide biosynthetic pathway with a number of unusual structural and enzymic features. These include the integrated synthesis of a β-amino-pentaketide precursor and the formation of β-

and γ-carboxyl-peptide bonds. Other features of this complex system also observed in diverse related biosynthetic clusters are integrated C- and N-methyltransferases, an integrated aminotransferase, and an associated O-methyltransferase and a racemase acting on acidic amino acids [28].

Studies revealed several structural variants of microcystin: MCYST-RR (microcystin with Arg and Arg, indicated by *m/z* 1038 and confirmed by PSD revealing a *m/z* = 135 fragment deriving from the Adda side chain), and MCYST-FR (microcystin with Phe and Arg, indicated by *m/z* = 1015). The presence of [Asp(3)]-MCYST-LR (microcystin with Leu and Arg, Asp non-methylated, indicated by *m/z* 981), and [Asp(3)]-MCYST-YR (microcystin with Tyr and Arg, Asp non-methylated, indicated by *m/z* 1,031) were likely.

The relative amounts of the peptides varied between february, april, and may. The serin-proteases trypsin, plasmin, elastase were inhibited, assumable due to the cyanopeptolins found. Elastase and the cysteine-protease papain were not inhibited, inhibitions of protein kinase and glutathione S-transferase (GST) was low. Strong inhibition has been observed with protein-phosphatase-1, likely due to the microcystins present in the samples [29].

A toxin has been obtained from the cyanobacterium *M. aeruginosa* PCC 7941 by extracting freeze-dried cells with water-saturated, acidified *n*-butanol, di-Et ether-water distribution, reversed-phase TLC, and silica HPLC. The following amino acids were identified: β-methyl-Asp, Thr, Glu, Ala, Val, Leu, Phe, Arg, N-methyldehydro-Ala, and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. The mass spectroscopic data showed that the fraction was still composed of several, most likely cyclic peptides that did not stain with ninhydrin [30]. The structures of these hepatotoxins of general name cyanoginosins-XY are proposed to be cyclo-D-Ala-L-X-erythro-β-methyl-D-isoAsp-L-Y-Adda-D-isoGlu-N-methyldehydroAla, were X and Y represent variable amino acids and Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic [31].

Also the toxin cyanoginosin-LA, has been isolated from *Microcystis aeruginosa*. The structure of cyanoginosin-LA has been determined to be cyclo(D-Ala-L-Leu-erythro-β-methyl-D-isoAsp-L-Ala-Adda-D-isoGlu-N-methyldehydroAla),(Adda = 3-amino-9- methoxy -2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid residue) studied by degradation and spectral methods [32]. The amino-terminal sequence of microcystin AL is as follows: Val-Leu-Ala-Ser-Leu-Val-Ser-Thr-Ser-Gln-Ala-Gly-Ser-Leu-Glu-Leu-Leu-Ala [33].

Microcystin-LR, a hepatotoxic cyclic peptide associated with most strains of the blue-green algae *Microcystis aeruginosa,* that proves to be a potent inhibitor of type 1 ($IC_{50}$ = 1.7 nM) and type 2A ($IC_{50}$ = 0.04 nM) protein phosphatases. Microcystin-LR inhibited the activity of both type 1 and type 2A phosphatases >10-fold more potently than okadaic acid under the same conditions. Type 2A protein phosphatases in diluted mammalian cell extracts were found to be completely inhibited by 0.5 nM microcystin-LR while type I protein phosphatases have been only slightly affected at this concentration. Thus, microcystin-LR may prove to be a useful probe for the study and identification of cellular processes which are mediated by protein phosphatases [34]. Microcystin-LR peptide which on hydrolysis has been shown to consist of equimolar amounts of L-methionine, L-tyrosine, D-alanine, D-glutamic acid, erythro-β-Me aspartic acid and methylamine.

Ten microcystins were identified (cyclic heptapeptidic toxins) of the hepatotoxic cyanobacterium *Microcystis aeruginosa* PCC 7820. Three new variants were identified: desmethylated microcystin LW (MCYST-dMeLW), desmethylated microcystin LF (MCYST-dMeLF), and microcystin LL (MCYST-LL). The concentration of intracellular microcystins reached 2-8 mg/g of dried cells, with a equivalent of 1-5 mg/g by protein phosphatase 2A (PP2A) inhibition assay. Toxin produced can be correlated to biomass increase up to the middle of the exponential phase of growth and ceases thereafter. Toxin release occurred during the stationary phase, and extracellular microcystin concentration reached 0.25 mg/L. Intracellular microcystin pool component (MPC) was constant with 51 ± 2% MCYST-LR, whereas this toxin stood for only 29 ± 3% of extracellular MPC. MCYST-LR, the less hydrophobic microcystin, diffuses less easily across membranes. Hydrophobicity might play a key role in microcystin release process [35].

Five microcystins have been obtained from *M. aeruginosa* strain CALU 972 isolated from a hepatotoxic water bloom collected in Lake Kroshnosero (Russia). The structure of a new toxin has been identified as [Dha7]microcystin-YR by amino acid analyses and fast atom bombardment mass spectrometry, and the other toxins were assigned the structures as [Dha7]microcystin-LR, [D-Asp3,Dha7]microcystin-LR, [Dha7]microcystin-RR, and [D-Asp3,Dha7]microcystin-RR [36].

Seven microcystins (MCYSTs), three major and four minor ones, have been isolated from a cultured *Microcystis aeruginosa* strain M.TN-2. MCYST-LR, -RR, -RA, -FR, -WR,

[D-Asp3] MCYST-FR, and [D-Asp3] MCYST-WR have been characterized by amino acid analyses, MALDI-TOF mass spectrometry, 1H and DQF-COSY NMR spectroscopy and UV spectroscopy. Among them, [D-Asp3] MCYST-FR and [D-Asp3] MCYST-WR, compounds, were the desmethyl variants of MCYST-FR and MCYST-WR, respectively [37]. Also have been identified [D-Asp3]microcystin-YR and [D-Asp3]microcystin-RR [38]. Two toxic heptapeptides have been isolated from an axenic *M. aeruginosa* strain, K-139. Using mainly a nondestructive NMR method, the structure of the major toxin has been identified as 7-desmethylmicrocystin LR which lacks an N-Me group of the dehydroalanine moiety of microcystin LR. Amino acid analyses yielded D-glutamic acid, D-erythro-β-methylaspartic acid and D-alanine in equimolar and L-arginine in two-fold molar ratios. Investigation of the toxin with fast atom bombardment mass spectrometry showed a nominal relative mol mass of 1023. 3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) has been identified by 1H NMR and 1H, 1H COSY spectroscopy. The presence of dehydroalanine has been confirmed by hydrogenation and subsequent amino acid analyses. The minor toxin has been deduced to be 3,7-didesmethylmicrocystin LR [39].

The autoxidative degradation is responsible for the inactivation of the unstable Microcystis toxin HBP-Tx. The purified toxin has been similar in its properties to the "fast-death-factor" in *Microcystis*, described as a cyclic peptide. The apparent presence of an entirely different toxin has been simulated by the partially inactivated HBP-Tx. A number of associated fluorescent compounds have been identified as the non-toxic degradation products of the toxin [40].

The characterize the two toxins (P-1 and P-2) isolated from the blue-green alga *Microcystis aeruginosa*, by amino acid analysis, mass spectrometry, 1H- and 13C-NMR. P-2, the major toxin, had a molecular weight of 1044, and consisted of one molecule each of beta-methylaspartic acid, D-Glu, D-Ala, L-Arg, L-Tyr, N-methyldehydroalanine, and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda). P-1, with a molecular weight of 994, appeared to have almost the same composition except that it contained L-Leu instead of L-Tyr in P-2. Mass spectrometric studies, along with a negative ninhydrin reaction, indicated that each toxin was a cyclic peptide. P-2 showed an $LD_{99}$ of 70 micrograms/kg mice when injected i.p. [41].

The bloom of *Microcystis* sp. contained both non-toxic (cyanopeptolin-type) and hepatotoxic (microcystin-type) peptides. The cyanopeptolin structure of the non-toxic peptides (called cyanopeptolin VW-1 and VW-2) has been revealed by matrix assisted laser desorption ionization mass spectrometry (MALDI-TOF-MS) of whole cells, showing dominant molecular ions at *m/z* = 975 and *m/z* 995. On post source decay (PSD), both cyanopeptolins showed fragments deriving from Ahp-Phe-MTyr (3-amino-6-hydroxy-2-piperidone), the characteristic partial structure of cyanopeptolins. The amounts of each of the two cyanopeptolins could only roughly be to be >0.1% of bloom material dry weight [42].

The amino acid sequence of the hepatotoxic peptide Akerstox (Microcystin-LR) isolated from a bloom of the blue-green alga *M. aeruginosa* in a Norwegian freshwater lake, has been identified as by fast atom bombardment-mass spectrometry (FAB-MS) and mass spectrometry (MS) methods. The peptide, containing alanine, arginine, glutamate, leucine, β-methylaspartate, *n*-methyldehydroaniline, and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoate, was cyclic [43].

Two toxins, with $LD_{50}$ (i.p., mouse) of 40 and 150 mg/kg, were isolated using gel filtration and HPLC from cyanobacterium *M. aeruginosa.* The amino acid compound and molecular weight (994) of the 40 mg/kg toxin was the same as that for microcystin-LR, while the 150 mg/kg toxin had an amino acid compound and molecular weight (1048) different from any of the reported cyanobacteria heptapeptide toxins reported [44].

Aeruginoguanidines 98A, 98B, and 98C (I,II, and III, respectively) were isolated from the cyanobacterium *Microcystis aeruginosa* (NIES-98). The absolute stereochemical of I, consisting of Hphpa trisulfate (1-(4-hydroxy-3-hydroxymethyl)phenyl-1-hydroxy-2-propylamine 4',3',1-tri-O-sulfate), MpArg (Nα-methyl-Nω-prenylarginine) and MgArg ((Z)-Nα-methyl-Nω-geranylarginine).These compounds showed moderate cytotoxicity against the P388 murine leukemia cells [45].

Five protease inhibitors, related to aeruginosins 298-A, 98-A and 98-B, have been isolated from the cyanobacterium *Microcystis aeruginosa*. Aeruginosins 98-C and 101 differed from 1 in the Hpla units. Aeruginosin 298-B lacked the argininol unit in 5. Aeruginosins 89-A and 89-B have been observed as the tautomers in HPLC because of the presence of the argininal units [46].

Radiosumin B, an N-Me dipeptide containg 2 unusual amino acid residues, has been isolated from the cyanobacterium *Microcystis aeruginosa* Kutzing [47]. Microcystins and peptides are given in Table 4.

**Nitrogen compounds**

*Microcystis pulverea*, and *M. aeruginosa* contained alanine, aspartic acid, arginine, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, proline, serine and glycine, threonine, tryptophan, tyrosine and valine.Glutamic acid, threonine, serine and glycine are quantity predominant. The compounds of amino acids in green algae cultivated in an inorganic medium did not differ significantly from cultures in an organic medium [48].

Cellular and extracellular levels of cyclic AMP (cAMP) were analyzed in the blue-green algae *Microcystis aeruginosa, Anabaena flos-aquae,* and *Synechococcus leopoliensis,* and *the green algae Chlorella pyrenoidosa, Cosmarium botrytis, Pandorina morum, Scenedesmus communis,* and *Pediastrum biradiatum*. On the basis of chromatographic analyses and several biochemical assays, each alga produced cAMP and released it into the medium. Cellular cAMP (92-394 pmol/g) and extracellular cAMP (8-440 pmol/L) varied greatly among species [49].

Cytochrome c6, is a soluble hemoprotein that serves as a photosynthetic electron transport component in cyanobacteria and algae, carrying electrons from the cytochrome bf complex to photosystem I. [50].

Methods, including freeze-thawing, (NH4)2SO4 presipitation, and ion-exchange chromatography, are used for the isolation of ferredoxins I and II, cytochrome c553, cytochrome f, cytochrome c550, and plastocyanin from large quantities of various cyanobacteria. There is a variation in the relative amounts of these proteins in different batches of cells which may be related to the nutritional status of the organisms [51]. It is a low-potential, autoxidizable cytochrome. This cytochrome should not be confused with a degradation product of cytochrome f, which may be formed during the isolation of the latter protein. Cytochromes c550 are distinctive in size, amino acid compound, and N-terminal amino acid sequence [52].

**TABLE 4**
**Microcystins and other peptides from *Microcystis aeruginosa***

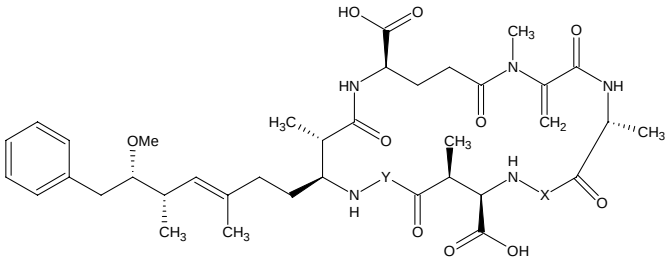
| Structure | Properties |
|---|---|
|  <br>X = L-Leu, Y= L-Ala<br>Microcystin LA<br><br>X = L-Leu, Y= L-Arg<br>Microcystin LR<br><br>X = L-Tyr, Y= L-ARg<br>Microcystin YR<br><br>X = L-Tyr, Y= L-Ala<br>Microcystin YA<br><br>X = L-Tyr, Y= L-Met<br>Microcystin YM<br><br>X = L-Arg, Y= L-Arg<br>Microcystin RR<br><br>X = L-Leu, Y= L-MeAla<br>Microcystin Laba<br><br>X = L-Phe, Y= L-Arg<br>Microcystin FR<br><br>X = L-Ala, Y= L-Arg<br>Microcystin Ar | Microcystins also known as cyanoginosins. These are hepatotoxin known to be the cause of animal and human deaths, is produced by the bloom-forming cyanobacterium *Microcystis aeruginosa* in freshwater bodies worldwide (32)<br>These cyclic peptides are potent inhibitors of eukaryotic protein phosphatases type 1 and 2A (31)<br>Microcystins are potent liver toxins and tumor promoters produced by several cyanobacteria genera (74)<br>Microcystin-LR inhibited the activity of both type 1 and type 2A phosphatases >10-fold more potently than okadaic acid under the same conditions. It is a a hepatotoxic cyclic peptide (34)<br>Histologic evidence of dose-dependent hepatic inflammation was seen, including infiltration of centrilobular regions by lymphocytes, macrophages, and neutrophils, centrilobular fibrosis, apoptosis, and steatosis (71)<br>Microcystin LR and -LA are more toxic than microcystin-LY and -RR in adult mice (29, 70)<br>Microcystin LR induces rapid and characteristic deformation of isolated rat hepatocytes, is a potent, rapid-acting, direct hepatotoxin, with the immediate cause of death in acute toxicities being hemorrhagic shock secondary to massive hepatocellular necrosis and collapse of hepatic parenchyma (71,72) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides from *Microcystis aeruginosa***

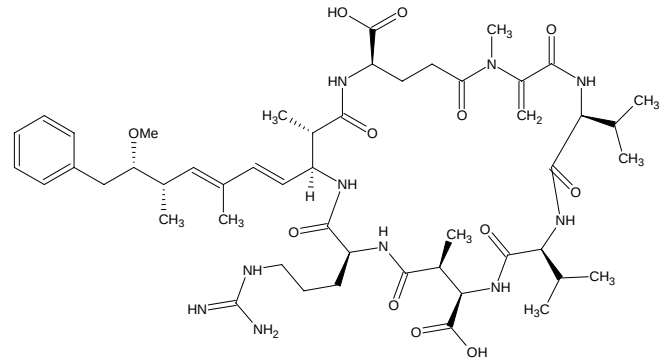
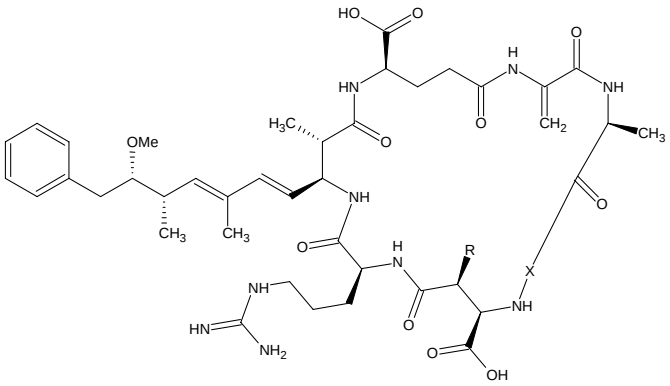
| Structure | Properties |
|---|---|
|  [D-Leu1]Microcystin-LR | It is similar to that of the commonly occurring microcystin-LR (69) |
|  R = Me, X= L-Leu [Dha7]microcystin-LR<br><br>R = H, X= L-Leu [D-Asp3,Dha7]microcystin-LR<br><br>R = Me, X= L-Arg [Dha7]microcystin-RR<br><br>R = H, X= L-Leu [D-Asp3,Dha7]microcystin-RR | Hepatotoxic cyanobacterial were obtained from *M. aeruginosa* (68,92) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides from *Microcystis aeruginosa***

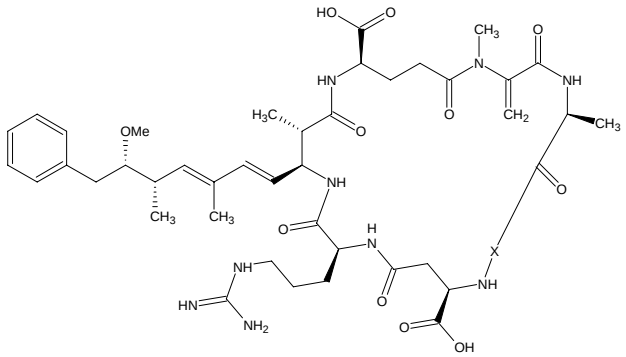
| Structure | Properties |
|---|---|
|  | Microcystins with properties hepatotoxic (36, 73) |
| X = L-Arg<br>[D-Asp3]Microcystin-RR<br><br>X = L-Tyr<br>[D-Asp3]Microcystin-YR | |
| 7-desmethylmicrocystin LR | Lacks an N-Me group of the dehydroalanine moiety of microcystin LR. Amino acid analyses yielded D-glutamic acid, D-erythro-β-methylaspartic acid and D-alanine in equimolar and L-arginine in two-fold molar ratios (39) |
| Microcystin WR<br>Desmethylated Microcystin LF<br>Desmethylated Microcystin LW<br>Microcystin LL<br>Microcystin AL | Microcystins with properties hepatotoxic (35) |
| 3,7-didesmethylmicrocystin LR | Microcystin with properties hepatotoxic (39) |

**TABLE 4(CONTINUED)**
**Microcystins and other peptides from *Microcystis aeruginosa***

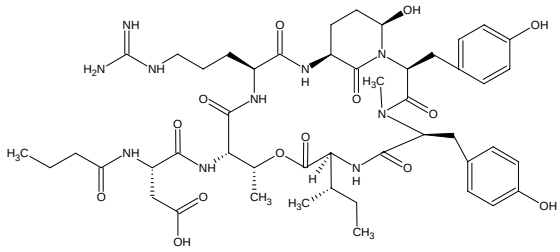
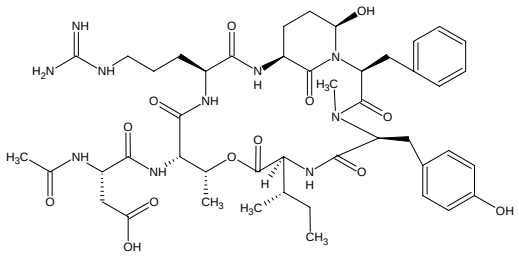
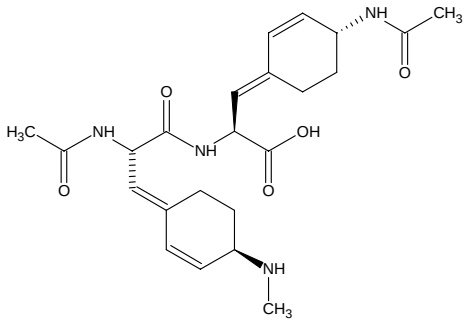
| Structure | Properties |
|---|---|
|  Micropeptin EI 992 | Trypsin inhibitors (91-92) |
|  Micropeptin EI 964 | Trypsin inhibitors (91-92) |
| Toxin P-1 | Cyclic peptide (41) |
| Toxin P-2 | Cyclic peptide (41) |
|  Radiosumin B | Showed a remarkable antiviral activity against influenza A virus (47) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

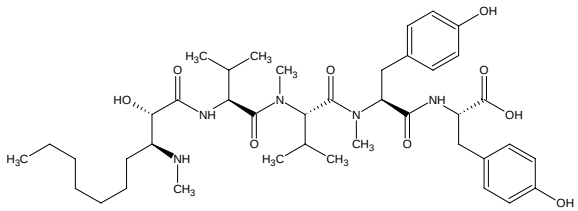
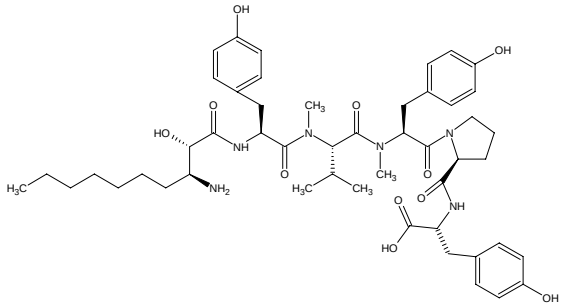
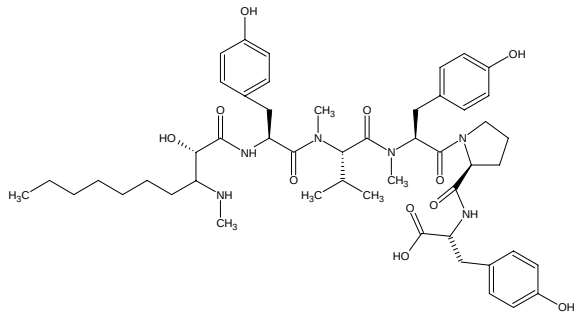
| Structure | Properties |
|---|---|
|  Microginin 478 | Inhibited aminopeptidase M and angiotensin-converting enzyme (88) |
|  Microginin 51-A | Inhibited aminopeptidase M and angiotensin-converting enzyme (88) |
|  Microginin 51-B | Inhibited aminopeptidase M and angiotensin-converting enzyme (88) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

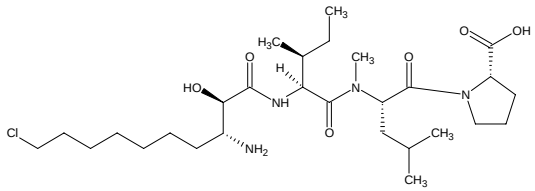
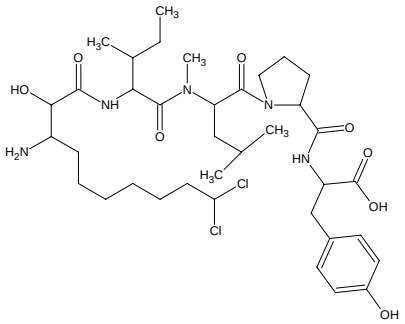
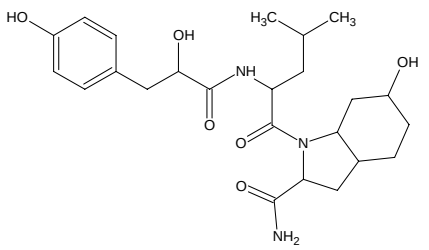
| Structure | Properties |
|---|---|
|  Microginin 91-A | Inhibited aminopeptidase M and angiotensin-converting enzyme (88) |
|  Microginin 91-E | Inhibited aminopeptidase M and angiotensin-converting enzyme (88) |
|  Aeruginosin EI 461 | Linear peptide (91-92) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

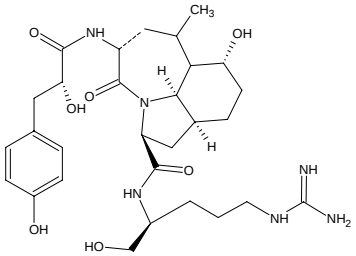
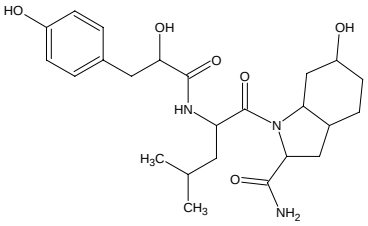
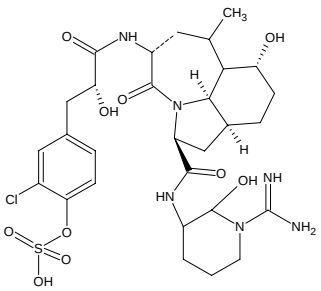
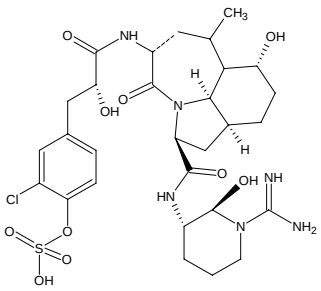
| Structure | Properties |
|---|---|
|  Aeruginosin 298-A | Protease inhibitor (46) |
|  Aeruginosin 298-B | Protease inhibitor (46) |
|  Aeruginosin 89-A | Protease inhibitor (46) |
|  Aeruginosin 89-B | Protease inhibitor (46) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

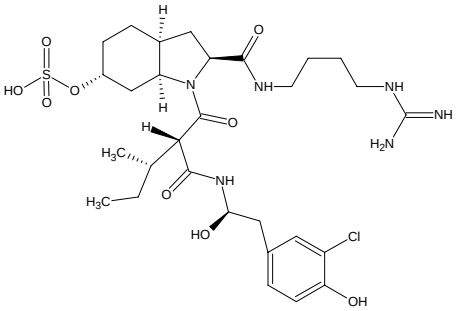
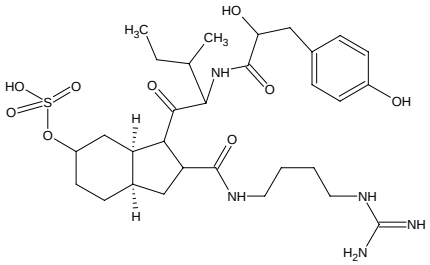
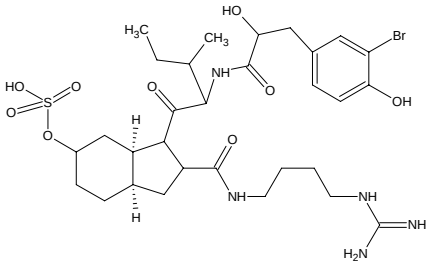
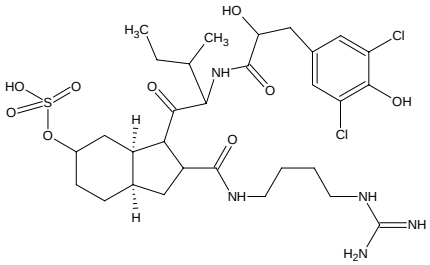
| Structure | Properties |
|---|---|
|  Aeruginosin 98-A | Protease inhibitor (46) |
|  Aeruginosin 98-B | Protease inhibitor (46) |
|  Aeruginosin 98-C | Protease inhibitor (46) |
|  Aeruginosin 101 | Protease inhibitor (46) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

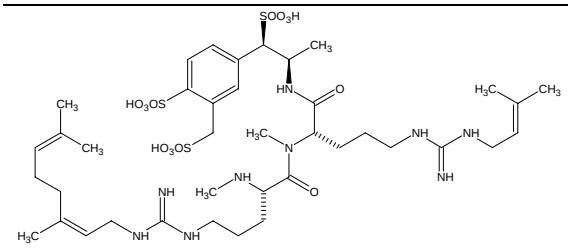
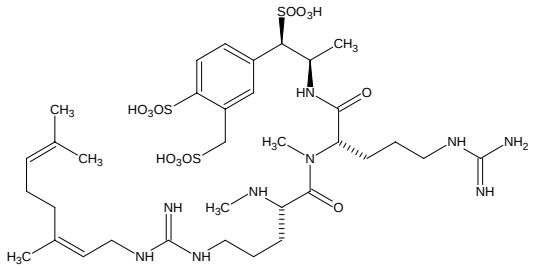
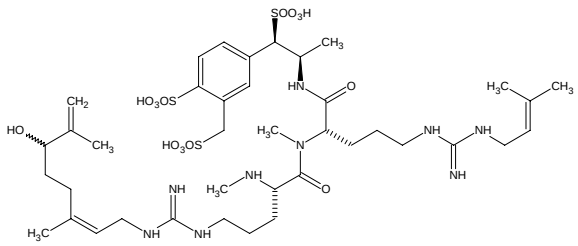
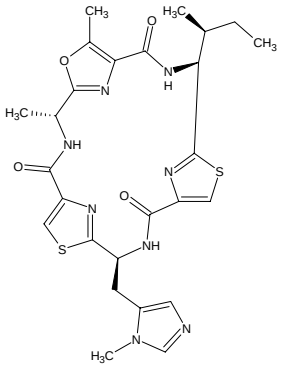
| Structure | Properties |
|---|---|
|  Aeruginoguanidines 98A | Showed moderate cytotoxicity against the P388 murine leukemia cells (45) |
|  Aeruginoguanidines 98B | Showed moderate cytotoxicity against the P388 murine leukemia cells (45) |
|  Aeruginoguanidines 98C | Showed moderate cytotoxicity against the P388 murine leukemia cells (45) |
|  Microcyclamide | Showed a cytotoxicity against the lymphocytic mouse leukemia and showed an anticyanobacterial activity against *Anabaena* sp. (86) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

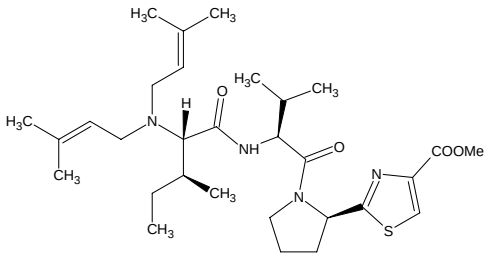
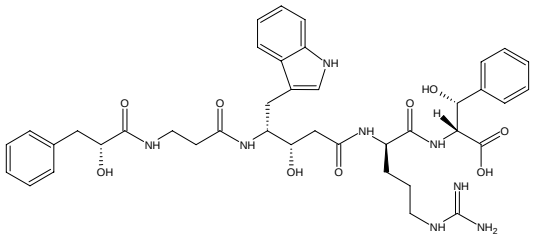
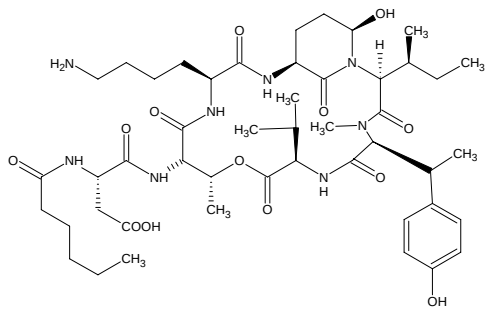
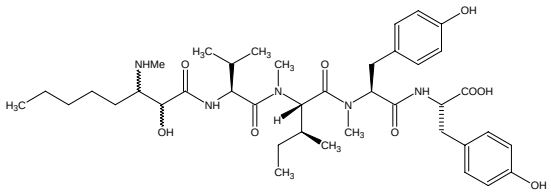
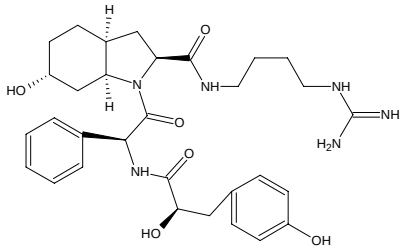
| Structure | Properties |
|---|---|
| Cyanopeptolin VW-1 | Non-toxic peptides (42) |
| Cyanopeptolin VW-2 | Non-toxic peptides (42) |
|  Aeruginosamide | Showed mild cytotoxicity to human ovarian tumor and leukemia cells (75) |
|  Kasumigamide | Kasumigamide, a antialgal tetrapeptide contaigning an N-terminal α-hydroxy acid, was isolated from *Microcystis aeruginosa* (NIES-87). This peptide showed an antialgal activity against the green alga *Chlamydomonas neglecta* (89) |
|  Micropeptin SD 944 | Serine-protease inhibitor (90) |

**TABLE 4 (CONTINUED)**
**Microcystins and other peptides *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
| Micropeptin SD 979 | Protease inhibitor (90) |
| Micropeptin SD 999 | Protease inhibitor (90) |
| Micropeptin SD 1002 | Serine-protease inhibitor (90) |
|  Microginin SD 755 | Inhibit amino-proteases (90) |
|  Microcin SF 608 | Serine-protease inhibitor (90) |

Cytochrome c550 contains 135 amino acid residues with the Cys-X-X-Cys-His (where X = a amino acid) heme-binding site at residues 37-41. The sequence from residue 28 to 45 shows similarity to cytochrome c553 residues 1-18 when the heme-binding sites are aligned. Another region of similarity is in the C-terminal regions of these 2 proteins. The 2 aligning regions of cytochrome c553 correspond to helical segments in other related cytochromes. The single methionine residue in cytochrome c550 of *M. aeruginosa* occurs at position 119 but there is no methionine in this region in the *A. flos-aquae* cytochrome,

indicating that methionine is not the 6th ligand to the heme Fe. Histidine 92 is a possible 6th ligand in M. aeruginosa cytochrome c550. The far-UV CD spectrum indicates that this protein is approximated of 17% α-helix, 42% β-pleated sheet, and 41% random coil [53].

Cytochrome c553 has been purified from 5 cyanobacteria, and the structures of the ferrocytochromes have been studied by 1H-NMR spectroscopy. 1H-NMR spectra were used to assign in the spectrum of the aphanizomenon flos-aquae protein 18 resonances to specific amino acid residues and 12 resonances to specific heme protons. The spectrums indicate that a tyrosine and methionine are located near pyrrole ring IV of the heme and that a phenylalanine ring is near the heme α-mesoproton. The general folding of the cytochrome c553 protein backbone appears to resemble that of *Pseudomonas aeruginosa* cytochrome c553, but the chirality of the cytochrome c553 axial methine S atom is R, the same as that of horse heart cytochrome c [54].

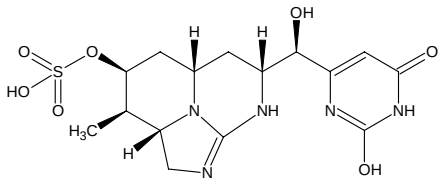
Cytochrome c553 is an electron donor to P700 in the photosynthetic electron transfer chain of cyanobacteria and eukaryotic algae. Cyanobacteria and algae also contain cytochrome c550 (Mr 15,000) which is quite different from cytochrome c553 (Mr 10,500) [55].

Polyamines have been determined in a variety of cyanobacteria including N fixing and nonfixing species. All cyanobacteria capable of fixing N contained sym-homospermidine as the major polyamine. The concentration of putrescine, spermidine, and spermine has been extremely low in these cyanobacteria. The cyanobacteria which normally fail to fix N contained spermidine as the major polyamine, whereas the sym-homospermidine content has been very low or under the limits of detection. Apparently there is a close relation between the sym-homospermidine content and the ability to fix N in cyanobacteria [56].

Easily solubilized carotenoid-containing proteins have been found in aquous extracts from 3 genera of cyanobacteria. The 3 proteins were purified, and the absorption spectra were virtually identical with absorption maxime at 495 and 465 nm. During the purification, the orange protein spontaneously changed to a red protein with a single, broad absorption maxima at 505 nm. The orange protein showed a molecular weight of 47,000 on gel filtration, whereas that of the red protein was 26,700. SDS-polyacrylamide gel electrophoresis indicated a single polypeptide of molecular weight 16,000 in both the red

and orange forms, but this method removed the chromophore from the proteins. The main carotenoid component of the complex has been 3'-hydroxy-4-keto-β,β-carotenoid or 3'-hydroxyechinenone. The number of carotenoid mols/mol of orange protein of molecular weight 47,000 was 20-40. The stoichiometry of carotenoid/protein seemed reasonably constant [57]. Nitrogen compounds are shown in Table 5.

**TABLE 5**
**Nitrogen compounds *Microcystis aeruginosa***

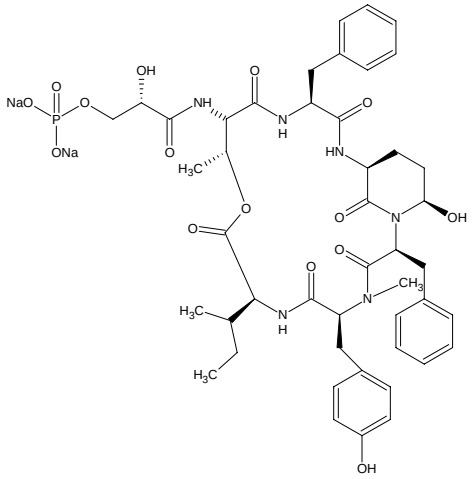
| Structure | Properties |
|---|---|
| Contained alanine, aspartic acid, arginine, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, proline, serine and glycine, threonine, tryptophan, tyrosine and valine. | Glutamic acid, threonine, serine and glycine are quantity predominant (48) |
| cAMP | Cellular cAMP (92-394 pmol/g) and extracellular cAMP (8-440 pmol/L) varied greatly among species (49) |
| Cytochrome c550, cytochrome f, Cytochrome c6, and cytochrome c553 | Are soluble hemoprotein that serves as a photosynthetic electron transport component in cyanobacteria and algae, carrying electrons from the cytochrome bf complex to photosystem I (50, 52, 53,55) |
| Plastocyanins | Plastocyanins, each containing between 97 and 104 amino acids. Involved in electron transport between photosystems II and I in higher plants and algae (51) |
| Polyamines | All cyanobacteria capable of fixing N contained sym-homospermidine as the major polyamine (57) |
| Carotenoid-containing proteins | The main carotenoid component of the complex was 3'-hydroxy-4-keto-β,β-carotenoid or 3'-hydroxyechinenone. The number of carotenoid mols /mol of orange protein of mol. wt. 47,000 was 20-40 (57) |
|  Cylindrospermopsin | Hepatotoxic alkaloid (48) |

**Organic phosphorus**

Inositol polyphosphate esters comprised a major fraction of the residual organic phosphorus in the extracts of 3 macrophyte species (*Myriophyllum, Valisneria,* and *Ceratophyllum*), an aquatic angiosperm (Lemna), and a blue-green alga (*Microcystis aeruginosa*), and may represent the largest distinct class of acid-resistant organic phosphorus compounds in aquatic plants. The inositol di-through tetraphosphate esters have been present in higher concentrations than the penta- plus hexaphosphate esters. Such enrichment of the lower phosphate esters in the plant extract is quite similar to the lower ester enrichment reported in lake sediments [58].

Micropeptin T-20, a glyceric acid 3-O-phosphate and 3-amino-6-hydroxy-2-piperidone-containg cyclic depsipeptide, has been isolated from a cyanobacterium *Microcystis aeruginosa*. Micropeptin T-20 inhibited chymotrypsin [59]. Table 6 shows the organic phosphorus obtained from *M. aeruginosa.*

**TABLE 6**
**Organic phosphorus from *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
| Inositol polyphosphate esters | The inositol di-through tetraphosphate esters were present in higher concentrations than were the penta- plus hexaphosphate esters (58) |
|  Micropeptin T-20 | Inhibited chymotrypsin (59) |

**Pigments**

The specific photosynthetic rate (µg C fixed/µg of chlorophyll a/h) was a good measure of the physiologic state of *Microcystis aeruginosa* because this quantity increased just before each population increase and decreased before algal densities diminished. Although some evidence of enhanced utilization of low light levels was found in the period from July to October, when high algal densities attenuated incoming radiation, this was not due to increasing chlorophyll and phycocyanin contents. There has been a decrease in the phycocyanin content of the algae during this period, perhaps related to the availability of inorganic nitrogen [60].

*Microcystis aeruginosa* by comparative determinations showed that the spectrophotometric methods overestimate chlorophyll a and pheophytin a [61]. Also Phycocyanin, phycobilin and allophycocyanin have been isolated from the cyanobacterium *Microcystis aeruginosa* [62]. Pigments are given in Table 7.

**Sulfur compounds**

Volatile organic sulfur compounds produced by *Microcystis* isolated from inland waters of Japan were identified. Compounds with an unpleasant smell were detected that came from 7 strains of *Microcystis aeruginosa* and 3 strains of *M. wesenbergii*. Iso-PrSH was detected in all strains and iso-$Pr_2S_2$in 5 strains. Me isothiocyanate, iso-Pr Me sulfide, and iso-Pr Me disulfide were also found in some strains. Iso-PrSH and Iso-$Pr_2S_2$ were decompose by chlorination, with the formation of iso-Pr sulfonyl chloride. Iso-Pr sulfonyl chloride exhibited mutagenic activity for strain TA 98 in the presence of S9 mix and for strain TA 100 with and without the S9 mixed. Some chlorinated algal cultures showed mutagenic activity with strains TA 98 and TA 100, with and without the S9 mix [63].

Odorous sulfur compounds produced in decaying blue-green algal cultures and reservoir waters containing blue-green algal blooms included MeSH, $Me_2S$, iso-BuSH, and BuSH [64]. Table 8 shows the sulfur compounds isolated from *M. aeruginosa.*

**TABLE 7**
**Pigments from *Microcystis aeruginosa***

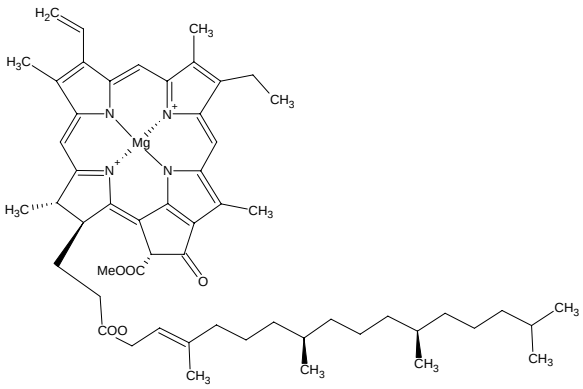
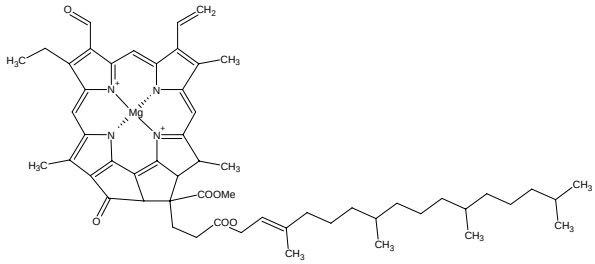
| Structure | Properties |
|---|---|
|  Chlorophyll a | This quantity increased just before each population increase and decreased before algal densities diminished (61) |
|  Chlorophyll b | This quantity increased just before each population increase and decreased before algal densities diminished (61) |
| Phycocyanin | Was isolated from the cyanobacterium (62) |
| Pheophytin a | Was isolated from the cyanobacterium (62) |
| Phycobilin | Was isolated from the cyanobacterium (62) |
| Allophycocyanin | Was isolated from the cyanobacterium (62) |

**TABLE 8**
**Sulfur compounds from *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
| Iso-PrSH | Volatile organic sulfur compounds (63) |
| Iso-$Pr_2S_2$ | Volatile organic sulfur compounds (63) |
| Iso-Pr Me sulfide | Volatile organic sulfur compounds (63) |
| Iso-Pr sulfonyl chloride | Volatile organic sulfur compounds (63) |
| MeSH | Odorous sulfur compounds (64) |
| $Me_2S$ | Odorous sulfur compounds (64) |
| iso-BuSH | Odorous sulfur compounds (64) |
| BuSH | Odorous sulfur compounds (64) |

**Miscellaneous compounds**

Ferredoxins of eukaryotic algae and higher plants and is dissimilar to those of the green and purple photosynthetic bacteria [65]. Ferredoxins from plant sources contain 2 Fe/molecule plus up to 6 cystein residues.

The compounds okadaic acid, calyculin A and tautomycin, found in the marine sponges *Halichondria okadai* and *Discoderma calyx* also has been isolated from blue-green alga *M. aeruginosa.* Shows cytotoxic properties against human epidermoid carcinoma. While okadaic acid was a more effective inhibitor of protein phosphatase 2A ($IC_{50}$, 0.07 nM) than protein phosphatase 1 ($IC_{50}$, 3.4 nM), other compounds of the okadaic acid class have been equally effective against the two protein serine/threonine phosphatases. The order of potency has been microcystin > calyculin A > tautomycin, and the $IC_{50}$ ranged from 0.1 to 0.7 nM. None of the okadaic acid class compounds inhibited protein tyrosine phosphatase 1 activity at concentrations up to 0.01 mM. These results indicate that the compounds of the okadaic acid class are selective inhibitors of protein serine/threonine but not tyrosine phosphatases [66].

A β-carotene oxygenase is described which occurs in the *cyanobacterium Microcystis*. It cleaves β -carotene and zeaxanthin specifically at the positions 7-8 and 7'-8'; echinenone and myxoxanthophyll are not affected. The oxidative cleavage of β-carotene leads to the formation of β -cyclocitral and crocetindial and that of zeaxanthin to hydroxy-β-cyclocitral and crocetindial in nearly stoichiometric amounts. The oxidant is O, as demonstrated by a high incorporation (86%) of $^{18}O$ into β -cyclocitral [7]. Miscellaneous compounds are shown in the Table 9.

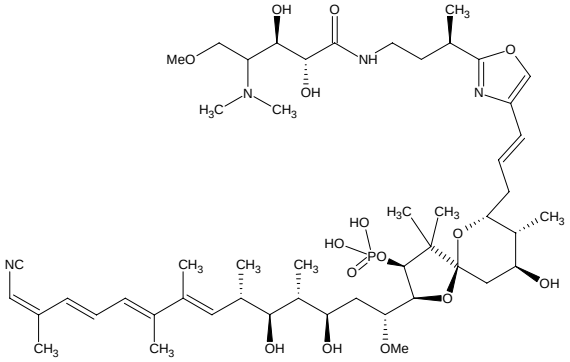
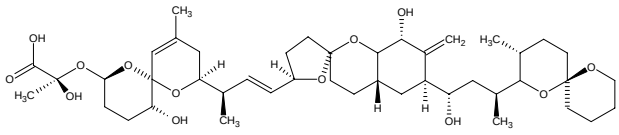
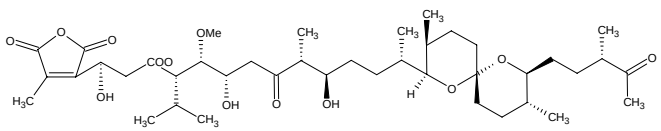
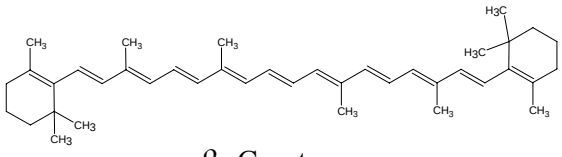
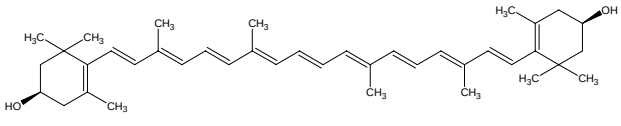
**Biological studies**

Experimental pharmacology

Microcystin LR (MCYST-LR) is a naturally occurring protein phosphatase inhibitor and potent hepatotoxin produced by strains of *Microcystis aeruginosa*. Histologic evidence of dose-dependent hepatic inflammation was seen, including infiltration of centrilobular regions by lymphocytes, macrophages, and neutrophils, centrilobular fibrosis, apoptosis, and steatosis. Analysis of lipid peroxidation products revealed a dose-dependent increase in malondialdehyde concentrations with an approximate 4-fold increase in the livers of the high-dose rats over those of the saline-treated controls. Livers from MCYST -exposed rats were more sensitive than those of controls to the cytotoxic effects of the organic oxidizing agent tert-butyl hydroperoxide, based on an MTT (3-[dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) viability assay. These histopathologic and biochemical findings indicate that oxidative stress may play a significant role in the pathogenesis of chronic MCYST toxicosis [67].

Cross-bred, anesthetized female swine were given intravascularly a lethal (72 μg/kg) or toxic-sublethal (25 μg/kg) dose of microcystin-LR (MCYST-LR), from *Microcystis aeruginosa*, or the vehicle. At the high dose, from 12 to 18 min after administration, central venous pressure and hepatic perfusion were significantly lower, and shortly thereafter, portal venous pressure was significantly higher and aortic mean pressure was significantly lower than controls.

**TABLE 9**
**Miscellaneous from** *Microcystis aeruginosa*

| Structure | Properties |
|---|---|
| Ferredoxins | Contain 2 Fe/molecule plus up to 6 cystein residues (65) |
|  Calyculin A | Selective inhibitors of protein serine/threonine but not tyrosine phosphatases (93) |
|  Okadaic acid | Effective inhibitor of protein phosphatase 2A ($IC_{50}$, 0.07 nM), (66) |
|  Tautomycin | Selective inhibitors of protein serine/threonine but not tyrosine phosphatases (66) |
|  β -Carotene | The oxidative cleavage of β-carotene leads to the formation of β-cyclocitral and crocetindial (7) |
|  Zeaxanthin | The oxidative cleavage of zeaxanthin leads to the formation of hydroxy-β -cyclocitral and crocetindial (7) |

By 45 min postdosing, serum bile acids, lactate, potassium, and total bilirubin, as well as blood $pO_2$, were significantly higher, while hematocrit, platelet count, and blood bicarbonate, $pCO_2$, and base excess were significantly lower than controls. By 90 min, serum arginase, urea nitrogen, inorganic phosphorus, and creatinine were significantly higher, while glucose and blood pH were significantly lower than in controls [68].

[D-Leu1]Microcystin-LR has been isolated from a hepatotoxic *Microcystis* bloom from brackish waters in the Patos Lagoon estuary, southern Brazil. Toxicity of [D-Leu1]Microcystin-LR according to bioassay and protein phosphatase inhibition assay, was similar to that of the commonly occurring microcystin-LR [69].

Microcystin (cyanoginosin)-LR and -LA are more toxic than microcystin-LY and -RR in adult mice. They induce different degrees of thrombocytopenia and leukopenia, and the lethalities of their binary and ternary mixtures are additive. Postnatal mice are resistant to doses of microcystin-LR that are lethal to adults but they are susceptible to higher doses. Substitution of a single L-amino acid for another in a microcystin markedly affects the dosimetric potency, but not the pathophysiology of its toxicity [70].

The cyclic heptapeptide microcystin LR induces rapid and characteristic deformation of isolated rat hepatocytes. The onset of blebbing has been accompanied neither by alteration in intracellular thiol and $Ca^{2+}$ homeostasis nor by ATP depletion. The irreversible effects were insensitive to protease and phospholipase inhibitors and also to thiol-reducing agents, excluding the involvement of enhanced proteolysis, phospholipid hydrolysis, and thiol modification in microcystin-induced blebbing. In contrast, the cell shape changes have been associated with a remarkable reorganization of microfilaments as visualized both by electron microscopy and by fluorescent staining of actin with rhodamine-conjugated phalloidin. The morphologic effects and the microfilament reorganization have been specific for microcystin LR and could not be induced by the microfilament-modifying drugs, cytochalasin D or phalloidin. Using inhibition of DNase microcystin LR as an assay for monomeric actin, microcystin LR-induced reorganization of hepatocyte microfilaments was not due to actin polymerization. On the basis of the rapid and microfilament reorganization and the specificity of the effects, it is suggested that microcystin LR constitutes a novel microfilament-perturbing drug with features that are clearly different from those of cytochalasin D and phalloidin [71].

The cyclic heptapeptide (mol. wt. 994) termed microcystin-LR (also known as cyanoginosin-LR). In time course histopathology studies with mice, significant liver damage, with an absence of pulmonary emboli, were observed after 15 min. Pulmonary emboli did not appear until 1 h. In rats, significant liver damage and the presence of occasional emboli were observed at 20 min. Measurements of rat femoral arterial, jugular venous, and hepatic portal venous blood pressures during the course of toxicity revealed a slowly declining arterial pressure and stable, normal venous pressures. In the mouse and rat, microcystin-LR is a potent, rapid-acting, direct hepatotoxin, with the immediate cause of death in acute toxicities being hemorrhagic shock secondary to massive hepatocellular necrosis and collapse of hepatic parenchyma [72].

The $LD_{50}$ value (i.p. mouse) of [Dha7]microcystin-RR, has been 180 µg/kg. The 48 h lethal concentration (48-h-$LC_{50}$) of the toxin for larvae of the yellow fever mosquito, *Aedes aegypti*, was 14.9 µg/mL [73].

On strain of *M. aeruginosa* contained a high amount cyclic peptide toxins as microcystin (cyanoginosin) YR and a lesser amount of LR. Three toxins, microcystin-RR, -YR and -LR, were detected in two strains of *M. aeruginosa* and four of *M. viridis*. The main component of the toxins of these strains has been microcystin-RR. $LD_{50}$ values of the purified toxins of YR and LR were similar, while a lower toxicity was estimated for RR. This explains the relatively weak toxicity of *M. viridis* whose main component is microcystin-RR [74].

Aeruginosamide a peptide isolated from a bloom of *M. aeruginosa* showed mild cytotoxicity to human ovarian tumor and leukemia cells [75].

A Microcystin-LR peptide which on hydrolysis has been shown to consist of equimolar amounts of L-methionine, L-tyrosine, D-alanine, D-glutamic acid, erythro-β-Me aspartic acid and methylamine has been isolated from a bloom of *M. aeruginosa*. The peptide was toxic to mice, rats and sheep when administered orally or i.p. (LD50 in mice = 0.056 mg/kg, i.p.). The liver has been the target organ, by electron microscopy changes could 1st be observed 15 min after injection, death, which followed within 1-3 h, has been due to the massive pooling of blood in the liver, following destruction of the sinusoids. Repeated inoculations of mice with sublethal doses of the peptide led to hepatocyte necrosis. *In vitro*, the purified toxin had no hemagglutination activity and no specific effect on major metabolic functions.

Incubation of freshly isolated hepatocytes with the peptide toxin caused the cells to lose their spherical shape and to become deformed; characteristic protrusions on the surface of the cells could be seen by light as well as by electron microscopy. The deformation was 1st seen 5 min after addition of toxin to hepatocytes, and it increased with time. The response was also dose dependent; 30 ng/mL was sufficient to cause the deformation of half the cells. The affected hepatocytes did not release aspartate aminotransferase into the suspension medium, nor did they show increased trypan blue uptake or cell lysis. This rapid, *in vitro* effect will facilitate the study of the mechanism of action of the peptide toxin from *M. aeruginosa* [76]. Parenteral administration of the purified toxin into mice produced extensive liver lobular hemorrhage and death within 1-3 h. Repeated inoculation of sublethal doses daily over some weeks produced progressive hepatocyte degeneration and necrosis and the development of fine hepatic fibrosis [77]. Also induced thrombocytopenia, pulmonary thrombi, and hepatic congestion. The lethality of the toxin has been unaffected by several anticoagulants. The acute liver damage that follows injection of the toxin has been attributed to direct action on liver cells, but may be due to hypoxemia, heart failure, and shock [78].

Whereas sheep treated intraluminally with 990-1040 or 1040-1840 mg *M. aeruginosa*/kg showed changes in hematology or serum biochemical parameters. The serum enzyme changes in the poisoned sheep suggest liver damage in the sheep. The marked decrease in serum glucose in lethally poisoning sheep is probably associated with the failure of hepatic gluconeogenesis to meet tissue glucose demand. Hepatic insufficiency rather the purely circulatory dysfunction may be responsible for the death of *M. aeruginosa*-poisoned sheep [79].

A diarrhea-producing toxin from a blue-green alga, *M. aeruginosa* Kuetzing, has been obtained from standing laboratory cultures. The nondialyzable fraction of the lysate from whole cells produced fluid accumulation in the ligated small intestinal loops in guinea pigs [80].

Laboratory cultures of a toxic strain of *M. aeruginosa* (WR 70) have been supplemented by various concentrations of agents known either to eliminate plasmids (acridine Orange) or to select for plasmid-free cells (Na dodecyl sulfate) in bacteria. Toxicity of the cultures was monitored by i.p. injection of disrupted cells into mice. Cultures of toxic *M.*

*aeruginosa* became nontoxic after growth in suitable concentratios of acridine Orange, streptomycin, Na dodecyl sulfate, and chloramphenicol. These results indicate a possibility of plasmid involvement in the toxicity of *M. aeruginosa* (WR 70) [81].

When the peptide hepatotoxin was added to hepatocyte suspensions it produced deformation of the cells, as shown by scanning electron microscopy. This has been apparent within 5 min of addition of toxin to the cells and the response has been dose dependent: 30 ng of toxin was sufficient to cause deformation in 58 +/- 9% of 1.4 x $10^6$ hepatocytes/ml of incubation. The deformation did not lead to cell death as measured by Trypan blue uptake within 120 min. Deoxycholate, cholate bromosulphophthalein, and rifampicin were found to prevent the deformation of hepatocytes by *Microcystis aeruginosa* toxin in a dose dependent manner, analogous to the effect of these agents on the response of hepatocytes to added phalloidin. This suggests that *Microcystis aeruginosa* toxin is transported into hepatocytes in the same way as phalloidin; namely sharing a transport system for bile acids on the hepatocyte plasma membrane [82]. The effects of the cyclic peptide toxin, on erythrocytes and fibroblasts, the toxin caused no morphologic alterations. In hepatocytes, the toxin induced marked morphologic alterations at a concentration of approximated 50 nM. In erythrocytes and fibroblasts, no effects on ion transport were observed. In hepatocytes, the toxin induced a significant increase in both phosphate and K efflux at concentrations far below the concentration causing morphologic alterations (0.1 and 1 nM, resp.). Apparently, the cytotoxicity of the toxin is not due to a nonspecific interaction with the plasma membrane; the effects of the toxin in hepatocytes are probably due to an interaction of the toxin with cytoskeletal elements [83, 84].

Three cyclic heptapeptide toxins (MCYST-RR, -RA, -FR) showed a $LD_{50}$ in the rat and mouse of approximately 50, 500 and 1000 micrograms/kg, respectively. Hepatic insult of the toxins at concentrations of 0.5-4.0 times the rat i.p. lethal dose were assessed by monitoring bile flow, accumulation of total protein in the perfusate, release of intracellular enzymes and histopathologic examination of perfused liver tissue. One hundred micrograms of microcystis RR toxin produced cessation of bile flow during a 1 hr perfusion period. Hepatic cell membranes remained intact during the perfusion since release of enzymes and proteins into the perfusate has been similar for toxin treated and control livers, and histopathologic examination of Trypan Blue infused livers revealed

exclusion of the dye from the intracellular compartment of the parenchyma. Histopathologic findings for all three toxins showed hepatocellular disassociation that increased with toxin concentration. At the ultrastructural level, all three toxins caused dose-dependent vesiculation of rough endoplasmic reticulum, formation of concentric whorls composed of rough-ER, mitochondrial swelling, large cytoplasmic vacuoles and altered bile canaliculi [85].

Microcyclamide, a cytotoxic cyclic hexapeptide, has been isolated from the cultured cyanobacterium *Microcystis aeruginosa* (NIES-298). This peptide showed a moderate cytotoxicity against P388 murine leukemia cells [86]. The absolute configuration of microcyclamide possessing Tzl-amino acids has been determined by the advanced Marfey's method combined with flash hydrolysis. At 13.7 and 26.6 μg/mL ($IC_{50}$). Microcyclamide showed a cytotoxicity against the lymphocytic mouse leukemia and showed an anticyanobacterial activity against *Anabaena* sp. [87].

Eight new linear peptides, microginins 478, 51-A, 51-B, and 91-A to 91-E, congeners of microginin, have been isolated from *M. aeruginosa*. These peptides inhibited aminopeptidase M (I, 51-A, and 91-C, D, and E) and angiotensin-converting enzyme [88].

Kasumigamide, a novel antialgal tetrapeptide contaigning an N-terminal α-hydroxy acid, has been isolated from the freshwater cyanobacterium *Microcystis aeruginosa* (NIES-87). This peptide showed an antialgal activity against the green alga *Chlamydomonas neglecta* (NIES-439) [89].

Five protease inhibitors, micropeptins SD944, SD979, SD999 and SD1002 and microginin SD755 have been isolated along with two known inhibitors, micropeptin SD1002 and microcin SF608, from the hydrophilic extract of *Microcystis aeruginosa*. Compounds SD944, SD1002, and SF608 are serine-protease inhibitors while compound SD755 was found to inhibit amino-proteases [90].

Two new trypsin inhibitors, micropeptins EI992 and EI964 and a modified linear peptide aeruginosin EI461 have been isolated from the hydrophilic extract of two samples of *Microcystis aeruginosa*, collected from the Einan Reservoir in Israel. Aeruginosin EI461 differs from the 14 known aeruginosins in the relative and absolute stereochemical of the Choi-6-hydroxyl substituent [91-92].

The analysis of methanolic extracts of cultured strains of genus *Microcystis* ranged

between 20.0 µg to 79.0 µg revealed a remarkable antiviral activity against influenza A virus. The observed antiviral activity has been associated with protease inhibitory activity of approximately 90% and suggests that protease inhibitory activity may be responsible for reducing virus replication. These results show that cyanobacteria are able to produce compounds with biological activity that may be of potential clinical interest [93].

When tested Microcystin-LR against certain green algae, cyanobacteria, heterotrophic bacteria and fungi, the toxin inhibited growth of only green algae and cyanobacteria. Purified toxin at a concentration of 50 µg/ ml caused complete inhibition of growth followed by cell lysis in Nostoc muscorum and Anabaena BT1 after 6 days of toxin addition. Addition of toxin (25 µg/ml) to the culture suspensions of the *Nostoc* and *Anabaena* strains caused instant and drastic loss of $O_2$ evolution. Furthermore a marked reduction (about 87%) in the $^{14}CO_2$ uptake was also observed at a concentration. of 50 µg/ ml. Besides its inhibitory effects on photosynthetic processes, *M. aeruginosa* toxin (50 µg/ ml) also caused 90% loss of nitrogenase activity after 8 h of its addition. These results demonstrate that the toxin is strongly algicidal and point to the possibility that it may have an important role in establishment and maintenance of toxic blooms of *M. aeruginosa* in freshwater ecosystems [94].

**Compound used by control of *Microcystis aeruginosa***

Copper sulfate at 1.5 ppm completely controlled *Microcystis* species in ponds, without affecting the other algae [95]. Toxicity trials conducted with the algicide Algistat (active ingredient 2,3-dichloro-1,4-naphthoquinone) indicated that a dose of 0.66 ppm has been highly toxic to fish and 0.5 ppm was the general lethal level for blue-green algae, *Oscillatoria, Microcystis,* and *Anabaena* [37]. The toxicities of KMnO4 and $CuSO_4$.5H2O for prevent the growth of 8 algae species (*Microcystis aeruginosa*, *Anabaena circinalis*, *Gloeotrichia echinulata*, *Oscillatoria rubescens*, *O. chalybia*, *Hydrodictyon reticulatum*, *Dictyosphaerium pulchellum*, and the *diatom genus* (Gomphonema) the concentration required to kill the algae with a 4-, 12-, 24-, 48-, or 72-hr. is treatment with 1-5 ppm. KMnO4 has been about as effective against all 8 algae species after 4 hrs of treatment as after 72 [96].

The effects of different concentrations of panacide on bloom-forming noxious algae

have been compared with the relative amounts required to prevent the growth of algae and the optimum concentrations and treatment times for killing algae. Concentrations of 0.1 to 10 ppm have been algistatic. The algicidal concentrations with 3-h treatment ranged between 10 and 50 ppm. The same effect could also be achieved by prolonging the treatment time to 6 h and reducing the dosages of panacide [97].

Cutrine at 1-3 ppm caused 100% kill of fishery waters algae (*Microcystis aeruginosa, Anabaena spiroides, Peridinium inonspirum,* and *Spirogyra species*) without poisoning fish even at concentratios of 10 ppm [98].

The aquous extract of the fruits of *Acacia nilotica* showed algicidal activity against species of: *Rivularia, Spirogyra, Oscillatoria, Pediastrum, Coelastrum, Spirulina, Chroococcus, Microcystis, Cyclotella, Euglena, Cosmarium,* and *Closterium*. Due to the high content of tannins in the fruits (18-23%), the algicidal properties of this plant may well due to these compounds [99].

Bayluscide at 0.032 and 0.32 ppm activated cell division in *Scenedesmus opaliensis* and *Coelastrum* microporum, at 0.056, 0.1, and 0.18 ppm caused deformation and disintegration of cell content in *S. opaliensis* and at 1.0 ppm caused deformation and disintegration of cell content in *C. microporum.* The morphologic effects of bayluscide on *M. flos-aquae* and *O. amphibia* has been noted at 0.56 and 1.8 ppm, response. Hg exerted morphologic effects at 0.0056 ppm in *S. opaliensis* and at 0.018 ppm in the other algae. Fe toxicity has been noted at 1.8 ppm in *C. microporum* and at 10 ppm in the other algae. Also affected *Microcystis aeruginosa* at 5.6 ppm and the other algae at 18 ppm [100].

Algimycin-400 or Algimycin-400 E at 1 mg/L, or the Cu triethanolamine chelates swimfree and swimetrine at 3 and 6 µg/L, response, totally inhibited the growth of *Chlorella pyrenoidosa* in the laboratory, but were less active against *Phormidium inumdatum*. Algimycin-400, swimfree, and swimtrine (2-6 mg/L) prevented the growth of *Coccochloris* and diatoms. Algimycin-400 has been the most active against mustard algae. Algicidal activity against *C. pyrenoidosa* and *P. inundatum* has been shown by algimycin-400 and algimycin 400 E only. The Cu-containing algicides CuSO4, algimycin PL5-C, Mariner A, and cutrine plus were extremely active against the planktonic blue-green algae *Oscillatoria rubescens*, *Microcystis aeruginosa*, and *Gloeotrichia echinulata* [101, 102].

Threshold toxicities under laboratory conditions for *Ankistrodesmus sp., Raphidiopsis sp.,* and *Microcystis sp.* have been between 0.2 and 0.3, 0.0 and 0.1, and 0.0 and 0.05 mM, response $H_2O_2$ concentrations of 0.5, 0.2, and 0.05 mM reduced the optical densities of chlorophyll extracts to ≤5% of the controls for *Ankistrodesmus, Raphidiopsis,* and *Microcystis,* response [103].

*Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae.* The inhibitors have been identified as eugeniin, 1-desgalloyleugeniin, a mixture of epicatechin 3-gallate and catechin 3-gallate, gallic acid, quercetin, quercitrin, and avicularin [104].

Spiroidesin, a D-amino acid-containning linear lipopeptide, has been isolated from water blooms of A. spiroides. Spiroidesin inhibited cell growth of the toxic cyanobacterium *M. aeruginosa* ($IC_{50}$, 1.6 X $10^{-6}$ M), [105].

The anticyanobacterial compound (Activity *against Microcystis). Sphingomonas* sp. produces argimicin A, a novel pentapeptide exhibiting high algicidal activity against *Microcystis aeruginosa* [106]. Table 10 shows the compounds used to control *M. aeruginosa.*

**Environmental toxicology**

Suspended algae, or phytoplankton, are the prime source of organic matter supporting food webs in freshwater ecosystems [107]. Phytoplankton productivity is reliant on adequate nutrient supplies; however, increasing rates of nutrient supply, much of it manmade, fuels accelerating primary production or eutrophication. An obvious and problematic symptom of eutrophication is rapid growth and accumulations of phytoplankton, leading to discoloration of affected waters. These events are termed blooms. Blooms are a prime agent of water quality deterioration, including foul odors and tastes, deoxygenation of bottom waters (hypoxia and anoxia), toxicity, fish kills, and food web alterations. Toxins produced by blooms can adversely affect animal (including human) health in waters used for recreational and drinking purposes. Numerous freshwater genera within the diverse phyla comprising the phytoplankton are capable of forming blooms; however, the blue-green algae (or cyanobacteria) are the most notorious bloom formers.

**TABLE 10**
**Compounds used by control of *Microcystis aeruginosa***

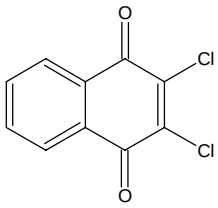
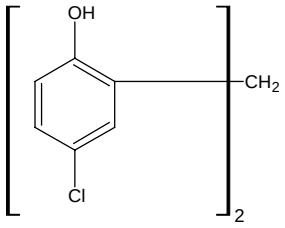
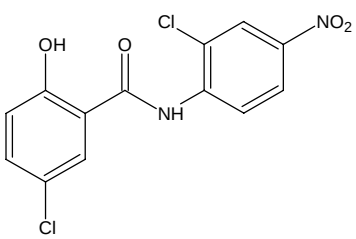
| Structure | Properties |
|---|---|
| $CuSO_4.5H_2O$<br>Copper sulfate | At 1.5 ppm completely controlled *Microcystis* species in ponds, without affecting the other algae (95,96) |
|  <br>Algistat | A dose of 0.66 ppm was highly toxic to fish and 0.5 ppm was the general lethal level for blue-green alga (98) |
| KMnO4<br>Potasium permanganate | Pevent the growth of some algae species (96) |
|  <br>Panacide | Concentrations of 0.1 to 10 ppm were algistatic (102) |
| Tannins | Tannins of the fruits of *Acacia nilotica* showed algicidal activity (99) |
|  <br>Bayluscide | Affected *Microcystis aeruginosa* at 5.6 ppm. (100) |

**TABLE 10 (CONTINUED)**
**Compounds used by control of *Microcystis aeruginosa***

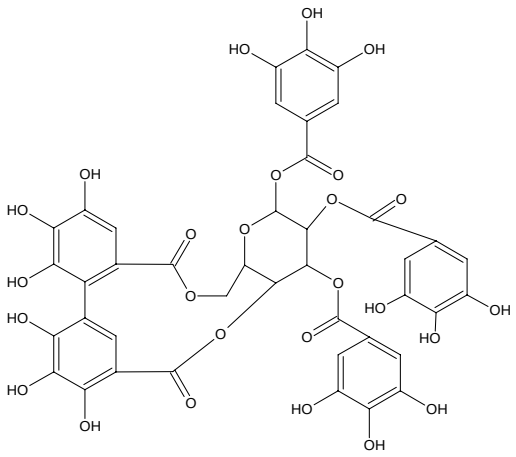
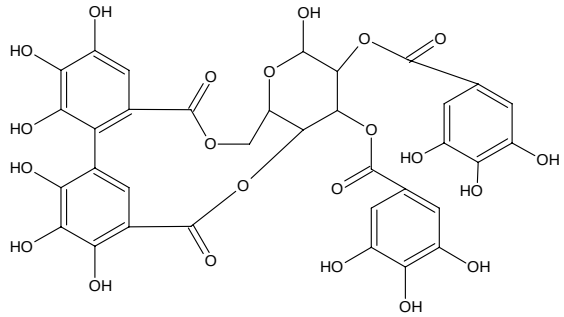
| Structure | Properties |
|---|---|
| Algimycin PL5-C<br>Algimycin-400<br>Algimycin-400 E | At 1 mg/L, totally inhibited the growth of some algae (101) |
| Swimfree, and Swimtrine | Extremely active against the planktonic blue-green (101) |
| Mariner A | Extremely active against the planktonic blue-green (101) |
| Cutrine | At 1-3 ppm caused 100% kill of waters algae (101) |
|  <br>Eugeniin | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  <br>1-desgalloyleugeniin | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |

**TABLE 10 (CONTINUED)**
**Compounds used by control of *Microcystis aeruginosa***

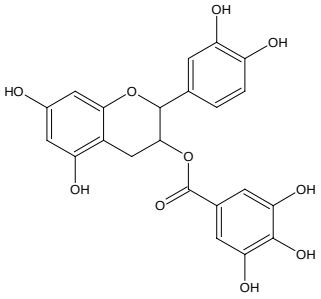
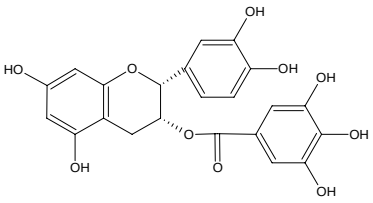
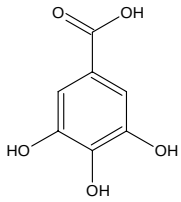
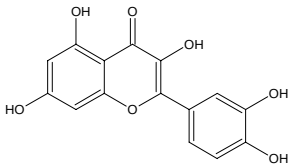
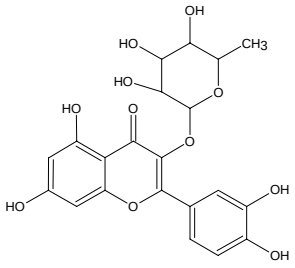
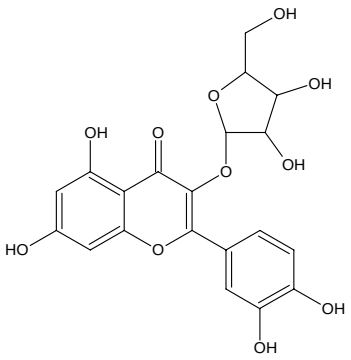
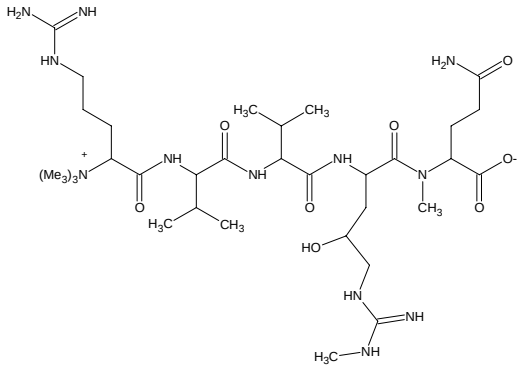
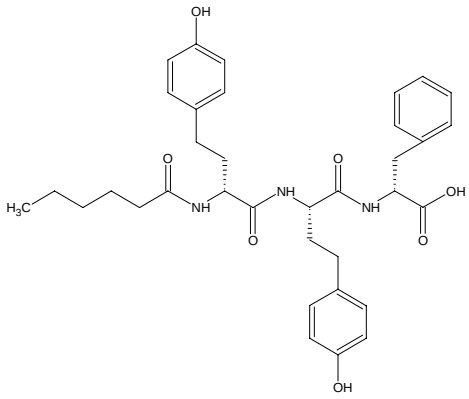
| Structure | Properties |
|---|---|
|  Epicatechin 3-gallate | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  Catechin 3-gallate | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  Gallic acid | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  Quercetin | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  Quercitrin | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |

**TABLE 10 (CONTINUED)**
**Compounds used by control of *Microcystis aeruginosa***

| Structure | Properties |
|---|---|
|  Avicularin | Inhibitor found in *Myriophyllum brasiliense* showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* and *Anabaena flos-aquae* (104) |
|  Argimicin A | Pentapeptide exhibiting high algicidal activity against *Microcystis aeruginosa* (106) |
|  Spiroidesin | Spiroidesin inhibited cell growth of the toxic cyanobacterium *M. aeruginosa* (105) |

This is especially true for harmful toxic, surface-dwelling, scum-forming genera (*Anabaena, Aphanizomenon, Nodularia, Microcystis)* and some subsurface bloom-formers (*Cylindrospermopsis*, *Oscillatoria*) that are adept at exploiting nutrient-enriched conditions. They thrive in highly productive waters by being able to rapidly migrate between radiance-rich surface waters and nutrient-rich bottom waters. Furthermore, many harmful species are tolerant of extreme environmental conditions, including very high light levels, high temperatures, various degrees of desiccation, and periodic nutrient deprivation. Some of the most noxious cyanobacterial bloom genera (*Anabaena, Aphanizomenon, Cylindrospermopsis, Nodularia*) are capable of fixing atmospheric nitrogen (N2), enabling them to periodically dominate under nitrogen-limited conditions. Cyanobacteria produce a range of organic compounds, including those that are toxic to higher-ranked consumers, from zooplankton to further up the food chain [108].

The toxicity of natural blooms of *Microcystis* is due to: (a) *Microcystis*-bacteria interactions, (b) an environmental effect, or (c) the presence of >1 strain or species of *Microcystis*. Partial characterization of bacteria associated with unialgal cultures and natural blooms indicated that no relation exists between *Microcystis* and the occurrence of ≥ 1 types of bacteria. Freshwater cyanobacteria (blue-green algae) can produce numerous potent toxins and represent an increasing environmental hazard. Environmental parameters had an influence on the toxicity of toxic isolates, but even the highest possible dose of a nontoxic isolate injected (800 mg/kg) was innocuous whereas 20 mg/kg from a toxic isolate generally killed mice [109]. The presence of blue-green algae (BGA) toxins in surface waters used for drinking water sources and recreation is receiving increasing attention around the world as a public health concern. However, potential risks from exposure to these toxins in contaminated health food products that contain BGA were largely ignored. BGA products are commonly consumed in the United States, Canada, and Europe for their putative beneficial effects, including increased energy and elevated mood. Many of these products contain *Aphanizomenon flos-aquae*, a BGA that is harvested from Upper Klamath Lake (UKL) in southern Oregon, where the growth of a toxic BGA, Microcystis aeruginosa, is a regular occurrence. *M. aeruginosa* produces compounds called microcystins, which are potent hepatotoxins and probable tumor promoters. Because *M. aeruginosa* coexists with *A. flos-aquae,* it can be collected inadvertently during the

harvesting process, resulting in microcystin contamination of BGA products. In fall 1996, the Oregon Health Division learned that UKL was experiencing an extensive *M. aeruginosa* bloom, and an advisory was issued recommending against water contact. The advisory prompted calls from consumers of BGA products, who expressed concern about possible contamination of these products with microcystins. In response, the Oregon Health Division and the Oregon Department of Agriculture established a regulatory limit of 1 µg/g for microcystins in BGA-containing products and tested BGA products for the presence of microcystins [110].

A toxic incident resulting in the death of 76 people in Brazil in 1996 was due to microcystins in water used for hemodialysis. An outbreak of acute liver failure occurred at a dialysis center in Caruaru, Brazil 134 km from Recife, the state capital of Pernambuco. At the clinic, 116 (89%) of 131 patients experienced visual disturbance, nausea, and vomiting after routine hemodialysis treatment on 13-20 February 1996. Subsequently, 100 patients developed acute liver failure; 76 of these died. In December, 52 of the deaths were attributed to a common syndrome called Caruaru syndrome. Examine of phytoplankton from the dialysis clinic water source, analyses of the clinic water treatment system and serum and liver tissue of clinic patients led to the identification of 2 groups of cyanobacterial toxins, hepatotoxic cyclic peptide microcystins and the hepatotoxic alkaloid, cylindrospermopsin. The major contributing factor to death of dialysis patients was i.v. exposure to microcystins, specifically microcystin-YR, -LR, and -AR. From liver concentrations and exposure volumes, it was established that 19.5 µg/L microcystin was in the water used for dialysis treatments. This is 19.5 times the level set as a guideline for safe drinking water supplies by the World Health Organization [111].

In february 2000 the Swan-Canning estuary in Western Australia experienced a record bloom of the toxic cyanobacteria *Microcystis aeruginosa*. At its height, concentratios of *M. aeruginosa* reached integrated water column cell counts of 15,000/mL and formed bright green scums in sheltered bays, where counts of 130 million cells/mL were recorded. Due to public health concerns parts of the river were closed from 10 to 22 Feb. 2000. A number of methods to reduce bloom accumulations were tried, including an attempt to increase the salinity of the surface water above the critic 10 ppt level for *Microcystis*; using a bentonite clay and poly-aluminum chloride mixture to flocculate and sink the algae; and sucking up

scums using oil spill equipment. Over 900 tonnes of *M. aeruginosa* were removed and safely disposed using sewage treatment facilities. The bloom collapsed when the freshwater flush subsided and seawater intrusion from the Indian Ocean re-established itself, raising the salinities above the tolerance of *Microcystis* [112].

Eutrophication of reservoirs used for drinking water supplies is a very common problem, particularly in lowland reservoirs. Long water retention time (60-120 days) favors cyanobacterial bloom occurrence in Sulejow Reservoir, Poland. The localization of the water intake in a bay exposed to north-east winds favored the *Microcystis* bloom accumulation, which formed a 0.5-m thick dense scum for the 1st time in September 1999. Cyanobacterial hepatotoxins can pose a potential health problem because the presence of approximated 0.8 μg/L microcystins was detected in drinking water. A study of the efficiency of each stage of water treatment processes in the elimination of microcystins showed that pre-chlorination, coagulation, and rapid sand filtration were ineffective in removing microcystins from water. Significant elimination was observed after ozonization and chlorination. The concentration. of microcystins in bloom material was 12-860 μg/g dry weight of phytoplankton biomass [113].

In a shallow coastal lagoon in the city of Rio de Janeiro (Jacarepagua Lagoon). Fish (*Tilapia rendalli*) were collected every 2 week from August 1996 to November 1999. *Microcystins* were analyzed by HPLC in phytoplankton, fish liver and viscera while fish muscle tissue was analyzed by enzyme linked immunosorbent assay (ELISA). Microcystins can accumulate in fish tissue (0.04 μg kg-1 day). Human consumption of fish which are harvested from cyanobacterial blooms that contain cyanotoxins. Chronic and subchronic toxicity from exposure to microcystins, cyclic peptide liver toxins from certain cyanobacteria, poses an important hazard [114].

The water supply of Yokohama City, Japan, depends on the Sagami and Sakawa rivers and is characterized by pollution by oils, anionic surfactants, and blue-green algae (*Microcystis*) which clog filters and give the water a musty odor. The Sagami River water quality council monitors the water quality, reports on water pollution accidents, installs screening to prevent *Microcystis* intake [115].

In South African 1983, three of four white rhinoceroses died within 3 months of introduction into a game reserve. Post-mortem examination of one of the animals revealed

marked hepatomegaly with haemorrhage and severe necrosis of the liver as well as numerous ecchymoses and petechiae in the subcutaneous tissue and subserosa of the thorax, abdomen and diaphragm. Histologically, severe hepatic necrosis was the most significant finding. Algae recovered from the dam from which the animals drank were identified as *Microcystis aeruginosa* [116].

During the summer of 1995, about 20 spot-billed ducks died unnaturally in a pond (Shin-ike) in Nishinomiya, Hyogo Prefecture, Japan. The suspected cause was the sudden appearance of toxic freshwater bloom of cyanobacteria. However, no birds died in a nearby pond (Oo-ike) in which the cyanobacteria was also present. Morphological observation of these cyanobacteria by microscope revealed that they were almost unialgal and were both *Microcystis aeruginosa*. The lyophilized algal cell powder from Shin-ike contained large amounts of microcystins which showed acute toxicity for mouse, while that from Oo-ike had only a very small amount of microcystin-RR which did not show acute toxicity [117].

## CONCLUSIONS

*Microcystis aeruginosa* are capable of producing two kinds of toxin, the cyclic peptide hepatotoxin and the alkaloid neurotoxin. Serious illness such as hepatoenteritis, a symptomatic pneumonia and dermatitis may result from consumption of, or contact with water contaminated with toxin producing cianobacteria. Several blooms of cyanobacteria naturally occurring in freshwater reservoirs have been associated to numerous fatalities and cases of livestock and human poisoning conducted to research the efficacy of several methods in controlling algal growth of freshwater species. A total of 16 structural variants of the toxin were isolated from the *Microcystis aeruginosa,* with microcystin LR (MCYST-LR) as the most abundant making up 77%, MCYST -RR with 38%. They are involved in promoting primary liver tumors and a previous study showed that they might also be tumor initiators. Cyanobacteria is able to produce compounds with biological activity that may be of potential clinical interest cytotoxic cyclic peptides (microcyclamide and aeruginosamide), protease inhibitors related to aeruginosins, trypsin inhibitors (micropeptins) and inhibitors of serine proteases.

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