Review

Production of Secondary Metabolites by Freshwater Cyanobacteria

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Received April 30, 2004

Freshwater cyanobacteria produce lethal toxins such as microcystins and anatoxins. During the purification of microcystins in bloom samples we found that a toxic cyanobacterium produced not only microcystins but also other types-peptides in early 1990. Since then we have isolated approximately thirty peptides from freshwater cyanobacteria. In this manuscript we focused on the following topics concerning the isolated peptides: 1) how to isolate desired compounds and to determine their structures, 2) structural classification of isolated compounds, 3) isolation of similar peptides from laboratory strains and bloom materials, 4) structurally related peptides from freshwater and marine origins, 5) β-amino acid containing peptides from cyanobacteria, 6) comprehensive analysis system for the biosynthetic study of peptides produced by cyanobacteria, 7) biological activities of isolated compounds.

Key words cyanobacteria; microcystin; structural determination; non-toxic peptides; advanced Marfey's method; β-amino acid

Introduction

It is well known that freshwater cyanobacteria produce the following lethal toxins: 1) hepatotoxins, microcystins, nodularin and cylindrospermopsin and 2) neurotoxins, anatoxin-a, anatoxin-a(s) and saxitoxin (Fig. 1).[1] No cyanobacterial toxin has been discovered in freshwater cyanobacteria since the structure of cylindrospermopsin was determined in 1992.[2] Since the late 1980's we have studied isolation and analysis of microcystins in Japanese water bloom samples and laboratory isolates of toxigenic cyanobacteria. During the purification of microcystins using thin layer chromatography (TLC), we found that a toxic fraction from a cyanobacterium frequently contains other spots than those of microcystins (Fig. 2). Later we isolated and determined the structures of several compounds from Japanese *Microcystis aeruginosa* as aeruginopeptins.[3] At almost the same time, Martin *et al.* isolated similar compounds, cyanopeptolins, from *M. aeruginosa* PCC7806 (Fig. 3).[4] This was the first time that bioactive peptides other than microcystins were isolated from toxic freshwater cyanobacteria. Since then it has been recognized that cyanobacteria produce not only toxins but also other type-compounds. This fact encouraged us to isolate such compounds because of the following three reasons: 1) toxicological problems, 2) biosynthesis and 3) origins for biological active compounds.

1) There are so many incidents due to microcystins in cyanobacteria all over the world. In laboratory experiments i.p. injection gives very potent toxicity,[5] while less toxicity is

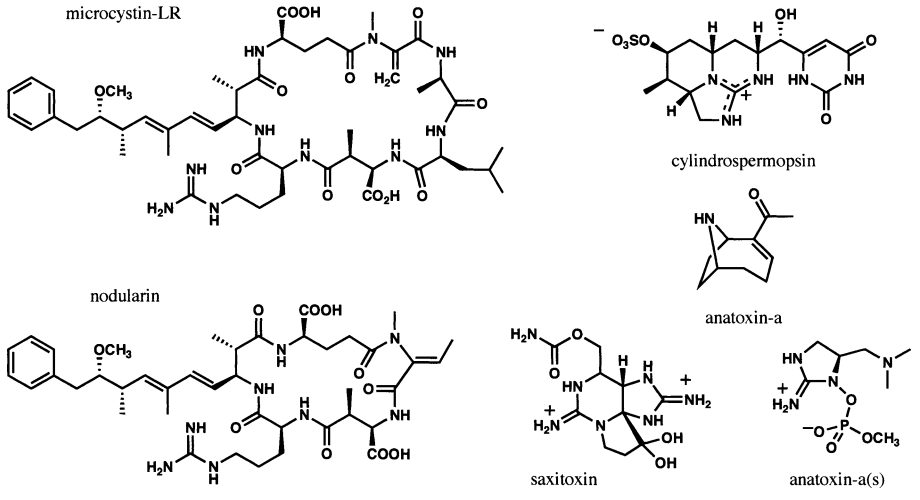

Fig. 1. Structures of Cyanobacterial Toxins

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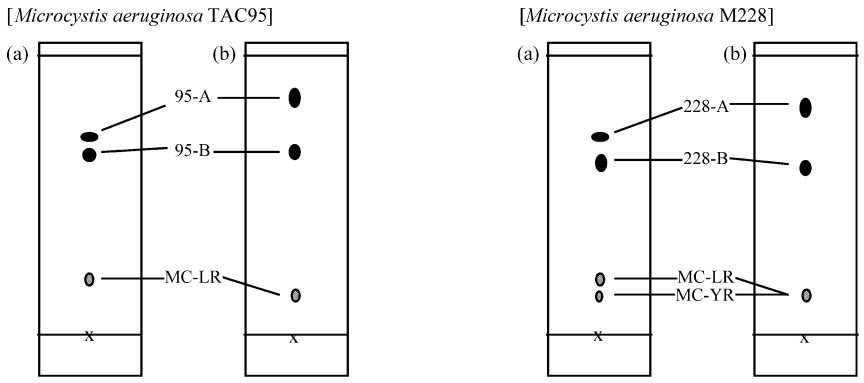


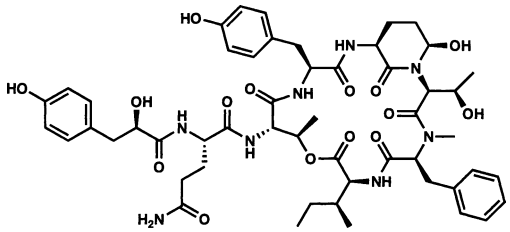
TLC conditions, plate: silica gel, mobile phase: a) $CHCl_3$/MeOH/H_2O (65:35:10, lower phase), b) AcOEt/*i*-PrOH/H_2O (4:3:7, upper phase), detection: UV254 nm, I_2 vapor.

Fig. 2. Thin Layer Chromatograms of Toxic Fractions from Japanese Cyanobacterial Strains

shown by oral administration.[6] However, under natural environmental conditions affected animals and human may ingest orally whole cyanobacteria, which contain microcystins and other type-compounds in most cases, through drinking water and die. How do we explain this contradiction? We hypothesized that co-produced compounds assist microcystin in facilitating its toxicity.

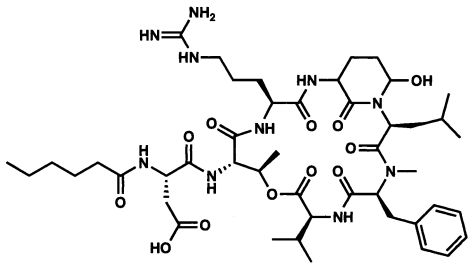
2) The second is the biosynthesis of secondary metabolites produced by cyanobacteria. As mentioned earlier, cyanobacteria produce versatile compounds. Molecular studies show that microcystins are formed using a multienzyme system.[7,8] How are other type-compounds produced in cyanobacteria? Also, is the production of microcystins and other type-compounds closely related biogenetically? Rouhiainen *et al.* reported the genes encoding synthetases of cyclic depsipeptides, anabaenopeptilides that are structurally related to aeruginopeptins.[9]

3) In the natural environment there are various microalgae, of which dinoflagellate and cyanobacteria have produced many secondary metabolites including toxins. It is easier to cultivate cyanobacteria in a laboratory compared with other microalgae. Many research groups have started to explore useful metabolites from cultured cyanobacteria since mid 1970. Carmichael classified these compounds into four groups: biotoxins, irritants, cytotoxins and miscellaneous.[10] According to a review by Moore *et al.*, six to ten percent of extracts of laboratory-cultured cyanobacteria are significantly cyto-



aeruginopeptin 228-A

Microcystis aeruginosa M228



cyanopeptolin A

Microcystis aeruginosa PCC7806

Fig. 3. Structures of Aeruginopeptin 228-A and Cyanopeptolin A

Ken-ichi Harada was born in Hokkaido on December 10, 1948. He received his bachelor's degree in pharmaceutical sciences from Chiba University in 1971. After the graduation of the master course of Chiba University, he joined the Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd in 1973. In 1976 he moved to Faculty of Pharmacy, Meijo University and obtained his Ph. D. degree from Tohoku University in 1983. He was promoted to assistant professor, associate professor and full professor of Meijo University in 1985, 1988 and 2002, respectively. He has received the encouraging award from the Mass Spectrometry Society of Japan in 1985. His interest is currently directed toward establishing a methodology for understanding environmental problems based on natural product chemistry. Particularly, he is establishing a practical method for regulation of outbreak of cyanobacteia and production of toxic compounds using microorganisms co-exiting in a freshwater ecosystem.

Ken-ichi Harada

Table 1. Cyanobacterial Strains Used in the Present Study

Hepatotoxic strains		Hepatotoxic strains	
Microcystis aeruginosa	TAC 95	*Nodularia spumigena*	AV 63
	M228		AV 1
	K-139		BY1
	NIES-298		HEM
Anabaena flos-aquae	NRC 525-17	Non-toxic strains	
Anabaena flos-aquae	CYA 83/1		
Anabaena sp.	202 A1	*Nodularia spumigena*	HKVV
	202 A2/41		AV 63
	90	*Oscillatoria agardhii*	2
	66		18
Oscillatoria agardhii	97	Neurotoxic strains	
	CYA 128		
Nostoc sp.	152	*Anabaena* sp.	37
			123
		Oscillatoria sp.	193

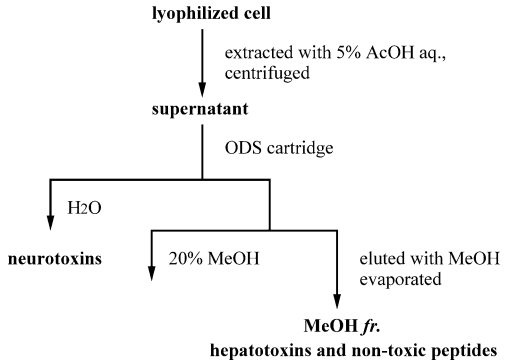


Fig. 4. Isolation Procedure of Secondary Metabolites from Cyanobacteria

toxic against human tumor cell lines.[11] Indeed, his group is developing cryptophycin related compounds for clinical use as solid tumor and tumor selective agents.[11] This indicates that cyanobacteria possess potential for production of useful lead compounds.

How to Isolate Desired Compounds and to Determine Their Structures

We have started to isolate other type-compounds than microcystins from freshwater cyanobacteria since the early 1990s. The used cyanobacteria were classified into three groups; hepatotoxic, neurotoxic and non-toxic and it was investigated what secondary metabolites were isolated from these cyanobacteria (Table 1). All *Microcystis* were derived from Japanese lakes and the remaining strains, except *Anabaena* 525-17,[12] were collected in Finland. How do we isolate desired compounds and determine their structures? This stage includes isolation, discrimination between toxic and non-toxic compounds, and structure determination including the absolute stereochemistry.

We have established an isolation method for microcystins from cyanobacteria.[13] This method shown below can be applicable to the isolation of other type-compounds (Fig. 4): 1) Lyophilized cells were extracted three times with 5% aqueous acetic acid for 30 min while stirring. 2) The combined extracts were centrifuged at 9300 ***g*** for 1 h and the supernatant was collected. 3) The supernatant was directly applied

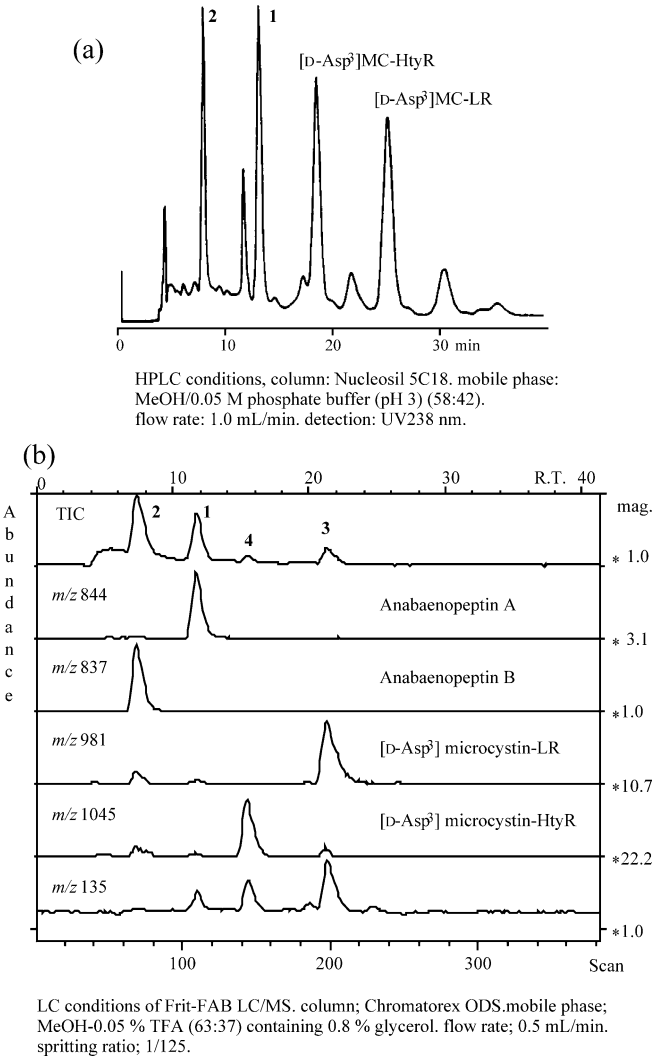


Fig. 5. (a) High Performance Liquid Chromatogram of a Toxic Fraction of *Anabaena flos-aquae* 525-17 and (b) Mass Chromatograms of Anabaenopeptins and Microcystins in a Toxic Fraction of *Anabaena flos-aquae* 525-17

to a preconditioned reversed phase silica gel column using an appropriate pump. 4) The column was washed with water, followed by water : methanol (8 : 2). 5) The desired compounds were eluted with water : methanol (1 : 9). 6) The desired fraction was analyzed by the TLC mentioned above using both mobile phases, chloroform : methanol : water (lower phase) and ethyl acetate : isopropanol : water (upper phase) for the subsequent separation using silica gel. 7) The desired fraction was chromatographed on silica gel with chloroform : methanol : water (lower phase). When the desired compounds were not purified by this step, the chromatography should be repeated with another mobile phase, ethyl acetate : isopropanol : water (upper phase). 8) Finally, the more purified fraction from the above separation was subjected to gel chromatography on TOYOPEARL HW-40 using methanol as the mobile phase.

This procedure was applied to *A. flos-aquae* 525-17 strain giving several peaks that were revealed in the LC chromatogram of the desired fraction as shown in Fig. 5a.[14] In the next step we have to differentiate microcystins from other type-compounds. For this purpose LC/MS is quite effective, because any microcystin gives a common characteristic

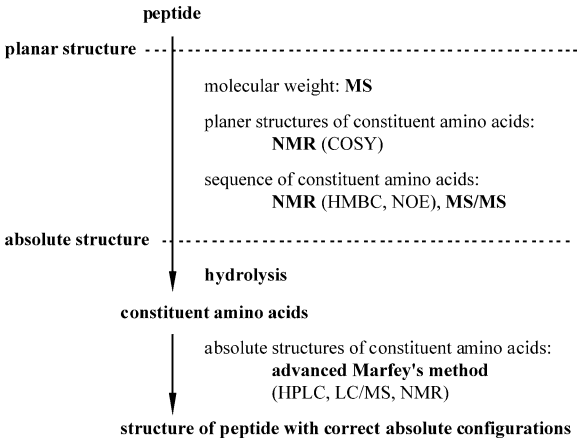


Fig. 6. General Procedure for the Structure Determination of Isolated Peptides

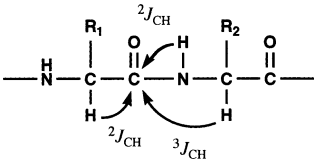


Fig. 7. Structure Determination Using HMBC (^{1}H-Detected Multiple-Bond Heteronuclear Multiple Quantum Coherrence Spectrum) Technique

fragment ion, *m*/*z* 135, which is derived from the Adda ((2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid) portion and this is available for the differentiation of microcystins from other type-compounds. Figure 5b shows the mass chromatograms of the desired fraction. Peaks 3 and 4 are microcystins, because these peaks are also detected in the mass chromatogram monitored at *m*/*z* 135. Therefore peaks 1 and 2 are not microcystins, but other type-compounds with molecular weights of 843 and 836, respectively.[15] This result indicated that this method can be applied for the screening of other type-compounds from freshwater cyanobacteria.

According to the screening mentioned above we isolated about 30 other type-compounds from freshwater cyanobacteria, almost all of which were peptides; their structures were determined as will be shown later. Figure 6 shows our general procedure for structure determination. Molecular weight was usually determined using fast atom bombardment (FAB) or electrospray ionization (ESI) mass spectrometry. Planar structures were determined by the combination of the following NMR spectral methods: ^{1}H–^{1}H COSY (correlation spectroscopy), HMBC (^{1}H-detected multiple-bond heteronuclear multiple quantum coherrence spectrum), HMQC (heteronuclear multiple quantum coherence spectrum) and NOESY (nuclear Overhauser effect spectroscopy). In particular, HMBC is quite useful for sequencing, when a peptide is treated (Fig. 7).

In order to carry out various studies on the isolated compounds such as toxicology, molecular biology, pharmacology, information about absolute stereochemistry is needed. When we started this study, no effective method had been developed so far. Figure 8 is our established method for the determination of absolute stereochemistry of constituent amino acids in a peptide. This method is named the "advanced Marfey's methods".[16,17] First of all, a peptide is subjected to the usual acidic hydrolysis to obtain each constituent amino acid. The reaction product is divided into two portions (samples 1 and 2), one of which is derivatized with L-FDLA (1-fluoro-2,4-dinitrophenylleucinamide) and another is derivatized with D-FDLA. The resulting derivatives are analyzed by LC/MS, noting that the L-derivative is usually eluted prior to the corresponding D-isomer. Although there are a few exceptions, we have already established a separation mechanism based on conformational analysis.[16] Therefore, this method can be applied to any amino acid even if a standard sample is not available.

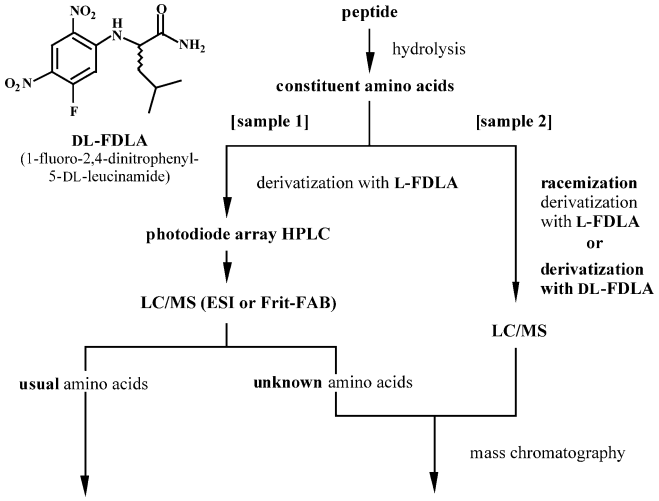


Fig. 8. A Procedure of Advanced Marfey's Method for the Determination of Constituent Amino Acids in a Peptide

As will be mentioned later we isolated nostophycin from a *Nostoc* species, which is 22-membered cyclic peptide including glycine, three L- and two D-amino acids plus a characteristic β-amino acid, 3-amino-2,5-dihydroxy-8-phenyloctanoic acid (Ahoa).[18] According to our established procedure, nostophycin was hydrolysed and then derivatized with FDLA. The HPLC chromatogram of the resulting derivatives is shown in Fig. 9. The ordinary amino acids were identified by direct comparison of corresponding standard samples. However this operation was laborious and it was also impossible to determine the stereochemistry of the primary amino group in Ahoa because a standard sample was not available. So the samples were subjected to the advanced Marfey's method.

Figures 10a and b show the mass chromatograms for the derivatives with L-FDLA and with L- and D-FDLA, respectively. In the mass chromatogram in Fig. 10b each peak of the constituent amino acids was presented at the corresponding *m*/*z* values and Ahoa was detected at *m*/*z* 560. The mass chromatogram shown in Fig. 10a shows the mixture of both enatiomers. The comparison of both chromatograms can lead to the conclusion that proline and phenylalanine have the L and *allo*isoleucine and glutamic acid have the D-configuration. Also, it was possible to determine the absolute configuration of Ahoa as R based on our proposed separation mechanism. Naturally, we have to determine the stereochemistry of these two secondary alcohols and we are also developing an effective method for secondary alcohols.[19,20]

Through the present study about 30 compounds were isolated, almost of which are peptides and determined their

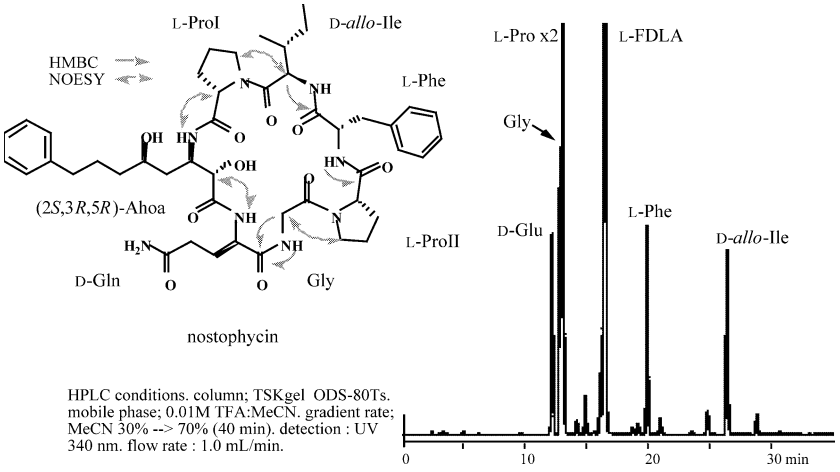
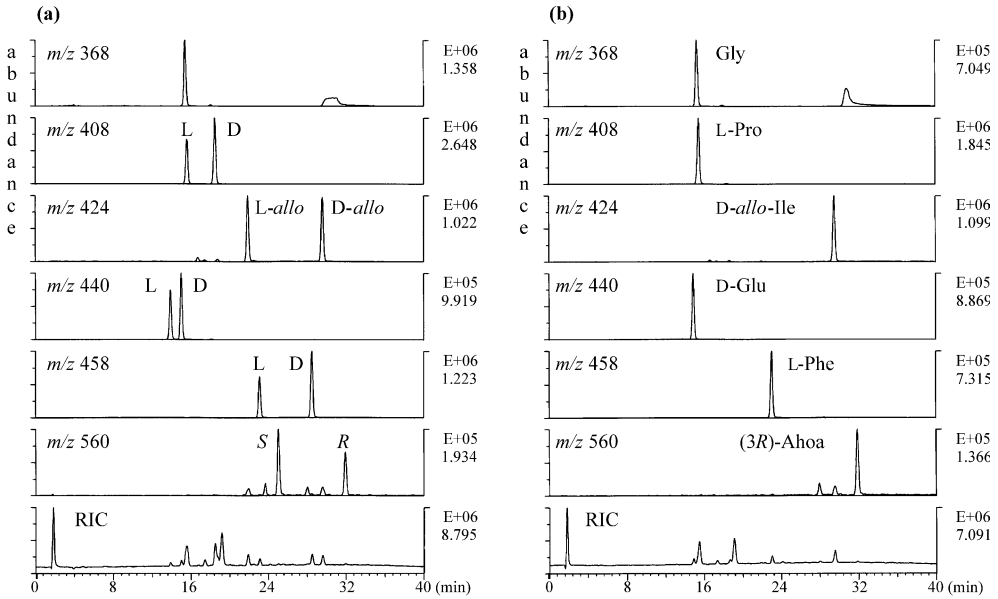


Fig. 9. High Performance Liquid Chromatogram of the DLA (2,4-Dinitrophenylleucinamide) Derivatives of Constituent Amino Acids from Nostophycin



LC conditions of ESI LC/MS. column; Develosil ODS-HG-5. mobile phase; MeCN-0.01 M TFA aq.
gradient rate; MeCN 30% --> 70% (40 min). flow rate; 0.2 mL/min.

Fig. 10. (a) Mass Chromatogram of the DL-DLA (2,4-Dinitrophenylleucinamide) Derivatives of Constituent Amino Acids from Nostophycin and (b) Mass Chromatogram of the L-DLA (2,4-Dinitrophenylleucinamide) Derivatives of Constituent Amino Acids from Nostophycin

structures. Here we would like to focus on the following topics on the isolated compounds: First, non-toxic compounds are structurally classified. Second, we have an interesting phenomenon concerning laboratory and bloom cyanobacteria. Third, several closely similar compounds were isolated from freshwater and marine origins. Fourth, we encounter frequently β-amino acid containing peptides from cyanobacteria. Fifth, a comprehensive analysis system for the biosynthetic study of peptides is developed. Finally, the biological activities of isolated compounds are summarized.

Structural Classification of Isolated Compounds

The first group is a 19-membered depsipeptide with Ahp (3-amino-6-hydroxy-2-piperidone), which are named as aeruginopeptin, anabaenopeptilide, micropeptin and oscillpeptilide (Fig. 11). However they have the same skeleton and approximately 60 components have been isolated so far. The second group is a 19-membered cyclic peptide possessing an ureido linkage; they are designated as anabaenopeptins, nodulapeptins and oscillamide.[21] Among the constituent amino acids in these compounds, Lys has only the D-configuration (Fig. 12). The third group includes several different peptides that are not classified into the groups mentioned above. Spumigins are linear peptides with arginine and related amino acids.[22] Ocsillacyclin is a typical cyclic peptide containing 9 amino acid residues. As mentioned earlier, nostophycin was a 22-membered cyclic peptide isolated from *Nostoc* sp. and has a β-amino acid residue. Microcyclamide is also a cyclic peptide with one oxazole and two thiazoles (Fig. 13).[23,24] Microviridine is a tricyclic peptide composed of only L-amino acids. Microviridines have been produced by both toxic and non-toxic cyanobacteria. We also isolated some of compounds from non-toxic strains, two of which are glycosidic compounds. Suomilide was isolated from non-

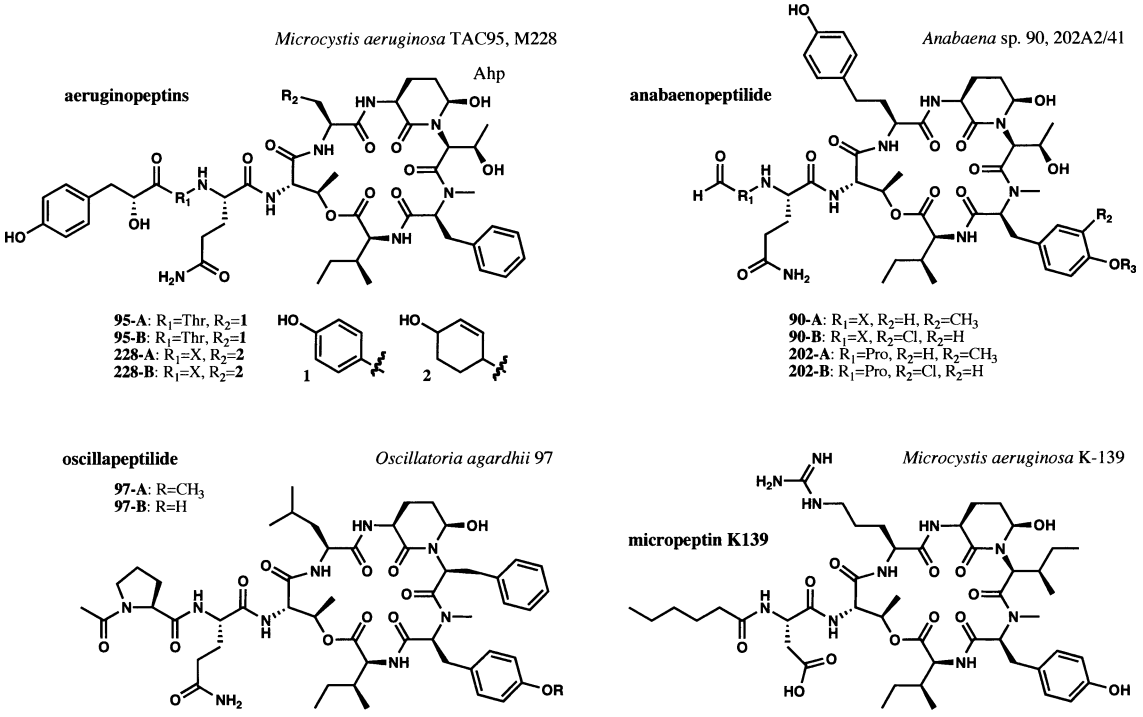
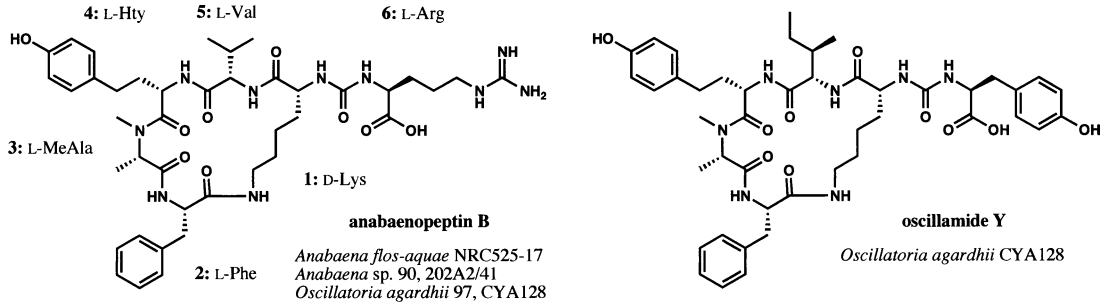


Fig. 11. Structures of 19-Membered Depsipeptides with Ahp (3-Amino-6-hydroxy-2-piperidone) from Toxic Cyanobacteria



peptide	4	5	6
anabaenopeptin A	Hty	Val	Tyr
anabaenopeptin B	Hty	Val	Arg
anabaenopeptin C	Hty	Val	Lys
anabaenopeptin D	Hty	Val	Phe
anabaenopeptin E	3'-MeHty	Val	Arg
anabaenopeptin F	Hty	*allo*-Ile	Arg
oscillamide Y	Hty	*allo*-Ile	Tyr

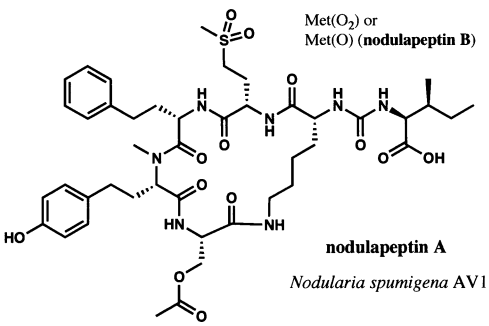


Fig. 12. Structures of 19-Membered Cyclic Peptides Possessing Ureido Linkage from Toxic Cyanobacteria

toxic *Nodularia* and has glucose and guanidinoamine moieties.[25] Non-toxic *Nodularia* also produce heterocystglycolipid (Fig. 14). Namikoshi and Rinehart reviewed bioactive compounds produced by freshwater cyanobacteria.[21]

From the results described above, we summarize the production of secondary metabolites from three kinds of cyanobactertia as follows: hepatotoxic strains always produce non-toxic peptides together with microcystins or nodularin. On the other hand neurotoxic strains produce anatoxin but do not produce any peptides except for the *Anabaena flos-aquae* 525-17 strain. We isolated two glycosidic compounds from non-toxic strains. Recently, the isolation of fatty acids and glycolipids has been reported[26]; we also isolated unsaturated fatty acids as an insecticidal agent against mosquito.[27] However, we consider that these compounds are not secondary metabolites.

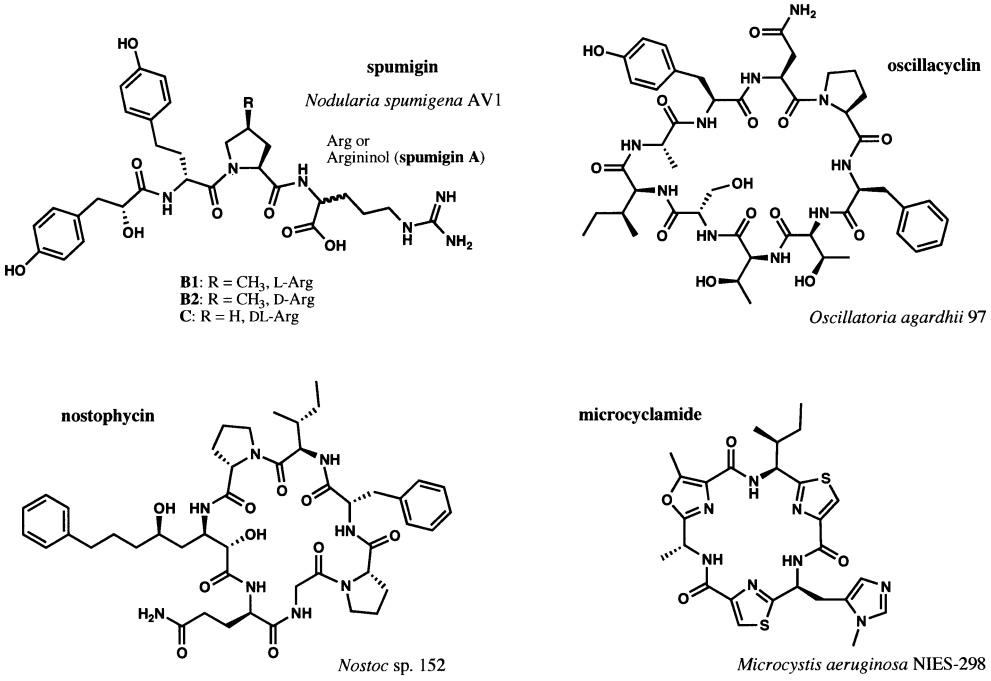


Fig. 13. Structures of Several Peptides from Toxic Cyanobacteria

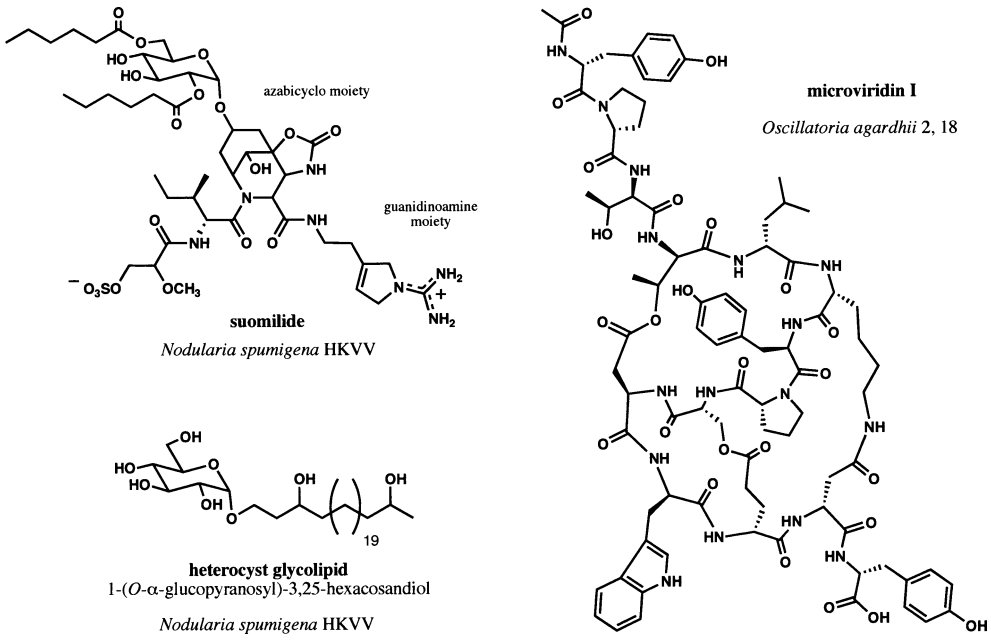


Fig. 14. Structures of Compounds from Non-toxic Cyanobacteria

Isolation of Similar Peptides from Laboratory Strains and Bloom Materials

During the isolation work we encountered the following interesting results. As described previously, we isolated four aeruginopeptins from the strains[3] and also isolated three different but structurally similar aeruginopeptins from bloom samples collected in Lake Suwa in 1991.[28] At that time we investigated the relationship between *Microcystis* species and production of microcystins and aeruginopeptins. From 1991 to 1994 we collected bloom samples every ten days in Lake Suwa in Japan and analyzed species composition and the microcystins and aeruginopeptins contents. In 1991 *M. aeruginosa* (large) was not always dominant but was most abundant during July. The seasonal change of the *M. aeruginosa* coincides well with the production of these two compounds (Fig. 15). On the other hand, no *M. aeruginosa* appeared but *M. viridis* was always dominant in 1992.[29] The amounts of microcystins were changed to around 500 μg per 1 g of dried cells, but no aeruginopeptins were detected. These results indicated that aeruginopeptins were mainly produced by *M. aeruginosa*.[28]

Microcyclamide was isolated from Japanese *M. aeruginosa* NIES-298 strain and a cyclic peptide with one oxazole and two thiazoles[23,24]: Very recently, we had an opportunity

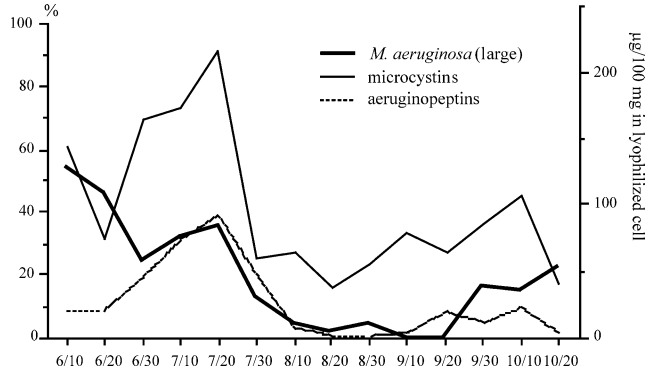
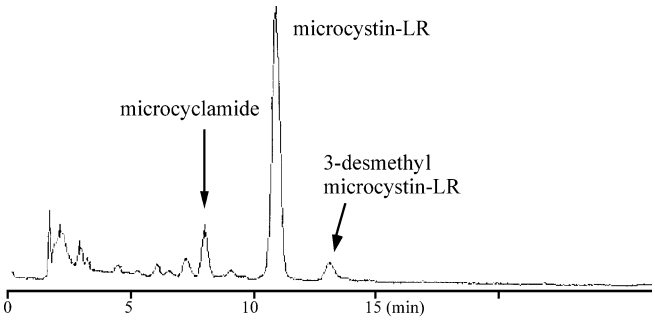


Fig. 15. Seasonal Change of *Microcystis* Species and Production of Microcystins and Aeruginopeptins 917S in Lake Suwa, Japan in1991 and 1992



HPLC conditions: column; COSMOSIL 5C18 AR-II. mobile phase; MeOH/0.05M phosphate buffer (pH 3.0) = 58:42. flow rate; 1.0 mL/min, detection; UV 238 nm

Fig. 16. High Performance Liquid Chromatogram of the Extract of Bloom Samples Collected in Philippine

to investigate a Philippine toxic bloom sample, which include *M. aeruginosa* as the main species. Figure 16 shows the HPLC chromatogram of the extract of this bloom sample, in which microcyclamide was detected together with microcystin-LR as the main product.[30] These experimental results clearly show that secondary metabolites from strains are also produced under natural environmental conditions.

Structurally Related Peptides from Freshwater and Marine Origins

As mentioned earlier, freshwater cyanobacteria produce the six toxins. Among them, saxitoxin is also known as the PSP (paralytic shellfish poisoning) toxin and to be also produced by *Alexandrium*. Motuporin was found in a marine sponge, *Theonella*, and is structurally similar to nodularin.[1] Through the present study we obtained further examples of structurally related peptides isolated from freshwater and marine origins. Two representative cyclic peptides, aeruginopeptins and micropeptins, were isolated from Japanese and Canadian cyanobacteria, respectively. Japanese natural product chemists had already isolated similar compounds with the same skeleton from marine organisms, which were named as konbamide[31] and keramamide A[32] (Fig. 17). There are many cyclic peptides possessing thiazole and oxazole rings such as microcyclamide from cyanobacteria.[23] Similar cyclic peptides were also isolated from marine origins as shown in Fig. 18; westellamide (cycloxazole) was discovered

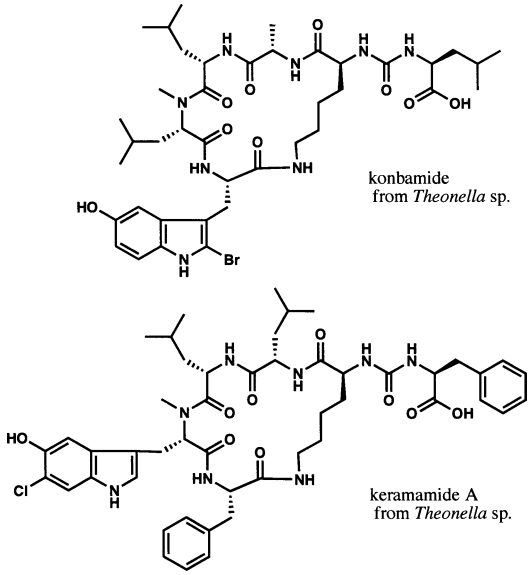


Fig. 17. Structures of Konbamide and Keramamide A

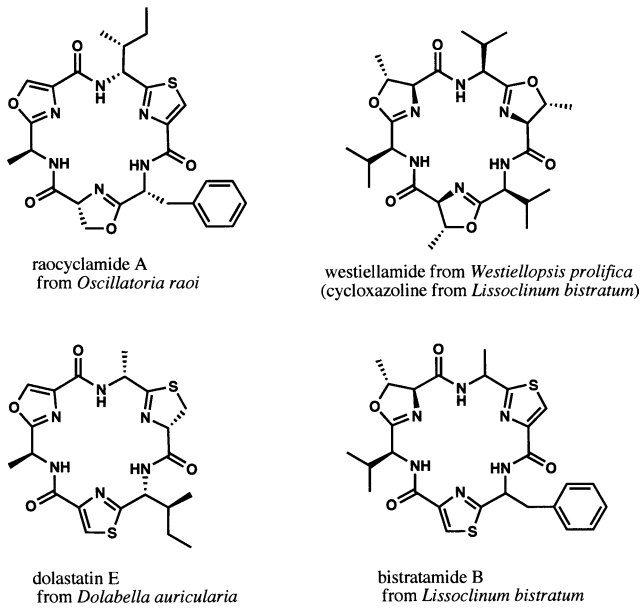
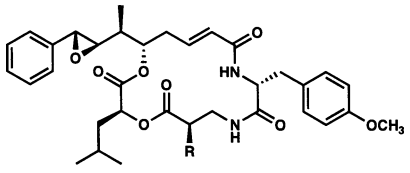


Fig. 18. Structures of Thiazole and Oxazole-Contaning Cyclic Peptides from Cyanobacterial and Marine Oridines

in both marine and freshwater origins. Cryptophycin was isolated from the lipophilic extract of terrestrial *Nostoc* sp. and shows potent cytotoxic properties.[11] On the other hand, Japanese scientists discovered a compound very similar to cryptophycin from Okinawan *Dysidea arenaria*, which was designated as arenastatin A (Fig. 19).[33] Recently, many semisynthetic cryptophycins are being developed as new anti-tumor drugs.[11]

As described above, we demonstrated that several kinds of bioactive compounds were isolated from both marine origins such as sponge, tunicate and seaweed and terrestrial cyanobacteria. These results remind us a problem what is the real producer of these compounds. Many organisms live in the marine ecocystem. Usually, lower organisms such as bacteria and cyanobacteria produce these metabolites, which are transferred to these macroscopic organisms using symbiosis,



cryptophycin B: R= CH_3
arenastatin A: R= H

Fig. 19. Structures of Cryptophycin B and Arenastatin A

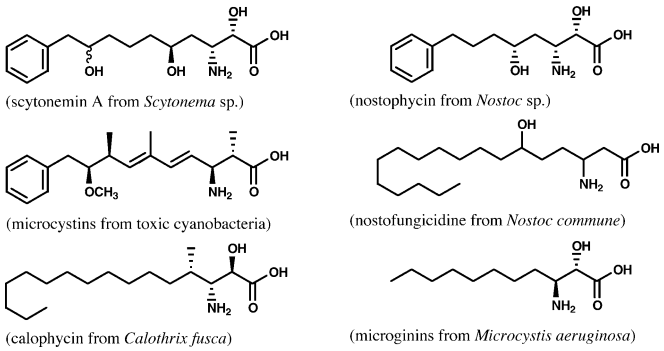


Fig. 20. Structures of β-Amino Acids Contained in Peptides Isolated from Cyanobacteria

association and the food chain.[34] Even if producing bacteria and cyanobacteria can be isolated, such microorganisms alone cannot produce bioactive compounds in most cases. On the other hand, no definite conclusion has been proposed so far for the transfer of secondary metabolites in the freshwater ecosystem.

β-Amino Acid Containing Peptides from Cyanobacteria

We isolated nostophycin together with microcystins from *Nostoc* species.[19] Surprisingly, this structure is closely similar to that of scytoneamine A isolated from a marine cyanobacterium *Scytonema*.[35] There are many β-amino acid-containing peptides from cyanobacteria. Apart from the stereochemistry, these compounds have characteristic β-amino acids (Fig. 20). We considered that a β-amino acid is one of the characteristic features in peptides produced by cyanobacteria. Moore *et al.* carried out a labeling experiment for microcystins using a ^{13}C-NMR spectroscopic technique.[36] According to this result, a β-amino acid moiety (Adda) of microcystin was formed with four molecules of acetic acid, four methyl groups from methionine and part of a phenylalanine moiety. This experimental result indicates that the Adda is biosynthesized by way of a hybrid pathway composed of polyketide and peptide.

Two groups have demonstrated the organization of the gene cluster for the five amino acid moieties in microcystin.[37,38] Very recently the polyketide synthetase gene coupled to the peptide synthetase involved in the biosynthesis of microcystin has been reported.[7,8] Although there are several problems yet to be solved, these results almost coincide with that of the previous labeling experiment.

Comprehensive Analysis System for the Biosynthetic Study of Peptides Produced by Cyanobacteria

Microcystins are non-ribosomally synthesized *via* a mixed polyketide synthase/non-ribosomal peptide synthetase system called microcystin synthetase.[7,8] As mentioned earlier, we have carried out the detection, isolation and structural determination of non-toxic peptides produced together with microcystins by toxic cyanobacteria, which are classified into several groups on the basis of their structures and some of these non-toxic peptides are also non-ribosomally synthesized as well as microcystins.[9] In order to correlate the secondary metabolic peptides produced by the hepatotoxic cyanobacteria with the corresponding peptide synthetase genes, an analytical method using ESI-LC/MS and photodiode array detection was developed for the exhaustive screening of cyanobacterial peptides and it was successfully applied to the peptide fractions extracted from these strains. The method was applied to a hepatotoxic cyanobacterium, *M. aeruginosa* K-139 and four types of peptides, microcystins (MC), micropeptin (Mip), microviridins (Miv) and aeruginosins (Aes) are clearly detected (Fig. 21). The established method was advantageous over conventional ones using the usual HPLC and MALDI-TOFMS,[39] because more structural information could be obtained and it is easier to distinguish microcystins from other peptides using this method. Small amounts of other peptides could also be detected by this method. The established method will contribute to the investigation of the relationship between genes encoding the peptide synthetase and secondary metabolic peptides.[40]

Biological Activities of Isolated Compounds

As mentioned in the introduction, we have shown a contradiction that the oral administration of microcystins causes less toxicity, whereas affected animals and human may ingest orally whole cyanobacteria in the natural environment. Originally, the present study had been started to verify a working hypothesis that co-produced compounds assist microcystin in facilitating its toxicity. Unfortunately, it was found that aeruginopeptins and anabaenapeptins did not assist microcystin-LR to enhance its toxicity in the preliminary experiment. Ito *et al.* have demonstrated that the intratracheal route is one of possibilities for the microcystin poisoning in the natural environment.[41]

Other type-compounds obtained in the present study show characteristic biological activities as shown in Table 2. They show serine protease inhibition activity, relaxation activity and cytotoxicity. Although the activity of these compounds is considerably weak, the present study indicated a possibility that cyanobacteria produce biological active metabolites available as lead compounds for medicine. For example, cryptophycins are particularly being developed as anti-cancer agents as shown above.[11] Cyanobacteria have an additional advantage that once we collect sponges and tunicates that contain useful compounds such as didemnin and arenastain A, it is difficult to find such marine organisms again. However, it is possible to cultivate easily cyanobacteria. Therefore, cyanobacteria are a much promising origin compared with higher microalgae and other organisms.

Conclusion and Outlook

Microcystins still threaten human health and life. Although experimental results from many studies have been accumulating to clarify a mechanism for microcystin poisoning, no effective treatment for it has as yet been established.

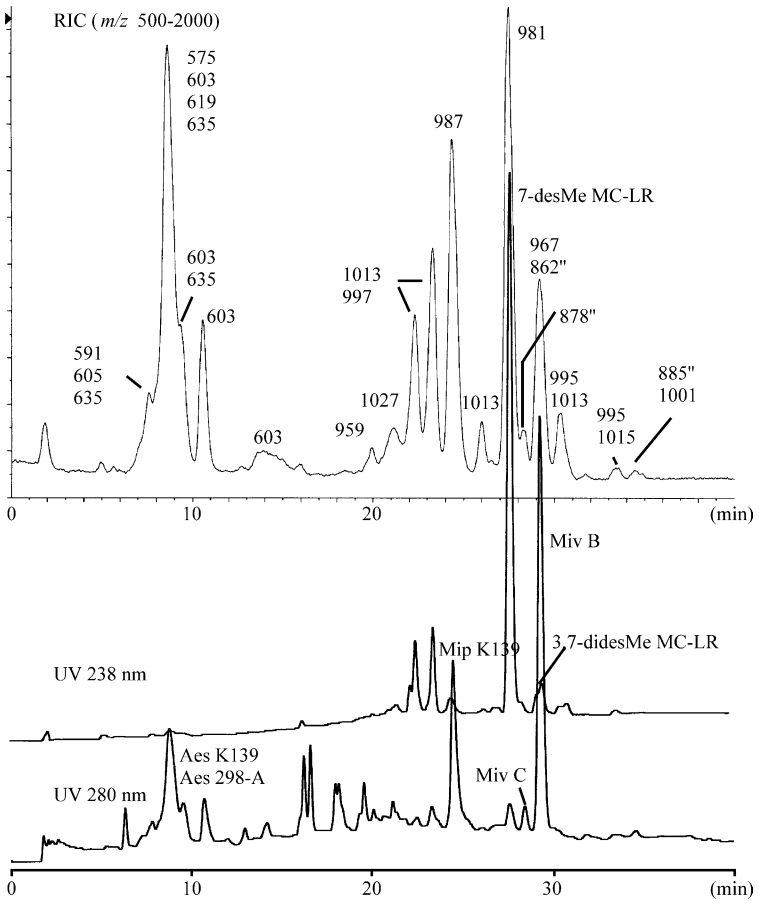


Fig. 21. High Performance Liquid Chromatograms Monitored at UV 238 and 280 nm, and RIC Chromatogram (*m/z* 500—2000) by ESI-LC/MS Analysis of the Extract from *M. aeruginosa* K-139

Table 2. Biological Activities of Compounds Obtained in the Present Study

Serine protease inhibition activity	
	Anabaenopeptilides (chymotrypsin)
	Spumigins (thrombin, trypsin, plasmin)
	Microviridin I (elastase)
	Suomilide (thrombin, trypsin, plasmin)
Relaxation activity	
	Nostophycin (against the lymphocytic mouse leukemia L1210)
Cytotoxic activity	
	Anabaenopeptins (to norepinephrine-induced constraction of rat aortic preparations)

Further, an effective method for the control of a cyanobacteria outbreak in a lake has not yet been established. These undesirable aspects concerning cyanobacteria may be emphasized at the moment. However, an ability to produce microcystins is fascinating in a sense, because this means that toxic cyanobacteria have the potentiality to produce useful secondary metabolites. Indeed, we have shown that freshwater cyanobacteria produce many peptides with diverse skeletons. Recent molecular biological studies have accelerated the clarification of the biosynthesis of these secondary metabolites. These metabolites will be changed to useful compounds using information obtained from the molecular studies.

Acknowledgements This research was supported by the following researchers and their helpful contribution was greatly appreciated: Professor M. Suzuki and Dr. K. Fujii (Meijo University), Professor W. W. Carmichael (Wright State University), Professor K. Sivonen (University of Helsinki), Professor M. Shirai (Ibaraki University), Dr. H.-D. Park (Shinshu University), and Professor M. F. Watanabe (Risshou University).

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