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Isolation and Characterization of a Variety of Microcystins from Seven Strains of the Cyanobacterial Genus *Anabaena*

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Hepatotoxins (microcystins) from seven freshwater *Anabaena* strains originating from three different Finnish lakes and one lake in Norway were isolated by high-performance liquid chromatography and characterized by amino acid analysis and fast atom bombardment mass spectrometry. All strains produced three to seven different microcystins. A total of 17 different compounds were isolated, of which 8 were known microcystins. The known compounds identified from six strains were MCYST (microcystin)-LR, [D-Asp^3]MCYST-LR, [Dha^7]MCYST-LR, [D-Asp^3,Dha^7]MCYST-LR, MCYST-RR, [D-Asp^3]MCYST-RR, [Dha^7]MCYST-RR, and [D-Asp^3,Dha^7]MCYST-RR. With the exception of MCYST-LR and [D-Asp^3]MCYST-LR, this is the first time that isolation of these toxins from *Anabaena* strains has been reported. Three of the strains produced one to three toxins as minor components which could not be identified. *Anabaena* sp. strain 66 produced four unidentified toxins. The other *Anabaena* strains always contained both MCYST-LR and MCYST-RR and/or their demethyl variants. Quantitative differences between toxins within and between strains were detected; at times MCYST-LR and at other times MCYST-RR or demethyl derivatives thereof were the most abundant toxins found in a strain.

Toxin-producing cyanobacteria commonly occur in fresh or brackish water environments (2, 32, 33, 36, 38). Two main types of toxins produced by cyanobacteria are known: cyclic peptide hepatotoxins and alkaloid neurotoxins. Hepatotoxic water blooms are more common and widespread than neurotoxic water blooms (5, 6, 8). In brackish water environments like the Baltic Sea and brackish water lakes and estuaries in New Zealand and Australia, *Nodularia spumigena* has been found to be responsible for the hepatotoxicity of water blooms, and the toxin was shown to be a cyclic pentapeptide called nodularin (molecular weight [MW] 824) (34, 36). In freshwater habitats, the most common hepatotoxin-producing cyanobacterium is *Microcystis* sp. (5, 6, 8). More recently, the production of hepatotoxins by *Anabaena* (15, 19), *Oscillatoria* (20, 25, 30), and *Nostoc* (28, 35) species has been reported. In Scandinavia, *Anabaena* spp. are as common as *Microcystis* spp. in hepatotoxic blooms, and these organisms together with *Oscillatoria agardhii* cause most of the hepatotoxic freshwater blooms in that area (2, 38).

The freshwater cyanobacterial hepatotoxins, called microcystins, found to date are all cyclic heptapeptides (7) (Table 1). The general structure of microcystins is characterized as cyclo(D-Ala^1–X^2–D-MeAsp^3–Z^4–Adda^5–D-Glu^6–Mdha^7-), in which X and Z are variable L amino acids, D-MeAsp is D-*erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine (7) (Table 1). The most unusual structural feature of these toxins is the presence of Adda, (2*S*, 3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (3, 27, 34), which is important to the toxicity of these compounds (10, 14). Twenty-nine different varieties of these toxins have been reported in the scientific literature (Table 1). In addition to the variable L amino acids, common differences include demethylation of amino acids 3 and/or 7. We have recently reported some new variants of microcystins which have modified Adda (ADMAdda and DMAdda) (28, 29, 37), D-serine in place of D-alanine (37), *N*-methylserine (Mser) in place of *N*-methyldehydroalanine (29, 37), and D-Glu(OC_3H_7O) instead of D-glutamic acid (29). Two unusual L amino acids are found: in *Nostoc* sp., L-arginine was found to be replaced by L-homoarginine (Har) (28, 35, 37), and in an *A. flos-aquae*, L-tyrosine was found to be replaced by L-homotyrosine (Hty) (15) (Table 1).

The cyanobacterial hepatotoxins have caused repeated animal poisonings worldwide (1, 5, 39). Toxins ingested are thought to enter the liver via the bile acid route and cause hepatocyte necrosis, which leads to pooling of blood in the liver. In acute poisoning, death is the result of hypovolemic shock (1). The molecular mechanism of action for microcystins is not known at present but seems to be connected with protein phosphorylation, since certain microcystins and nodularin have been shown to inhibit protein phosphatases 1 and 2A (22, 24). They are also suspected to be tumor promoters (12, 45).

There are two *A. flos-aquae* strains from which toxins have been isolated and characterized. They both originate from lakes in Canada. *A. flos-aquae* S-23-g-1 was found to produce MCYST (microcystin)-LR (MW 994) and [D-Asp^3]MCYST-LR (MW 980) (19). The other strain, NRC 525-17, produced the neurotoxin anatoxin-a(S) (23) together with four hepatotoxins, [D-Asp^3]MCYST-LR (MW 980), MCYST-HtyR (MW 1058), [D-Asp^3]MCYST-HtyR (MW 1044), and a partially characterized microcystin having an MW of 1014 (15).

To expand our understanding of the occurrence and distribution of hepatotoxins from cyanobacteria, we examined toxins from seven hepatotoxic Scandinavian *Anabaena* strains. More information on toxin production of *Anabaena* strains could be obtained by studying whether there is a

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TABLE 1. Microcystins reported in the scientific literature

Microcystin[a]	MW	Formula	Organism	Reference(s)
MCYST-LA	909	$C_{46}H_{67}N_7O_{12}$	*M. aeruginosa*[b]	3
MCYST-LAba	923	$C_{47}H_{69}N_7O_{12}$	*M. aeruginosa*[b]	13
MCYST-AR	952	$C_{46}H_{68}N_{10}O_{12}$	*Microcystis* spp.[c]	29
MCYST-YA	959	$C_{49}H_{65}N_7O_{13}$	*M. aeruginosa*[b]	4
[D-Asp^3,Dha^7]MCYST-LR	966	$C_{47}H_{70}N_{10}O_{12}$	*M. aeruginosa*[b]	16
[D-Asp^3]MCYST-LR	980	$C_{48}H_{72}N_{10}O_{12}$	*A. flos-aquae*,[b] *M. viridis*[c]	14, 19
[Dha^7]MCYST-LR	980	$C_{48}H_{72}N_{10}O_{12}$	*M. aeruginosa*[b]	16
[DMAdda^5]MCYST-LR	980	$C_{48}H_{72}N_{10}O_{12}$	*Microcystis* spp.,[c] *Nostoc* sp.[b]	29, 37
MCYST-LR	994	$C_{49}H_{74}N_{10}O_{12}$	*M. aeruginosa*,[b] *A. flos-aquae*[b]	4, 19, 34
MCYST-LY	1,001	$C_{52}H_{71}N_7O_{13}$	*M. aeruginosa*[b]	41
[D-Asp^3,ADMAdda^5]MCYST-LR	1,008	$C_{49}H_{72}N_{10}O_{13}$	*Nostoc* sp.[b]	28
[D-Asp^3,Dha^7]MCYST-RR	1,009	$C_{47}H_{71}N_{13}O_{12}$	*O. agardhii*[c]	20
[Mser^7]MCYST-LR	1,012	$C_{49}H_{76}N_{10}O_{13}$	*Microcystis* spp.[c]	29
[ADMAdda^5]MCYST-LR	1,022	$C_{50}H_{74}N_{10}O_{13}$	*Nostoc* sp.[b]	28
[D-Asp^3,ADMAdda^5]MCYST-LHar	1,022	$C_{50}H_{74}N_{10}O_{13}$	*Nostoc* sp.[b]	37
[D-Asp^3]MCYST-RR	1,023	$C_{48}H_{73}N_{13}O_{12}$	*O. agardhii*[b]	25
[Dha^7]MCYST-RR	1,023	$C_{48}H_{73}N_{13}O_{12}$	*M. aeruginosa*[b]	18
MCYST-FR	1,028	$C_{52}H_{72}N_{10}O_{12}$	*Microcystis* spp.[c]	29
MCYST-M(O)R	1,028	$C_{48}H_{72}N_{10}O_{13}S$	*Microcystis* spp.[c]	29
MCYST-YM(O)	1,035	$C_{51}H_{69}N_7O_{14}S$	*M. aeruginosa*[c]	4
[ADMAdda^5]MCYST-LHar	1,036	$C_{51}H_{76}N_{10}O_{13}$	*Nostoc* sp.[b]	28
MCYST-RR	1,037	$C_{49}H_{75}N_{13}O_{12}$	*M. aeruginosa*,[b] *M. viridis*[b]	21, 31, 43
[D-Ser^1,ADMAdda^5]MCYST-LR	1,038	$C_{50}H_{74}N_{10}O_{14}$	*Nostoc* sp.[b]	37
[ADMAdda^5,Mser^7]MCYST-LR	1,040	$C_{50}H_{76}N_{10}O_{14}$	*Nostoc* sp.[b]	37
MCYST-YR	1,044	$C_{52}H_{72}N_{10}O_{13}$	*M. aeruginosa*,[b] *M. viridis*[b]	4, 43
[D-Asp^3]MCYST-HtyR	1,044	$C_{52}H_{72}N_{10}O_{13}$	*A. flos-aquae*[b]	15
[D-Glu(OC$_3$H$_7$O)^6]MCYST-LR	1,052	$C_{52}H_{80}N_{10}O_{13}$	*Microcystis* spp.[c]	29
MCYST-HtyR	1,058	$C_{53}H_{74}N_{10}O_{13}$	*A. flos-aquae*[b]	15
MCYST-WR	1,067	$C_{54}H_{73}N_{11}O_{12}$	*Microcystis* spp.[c]	29

[a] Aba, aminoisobutyric acid; Dha, dehydroalanine; DMAdda, *O*-demethylAdda; ADMAdda, *O*-acetyl-*O*-demethylAdda; Mser, *N*-methylserine; Har, homoarginine; M(O), methionine *S*-oxide; Hty, homotyrosine.
[b] Toxins isolated from strain samples.
[c] Toxins isolated from bloom samples.

difference between strains isolated from different lakes or strains isolated at different time intervals from the same lake. The results are presented in this report.

MATERIALS AND METHODS

Organisms. Six *Anabaena* strains used in this study were isolated from three different lakes in Finland, with three of the strains coming from the same lake but collected at different times (Table 2). From bloom sample 202, two parallel *Anabaena* isolations were made. All Finnish strains came from the bloom samples, which were found to be hepatotoxic by the intraperitoneal mouse bioassay (38). A seventh strain, *A. flos-aquae* (Breb.) NIVA-CYA 83/1, isolated from L. Edlandsvatn, Roagland, Norway, in 1981 (9), was a gift from Olav M. Skulberg (Norwegian Institute for Water Research, Oslo, Norway). All *Anabaena* strains were isolated and cultured on Z8 medium minus nitrogen as detailed earlier (38). Strains were grown at ~22°C with continuous illumination of about 50 microeinsteins/m^2 per s. Cells were harvested after 12 to 14 days of incubation and lyophilized prior to toxicity testing and toxin isolation.

Isolation of toxins. Toxins were extracted twice (2 h and overnight) from lyophilized *Anabaena* cells (amounts of cells used are shown in Table 2) with 1-butanol–methanol–water (1:4:15 [vol/vol]). Supernatants were combined, evaporated to one-half of the original volume, and applied on preconditioned C_{18} silica gel columns or cartridges (Bond Elut, Analytichem), depending on the original amount of cells used in extraction. The toxins were eluted from the column or cartridges by using 80 to 100% methanol and then evaporated to dryness. The toxic fractions were then separated

TABLE 2. *Anabaena* strains and toxins isolated in this study

Organism	Isolation date	Place	Amt (g) of cells used for extraction	Component toxins (%)
Anabaena sp. strain 60	7/21/86	Lake Ylä-Keyritty[a]	1.5	A (45), B (10), E (35), F (10)
Anabaena sp. strain 66	7/22/86	Lake Kiikkara[a]	1.5	I (20), J (55), K (15), L (10)
A. flos-aquae CYA 83/1	1981	Lake Edlandsvatn[b]	5.0	A (36), B (18), E (12), F (24), M (5), N (5)
Anabaena sp. strain 90	7/30/86	Lake Vesijärvi[a]	0.6	A (57), B (15), E (28)
Anabaena sp. strain 141	8/25/86	Lake Vesijärvi	1.0	A (14), B (28), E (20), F (38)
Anabaena sp. strain 202A1	7/23/87	Lake Vesijärvi	10.0	C (35), D (5), G (45), H (5), O (4), P (4), Q (2)
Anabaena sp. strain 202A2	7/23/87	Lake Vesijärvi	1.4	C (35), D (10), G (45), P (10)

[a] Finland.
[b] Norway.

by high-performance liquid chromatography (HPLC), using either a Varian Vista model 5560 solvent delivery system with a Varian model 200 UV detector plus a semipreparative C_{18} silica gel column (μBondapak; 19 by 150 mm; Waters) (strains 202A1, 202A2, and CYA 83/1) or a Beckman model 421 solvent delivery system with a Beckman model 165 UV detector plus a semipreparative C_{18} silica gel column (μBondapak; 19 by 300 mm; Waters) (strains 60, 66, 90, and 141). The mobile phase, acetonitrile–10 mM ammonium acetate (26:74), was used at a flow rate of 4 ml/min, and toxins were detected by UV at 238 nm (absorption maximum of the toxins). The toxin fractions were further purified with the same Beckman HPLC system by using a methanol gradient from 0 to 50% over 25 min in 10 mM phosphate buffer (pH 6.8). The third purification step was accomplished by using a Beckman model 406 HPLC with a model 167 UV detector and an analytical C_{18} silica gel column (Alltech; 4.6 by 250 mm) with acetonitrile–20 mM ammonium acetate (pH 5, 25:75) as the mobile phase at 1 ml/min. The fourth purification step, if necessary, was performed under similar conditions with a mobile phase of methanol–50 mM sodium sulfate (1:1). The purity of the toxins was monitored, and toxins were identified in the isolated fractions after the second purification step by their UV spectra, using a Waters 600E HPLC system with a 990 photodiode array detector. An ISRP column (4.6 by 250 mm; Regis Pinkerton) and a mobile phase of acetonitrile–100 mM phosphate buffer (pH 6.8, 15:85) with a flow rate of 1 ml/min were used. The purified toxins were desalted and stored at −20°C prior to amino acid analysis and fast atom bombardment mass spectroscopy (FABMS).

Amino acid analysis. Isolated compounds were hydrolyzed with 6 N HCl at 110°C for 21 h, and the amino acids obtained, after pre-column derivatization with phenyl isothiocyanate, were analyzed with a Waters Pico Tag HPLC system. The derivatives were separated on a C_{18} silica gel column (3.9 by 150 mm) by using Pico Tag eluents A and B over 13 min and were detected by UV absorption.

FABMS. FABMS was run on either a VG ZAB-SE (LRFABMS) or a VG 70-SE4F (HRFABMS) mass spectrometer, using xenon atoms of 8 keV energy and a matrix of dithiothreitol-dithioerythritol (magic bullet) (44). Approximately 5 to 10 μg of each sample was applied in a methanol solution.

Toxicity testing. Toxicity of the cells and fractions after the first HPLC purification step were tested by mouse bioassay. Aqueous cell suspensions and fractions from HPLC were injected intraperitoneally into mice (20- to 25-g female NMRI mice at the University of Helsinki; 20- to 25-g male ICR Swiss mice at Wright State University). Mice were monitored for 4 h. The typical signs of poisoning by the hepatotoxic microcystins were lethargy, pallor of ears, tail, and feet, death in 45 to 90 min, and swollen blood-engorged liver found upon necropsy.

RESULTS

All *Anabaena* strains used were hepatotoxic by the mouse bioassay. Typical signs and autopsy findings for cyanobacterial hepatotoxins were found. Strains isolated from Finnish lakes were described on the genus level and defined by strain number. The preliminary identification placed them in the *A. flos-aquae/lemmermannii/circinalis* species group. The cells and heterocysts of strains 90 and 141 were round. In the rest of the strains, they were oval to rectangular and resembled the cells of Norwegian *A. flos-aquae* strain CYA 83/1.

Table 2 shows the relative amounts of toxins (coded from A to Q) isolated from the seven *Anabaena* strains. Fractions collected during the first HPLC run were tested for hepatotoxicity by the mouse bioassay, but in subsequent HPLC purifications, the toxic peaks were monitored by UV spectra (maximum absorbance at 238 nm) obtained by a photodiode array detector in order to reduce the amount of compound consumed in the mouse bioassay. From the *Anabaena* cultures studied, 32 toxins were isolated: 3 from strain 90, 4 each from strains 60, 66, 141, and 202A2, 6 from strain CYA 83/1, and 7 from strain 202A1 (Table 2). The number of different toxins isolated per strain was greater when a larger amount of cells (5 to 10 g) was used for extraction (Table 2).

Results of amino acid analysis and molecular weights determined by FABMS of the isolated toxins are given in Table 3. Eight (A to H) of the seventeen toxins isolated were known microcystins (Tables 1 and 3; Fig. 1). Their structures were confirmed by direct comparison of their physicochemical properties with those of authentic samples. The known microcystins contained L-leucine plus L-arginine or two L-arginines as the variable L amino acid components. Methylation or demethylation on amino acids 3 and/or 7 was the other difference found among these toxins. Identities of compounds were assigned as follows: A, MCYST-LR; B, [D-Asp^3]MCYST-LR; C, [Dha^7]MCYST-LR; D, [D-Asp^3, Dha^7]MCYST-LR, E, MCYST-RR, F, [D-Asp^3]MCYST-RR; G, [Dha^7]MCYST-RR; and H, [D-Asp^3,Dha^7]MCYST-RR (Fig. 1). Nine compounds (I to Q) were new toxins having either an unidentified amino acid or differing in molecular weight (Table 3) from previously identified toxins (Table 1). The identification of these toxins will be completed at a later date.

DISCUSSION

Identification of the laboratory-cultured *Anabaena* strains used in this study was difficult since in culture the strains lost the characteristic morphological features used by phytoplankton taxonomists. According to the criteria of Starmach (40) and Tikkanen (42), strain 60 is most likely *A. flos-aquae* (rectangular-oval cells) and strains 90 and 141 are likely *A. circinalis* (round cells). Isolates from sample number 202 were initially thought to be *A. flos-aquae* (202A1) and *A. lemmermannii* (202A2), but reexamination of these strains after 2 years in culture did not show any difference in morphology. Strain 66 was tentatively identified as *A. lemmermannii* (38, 40). Because of uncertainties in taxonomy, we simply assigned the various *Anabaena* clones strain numbers.

When more material was used for toxin extraction, a greater number of toxins was found. This means that the differences between strains from the same lake isolated in the same year (90 and 141) or from the same water bloom sample (202A1 and 202A2) might not be real differences between the strains but rather a reflection of the amount of cells used in the toxin isolation. The toxin profiles of strains 90 and 141 were different; however, in strain 90, the main toxins found were MCYST-LR and MCYST-RR, but in strain 141, [D-Asp^3]MCYST-LR, and [D-Asp^3]MCYST-RR were more abundant. Since strains 202A1 and 202A2 originate from the same water bloom sample, it was not surprising that their major toxins are the same.

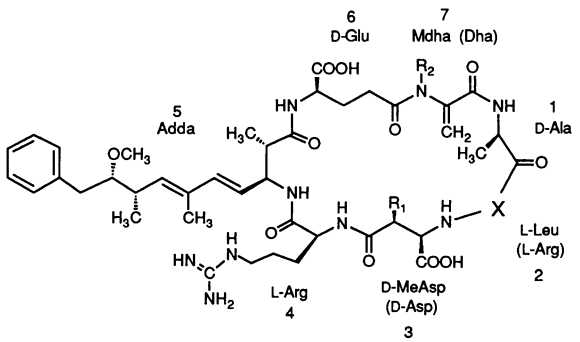
It is interesting that strains isolated in different years and even after a 1-month interval from Lake Vesijärvi did not produce the same toxins. There were also morphological differences between the strains. In the laboratory, toxins

TABLE 3. FABMS data and amino acid components of the toxins isolated from *Anabaena* spp.

Toxin	R_t[a]	M + H, *m/z*[b]	Ion formula[c]	Amino acids[d]
A	5.3	995	$C_{49}H_{75}N_{10}O_{12}$	D-Ala, L-Leu, D-MeAsp, L-Arg, D-Glu, $MeNH_2$
B	4.9	981	$C_{48}H_{73}N_{10}O_{12}$	D-Ala, L-Leu, D-Asp, L-Arg, D-Glu, $MeNH_2$
C	5.0	981	$C_{48}H_{73}N_{10}O_{12}$	D-Ala, L-Leu, D-MeAsp, L-Arg, D-Glu
D	4.8	967	$C_{47}H_{71}N_{10}O_{12}$	D-Ala, L-Leu, D-Asp, L-Arg, D-Glu
E	6.3	1038	$C_{49}H_{76}N_{13}O_{12}$	D-Ala, L-Arg, D-MeAsp, L-Arg, D-Glu, $MeNH_2$
F	6.1	1024	$C_{48}H_{74}N_{13}O_{12}$	D-Ala, L-Arg, D-Asp, L-Arg, D-Glu, $MeNH_2$
G	6.0	1024	$C_{48}H_{74}N_{13}O_{12}$	D-Ala, L-Arg, D-MeAsp, L-Arg, D-Glu
H	7.1	1010	$C_{47}H_{72}N_{13}O_{12}$	D-Ala, L-Arg, D-Asp, L-Arg, D-Glu
I	5.9	1063	$C_{52}H_{75}N_{10}O_{14}$	D-Ala, unk,[e] D-MeAsp, L-Arg, D-Glu, L-Ser
J	6.8	1045	$C_{52}H_{73}N_{10}O_{13}$	D-Ala, unk,[e] D-MeAsp, L-Arg, D-Glu
K	6.3	1031	$C_{51}H_{71}N_{10}O_{13}$	D-Ala, unk,[e] D-Asp, L-Arg, D-Glu
L	7.9	1029	$C_{52}H_{73}N_{10}O_{12}$	D-Ala, unk,[f] D-MeAsp, L-Arg, D-Glu
M	9.4	1009	$C_{50}H_{77}N_{10}O_{12}$	D-Ala, L-Leu, D-MeAsp, L-Arg, D-Glu, $MeNH_2$
N	8.7	995	$C_{49}H_{75}N_{10}O_{12}$	D-Ala, L-Leu, D-Asp, L-Arg, D-Glu, $MeNH_2$
O	5.6	1042	$C_{48}H_{76}N_{13}O_{13}$	D-Ala, L-Arg, D-MeAsp, L-Arg, D-Glu, L-Ser
P	4.5	999	$C_{48}H_{75}N_{10}O_{13}$	D-Ala, L-Leu, D-MeAsp, L-Arg, D-Glu, L-Ser
Q	4.6	999	$C_{48}H_{75}N_{10}O_{13}$	D-Ala, unk,[g] D-Asp, Arg, D-Glu, L-Ser

[a] R_t, Retention time in HPLC (ISRP column [4.6 by 250 mm; Regis Pinkerton], acetonitrile–100 mM phosphate buffer [pH 6.8, 15:85], flow rate of 1 ml/min).
[b] Obtained by a VG ZAB-SE mass spectrometer.
[c] Obtained by a VG 70-SE4F mass spectrometer.
[d] Waters Pico Tag HPLC system and gas chromatography with a Chirasil Val III column (29, 37). D-MeAsp, *erythro*-β-methylaspartic acid; $MeNH_2$, methylamine generated from the *N*-methyldehydroalanine unit by acid hydrolysis; unk, unknown amino acid.
[e] 195 Da, $C_{10}H_{13}NO_3$.
[f] 179 Da, $C_{10}H_{13}NO_2$.
[g] 145 Da, $C_7H_{15}NO_2$.

were extracted from cultured cells after 12 to 14 days, and all strains were cultured under similar conditions; therefore, the differences probably are not due to culture conditions. Even in the same lake there appear to exist many closely related *Anabaena* strains. The dominance of certain strains and hence the main toxins present might vary from sample to sample. Namikoshi and coworkers (29) have isolated 12 hepatotoxins from a *Microcystis* spp. bloom. These results indicate that the use of HPLC as a method to detect and quantitate toxin content of natural blooms will prove difficult unless a full range of toxin standards is available for comparison and identification.



toxin	assignment	X	R_1	R_2
A	MCYST-LR	L-Leu	CH_3	CH_3
B	[D-Asp^3]MCYST-LR	L-Leu	H	CH_3
C	[Dha^7]MCYST-LR	L-Leu	CH_3	H
D	[D-Asp^3,Dha^7]MCYST-LR	L-Leu	H	H
E	MCYST-RR	L-Arg	CH_3	CH_3
F	[D-Asp^3]MCYST-RR	L-Arg	H	CH_3
G	[Dha^7]MCYST-RR	L-Arg	CH_3	H
H	[D-Asp^3,Dha^7]MCYST-RR	L-Arg	H	H

FIG. 1. Structures of microcystins isolated from six strains of *Anabaena* spp.

The same toxins were isolated from strains in two of the lakes: strain 60 from Lake Ylä-Keyritty (Rautavaara, Finland) and strains 141 and 90 from Lake Vesijärvi (Lahti, Finland). These lakes are about 400 km apart, and there is no connection between them. Lake Vesijärvi is in the southern part of Finland, and since 1928 it has been known to produce toxic cyanobacteria blooms (17). Lake Ylä-Keyritty is a small lake in the central part of Finland with no known history of toxic cyanobacteria. The main toxins from strains 60 (MCYST-LR) and 141 ([D-Asp^3]MCYST-RR) were different, but in strains 60 and 90 they were the same.

Known toxins isolated from *Anabaena* strains used in this study were MCYST-LR and MCYST-RR and/or their demethyl analogs except for strain 66, which produced only unknown compounds. The Norwegian *A. flos-aquae* strain CYA 83/1 contained the same toxins as did strains 60, 90, and 141 plus two toxins which could not be identified by amino acid and FABMS data. Strains 202A1 and 202A2 did not produce methylated microcystins but instead produced demethylated forms (demethylation in amino acid 7 or in both amino acids 3 and 7) different from those produced by the rest of the strains (demethylation in amino acid 3) plus a few unknown compounds.

These results plus those of Krishnamurthy et al. (19) and Harada et al. (15) show that strains of *Anabaena* produce hepatotoxins in certain lakes of Scandinavia and Canada. Interestingly, the toxin production patterns from the majority of these strains have some common features. Toxins produced by all of the *Anabaena* strains studied so far except strain 66 contain MCYST-LR and/or its demethylated forms (aspartic acid and/or dehydroalanine) (15, 19; this study). MCYST-LR has been found to be the most commonly occurring heptapeptide hepatotoxin (5, 6) (Table 1). It has been found in many different *Microcystis* strains from

different parts of the world (5, 6, 8); its demethyl derivatives have been isolated from a *Microcystis* strain (16) and a bloom sample from Japan (14), and its homologs have been isolated from a *Nostoc* strain (28, 37). Most of the Scandinavian *Anabaena* strains (except strain 66) contain also MCYST-RR or its demethyl variants. MCYST-RR has also been found in *Microcystis* spp. in Japan and the United States (21, 29, 31, 43); its demethyl derivatives have been isolated from a Scandinavian *Oscillatoria* strain and water bloom (20, 25) and, in one case, from a Finnish *Microcystis aeruginosa* strain (18).

In summary, 17 different compounds were isolated from seven *Anabaena* strains from Scandinavia. Toxins were identified by their amino acid analysis and FABMS data and confirmed by direct comparison with authentic samples. Eight of these compounds were found to be known microcystins, and six of the eight are reported here for the first time as occurring in *Anabaena* isolates. The other nine compounds are new microcystins, and the structure assignments of these microcystins are in progress. Little is known about the ecological significance or biogenesis of these toxins. A recent study by DeMott et al. (11) showed that these toxins were poisonous to zooplankton, which might indicate that the toxins are produced by cyanobacteria in order to give them a competitive advantage. The only published study on the biogenesis of these compounds investigated the incorporation of radiolabeled precursors into the carbon backbone of the unusual amino acids, Adda and Masp, of microcystins (26). More research is needed to elucidate the genetics and biochemistry of toxin production.

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