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Identification of microcystins in a Lake Victoria cyanobacterial bloom using LC–MS with thiol derivatization☆

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ABSTRACT

Microcystins are cyclic heptapeptides from cyanobacteria which are responsible for poisonings of livestock and humans. Cyanobacteria also produce a range of peptides and other compounds that can result in complex chromatograms when samples are analysed by LC–MS. Thiol derivatization of the α,β-unsaturated amide present in most microcystins was recently shown to simplify analysis of LC–MS chromatograms of a *Microcystis* culture, making it easier to identify peaks corresponding to microcystins in complex mixtures. This method was applied to analysis of extracts taken from a natural cyanobacteria bloom in Mwanza Gulf, Lake Victoria, Tanzania, in 2010, revealing the presence of numerous putative microcystin analogues in the sample. Results were verified using LC–MS^2, LC–MS/MS with precursor-ion scanning, and LC–HRMS, leading to identification of 8 major and 17 minor microcystins in the sample, including analogues of microcystin-RY, -RL and -RA. Microcystin-YR (**2**), -RR (**3**), and -RY (**9**) were isolated from bloom material from Lake Victoria, and the structure of **9** was confirmed by NMR spectroscopic analysis and NMR spectral comparison with **2** and **3**. Confirmation of the structure of MC-RY (**9**) facilitated detailed analysis of its MS^2 spectrum, thereby supporting the structures of related analogues tentatively established on the basis of MS analyses.

1. Introduction

Microcystins (Fig. 1) are a group of more than 80 cyclic heptapeptide hepatotoxins produced by some freshwater cyanobacteria in the genera *Microcystis*, *Anabaena*, *Nostoc*, and *Planktothrix* (Codd et al., 1999; Sivonen and Jones, 1999; Welker and von Döhren, 2006). Microcystins are usually cell-bound in healthy cyanobacterial cells, but cell lysis can occur in senescent blooms leading to release of toxins into the surrounding water. Poisoning of wild and domesticated animals and humans has occurred due to the ingestion of microcystins. Microcystins can therefore be found in raw

Abbreviations: Aba, aminobutyric acid; Adda, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid; Apa, aminopropionic acid; Dha, dehydroalanine; Dhb, dehydrobutyrine; HRMS, high-resolution mass spectrometry; Mdha, *N*-methyldehydroalanine; Mdhb, *N*-methyldehydrobutyrine; MEMHEG, *O*-(2-mercaptoethyl)-*O*′-methyl-hexa(-ethylene glycol); Mser, *N*-methylserine.

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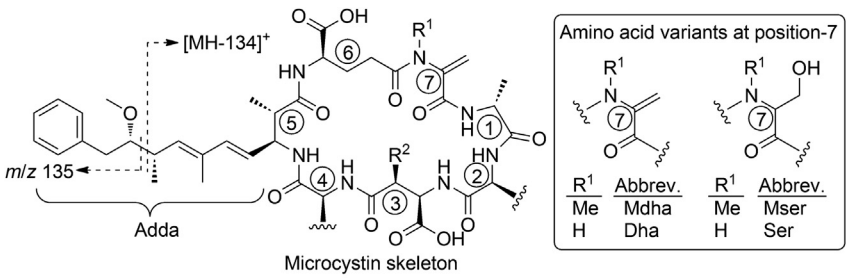

		②	④	⑦	R^1	R^2	$[MH]^+$ *m/z*
1	MC-LR	Leu	Arg	Mdha	Me	Me	995.5
2	MC-YR	Tyr	Arg	Mdha	Me	Me	1045.5
3	MC-RR	Arg	Arg	Mdha	Me	Me	1038.5
4	MC-LA	Leu	Ala	Mdha	Me	Me	910.5
5	MC-LF	Leu	Phe	Mdha	Me	Me	986.5
6	MC-LY	Leu	Tyr	Mdha	Me	Me	1002.5
7	MC-LW	Leu	Trp	Mdha	Me	Me	1025.5
8	[Dha^7]MC-LR	Leu	Arg	Dha	H	Me	981.5
9	MC-RY	Arg	Tyr	Mdha	Me	Me	1045.5
10	MC-RA	Arg	Ala	Mdha	Me	Me	953.5
11	MC-HilR	Hil	Arg	Mdha	Me	Me	1009.5
12	MC-FR	Phe	Arg	Mdha	Me	Me	1029.5
13	MC-RF	Arg	Phe	Mdha	Me	Me	1029.5
14	[$Mser^7$]MC-YR	Tyr	Arg	Mser	Me	Me	1063.5
15	[$Mser^7$]MC-LR	Leu	Arg	Mser	Me	Me	1013.5
16	[Asp^3]MC-RY	Arg	Tyr	Mdha	Me	H	1031.5
17	[Asp^3]MC-LR	Leu	Arg	Mdha	Me	H	981.5
18	MC-LY(OMe)	Leu	MeOTyr	Mdha	Me	Me	1032.5
19	MC-LL	Leu	Leu	Mdha	Me	Me	952.5
20	[Asp^3]MC-YR	Tyr	Arg	Mdha	Me	H	1031.5
21	[Dha^7]MC-YR	Tyr	Arg	Dha	H	Me	1031.5
22	[$Mser^7$]MC-RY	Arg	Tyr	Mser	Me	Me	1063.5
23	[Dha^7]MC-RY	Arg	Tyr	Dha	H	Me	1031.5
24	MC-RY(OMe)	Arg	MeOTyr	Mdha	Me	Me	1075.5
25	MC-RAba	Arg	Aba	Mdha	Me	Me	967.5
26	MC-RApa(1)	Arg	Apa	Mdha	Me	H	981.5
27	MC-RApa(2)	Arg	Apa	Dha	H	Me	981.5
28	MC-RL	Arg	Leu	Mdha	Me	Me	995.5
29	MC-YA	Tyr	Ala	Mdha	Me	Me	960.5
30	MC-YAba	Tyr	Aba	Mdha	Me	Me	974.5
31	MC-LAba	Tyr	Aba	Mdha	Me	Me	924.5

Fig. 1. Structures of microcystin analogues discussed in the text, with the seven amino acid residues labelled 1–7, and showing the Adda-fragmentation (*m/z* 135 and $[MH-134]^+$). Amino acid variations in the structures were present at sites 2–4 and 7, and these are indicated with standard 3-letter amino acid abbreviations (with the exception of (MeO)Tyr = methoxytyrosine at position 4, Adda = 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid at position 5, and Mdha = *N*-methyldehydroalanine, Dha = Dehydroalanine, and Mser = *N*-methylserine at position 7). **1**–**8** were available commercially as standards, and **9** was isolated and its structure verified by NMR spectroscopy.

and treated water samples, bloom material, fish and other animal tissues, as well as other types of biological materials (Sivonen and Jones, 1999). This can make microcystin analysis more difficult due to the presence of compounds from a range of cyanobacteria and algae, as well as the matrix components of the sample being analysed.

Derivatization of cyanobacterial samples with appropriate thiols has been shown to be useful in identifying [$Mdha^7$]- and [Dha^7]-microcystins during LC–MS analysis (Miles et al., 2012). Reaction with either mercaptoethanol or *O*-(2-mercaptoethyl)-*O′*-methyl-hexa(ethylene glycol) (MEMHEG) (**a** and **b**, respectively, in Fig. 2) proceeded rapidly and specifically, labelling reactive microcystins with extra mass (78 and 356 Da, respectively) in residue-7 (Fig. 2). The Mdhb-containing cyanotoxin nodularin did not react, suggesting that this procedure may be capable of differentiating between microcystins containing the isobaric amino acids Mdha (thiol-reactive) and Dhb (unreactive) at position-7 by LC–MS—without the need to resort to purification followed amino acid analysis or NMR spectroscopy. Mass spectral fragmentation of both the underivatized and thiol-derivatized microcystins was shown to give structurally informative fragments, including a fragment with *m/z* of $[MH-134]^+$ (i.e. Adda fragmentation, Fig. 1), during LC–MS^2 analysis with an ion trap mass spectrometer.

The potential utility of this approach was illustrated during method development, where application of the thiol-derivatization procedure resulted in identification of