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CYANOVIRIDIN RR, A TOXIN FROM THE CYANOBACTERIUM (BLUE-GREEN ALGA) MICROCYSTIS VIRIDIS

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Abstract. A toxin, named as cyanoviridin RR (1), has been isolated from the cyanobacterium *Microcystis viridis*. The structure of the toxin has been determined by modern NMR techniques such as the HMBC spectrum.

The occurrence of water blooms of cyanobacterium (blue-green alga) *Microcystis* in water bodies for public use are becoming a serious problem on the water management and public health, because some causative species are reported to produce toxins. The toxins, whose structures were clarified, were isolated from only *Microcystis aeruginosa* regarded as the most frequent producer of toxins.[1] In Japan, *M. viridis* is getting much attentions, because (i) it is the most predominant water-bloom alga at some of the important lakes,[2] which are utilized as reservoirs for water to drink, and (ii) the toxicity of the algal bodies was recently recognized.[3] Herein, we would describe the structure elucidation of a toxin, cyanoviridin RR (1), which was isolated from *M. viridis*.

An axenical clonal strain of *M. viridis* (NIES-102), isolated from a bloom on the Kasumigaura Lake,[4] was cultivated in MA medium, and the dried cells (100 g) were extracted with butanol. The extract was chromatographed monitoring toxicity against mice, to afford three toxic peptides, tentatively named as toxins A (70 mg), B (40 mg), and C (20 mg).

Acid hydrolysis of toxin A , $C_{49}H_{75}N_{13}O_{12}$, *m/z* 1039 (M^{+}+1) (FAB-MS),

dp 235 °C, $[\alpha]_D$ -95.2 (c = 0.104, H_2O), λ_{max} 239 nm (log ε 4.49), yielded a mixture of amino acids, in which alanine (Ala; 1 eq), glutamic acid (Glu; 1 eq), arginine (Arg; 2 eq), and β-methylaspartic acid (Masp; 1 eq) were de-

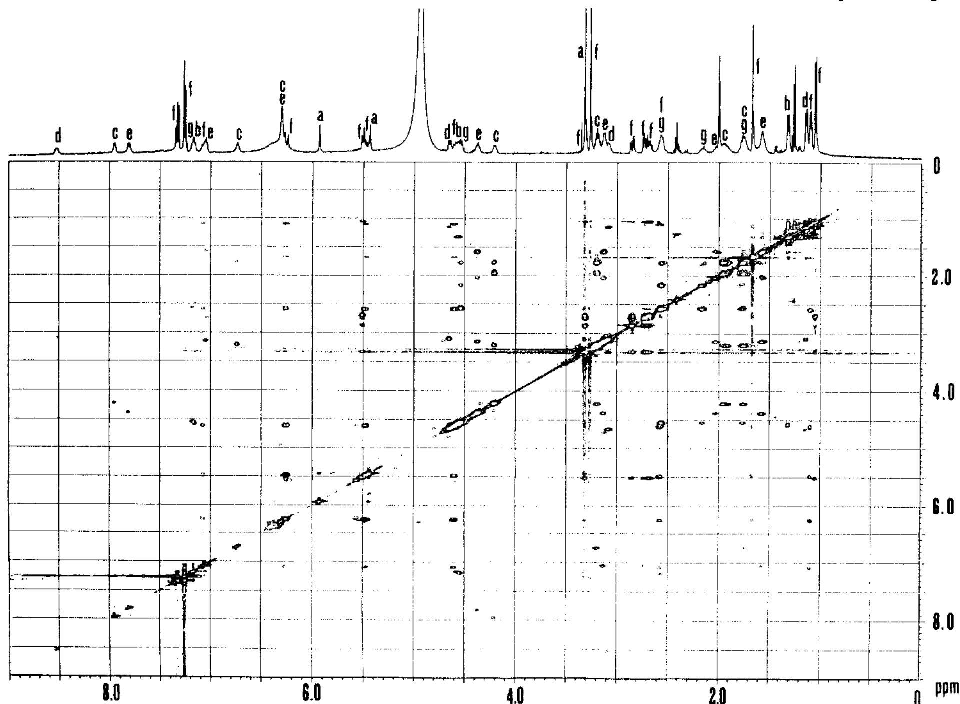
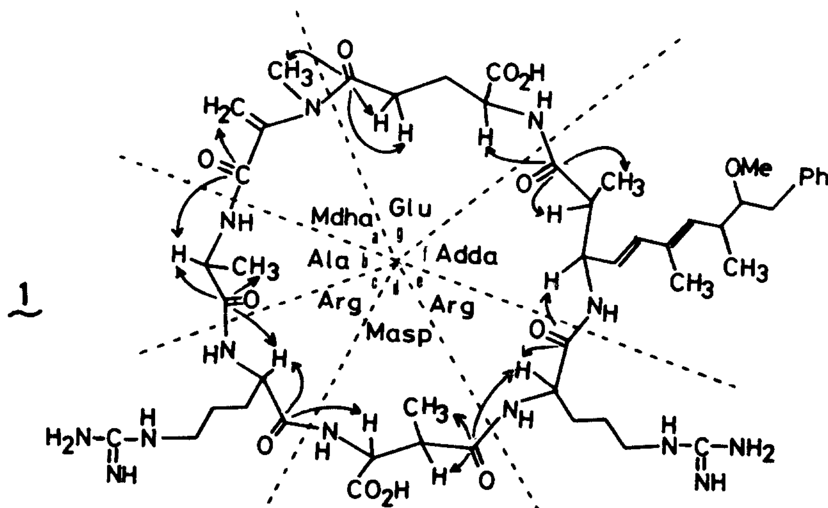


Fig. 1. The phase sensitive HOHAHA spectrum of 1 (6 mM in CD_3CN-TFA) measured on a Bruker AM-500 spectrometer; 2 x 512 x 2048 data matrix size; 24 scans per t_1 value; mixing time = 70 msec with a low-level transmitter. Letters a-g are for assignment of the protons of the amino acids denoted in 1



tected. Although the ^{1}H NMR spectrum of toxin A in D_2O exhibited sharp signals of the amino-acid components, it lacked the signals of the amide proton that were necessary to determine the amino-acid sequence by NMR spectrometry. Use of DMSO-d_6 as a solvent resulted only in broad and unresolved ^{1}H NMR signals. Acetonitrile-d_3 containing a minute amount of trifluoroacetic acid (TFA) was finally found to be an excellent solvent for toxin A. The HOHAHA spectrum[5] (Fig. I) taken in this solvent allowed us quick assignment of all the correlation peaks and, at the same time, the structure of all of the seven amino-acid fragments; Adda, Ala, Glu, Masp, Arg, Arg, and Mdha (N-methyl- -alanine). Of these, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) was reminiscent of cyanoginosins.[1]

Next, attention was focused on finding NOEs from the amide protons to the

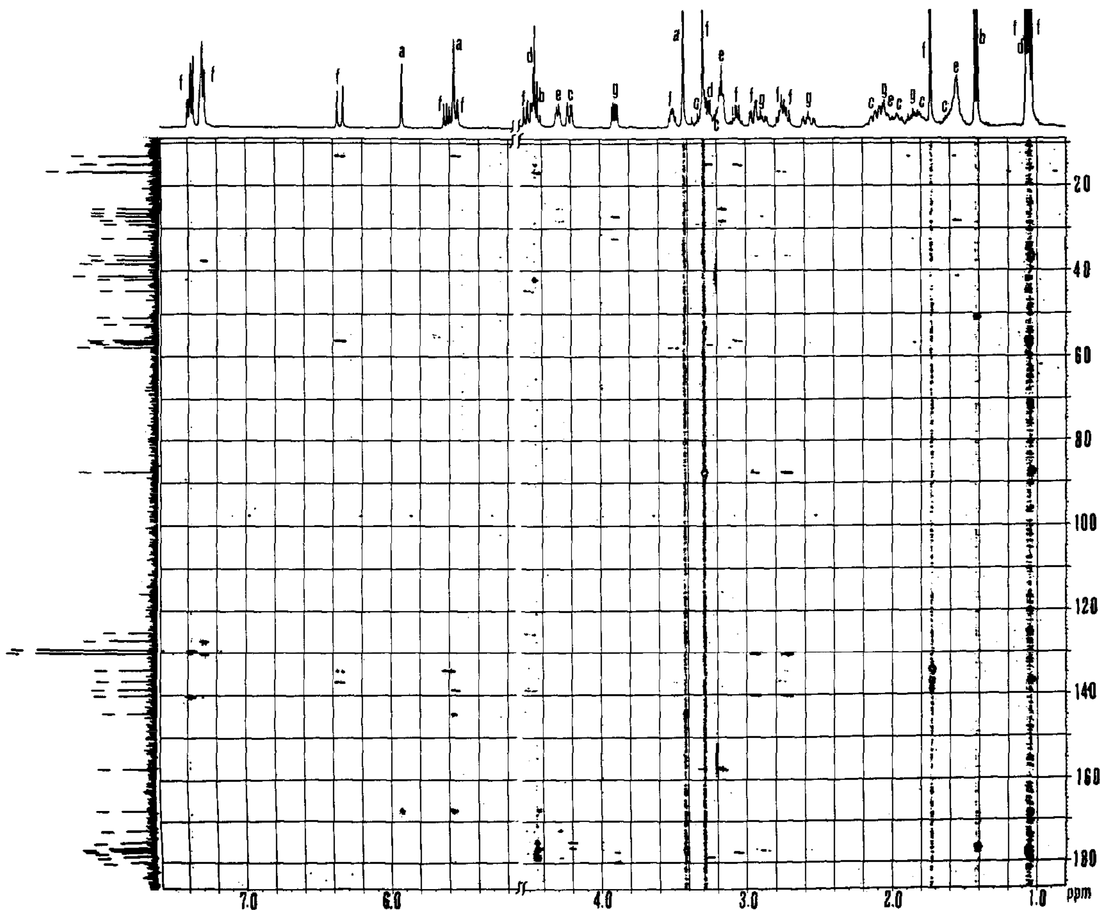
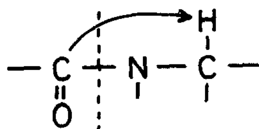


Fig. II. The 2D HMBC spectrum of 1 (6 mM in D_2O) measured on a Bruker AM-400 spectrometer using an inverse probe: 860 x 2048 data matrix size; 96 scans per t_1 value.

protons of the neighboring amino-acid units. The ROESY experiment[6] carried out in CD_3CN-TFA yielded a good amount of the NOE-correlation peaks from which the amino-acid arrangement of toxin A could be deduced as in 1.

There was, however, slight ambiguity in the sequence because of weak intensities of some cross peaks. A 2D-COLOC experiment[7] done to confirm the strucutre was unsuccessful, since the solubility of toxin A in the solvent was poor (6 mM) and the sample slowly decomposed in the acidic medium.

The HMBC spectrum[8] (Fig. II) exhibited the cross peaks due to the long-range couplings through the amide bonds from the carbonyl carbons to the protons of the neighboring amino acids as depicted in



1, which enabled us to determine the amino-acid sequence (1) without ambiguity. Referring to the designation of cyanoginosins made by Botes,[1] we named toxin A (1) as cyanoviridin RR. This is the first toxin isolated from *M. viridis*. Study on the stereochemistry of cyanoviridin RR, together with the structures of toxins B and C is in progress.

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