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Argimicin A, a Novel Anti-cyanobacterial Compound Produced by an Algae-lysing Bacterium

Sir:

Many problems caused by blooms of cyanobacteria have been reported[1] and the frequency of the blooms seems to be increasing. From investigation of aquatic ecosystems, it has been disclosed that microorganisms called algae-lysing bacteria have the ability to kill the organisms of water blooms[2]. In the course of our studies on the interactions between blue-green algae and algae-lysing bacteria, we found a strain of *Sphingomonas* sp. that produced a novel pentapeptide exhibiting high algaecidal activity against *Microcystis* spp. This communication describes the isolation and the structural elucidation of the active compound, argimicin A (**1**).

The producer was isolated from a water sample containing colonies of *Microcystis* sp. collected at Lake Biwa, and was classified into *Sphingomonas* sp. by taxonomic studies and 16S rDNA analysis. The fermentation of the strain was carried out in 1/10 Trypto-soy medium (Tryptone 1.5 g, soypeptone 1.5 g, NaCl 5 g, distilled water 1 liter) at 30°C with agitation and aeration for 48 hours. The filtrate of cultured broth was adjusted to pH 9 and passed through a Diaion HP-20 column. The column was washed with distilled water and the compound eluted with 70% aqueous MeOH containing 1% acetic acid. The active fractions were combined, concentrated to 10 ml *in vacuo*, and applied on a Toyopearl HW-40 column. The active principle was absorbed on the gel filtration resin, and was desorbed with a 0.1% trifluoroacetic acid aqueous solution. The eluate was evaporated to dryness under reduced pressure to yield a brown syrup. Further HPLC purification was carried out using an ODS column with 3.5% acetonitrile containing 0.01% trifluoroacetic acid as the solvent system. The fraction containing **1** was concentrated and dried in a vacuum oven at 35°C over night, and 10 mg of an colorless amorphous material was obtained.

The FAB-MS spectrum of **1** displayed a $[M+H]^+$ ion at *m/z* 743.5. From the data of the HRFAB-MS [found: 743.4888, calcd for $C_{32}H_{63}N_{12}O_8$: 743.4892] and the ^{13}C-NMR, the molecular formula of **1** was determined to be $C_{32}H_{62}N_{12}O_8$. **1** showed only end absorption in the UV spectrum. The data of IR spectrum [ν_{max} (KBr cm^{-1}); 3600(br.), 2924, 1745~1635, 1558, 1541] suggested the existence of a peptide skeleton. Furthermore, the signals of several tertiary sp^3 carbons and several non-protonated sp^2 carbons were also recorded in the ^{13}C-NMR spectrum around 60~70 and 170~180 ppm, respectively. Since these signals were attributable to be α-carbons of amino acids and carbonyl carbons of amide bonds, respectively, **1** was considered to be a pentapeptide or a hexapeptide. Two *N*-methyl carbon signals (28.4 and 33.2 ppm) and overlapping methyl carbon signals (53.4 ppm) were observed in the ^{13}C-NMR spectrum of **1**. The signal at 53.4 ppm was clarified to be the three *N*-methyl carbon signals from HMQC data (δ_H 3.22, 9H), and the existence of an *N*-trimethylammonium group was confirmed. The correlation data in the ^1H-^1H COSY, TOCSY spectra indicated the partial structures of the five side chains of the peptide, and two of these were attributed to Val residues as shown in Fig. 1. From the data of ^{13}C-NMR, HMQC and HMBC spectra in D_2O, the backbone of the pentapeptide including an *N*-trimethylammonium group as the *N*-terminal was unveiled.

In the TOCSY spectrum in DMSO-d_6, two protons correlated each other were observed at 6.68 and 7.08 ppm and the existence of a primary amide was disclosed. The position of the amide group was determined by pH shifts of ^{13}C-NMR signals in D_2O. The ^{13}C signals of α-carbonyl, α-, and β-carbon of the *C*-terminal residue were upfield shifted from 177.6, 60.0, and 25.6 ppm at neutral condition (pH 6.8), respectively, to 175.5, 58.1, and 24.7 ppm at pH 2.7. On the other hand, the γ-carbonyl carbon signal did not change its chemical shift, thus, *N*-methyl-Gln was comfirmed as the *C*-terminal. The remaining formula was $C_3H_{11}N_6$, including an *N*-methyl group, suggesting the existence of two guanidino groups. Correlation signals were observed in the HMBC spectrum between δ-proton signals of the two unusual amino acids and the remaining sp^2 carbons at 157.7 and 158.0 ppm, respectively. The latter carbon signal also correlated with the protons of the remaining *N*-methyl group. These results and comparison with the ^{13}C-NMR chemical shifts of Arg indicated that **1** consisted of a *N*-trimethyl-Arg as the *N*-terminal residue, two Val, 4-hydroxy-NG-methyl-Arg, and *N*-methyl-Gln as the *C*-terminal residue. Finally, the position of the NG-methyl group was proposed to connect to the terminal nitrogen atom by the correlation signal in DMSO-d_6 between the δ-proton and NH proton in the ^1H-^1H COSY spectrum. Thus, the structure of **1** was determined as shown in Fig. 2, and the assignments of all the ^{13}C-NMR signals are listed in Table 1.

Argimicin A exhibits strong activity against the toxic blue-green algae *Microcystis viridis* NIES-102 and *M.*

Fig. 1. The partial structures of side chain of amino acid residues.

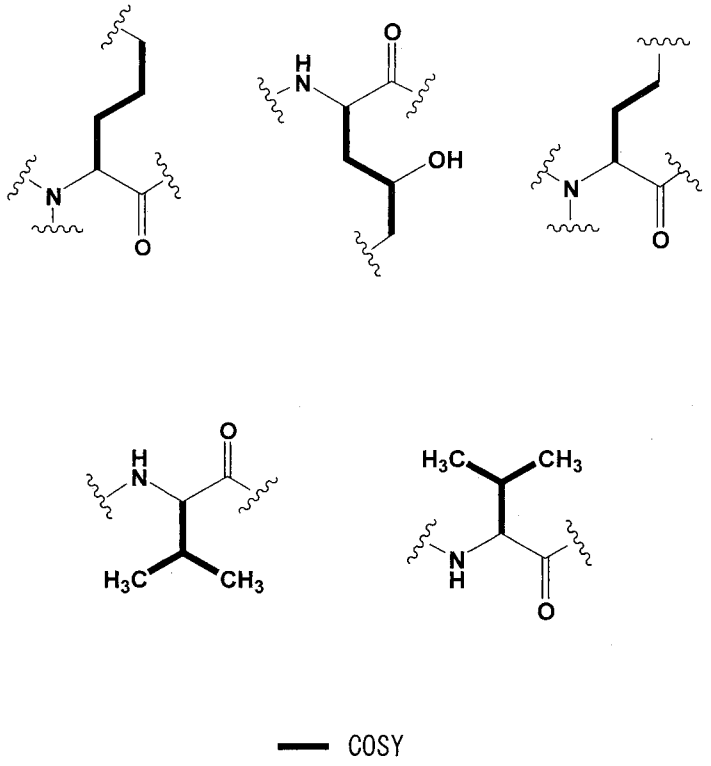
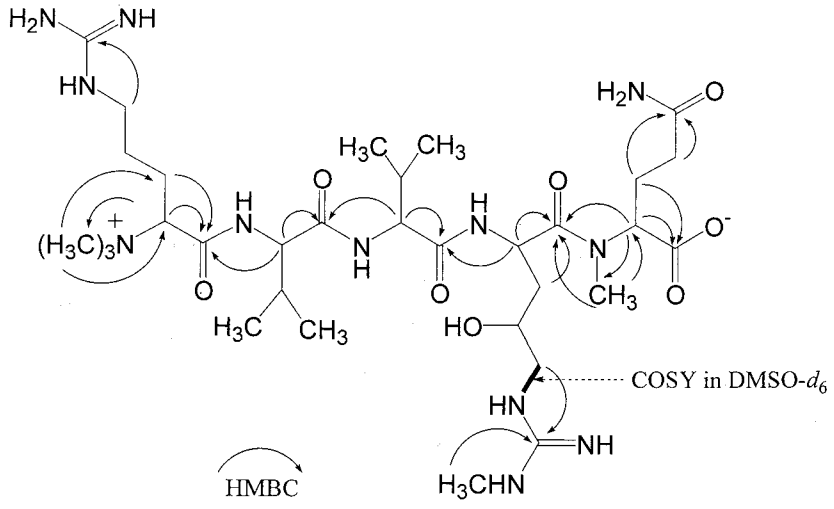


Fig. 2. The structure of argimicin A (**1**).



aeruginosa NIES-298 at the concentrations of 12 ng/ml and 100 ng/ml, respectively. On the other hand, it showed no effects on *Escherichia coli* IAM12119, *Bacillus subtilis* IFO3027, and chlorophyta *Chlorella vulgaris* IAMC-27. Furthermore, since argimicin A was a peptide and seemed to be easily decomposed by aquatic bacteria, the activities against non-axenic cyanobacteria isolated from the environment were tested. The compound was also active at 100 ng/ml against an unidentified filamentous cyanobacterium and the colony forming *Microcystis* sp.

Table 1. Assignments of ^{13}C-NMR signals (125 MHz, in D_2O, pH 6.8).

Carbon No.		^{13}C chemical shift (ppm)	
Me_3Arg	CO	167.44	s
	α	75.02	d
	β	24.54	t
	γ	25.36	t
	δ	41.40	t
	guanidino-C	157.69	s
	N-Me_3	53.44	q
Val[1]	CO	173.38	s
	α	61.04	d
	β	30.80	d
	β-Mea	19.18	q
	β-Meb	19.34	q
Val[2]	CO	173.64	s
	α	60.27	d
	β	31.27	d
	β-Mea	18.66	q
	β-Meb	19.34	q
OHMeArg	CO	173.99	s
	α	48.02	d
	β	36.07	t
	γ	67.48	d
	δ	47.68	t
	guanidino-C	158.04	s
	Ng-Me	28.43	q
MeGln	CO	177.60	s
	α	60.04	d
	β	25.60	t
	γ	32.09	t
	γ-CO	179.43	s
	N-Me	33.21	q

isolated from water bloom. The activities of known algaecides produced by algae-lysing bacteria were considered to be too weak to work in actual ecosystem. For example, the minimum inhibitory concentration of L-lysine produced by an aquatic actinomycete against *Microcystis* spp. has been reported to be 5 μg/ml[3]. Argimicin A might be one of the algaecides which have enough potency to show activity in a natural aquatic environment. Further studies on the stereochemistry and the ecological role in the aquatic ecosystem of **1** are under way.

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