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COMMUNICATIONS TO THE EDITOR

Argimicins B and C, New Anti-cyanobacterial Compounds Produced by *Sphingomonas* sp. M-17

Sir:

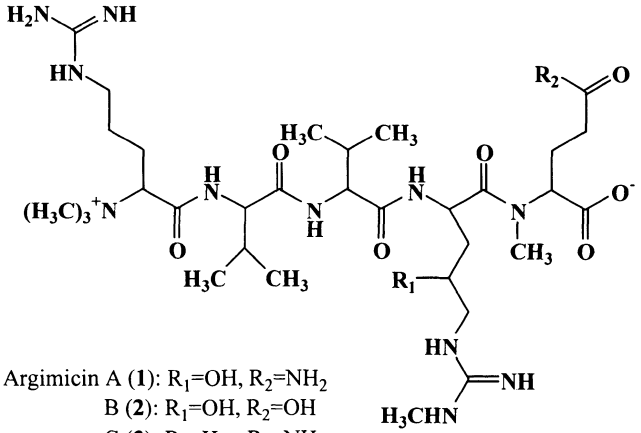
Blooms of cyanobacteria are widespread in lakes and reservoirs throughout the world. Based on knowledge of aquatic microbial ecosystem[1,2], we have surveyed the interactions between algae and algae-lysing bacteria[3]. In the course of the study, *Sphingomonas* sp. M-17 was found to produce a unique anti-cyanobacterial compound, argimicin A (**1**)[4], which exhibited potent and selective activities against cyanobacteria[5]. The strain produced mainly argimicin A. Other minor anti-cyanobacterial constituents produced were not enough for structure determination. Modifications of culture conditions improved the amounts of argimicins B and C, the two minor components. This communication describes the isolation, the structural elucidation and the biological activities of argimicins B and C.

In the original condition, strain M-17 was cultured in 1/10 Tryptosoy 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 pH increased to 9.6 with culture time and that was over a temperature range for growth of the strain. Thus, pH was controlled at optimum pH 7.0 of the strain during the cultivation and the activity rose two times by pH control. 1-Propanol was added to the culture filtrate (20 liters, 1% v/v) and the solution was applied to an activated charcoal column filled with 1% aqueous 1-propanol solution. The column was washed with 1% aqueous 1-propanol solution and eluted with 60% aqueous acetone containing 0.1% trifluoroacetic acid. The active fractions were collected and concentrated *in vacuo*. The concentrate was diluted in distilled water, adjusted to pH 9.5, and it was applied to a Diaion HP-20 column filled with water adjusted to pH 9.5. After washed with distilled water, the active principle was eluted with 70% aqueous MeOH containing 0.1% trifluoroacetic acid. The active fraction was concentrated and then chromatographed on a column of Toyopearl HW-40F with a 0.1% aqueous trifluoroacetic acid as a mobile phase. Active fractions were combined and evaporated to dryness. Further purification was carried out by preparative HPLC using a Cosmosil 5C18-AR column with 5% acetonitrile containing 0.02% trifluoroacetic acid. Sixteen mg of the new active compound, argimicin B (**2**) was obtained from 60 liters cultured fluid.

The molecular formula of **2** was confirmed as $C_{32}H_{61}N_{11}O_9$ from HRFAB-MS [found *m/z* 744.4728, calcd for $C_{32}H_{62}N_{11}O_9$ *m/z* 744.4732] and the ^{13}C-NMR data. Since the ^{1}H-NMR spectra of **1** and **2** were quite similar each other[4], **2** was suggested to be closely related to **1**. In the ^{13}C-NMR spectrum, three *N*-methyl carbon signals (28.5 and 33.4, 53.5 ppm) and two guanidino carbon signals (157.6 and 157.9 ppm) were observed. The signal at 53.5 ppm was clarified to be three *N*-methyl carbon signals from HSQC data (δ_H 3.22, 9H), and the existence of an *N*-trimethylammonium group was established. From ^{1}H-^{1}H COSY and TOCSY data, five partial structures were indicated and were connected to be a pentapepteide moiety by HMBC data. This partial structure was the completely same as that of **1**. However, although the remains were two hydrogen, one nitrogen and one oxygen atoms in the case of **1**, those were one hydrogen and two oxygen atoms in the case of **2**. These atoms were joined to two carbonyl carbon (175.2 and 178.0 ppm) as OH and O^-, respectively. Therefore, the structure of **2** was determined as shown Fig. 1 and the assignment of the ^{1}H and ^{13}C-NMR signals were listed in Table 1. As a result, a difference between **1** and **2** was only in the *C*-terminal amino acid, *N*-methyl-Gln and *N*-methyl-Glu in **1** and **2**, respectively.

Modification of culture conditions to improve the

Fig. 1. Structures of argimicins.



Argimicin A (**1**): R_1=OH, R_2=NH_2
B (**2**): R_1=OH, R_2=OH
C (**3**): R_1=H, R_2=NH_2

productivity of the strain had been continued aside from the isolation and the structural determination of **2**. As the results of efforts, the cultivation in 702 medium (polypeptone 10 g, yeast extract 2 g, $MgSO_4 \cdot 7H_2O$ 1 g, distilled water 1 liter) at 30°C for 96 hours with controlling pH 7.0 arose the activity 4 times more than that in above mentioned conditions. Furthermore, a novel constituent was recognized by HPLC analysis. The isolation method of the compound was the same as that of **2** except the mobile phase of HPLC, *i.e.*, 3.5~6.5% acetonitrile containing 0.02% trifluoroacetic acid in a linier gradient system. Five mg of argimicin C (**3**) was obtained from 1 liter cultured fluid.

From the data of HRFAB-MS [found *m/z* 727.4956, calcd for $C_{32}H_{63}N_{12}O_7$ *m/z* 727.4943] and ^{13}C-NMR, the molecular formula of **3** was decided to be $C_{32}H_{62}N_{12}O_7$. The ^{1}H-NMR spectrum of **3** was also similar to that of **1**[4] and it indicated **3** was an analogue of **1**, too. From ^{13}C-NMR and HSQC data, two *N*-methyl groups, two guanidine groups and *N*-trimethylammonium group were confirmed. The ^{1}H-^{1}H COSY spectrum of **3** established the proton-proton correlations of four partial structures attributed to *N*-Me_3-Arg, two Val and *N*-Me-Gln moieties in **1**. The correlations attributable to a partial structure consisting of α-methine and three methylenes were observed. This partial structure, one guanidine and one *N*-methyl groups were combined to be a NG-methyl-Arg moiety by the data of HMBC spectrum. Furthermore, HMBC spectral data allowed to build up a pentapeptide moiety, and the remaining two hydrogen, one nitrogen and one oxygen atoms. These atoms joined to two carbonyl carbon (179.4 and 177.1 ppm) as NH_2 and O^-, respectively. Thus, the structure of **3** was determined as shown Fig. 1, and the assignment of the ^{13}C-NMR signals were listed in Table 1. The structure of **3** contains NG-methyl-Arg moiety instead of 4-OH-NG-methyl-Arg in **1**.

The productions of **2** and **3** are observed only by cultivation in 1/10 Tryptosoy and 702 media, respectively, although **1** is produced in both media. Two new compounds exhibit strong activities against all tested cyanobacteria as

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

Argimicin B (**2**)			Argimicin C (**3**)		
Carbon		δ_C(ppm)	Carbon		δ_C(ppm)
Me_3Arg	CO	167.3	Me_3Arg	CO	167.4
	α	75.0		α	75.0
	β	24.6		β	24.5
	γ	25.4		γ	25.3
	δ	41.4		δ	41.4
	guanidino-	157.6		guanidino-	157.4
	N-Me_3	53.5		N-Me_3	53.4
Val[1]	CO	173.2	Val[1]	CO	173.3
	α	61.1		α	61.0
	β	30.9		β	30.8
	β-Me_a	18.7		β-Me_a	18.7
	β-Me_b	19.3		β-Me_b	19.2
Val[2]	CO	173.5	Val[2]	CO	173.4
	α	60.2		α	60.3
	β	31.4		β	31.1
	β-Me_2	19.4		β-Me_2	19.3
OHMeArg	CO	174.2	MeArg	CO	174.0
	α	47.8		α	50.5
	β	36.1		β	28.7
	γ	67.5		γ	25.3
	δ	47.8		δ	41.4
	guanidino-	157.9		guanidino-	157.7
	NG-Me	28.5		NG-Me	28.7
MeGlu	CO	175.2	MeGln	CO	177.1
	α	58.9		α	59.8
	β	23.9		β	33.1
	γ	31.2		γ	25.4
	γ-CO	178.0		γ-CO	179.4
	N-Me	33.4		N-Me	32.3

Table 2. Antimicrobial activity of argimicins B (**2**) and C (**3**).

Test organisms	MIC (μg/ml)	
	Argimicin B (**2**)	Argimicin C (**3**)
Cyanobacteria		
Microcystis viridis NIES-102	0.098	0.190
Microcystis aeruginosa NIES-298	0.049	0.190
Synechocystis sp. PCC6803	0.780	0.780
Merismopedia tenuissima NIES-230	6.250	3.130
Spirulina platensis NIES-45	3.130	3.130
Aphanizomenon flos-aquae NIES-81	0.098	0.049
Fischerella major NIES-592	50	50
Green Algae		
Chlorella vulgaris IAM C-27	>200	>200
Chlorella kessleri IAM C-143	>200	>200
Scenedesmus sp. 1032	>200	>200
Scenedesmus sp. 1034	>200	>200
Scenedesmus sp. 1039	>200	>200
Bacteria		
Escherichia coli NBRC3301	>1000	>1000
Bacillus subtilis NBRC3027	>1000	>1000
Yearst		
Saccharomyces cerevisiae DKD-5D	>1000	>1000

demonstrated in Table 2. On the other hand, they have no activity against *Escherichia coli* NBRC3301, *Bacillus subtilis* NBRC3027, *Chlorella vulgaris* IAM C-27, *Chlorella kessleri* IAM C-143, *Scenedesmus* sp. and *Saccharomyces cerevisiae* DKD-5D. From these results, they show also potent and selective activities against cyanobacteria. However, it is noteworthy that the anti-cyanobacterial activities of these compounds are 2 to 10 times weaker than those of **1**. Therefore, the amino group of the *C*-terminus and the hydroxyl group of the next residue in **1** are important for its potent activities. The relationship between structure and activity and detailed action mechanisms of argimicins are under investigations.

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References

1) YAMAMOTO, Y.; T. KOUCHIWA, Y. HODOKI, K. HOTTA, H. UCHIDA & K.-I. HARADA: Distribution and identification of actinomycetes lysing bacteria in a eutrophic lake. J. Appl. Phycol. 10: 391~397, 1998
2) MITSUTANI, A.; A. UCHIDA & Y. ISHIDA: Occurrence of blue-green algae and algal lytic bacteria in Lake Biwa. Nihon Biseibutsu Seitai Gakkaiho 2: 21~28, 1987
3) IMAMURA, N.; I. MOTOIKE, N. SHIMADA, M. NISHIKIORI, H. MORISAKI & H. FUKAMI: An efficient screening approach for anti-*Microcystis* compounds based on knowledge of aquatic microbial ecosystem. J. Antibiotics 54: 582~587, 2001
4) IMAMURA, N.; I. MOTOIKE, M. NODA, K. ADACHI, A. KONNO & H. FUKAMI: Argimicin A, a novel anti-cyanobacterial compound produced by an algae-lysing bacterium. J. Antibiotics 53: 1317~1319, 2000
5) HIBAYASHI, R. & N. IMAMURA: Action mechanism of a selective anti-cyanobacterial compound, argimicin A. J. Antibiotics 56: 154~159, 2003

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