Pergamon

PII: S0031-9422(96)00767-4

Phytochemistry, Vol. 44, No. 8, pp. 1503–1505, 1997

0031-9422/97 $17.00+0.00

A 3-AMINO-10-CHLORO-2-HYDROXYDECANOIC ACID-CONTAINING TETRAPEPTIDE FROM *OSCILLATORIA AGARDHII*

TOMOHARU SANO* and KUNIMITSU KAYA

Environmental Chemistry Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan

(*Received* 23 *September* 1996)

Key Word Index—*Oscillatoria agardhii*; Cyanophyceae; Cyanobacterium; peptide; oscillaginin A.

Abstract—Oscillaginin A, a novel chlorine-containing linear tetrapeptide, was isolated from the freshwater toxic cyanobacterium, *Oscillatoria agardhii*. The structure was elucidated by chemical degradation and 2D NMR analyses. Oscillaginin B, a dechlorinated derivative of oscillaginin A, was also isolated from the same cells. Copyright © 1997 Elsevier Science Ltd

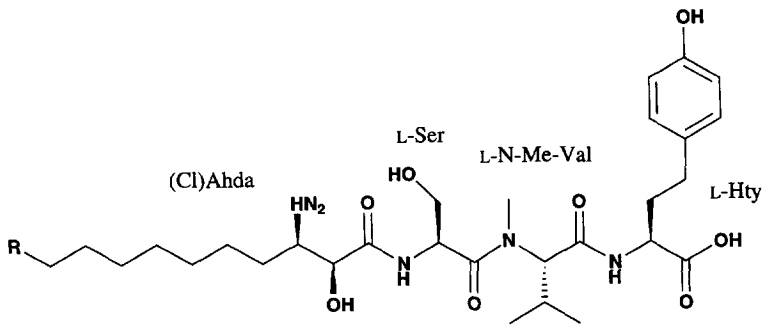
INTRODUCTION

The toxic strain of the cyanobacterium (blue-green alga), *Oscillatoria agardhii*, forms blooms in freshwater lakes and drinking water reservoirs, and produces heptacyclic peptide hepatotoxins, named microcystins [1]. During investigations of cyclic peptide toxins [2] of the toxic strain of *O. agardhii*, we found a novel chlorinated 3-amino-2-hydroxydecanoic acid-containing tetrapeptide (**1**). Furthermore, we also found a closely related nonchlorinated tetrapeptide (**2**). We describe herein the isolation and structural elucidation of the two new compounds.

RESULTS AND DISCUSSION

The chloroform–methanol–water (1:3:1) extract from freeze-dried cells was suspended in a 5% acetic acid aqueous solution. The suspension was filtered and the filtrate fractionated by solid-phase extraction using an ODS cartridge (Sep-Pak ODS). The compounds were further purified by reverse-phase HPLC. The yields of oscillaginin A (**1**) and oscillaginin B (**2**) were 3.0 and 1.0 mg, respectively.

Oscillaginin A (**1**) is an amorphous solid. A $[M+H]^+$ in the positive FAB-mass spectrum, using glycerol as a matrix, was observed at *m/z* 615; a $[M+H+2]^+$ was also observed at *m/z* 617. The intensity of the $[M+H+2]^+$ was equivalent to 32.6% of that of $[M+H]^+$, suggesting that compound **1** contained one chlorine atom in its molecule. The molecular formula of compound **1** was established as $C_{29}H_{47}O_8N_4Cl$ from high-resolution FAB-mass spectrometry data. The NMR data (Table 1) suggested that the compound is a peptide. The amino acids



1: R=Cl Oscillaginin A
2: R=H Oscillaginin B

* Author to whom correspondence should be addressed.

Table 1. ^{1}H* and ^{13}C† NMR data for oscillaginins A (**1**) and B (**2**) in DMF-d_7

Position		**1**			**2**		
		^{1}H	J (Hz)	^{13}C	^{1}H	J (Hz)	^{13}C
Hty	1			175.6			175.5
	2	4.22	*dd*, 4.9, 12.2	53.9	4.21	*m*	54.5
	3	2.01	*m*	35.2	2.03	*m*	35.2
		1.91	*m*		1.92	*m*	
	4	2.50	*m*	31.4	2.52	*m*	31.5
		2.46	*m*		2.48	*m*	
	5			132.9			132.9
	6,10	6.99	*d*, 8.2	129.9	7.01	*d*, 8.2	129.9
	7,9	6.72	*d*, 8.2	115.7	6.73	*d*, 8.2	115.7
	8			156.5			156.6
	NH	7.67	*d*, 7.0		7.68	*d*, 7.3	
N-Me-Val	1			169.7			169.8
	2	4.74	*d*, 11.0	63.4	4.76	*d*, 10.7	63.5
	3	2.27	*m*	26.9	2.28	*m*	26.9
	4	0.98	*d*, 6.4	20.1	1.00	*d*, 6.4	20.2
	4′	0.81	*d*, 6.7	19.1	0.82	*d*, 6.4	19.1
	N-Me	3.17	*s*	31.5	3.18	*s*	31.4
Ser	1			172.2			172.2
	2	5.02	*m*	52.5	5.03	*m*	52.5
	3	3.83	*m*	62.8	3.85	*m*	62.9
		3.74	*m*		3.75	*m*	
	NH	8.29	*d*, 8.0		8.31	*d*, 7.3	
Ahda(Cl)	1			172.9			173.0
	2	4.18	*br*	72.5	4.16	*d*, 3.7	72.7
	3	3.35	*br*	54.5	3.33	*m*	54.6
	4	1.71	*m*	31.9	1.72	*m*	32.2
		1.60	*m*		1.58	*m*	
	5	1.46	*m*	26.1	1.46	*m*	26.3
	6	1.28	*m*	29.9	1.27	*m*	29.9
	7	1.28	*m*	29.1	1.25	*m*	23.0
	8	1.40	*m*	27.2	1.25	*m*	23.0
	9	1.71	*m*	33.1	1.25	*m*	29.2
	10	3.62	*t*, 6.7	45.9	0.86	*t*, 7.0	14.2

* Recorded at 500 MHz (δ values).
† Recorded at 125 MHz (δ values).

detected by analysis of the hydrolysate (6 N HCl, 110°, 20 hr) were *N*-methylvaline (*N*-Me-Val), homotyrosine (Hty) and serine (Ser). Hty, N-Me-Val and Ser were shown to have the L-configuration by chiral GC analysis (Chirasil-L-Val capillary column, 0.25 mm i.d. × 25 m) of the *N*-trifluoroacetyl isopropyl ester derivatives of the hydrolysate [2]. Extensive NMR analyses using ^{1}H–^{1}H COSY and HMBC revealed the presence of a novel β-amino acid residue, 3-amino-10-chloro-2-hydroxydecanoic acid (ClAhda). The signal of H-10 in ClAhda was found at 3.62 ppm, and formed a triplet. The C-10 signal appeared at 45.9 ppm, and was shown to be a methylene carbon from the ^{13}C DEPT spectrum of compound **1**. These results showed that the chlorine atom was attached to C-10. The sequence of compound **1** was deduced mainly by HMBC correlations from N-H or N-Me to C-O.

Oscillaginin B (**2**) is also an amorphous solid. Its molecular formula was established to be $C_{29}H_{48}O_8N_4$ by positive high-resolution FAB-mass spectrometry. The spectral data (Table 1) of compound **2** were very similar to those of compound **1**, except for the end of Ahda. The detected three L-amino acids of oscillaginin B were the same as those of **1**. These results suggested that oscillaginin B was the nonchlorinated derivative of oscillaginin A. The sequence of compound **2** was deduced in the same way as compound **1**. The same β-amino acid residue has been found in microginin [3] and the configuration has been determined as [2*S*, 3*R*] [4]. The ^{1}H and ^{13}C NMR data of (Cl)Ahda in oscillaginin A and B resemble closely those of microginin. Therefore, the configuration of (Cl)Ahda in compounds **1** and **2** were determined to be the same as microginin.

Oscillaginin A (**1**) is a chlorinated 2-hydroxy-3-amino acid-containing tetrapeptide. Chlorinated 2-hydroxy-β-amino acid-containing cyclic peptides, the puwainaphytins, have been isolated from the cyanobacterium, *Anabaena* sp. [5]. The structures of the

oscillaginins thus resemble that of microginin, a Ahda-containing linear pentapeptide from the freshwater cyanobacterium, *Microcystis aeruginosa*.

EXPERIMENTAL

Cultivation of cyanobacterium. Oscillatoria agardhii (NIES-610 = CCAP 1459/22 = NIVA CYA 18) was provided by Dr John G. Day cultured in 10 l bottles with CT medium [6]. Cells were grown isothermally at 20° (light intensity, below 250 μmol photon m^{-2} s^{-1}; aeration rate, 1.5 l min^{-1}).

Extraction and isolation. The $CHCl_3$–MeOH–H_2O (1:3:1) extract from freeze-dried cells (6.6 g) was suspended in 5% HOAC aq. soln. The suspension was filtered and the filtrate fractionated by solid-phase extraction using an ODS cartridge (Sep-Pak ODS). The compounds were further purified by reverse-phase HPLC (Ultron ODS, 8 mm i.d. × 25 cm, flow rate 3 ml min^{-1}) with MeOH (55%) containing 0.05 M phosphate (pH 3). The yields of oscillaginin A (**1**) and oscillaginin B (**2**) were 3 mg and 1 mg, respectively.

Oscillaginin A (**1**). FAB HR-MS: *m/z* 615.3126, $[M+H]^+$ (Calcd for $C_{29}H_{48}O_8N_4{}^{35}Cl$: 615.3160); *m/z* 617.3122, $[M+H+2]^+$ (Calcd for $C_{29}H_{48}O_8N_4{}^{37}Cl$: 617.3131). UV λ_{max}^{MeOH} nm (log ε): 279 (3.2). $[\alpha]_D^{29}$ −90° (MeOH, *c* 0.5). ^1H and ^{13}C NMR: Table 1.

Oscillaginin B (**2**). FAB HR-MS: *m/z* 581.3586, $[M+H]^+$ (Calcd for $C_{29}H_{49}O_8N_4$: 581.3550). UV λ_{max}^{MeOH} nm (log ε): 279 (3.2). $[\alpha]_D^{29}$ −110° (MeOH, *c* 0.5). ^1H and ^{13}C NMR: Table 1.

Acknowledgements—The authors are grateful to Drs H. Ito, J. Hayashi and S. Serizawa for GC-mass spectrometry and high-resolution FAB-mass spectrometry. We also thank Dr John G. Day for providing the cyanobacterium and Dr Mark Bradley for gifts of L- and D-homotyrosine.

REFERENCES

1. Luukkainen, R., Sivonen, K., Namikoshi, M., Farding, M., Rinehart, K. L. and Niemela, S. I., *Applied Environmental Microbiology*, 1993, **59**, 2204.
2. Sano, T. and Kaya, K., *Tetrahedron Letters*, 1995, **36**, 8603.
3. Okino, T., Matsuda, H., Murakami, M. and Yamaguchi, K., *Tetrahedron Letters*, 1993, **34**, 501.
4. Matsuura, F., Hamada, Y. and Shioiri, T., *Tetrahedron*, 1994, **50**, 11303.
5. Moor, R. E., Bornemann, V., Niemczura, W. P., Gregson, J. M., Chen, J.-L., Norton, T. R., Patterson, G. M. L. and Helms, G. L., *Journal of the American Chemistry Society*, 1989, **111**, 6128.
6. Watanabe, M. M. and Nozaki, H., *List of Strains of Microalgae and Protozoa* (NIES-Collection, Fourth Edition). Microbial Culture Collection, Natl Inst. Environ. Stud., Tsukuba, Japan, 1994, p. 31.