Oscillapeptin G, a Tyrosinase Inhibitor from Toxic *Oscillatoria agardhii*

Tomoharu Sano* and Kunimitsu Kaya

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

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Oscillapeptin G, a tyrosinase inhibitor, was isolated from the freshwater toxic cyanobacterium *Oscillatoria agardhii*. The structure was determined to be **1** by chemical degradation and 2D NMR analyses.

Toxic strains of the cyanobacterium (blue-green alga) *Oscillatoria agardhii* Gomont (Oscillatoriales, Oscillatoriaceae) living in freshwater lakes and drinking-water reservoirs produce cyclic heptapeptide hepatotoxins named microcystins.[1] During investigations of cyclic peptide toxins of the toxic strain of *O. agardhii* we found a novel tyrosinase-inhibiting cyclic depsipeptide. We now describe the isolation and structure elucidation of oscillapeptin G (**1**) (Figure 1).

The $CHCl_3$–MeOH–H_2O (1:3:1, v/v) extract from freeze-dried cells (6.6 g) was suspended in aqueous 5% acetic acid solution, and the suspension was filtered. The filtrate, a potent tyrosinase inhibitor,[2] was subjected to solid-phase extraction and reversed-phase HPLC to give oscillapeptin G (**1**) in 0.24% yield. HRFABMS established the molecular formula $C_{53}H_{77}O_{17}N_9$. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) of **1** suggested that the compound is a peptide. The amino acids detected by amino acid analysis of the hydrolysate were homotyrosine (Hty), glutamic acid (Glu), threonine (Thr), leucine (Leu), *N*-methyltyrosine (*N*-Me-Tyr), and isoleucine (Ile). Glu, Thr, Leu, and Ile were shown to have the L-configuration by chiral GC analysis. Extensive NMR analysis by ^{1}H–^{1}H COSY and HMBC spectra revealed the spin systems of six amino acids but not Thr-2. The presence of an *N,N*-disubstituted derivative of Thr-2 was suggested due to the absence of the amido proton. On another Thr unit (Thr-1), the chemical shift of H-3 (δ 5.70 ppm) suggested that the hydroxyl group of Thr-1 was acylated. The structure of 3-amino-6-hydroxy-2-piperidone (Ahp) was deduced by COSY and HMBC spectra.[3,4] In the COSY spectra, the connectivities from NH (δ 7.57 ppm) to 6-OH (δ 6.23 ppm) of Ahp were determined. In addition, the chemical shifts of H-6 (δ 5.37 ppm) and C-6 (δ 76.5 ppm) suggested that C-6 was substituted with O and N. Furthermore C-2 correlated with H-3, H-6, and the α-H of the Thr-2 derivative, and C-6 correlated with the α-H of the Thr-2 derivative in an HMBC spectrum. Consequently, Ahp was deduced to be a part of a hemiaminol structure formed from glutamate γ-semialdehyde and Thr-2. The remaining unit was identified as glyceric acid by GC–MS, COSY and HMBC spectra, and chiral GC analysis of its isopropyl ester indicated the D-configuration. The sequence of **1** was mostly deduced by HMBC correlations from N–H to C=O (Figure 1). The HMBC correlation from β-H of Thr-1 to C=O of Ile confirmed the ester formation between Thr-1 and Ile (Figure 1). The methyl proton of *N*-Me-Tyr correlated with C=O of Thr-2 in the HMBC spectrum. Furthermore Thr-2 and Ahp were connected as a hemiaminol as mentioned above. From NMR and MS data, the Glu detected by amino acid analysis of the hydrolysate must have derived from glutamine (Gln) in **1**. These data established the structure of oscillapeptin G (Figure 1), where the G indicates the glyceric acid unit. Studies on the configurations of Hty, *N*-Me-Tyr and Ahp are now in progress.

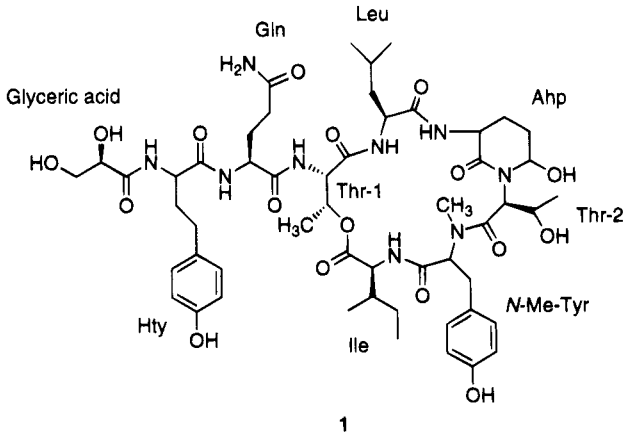

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Figure 1. Structure of oscillapeptin G.

Ahp-containing cyclic depsipeptides have been isolated from the sea hare *Dolabella auricularia*[3] and the blue-green alga *Microcystis aeruginosa*.[4–7] Oscillapeptin G is also an Ahp-containing cyclic depsipeptide, but it contains glyceric acid and homotyrosine as additional structural units.

Oscillapeptin G strongly inhibited tyrosinase activity to form melanin from tyrosine at 1×10^{-4} M. Microviridin, a tricyclic depsipeptide from the toxic cyanobacterium *Microcystis viridis*, also inhibits tyrosinase activity[8]; the tyrosinase inhibitory effect of oscillapeptin G was almost the same as that of microviridin.

Experimental Section

General Procedures. NMR spectra were recorded on a JEOL JNM A-500 spectrometer (500 MHz). ^{1}H- and ^{13}C-NMR chemical shifts are referenced to TMS. Homonuclear ^{1}H connectivities were determined by COSY and HOHAHA experiments, and heteronuclear ^{1}H-^{13}C connectivities were determined by HSQC and HMBC experiments. LRMS and HRMS were performed with a JEOL JMS-700 spectrometer. Specific rotation was obtained on an Atago POLAX-D polarimeter, and the IR spectrum was measured with a Shimadzu FTIR-8100M spectrometer.

Culture Conditions. *O. agardhii* was obtained from the National Institute for Environmental Studies Mi-

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Table 1. ^{1}H and ^{13}C-NMR Data for Oscillapeptin G in DMF-d_7

position		^{1}H	J (Hz)	^{13}C	HMBC correlations from C to
glyceric acid	1			173.2	Hty NH, glyceric acid H-3
	2	4.13	(dd, 4.0, 4.6)	74.3	
	3	3.78	(m)	65.3	
		3.73	(m)		
	2-OH	5.77	(br)		
	3-OH	4.93	(br)		
Hty	1			172.1	Hty H-2, Gln NH
	2	4.52	(dt, 4.9, 8.2)	52.9	
	3	2.12	(m)	34.2	
		1.98	(m)		
	4	2.61	(m)	30.4	Hty H-6,10
		2.54	(m)		
	5			128.4	
	6, 10	7.05	(d, 8.9)	130.0	
	7, 9	6.76	(d, 8.9)	116.2	
	8			156.6	
	NH	7.97	(d, 8.2)		
	OH	7.43	(br)		
Gln	1			173.0	Gln H-2, Thr-1 NH
	2	4.61	(m)	53.4	
	3	2.03	(m)	28.9	
		1.96	(m)		
	4	2.35	(m)	32.2	
		2.27	(m)		
	5			175.0	Gln H-4
	NH	8.18	(d, 7.6)		
	5-NH_2	9.30	(br)		
Thr-1	1			170.5	Thr-1 H-2, Leu NH
	2	4.87	(d, 9.2)	56.3	
	3	5.70	(q, 6.4)	72.8	
	4	1.33	(d, 6.4)	18.3	
	NH	8.30	(d, 9.2)		
Leu	1			173.7	Leu H-2, Ahp NH
	2	4.47	(m)	52.1	
	3	1.93	(m)	40.2	
		1.46	(m)		
	4	1.63	(m)	25.3	
	5	0.88	(d, 6.7)	21.3	
	5′	0.80	(d, 6.7)	23.6	
	NH	8.39	(d, 8.6)		
Ahp	2			170.5	Ahp H-3, H-6, Thr-2 H-2
	3	4.60	(m)	50.0	
	4	2.75	(m)	22.7	
		1.82	(m)		
	5	2.06	(m)	28.9	
		2.83	(m)		
	6	5.37	(br)	76.5	Thr-2 H-2
	NH	7.57	(d, 9.2)		
	OH	6.23	(br)		
Thr-2	1			172.3	Thr-2 H-2, *N*-Me-Tyr H-Me, H-2
	2	4.59	(d, 7.6)	55.4	
	3	3.63	(m)	66.5	
	4	0.46	(d, 6.1)	19.5	
N-Me-Tyr	1			170.4	*N*-Me-Tyr H-2, Ile N-H
	2	5.15	(dd, 11.8, 2.9)	61.9	*N*-Me-Tyr H-Me
	3	3.24	(dd, 14.5, 2.9)	34.1	*N*-Me-Tyr H-5,9
		2.78	(dd, 14.5, 11.8)		
	4			132.8	
	5, 9	7.07	(d, 8.9)	131.2	
	6, 8	6.75	(d, 8.9)	115.8	
	7			157.5	
	Me	2.79	(s)	30.4	
	OH	6.72	(br)		
Ile	1			173.7	Ile H-2, Thr-1 H-3
	2	4.75	(dd, 9.5, 5.8)	56.7	
	3	1.89	(m)	38.3	
	4	1.34	(m)	25.5	
		1.09	(m)		
	5	0.83	(t, 7.3)	11.5	
	6	0.90	(d, 6.7)	16.5	
	NH	7.83	(d, 9.2)		

crobial Culture Collection (NIES-610 = CCAP 1459/22 = NIVA CYA 18).[9] The strain was cultured in 10-L culture bottles with CT medium of the following composition: 15 mg $Ca(NO_3)_2 \cdot 4H_2O$, 10 mg KNO_3, 5 mg β-Na_2 glycerophosphate, 4 mg $MgSO_4 \cdot 7H_2O$, 0.01 mg vitamin B_{12}, 0.01 mg biotin, 1 mg thiamine HCl, 0.06 mg $FeCl_3 \cdot 6H_2O$, 0.01 mg $MnCl_2 \cdot 4H_2O$, 7 mg $ZnSO_4 \cdot 7H_2O$, 1.2 mg $CoCl_2 \cdot 6H_2O$, 0.75 mg $Na_2MoO_4 \cdot 2H_2O$, 0.3 mg

$Na_2EDTA \cdot 2H_2O$, 40 mg TAPS[*N*-tris(hydroxymethyl)-methyl-3-aminopropanesulfonic acid], and 100 mL distilled H_2O, pH 8.2. The cells were grown isothermally at 20 °C (light intensity, below 250 mmol photon m^{-2} s^{-1}; aeration rate, 1.5 L min^{-1}). After 2 weeks, the alga was harvested by centrifugation and freeze-dried. Yields of lyophilized alga averaged 0.08 g/L.

Extraction and Isolation. The $CHCl_3$–MeOH–H_2O (1:3:1, v/v, 500 mL) extract from freeze-dried cells (6.6 g) was suspended with aqueous 5% AcOH solution. The suspension was filtered and the filtrate was fractionated by solid-phase extraction using Sep Pak ODS cartridges. Further purification of the active fraction (0.23 g) by reversed-phase HPLC (Ultron ODS, 8 × 250 mm, flow rate, 3.0 mL/min) with MeOH (55%) containing 0.05 M phosphate (pH 3.0) yielded pure oscillapeptin G (**1**) (16 mg) as a colorless amorphous solid: retention time 6.7 min; $[\alpha]^{27}_D$ −86° (*c* 0.94, MeOH); UV (MeOH) λ max (log ϵ) 279 (3.5) nm; FABMS *m/z* $[M+Na]^+$ 1134, $[M-H]^-$ 1110; HRFABMS *m/z* 1134.5332 (calcd for $C_{53}H_{77}O_{17}N_9Na$, 1134.5335); IR (NaCl plate), ν_{max} 3374 (NH), 2967 (CH, aliphatic), 1736 (C=O, ester), 1653 (C=O, amide), 1539 (NH, amide), 1520 (NH, amide), 1453, 1242, 758 cm^{-1}.

Hydrolysis and Amino Acid Analysis. Oscillapeptin G (100 μg) in 6 N HCl was heated at 110 °C for 20 h. The amino acid hydrolysate was heated with 6 N HCl (0.2 mL) and *i*-PrOH (0.2 mL) at 110 °C for 1 h. The mixture was evaporated to dryness by gentle a stream of nitrogen (N_2). The residue was treated with trifluoroacetic anhydride (100 μL) and CH_2Cl_2 (100 μL) at 100 °C for 5 min and evaporated by N_2. The mixture in CH_2Cl_2 was analyzed by GC–MS using a Chirasil-L-Val capillary column (0.25 mm × 25 m) and the following conditions: column temperature 40–200 °C at 8 °C/min.

Tyrosinase Assay. Tyrosinase activity was measured in 3 mL of 50 mM Tris–HCl buffer (pH 6.2) at 25 °C in the presence of L-tyrosine (1.0×10^{-5} M) and 15 μg of tyrosinase (SIGMA, T-7755). After 2 min from the addition of tyrosine, the increase of absorbance at 280 nm was measured for 5 min. Oscillapeptin G (1.0×10^{-4} M) inhibited the tyrosinase activity to 55% of control.

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