Homocereulide, an Extremely Potent Cytotoxic Depsipeptide from the Marine Bacterium *Bacillus cereus*

Gui-Yang-Sheng Wang, Makoto Kuramoto, Kaoru Yamada,† Kazunaga Yazawa,† and Daisuke Uemura*
Faculty of Liberal Arts, Shizuoka University, Ohya, Shizuoka 422
†*Sagami Chemical Research Center, 4-4-1 Nishi-Ohnuma, Sagamihara, Kanagawa 229*

(Received June 6, 1995)

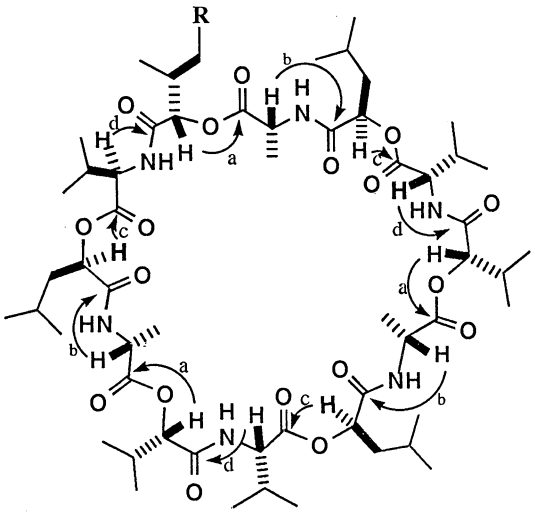
Homocereulide (**1**) and cereulide (**2**), isolated from the marine bacterium *Bacillus cereus* SCRC, showed extremely potent cytotoxicity. Their structures were elucidated by spectroscopic analysis and chemical degradation.

In the course of our ongoing search for natural potent antitumor products from marine organisms,[1] an extract from the marine bacterium *Bacillus cereus* SCRC-4h1-2 [2] was discovered to exhibit extraordinarily high cytotoxic activity. Two compounds, homocereulide (**1**) and cereulide (**2**), responsible for the bioactivity of the extract, were isolated. Both compounds showed highly potent activity against the murine leukemia cell line P388 and the Colon 26 tumor cell line (IC_{50}: **1**, 0.033 ng/ml and 0.0082 ng/ml; **2**, 0.0014 ng/ml and 0.035 ng/ml, respectively).

The lipophilic extract [$CHCl_3/CH_3OH$= 2/1 (v/v)] of mass-cultured *B. cereus* was fractionated by absorption chromatography on silica gel (10% $CH_3OH/CHCl_3$ elution), reversed-phase chromatography on ODS (CH_3OH elution), and HPLC on ODS (88% CH_3OH elution) to obtain **1** [colorless powder, $[\alpha]_D$ +10.5° (*c* 0.12, CH_3OH)] and **2** [colorless powder, $[\alpha]_D$ +10.4° (*c* 0.19, CH_3OH)]. Based on extensive 1-D and 2-D NMR analysis and HR-FABMS [$C_{57}H_{96}N_6O_{18}$, m/z 1175.6635 $(M+Na)^+$, 1191.6405 $(M+K)^+$], **2** was identified as cereulide.[3] We describe here the elucidation of the structure of the new lipopeptide homocereulide (**1**).

The molecular formula of homocereulide **1** was deduced to be $C_{58}H_{98}N_6O_{18}$ from FABMS [m/z 1167 $(M+H)^+$, 1189 $(M+Na)^+$ and 1205 $(M+K)^+$] and NMR data (Table 1). Compounds **1** and **2** showed similar ^{1}H-NMR spectra in $CDCl_3$, except for resonances around 2.0 and 5.0 ppm. An extensive analysis of NMR spectra (^{1}H-NMR, ^{13}C-NMR, DEPT, HH-COSY and CH-COSY) in $CDCl_3$ and a comparison of the NMR spectra of **1** with those of **2** suggested that homocereulide was composed of amino- and/or oxy-acids, namely three moles each of alanine, valine and α-hydroxyisocaproic acid (Hic), two moles of α-hydroxyisovaleric acid (Hiv) and one mole of α-hydroxy-β-methylvaleric acid (Hmv) (Table 1). The ^{1}H-NMR of homocereulide **1** indicated two protons at 4.99 ppm (d) and one proton at 5.05 ppm (d), corresponding to two Hiv and one Hmv, respectively. In the case of cereulide, a signal of three protons attributed to the α position of Hiv was observed at 5.02 ppm as a doublet. Furthermore, a slight difference in the three doublet proton signals of Ala (1.451 ppm, 1.442 ppm, and 1.438 ppm for each methyl group) was clearly recognized in the ^{1}H-NMR spectrum, since one Ala was connected with Hmv. In cereulide (**2**), amide N-H protons appeared at 7.84 ppm as a doublet (J= 7.0 Hz), whereas six amide protons of homocereulide (**1**) appeared as a multiplet in the ^{1}H-NMR spectrum due to the presence of Hmv instead of Hiv. The HMBC spectrum revealed a correlation of ^{1}H signals at 5.05 ppm (Hmv) and 4.99, 4.98 ppm (corresponding to 2 moles of Hiv) with the ^{13}C signal at 171.5 ppm (Ala) (arrow a in Figure 1), the 4.35 ppm proton signal (Ala) with the 171.9 ppm carbon signal (Hic) (arrow b in Figure 1), the 5.30 ppm proton signal (Hic) with the 170.4 ppm carbon signal (Val) (arrow c in Figure 1) and the 4.10 ppm proton signal (Val) with the 171.0 ppm (Hiv) and 170.9 ppm (Hmv) carbon signals (arrow d in Figure 1). The structure of **1** was clarified to be *cyclo*[-(Hic-Ala-Hiv-Val-)$_2$-Hic-Ala-Hmv-Val-] with a 36-membered ring. The hydrolysis of homocereulide in CH_3ONa-CH_3OH gave three compounds: L-Hiv-L-Val-OMe and D-Hic-D-Ala-OMe, which were identical to those from cereulide, including their absolute configuration,[3] and Hmv-Val-OMe. To determine the stereochemistry of Hmv-Val-OMe, we synthesized D-*allo*-Hmv-L-Val-OMe and L-Hmv-L-Val-OMe from L-isoleucine and L-Val-OMe in two steps.[4] Hmv-Val-OMe from **1** showed the same NMR and HPLC characteristics, and the same magnitude of optical rotation, but with an opposite sign, as D-*allo*-Hmv-L-Val-OMe.[4] Therefore, the stereochemistry of homocereulide was deduced to be *cyclo*[-(D-Hic-D-Ala-L-Hiv-L-Val-)$_2$-D-Hic-D-Ala-L-*allo*-Hmv-D-Val-].

Bacillus cereus produces the diarrheal and emetic toxins to bring food poisoning, and cereulide has been shown to be an emetic toxin.[3,5] Homocereulide may also be an emetic toxin. The *B. cereus* in our study was associated with the snail *Littorina*



1: R = CH_3

2: R = H

Figure 1. HMBC correlations in homocereulide (**1**).

Table 1. NMR data for homocereulide[a]

	H(ppm)	C(ppm)
NH	7.76 (6H, m)	
Ala	4.35 (3H, m)	171.5 (s)
	1.451 (3H, d, J= 7.0)	48.9 (d)
	1.442 (3H, d, J= 7.0)	15.7 (q)
	1.438 (3H, d, J= 7.0)	
Hic[b]	5.30 (3H, dd, J= 8.1, 4.7)	171.9 (s)
	1.76 (6H, m)	72.8 (d)
	1.68 (3H, m)	40.6 (t)
	0.92 (9H, d, J= 6.2)	24.4 (d)
	0.89 (9H, d, J= 6.2)	23.3 (q)
		21.3 (q)
Val	4.10 (3H, m)	170.4 (s)
	2.31 (3H, m)	59.3 (d)
	1.05 (9H, d, J= 6.6)	28.7 (d)
	0.95 (9H, d, J= 6.6)	19.3 (q)
		19.3 (q)
Hiv[b]	4.99 (1H, d, J= 3.3)	171.0 (s)
	4.98 (1H, d, J= 3.3)	78.8 (d)
	2.31 (2H, m)	30.5 (d)
	0.97 (12H, d, J= 7.3)	18.6 (q)
		16.9 (q)
Hmv[b]	5.05 (1H, d, J= 4.6)	170.9 (s)
	2.01 (1H, m)	78.3 (d)
	1.55 (1H, ddq)	37.2 (d)
	1.30 (1H, ddq)	24.5 (t)
	0.94 (3H, d, J= 7.0)	23.3 (q)
	0.91 (3H, d, J= 7.0)	15.1 (q)

[a]Spectra recorded in $CDCl_3$ with a JEOL JNM-GSX400 NMR spectrometer, J(HH) in Hertz. [b]Hic= α-hydroxyisocaproic acid, Hiv= α-hydroxyisovaleric acid, Hmv= α-hydroxy-β-methylvaleric acid.

sp., and it is unclear whether it causes the poisoning produced by the snail.

References and Notes

1 D. Uemura, in " Bioorganic Marine Chemistry," ed by P. J. Scheuer, Springer-Verlag, Berlin Heidelberg (1991), Vol. 4, p.1.

2 This bacterium was isolated from the surface of the snail *Littorina* sp. on the seashore of Shimoda in Izu Peninsula and deposited in the National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology, Japan, with the name *Bacillus cereus* under the accession No. SCRC-4h1-2.

3 a) N. Agata, M. Mori, M. Ohta, S. Suwan, I. Ohtani, and M. Isobe, *FEMS Microbiology Lett.*, **121**, 31 (1994). b) S. Suwan, M. Isobe, I. Ohtani, N. Agata, M. Mori, and M. Ohta, *J. Chem. Soc. Perkin Trans. 1*, **1995**, 765 .

4 a) L-Isoleucine was diazotized with sodium nitrite and hydrochloric acid, and then heated with water to give L-Hmv and D-*allo*-Hmv (approximately 3:1), b) two compounds, D-*allo*-Hmv-L-Val-OMe ($[\alpha]_D$ -51.2° (*c* 0.12, CH_3OH)) and L-Hmv-L-Val-OMe ($[\alpha]_D$ -4.0° (*c* 0.06, CH_3OH)), were obtained by the reaction of D-*allo*-Hmv and L-Hmv with L-Val-OMe and DCC, respectively. The value of $[\alpha]_D$ of L-*allo*-Hmv-D-Val-OMe from **1** was +50.5° (*c* 0.10, CH_3OH).

5 The structures of **1** and **2** resemble that of valinomycin, a particularly interesting potassium ionophore [H. K. Wipf, A. Olivier, and W. Simon, *Helv. Chim. Acta*, **53**, 1605 (1970)]. The activity of valinomycin against P388 and Colon 26 was 0.032 ng/ml and 0.21 ng/ml, respectively. The strong binding of **1** and **2** with K^+ was indicated in FABMS [**1**: $(M+K)^+$1191.6405, **2**: $(M+K)^+$ 1205].