Cyclohexadepsipeptides from the Filamentous Fungus *Acremonium* sp. BCC 2629

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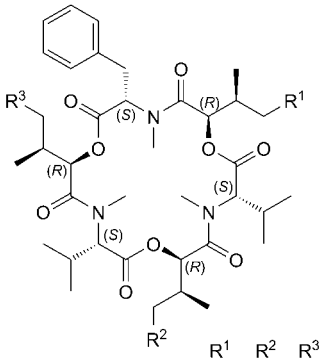
Seven new cyclohexadepsipeptides, beauvenniatins F, G_1, G_2, G_3, H_1, H_2, and H_3 (**1**–**7**, resp.), were isolated from cultures of the fungus *Acremonium* sp. BCC 2629. Their structures were elucidated by extensive spectroscopic analyses. The absolute configurations were addressed by HPLC analyses of their acid hydrolysates. Their biological activities were evaluated against *Mycobacterium tuberculosis* H37Ra and *Plasmodium falciparum* K1; the respective *MIC* and IC_{50} values were in the micromolar range.

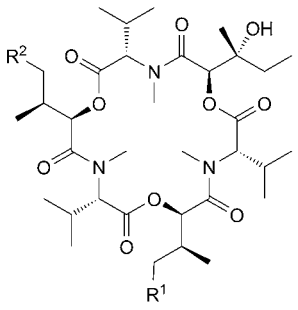
Introduction. – Cyclohexadepsipeptide antibiotics enniatins and beauvericins possess three units of *N*-methyl-L-amino acid which are alternately connected to three units of (*R*)-2-hydroxyisovaleric acid (D-Hiv) [1]. Various combinations of aliphatic amino acids, most commonly *N*-methyl-L-valine (*N*-Me-L-Val), *N*-methyl-L-leucine (*N*-Me-L-Leu), and *N*-methyl-L-isoleucine (*N*-Me-L-Ile), are found in enniatins, while, in beauvericins, three units of *N*-methyl-L-phenylalanine (*N*-Me-L-Phe) are usually observed. Some minor enniatins [2–4] and beauvericins [5][6] have one or more (*2R*,3*S*)-2-hydroxy-3-methylpentanoic acid (D-Hmp) units instead of D-Hiv.

We previously reported the isolation and structure elucidation of enniatins L, M_1, M_2, and N from the filamentous fungus *Acremonium* sp. BCC 2629 [7]. These are novel analogs which possess an OH group at the side chain of one of the D-Hmp residues. Recently, we found another *Acremonium* sp., strain BCC 28424, which produced new cyclohexadepsipeptides, beauvenniatins A–E, possessing scrambled aliphatic (enniatin-type) and aromatic (beauvericin-type) *N*-methyl-L-amino acids [8]. With this new information, we returned to the further investigation of strain BCC 2629, the culture extract of which exhibited a highly complex HPLC chromatographic profile, indicating the presence of several uncharacterized minor cyclodepsipeptides. We report here the investigation of all detectable depsipeptide constituents of the BCC 2629 mycelial extract, including the seven new analogs, beauvenniatins F, G_1, G_2, G_3, H_1, H_2, and H_3 (**1**–**7**, resp.). These new beauvenniatins possess one *N*-Me-L-Phe and two *N*-Me-L-Val residues, while they differ from each other in the structure and sequence of the D-2-hydroxy acid residues (*Fig. 1*).

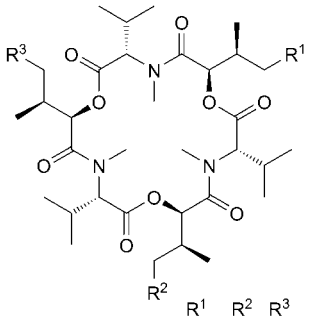
Results and Discussion. – 1. *Isolation and Structure Elucidation.* Compounds **1**–**17** were isolated from mycelia of BCC 2629 fermentation broth. The crude MeOH extract was subjected to *Sephadex LH-20* and silica-gel column chromatography to obtain a



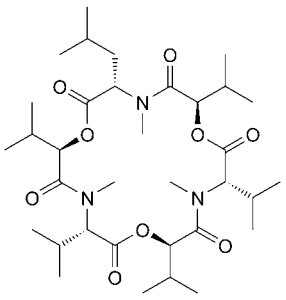
	R^1	R^2	R^3
Beauvenniatin F (**1**)	Me	Me	Me
Beauvenniatin G_1 (**2**)	Me	Me	H
Beauvenniatin G_2 (**3**)	Me	H	Me
Beauvenniatin G_3 (**4**)	H	Me	Me
Beauvenniatin H_1 (**5**)	Me	H	H
Beauvenniatin H_2 (**6**)	H	Me	H
Beauvenniatin H_3 (**7**)	H	H	Me
Beauvenniatin B (**8**)	H	H	H



	R^1	R^2
Enniatin L (**9**)	H	H
Enniatin M_1 (**10**)	Me	H
Enniatin M_2 (**11**)	H	Me
Enniatin N (**12**)	Me	Me



	R^1	R^2	R^3
Enniatin B (**13**)	H	H	H
Enniatin H (**14**)	Me	H	H
Enniatin I (**15**)	Me	Me	H
MK 1688 (**16**)	Me	Me	Me



Enniatin B_4 (**17**)

Fig. 1. *Structures of cyclodepsipeptides isolated from BCC 2629*

cyclodepsipeptide mixture (*Fig. 2*). This mixture was then separated by preparative HPLC by using a reversed-phase (RP) column to yield nine fractions (see *Fig. 2* and *Exper. Part*). Each fraction was subjected to further HPLC separation to obtain pure compounds, beauvenniatins F and B (**1** and **8**, resp.), enniatins L, N, B, H, I, and B_4 (**9**, **12**–**15**, and **17**, resp.), and MK 1688 (**16**). Beauvenniatins G_1, G_2, and G_3 (**2**–**4**, resp.) were inseparable under various chromatographic conditions; therefore, spectroscopic analysis was conducted for the mixture. Similarly, a mixture of beauvenniatins H_1, H_2, and H_3 (**5**–**7**, resp.), as well as a mixture of enniatins M_1 and M_2 (**10** and **11**, resp.), were obtained and characterized as in the case of the mixture of **2**, **3**, and **4**. Identification of compounds **9**–**17**, isolated from this fungus (BCC 2629), was reported in [7].

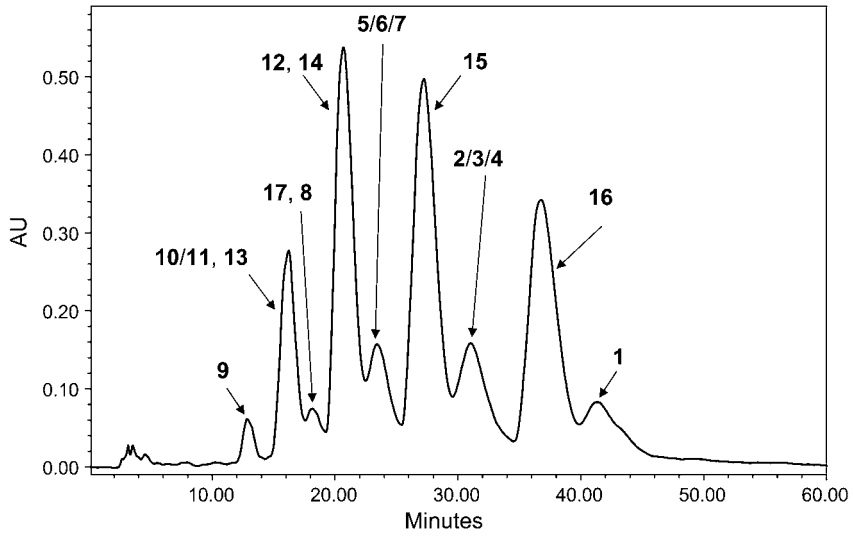


Fig. 2. *HPLC Chromatogram of a cyclodepsipeptide mixture from mycelia* (*Nova-Pak® C_{18}*, 3.9 × 150 mm, 4 μM; MeCN/H_2O 70:30; flow rate, 0.4 ml/min)

Spectroscopic data of **8** were identical to those of beauvenniatin B, isolated in our laboratory from *Acremonium* sp. BCC 28424 [8].

Beauvenniatin F (**1**) had a molecular formula of $C_{40}H_{63}N_3O_9$ as deduced from HR-MS (ESI-TOF). The IR spectrum of **1** showed amide and ester absorptions at $\tilde{\nu}_{max}$ 1658 and 1742 cm^{-1}, respectively, and additional absorptions of a monosubstituted benzene at 746 and 700 cm^{-1}, similarly to beauvenniatin B (**8**). Analysis of the ^{1}H- and ^{13}C-NMR, and 2D-NMR data (COSY, HMQC, and HMBC) revealed the presence of three 2-hydroxy-3-methylpentanoic acid (Hmp), two *N*-methylvaline (*N*-Me-Val), and one *N*-methylphenylalanine (*N*-Me-Phe) residues (*Table 1*). The ^{1}H- and ^{13}C-NMR signals of *N*-Me-Phe were observed at δ(H) 5.53–5.46 (*m*) and δ(C) 56.7, assigned to H- and C-atoms at the α-position (H–C(2)). Resonances at δ(H) 3.42 (*dd*, J = 14.5, 4.6), 3.11 (*dd*, J = 14.5, 12.7), and δ(C) 32.5 were assigned to the benzylic CH_2 group. Peaks at δ(H) 7.29–7.26 (*m*), 7.23–7.20 (*m*), and δ(C) 135.3, 127.1 (2×), 126.7 (2×), and 125.1 for a Ph group, and those at δ(H) 3.03 (*s*) and δ(C) 30.3 for an amide *N*-methyl were detected. The H-atom H–C(2) of *N*-Me-Phe showed COSY cross-signal with CH_2 H-atoms (CH_2(3)), which subsequently exhibited HMBCs with aromatic C-atoms C(4), C(5), and C(9). The HMBCs for *N*-Me-Phe was also observed from amide Me H-atoms (MeN (δ(H) 3.03)) to C(2) (δ(C) 56.7) and from H–C(2) to CO C-atom (at δ(C) 169.0) (*Fig. 3*). The NMR data of the three Hmp residues were similar to those of MK 1688 (**16**), while the NMR data of two *N*-Me-Val residues were similar to those of **8**. The sequence of the six residues was determined by analysis of HMBC and NOESY spectra (*Fig. 3*). Intense NOESY correlations were detected between MeN groups of *N*-methyl amino acids and H–C(2) of neighboring Hmp (δ(H) 3.03 (MeN of *N*-Me-Phe)/δ(H) 5.04 (H–C(2) of Hmp-1), δ(H) 3.19 (MeN of *N*-Me-Val-1)/δ(H) 5.47 (H–C(2) of Hmp-2), and δ(H) 3.22 (MeN of *N*-Me-Val-2)/δ(H) 5.24 (H–C(2) of

Hmp-3)). This result is consistent with known enniatins [9] in which the co-planarity between MeN groups of amino acid residues and α-H-atoms of neighboring 2-hydroxycarboxylic acid residues was observed. The alternate fashion of connectivity of three amino acids and three 2-hydroxycarboxylic acids was further confirmed by HMBCs from H–C(2) of Hmp (δ(H) 5.04, 5.47, and 5.24) to CO C-atoms of the neighboring *N*-methyl amino acids (C(1) (δ(C) 169.1, 168.2, and 169.0, resp.)) (*Fig. 3*).

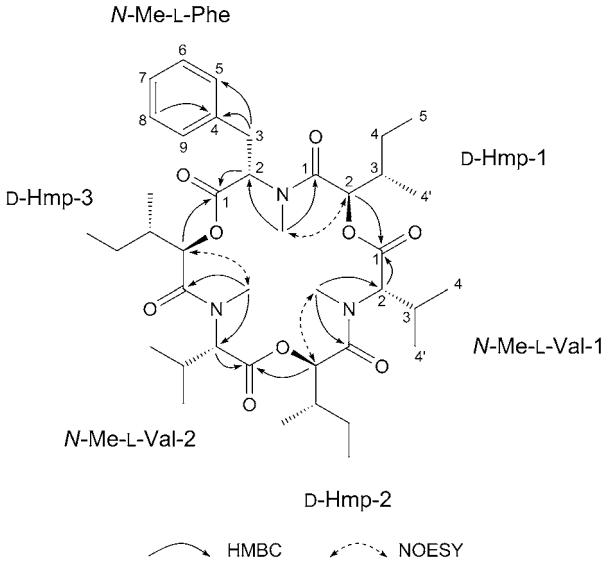


Fig. 3. *Selected HMBC and NOESY correlations for beauvenniatin F* (**1**)

The α-C-atom configuration of beauvenniatin F (**1**) was addressed by HPLC analysis of its acid hydrolysate (6N HCl, 110°, 15 h) by using a ligand exchange-type chiral column (*SUMICHIRAL OA-5000*; see *Exper. Part*). Co-injection of standard amino acids and 2-hydroxycarboxylic acids revealed that the hydrolysate of **1** consisted of *N*-Me-L-Val, *N*-Me-L-Phe, and (*2R*,*3S*)-2-hydroxy-3-methylpentanoic acid (D-Hmp).

Beauvenniatins G_1, G_2, and G_3 (**2**–**4**, resp.) were obtained as an inseparable mixture in a ratio of 1:1.4:1.5. The HR-ESI-TOF mass spectrum of the mixture displayed only one $[M+Na]^+$ ion peak at *m*/*z* 738.43, indicating that the three compounds possess identical molecular formula $C_{39}H_{61}N_3O_9$. The UV and IR spectra of this mixture were similar to those of compounds **1** and **8**. The ^{1}H- and ^{13}C-NMR data including COSY, HMQC, and HMBC indicated the presence of three Hiv, six Hmp, six *N*-Me-Val, and three *N*-Me-Phe residues. Therefore, it is most likely that each cyclohexadepsipeptide should be composed of the same six residues (a Hiv, two Hmp, two *N*-Me-Val, and an *N*-Me-Phe), only differing in the sequence. The ^{1}H- and ^{13}C-NMR signals, including COSY and NOESY correlations, of these six residues are similar to those of compound **1** and other known beauvenniatins (*Table 2*). The mixture was subjected to acid hydrolysis, followed by HPLC analysis, as described for beauvenniatin F (**1**). The HPLC analysis indicated that the hydrolysate of **2**/**3**/**4** was

Table 1. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of Beauvenniatin F* (**1**) *in CD_3OD.* δ in ppm, J in Hz.

Unit	Position	δ(H)	δ(C)
N-Me-L-Phe	C(1)	–	169.0[a])
	H–C(2)	5.53–5.46 (*m*)	56.7
	CH_2(3)	3.42 (*dd*, *J* = 14.5, 4.6), 3.11 (*dd*, *J* = 14.5, 12.7)	32.5
	C(4)	–	135.3
	H–C(5,9)	7.29–7.26 (*m*)	127.1[b]) (2×)
	H–C(6,8)	7.29–7.26 (*m*)	126.7[b]) (2×)
	H–C(7)	7.23–7.20 (*m*)	125.1
	MeN	3.03 (*s*)	30.3
N-Me-L-Val-1[c])	C(1)	–	169.1[a])
	H–C(2)	4.71 (*d*, *J* = 9.9)	60.5
	H–C(3)	2.32–2.21 (*m*)	26.4
	Me(4, 4′)	1.04 (*d*, *J* = 6.6), 0.93 (*d*, *J* = 7.1)	17.6[d]) (2×)
	MeN	3.19 (*s*)	29.6
N-Me-L-Val-2[c])	C(1)	–	168.2
	H–C(2)	4.99 (*d*, *J* = 10.0)	59.8
	H–C(3)	2.32–2.21 (*m*)	26.5
	Me(4, 4′)	1.08 (*d*, *J* = 6.6), 0.94 (*d*, *J* = 7.5)	17.5[d]), 17.2[d])
	MeN	3.22 (*s*)	29.4
D-Hmp-1	C(1)	–	169.4[a])
	H–C(2)	5.04 (*d*, *J* = 8.2)	73.3
	H–C(3)	1.65–1.60 (*m*)	34.5
	CH_2(4)	0.73–0.63 (*m*)	22.3
	Me(4′)	0.83 (*d*, *J* = 6.6)	12.4
	Me(5)	0.73–0.63 (*m*)	8.9
D-Hmp-2[e])	C(1)	–	168.7
	H–C(2)	5.47 (*d*, *J* = 6.8)	72.1
	H–C(3)	2.00–1.90 (*m*)	35.3
	CH_2(4)	1.53–1.41 (*m*), 1.32–1.17 (*m*)	23.4
	Me(4′)	1.05–0.92 (*m*)	12.3
	Me(5)	1.05–0.92 (*m*)	8.9
D-Hmp-3[e])	C(1)	–	169.6
	H–C(2)	5.24 (*d*, *J* = 6.9)	72.7
	H–C(3)	2.00–1.90 (*m*)	34.6
	CH_2(4)	1.53–1.41 (*m*), 1.32–1.17 (*m*)	23.4
	Me(4′)	1.05–0.92 (*m*)	12.0
	Me(5)	1.05–0.92 (*m*)	8.9

[a]) Assignments can be interchanged. [b]) Assignments can be interchanged. [c]) Two sets of H- and C-atom assignments for *N*-Me-L-Val-1 and *N*-Me-L-Val-2 can be interchanged. [d]) Assignments can be interchanged. [e]) Two sets of H- and C-atom assignments for D-Hmp-2 and D-Hmp-3 can be interchanged.

composed of D-Hiv, D-Hmp, *N*-Me-L-Val and *N*-Me-L-Phe in molar composition of 1:2:2:1, respectively.

Beauvenniatins H_1, H_2, and H_3 (**5**–**7**, resp.), with the same molecular formula $C_{38}H_{59}N_3O_9$ (HR-ESI-MS), were also inseparable and could be obtained as a mixture in the ratio of 1.3:1:1.2, respectively. Analysis of NMR data (*Table 3*) revealed that

Table 2. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of Beauvenniatins $G_1/G_2/G_3$* (**2**/**3**/**4**, resp.) *in $CDCl_3$*. δ in ppm, J in Hz.

Unit	Position	δ(H)	δ(C)
N-Me-L-Phe (3 units)	C(1)		169.3[a]) (3×)
	H–C(2)	4.82–4.70 (br. *m*)	61.5 (3×)
	CH_2(3)	3.47–3.41 (*m*), 3.24–3.13 (*m*)	34.9 (2×), 34.8
	C(4)		137.3 (3×)
	H–C(5,9)	7.30–7.26 (*m*)	129.1[b]) (3×), 129.0[b]) (3×)
	H–C(6,8)	7.30–7.26 (*m*)	128.5[b]) (6×)
	H–C(7)	7.23–7.18 (*m*)	126.7 (3×)
	MeN	2.91 (*s*), 2.88 (*s*), 2.87 (*s*)	35.2 (3×)
N-Me-L-Val (6 units)	C(1)		170.4[a]) (5×), 170.3[a])
	H–C(2)	5.02 (*d*, *J* = *ca.* 10), 5.00 (*d*, *J* = 10.3), 4.99 (*d*, *J* = 10.3), 4.65–4.61 (*m*)	62.4 (4×), 62.3 (2×)
	H–C(3)	2.36–2.00 (*m*)	27.8, 27.7 (2×), 27.5 (2×), 27.4
	Me(4,4′)	1.05–1.03 (*m*), 1.01–0.87 (*m*)	20.0 (3×), 19.8 (3×), 19.6, 19.5 (2×), 19.1, 19.0 (2×)
	MeN	3.17 (*s*), 3.16 (*s*), 3.15 (*s*), 3.14 (*s*), 3.13 (*s*)	31.9 (3×), 31.4, 31.3, 31.2
D-Hiv (3 units)	C(1)		169.6[c]), 169.5[c]) (2×)
	H–C(2)	5.32 (*d*, *J* = 8.5), 5.10 (*d*, *J* = 8.7), 4.85 (*d*, *J* = 8.9)	77.6, 75.4, 75.0
	H–C(3)	2.36–2.00 (*m*), 2.11–1.94 (*m*)	30.4, 29.8, 29.5
	Me(4,4′)	1.01–0.87 (*m*), 0.67 (*d*, *J* = 6.7)	18.9, 18.6, 18.4 (2×), 18.2, 18.0
D-Hmp (6 units)	C(1)		169.8[c]) (6×)
	H–C(2)	5.46 (*d*, *J* = 7.5), 5.43 (*d*, *J* = 7.6), 5.22 (*d*, *J* = 7.3), 4.97 (*d*, *J* = 7.9), 4.95 (*d*, *J* = 8.1)	76.5, 76.2, 74.1 (2×), 73.7, 73.6
	H–C(3)	2.11–1.94 (*m*), 1.80–1.71 (*m*)	36.6 (2×), 36.0 (2×), 35.6 (2×)
	CH_2(4)	1.49–1.39 (*m*), 1.19–1.12 (*m*)	25.2 (4×), 25.0, 24.9
	Me(4′)	1.01–0.87 (*m*), 0.85 (*d*, *J* = 6.6)	14.8 (2×), 14.6, 14.5, 14.4, 14.3
	Me(5)	1.01–0.87 (*m*), 0.78 (*t*, *J* = 7.2)	11.4 (2×), 11.3 (2×), 11.2, 11.1

[a]) Assignments can be interchanged. [b]) Assignments can be interchanged. [c]) Assignments can be interchanged.

Table 3. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, respectively) *of Beauvenniatins $H_1/H_2/H_3$* (**5**/**6**/**7**, resp.) *in $CDCl_3$*. δ in ppm, J in Hz.

Unit	Position	δ(H)	δ(C)
N-Me-L-Phe (3 units)	C(1)		169.5[a]) (3×)
	H–C(2)	4.78–4.66 (br. *m*)	61.8 (3×)
	CH_2(3)	3.47–3.42 (*m*), 3.23–3.15 (*m*)	35.1, 35.0, 34.9
	C(4)		137.4 (3×)
	H–C(5,9)	7.31–7.27 (*m*)	129.2[b]) (3×), 129.1[b]) (3×)
	H–C(6,8)	7.31–7.27 (*m*)	128.6[b]) (3×), 128.5[b]) (3×)
	H–C(7)	7.24–7.19 (*m*)	126.7 (3×)
	MeN	2.91 (*s*), 2.90 (*s*), 2.87 (*s*)	35.3 (3×)
N-Me-L-Val (6 units)	C(1)		170.5,[a]) (3×), 170.4[a]) (3×)
	H–C(2)	5.04 (*d*, *J* = 9.8), 5.03 (*d*, *J* = 10.2), 5.01 (*d*, *J* = 10.0), 4.65–4.61 (*m*)	62.5, 62.4 (2×), 61.2 (3×)
	H–C(3)	2.37–2.21 (*m*)	27.9 (2×), 27.8, 27.6, 27.5, 27.4
	Me(4,4′)	1.06–1.03 (*m*), 1.02–0.90 (*m*)	20.1 (3×), 19.9 (3×), 19.7 (2×), 19.5, 19.3, 19.2, 19.1
	MeN	3.18 (*s*), 3.17 (*s*), 3.15 (*s*)	32.1 (3×), 31.6, 31.5, 31.3
D-Hiv (6 units)	C(1)		169.6[c]) (3×), 169.5[c]) (3×)
	H–C(2)	5.34 (*d*, *J* = 8.7), 5.32 (*d*, *J* = 8.6), 5.11 (*d*, *J* = 8.6), 4.86 (*d*, *J* = 10.1), 4.84 (*d*, *J* = 9.5)	77.8, 77.6, 75.4 (2×), 75.1, 75.0
	H–C(3)	2.37–2.21 (*m*), 2.11–1.97 (*m*)	30.5 (2×), 29.9 (2×), 29.6 (2×)
	Me(4,4′)	1.02–0.90 (*m*), 0.73–0.68 (*m*)	19.0, 18.9, 18.7 (2×), 18.5 (4×), 18.3 (2×), 18.2, 18.1
D-Hmp (3 units)	C(1)		170.0[c]) (3×)
	H–C(2)	5.45 (*d*, *J* = 7.6), 5.22 (*d*, *J* = 7.3), 4.96 (*d*, *J* = 7.9)	76.5, 74.1, 73.7
	H–C(3)	2.11–1.97 (*m*), 1.80–1.72 (*m*)	36.6, 36.1, 35.6
	CH_2(4)	1.51–1.41 (*m*), 1.21–1.12 (*m*)	25.3, 25.2, 25.1
	Me(4′)	1.02–0.90 (*m*), 0.86 (*d*, *J* = 6.6)	14.9, 14.6, 14.4
	Me(5)	1.02–0.90 (*m*), 0.79 (*t*, *J* = 7.3)	11.4 (2×), 11.2

[a]) Assignments can be interchanged. [b]) Assignments can be interchanged. [c]) Assignments can be interchanged.

this mixture was composed of six Hiv, three Hmp, six *N*-Me-Val and three *N*-Me-Phe residues. Therefore, as in the previous case already described, beauvenniatins H_1 (**5**), H_2 (**6**), and H_3 (**7**) should be composed of the same six residues (two Hiv, an Hmp, two *N*-Me-Val and an *N*-Me-Phe) but alters only in the sequence. ^{1}H- and ^{13}C-NMR signals, including COSY and NOESY correlations, of the six residues in compounds **5**/**6**/**7** were closely related to compounds **1**, **2**/**3**/**4** and other known beauvenniatins. Configurations of all residues in compounds **5**/**6**/**7** were determined by using the same procedure as conducted for compounds **1** and **2**/**3**/**4**.

2. *Biological Properties.* Beauvenniatins F, $G_1/G_2/G_3$, and $H_1/H_2/H_3$ exhibited growth-inhibitory activities against *Mycobacterium tuberculosis* H37Ra with *MIC* values in the range of 1.07 – 4.45 μM, and proliferation inhibitions against the human malaria parasite (*Plasmodium falciparum* K1) with IC_{50} values in the range of 3.6 – 3.9 μM (*Table 4*). They also displayed cytotoxic activities toward cancer cell-lines (KB, BC, NCI-H187 cell-lines) with IC_{50} values ranging from 1.00 to 2.29 μM, as well as *Vero* cells with IC_{50} values in the range of 1.9 – 5.5 μM. The biological activities of these beauvenniatins possessing one *N*-Me-L-Phe residue were similar to those of enniatin B, the most commonly occurring analog. Biological activities of beauvenniatin B were recently reported [8].

Table 4. *Biological Activities of* **1** – **7** *and* **13**

Compound	Anti-malaria[a]), IC_{50} [μM]	Anti-TB[b]), *MIC* [μM]	Cytotoxicity[c]), IC_{50} [μM]			
			KB-cells	BC-cells	NCI-H187 cells	Vero cells
Beauvenniatin F (**1**)	3.8 ± 0.1	1.07	1.05 ± 0.05	1.00 ± 0.04	2.29 ± 1.26	5.5
Beauvenniatins $G_1/G_2/G_3$ (**2**/**3**/**4**)	3.9 ± 0.4	2.18	1.06 ± 0.06	1.40 ± 0.06	1.23 ± 0.49	4.1
Beauvenniatins $H_1/H_2/H_3$ (**5**/**6**/**7**)	3.6 ± 0.9	4.45	1.15 ± 0.11	1.17 ± 0.10	1.45 ± 0.38	1.9
Enniatin B (**13**)	5.2 ± 0.3	4.88	2.24 ± 0.44	6.36 ± 0.72	1.94 ± 0.08	13.0
Dihydroartemisinin	0.004					
Isoniazid		0.36				

[a]) *In vitro* antimalarial activity against *Plasmodium falciparum* K1. Each assay was performed in tripicate. The IC_{50} values were reported with mean ± S. D. ($N = 3$). [b]) *In vitro* antituberculous activity against *Mycobacterium tuberculosis* H37Ra. [c]) Assays were performed in tripicate, except for *Vero* cells. The IC_{50} values were reported with mean ± S. D. ($N = 3$).

Conclusions. – It has been demonstrated that the fungus *Acremonium* sp. BCC 2629 is a unique source of enniatin/beauvericin type cyclodepsipeptides, producing 17 compounds with various side-chain structures. In particular, the characteristic of both this strain and *Acremonium* sp. BCC 28424 is the production of beauvenniatins, which possess scrambled aliphatic/aromatic amino acids. These results also indicate that the recognition of the L-amino acid side-chain structure by the cyclodepsipeptide synthetase of these *Acremonium* species should be less specific, when compared to those of common fungal enniatin producers (*Fusarium* spp., *Verticillium* spp.) and beauvericin producers (*Beauveria* spp., *Isaria* spp., and *Cordyceps* spp.), which strictly

recognize aliphatic and aromatic L-amino acids, respectively. In addition, the cyclodepsipeptide synthetase of BCC 2629 also showed low specificity in recognition of the D-2-hydroxy acids, while the synthetase of BCC 28424 strictly recognized D-Hiv [8].

Experimental Part

General. Prep. HPLC: *Waters 600 controller*, *Waters 996 photodiode-array* detector with column *Prep Nova-Pak® HR-C18* (40 × 100 mm, 6 μM). M.p.: *Electrothermal IA9100* digital melting point apparatus. Optical rotations: *JASCO P-1030* digital polarimeter. UV and IR spectra: *Varian Cary 1E* UV-VIS spectrophotometer and a *Bruker VECTOR 22* spectrometer, resp. NMR Spectra (^{1}H, ^{13}C, DEPT, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC): *Bruker AV500D* spectrometer. ESI-TOF-MS: *Micromass LCT* spectrometer.

Fungal Material. The fungus used in this study was isolated and identified by Dr. *Nigel L. Hywel-Jones* from spore attached to the synnema of *Hirsutella formicarum* on ant, collected at Khao Sok National Park, Surat Thani, southern of Thailand. This fungus was deposited with the Thailand BIOTEC Culture Collection (BCC) and registered as BCC 2629. The colony-growth rate was moderate on potato dextrose agar, reaching a diameter of 50 mm in 20 d as pure white, diffused colony. The fungus was immersed with aerial mycelium, hyaline, septate, and branching, up to 3 μm with sporulation at 25° under normal day-light illumination. Anamorphic stage indicated the genus *Acremonium*, however, it was not possible to identify into species level, as this fungal genus is undergoing taxonomic revision at present [7].

Fermentation, Extraction, and Isolation. BCC 2629 was cultured in potato dextrose broth (10 l) under stationary condition for 40 d at 25°, thereafter the culture was filtered. The mycelial cakes were extracted with MeOH (1.5 l) and filtered. To the filtrate was added H_2O (100 ml), and the mixture was washed with hexane (800 ml). The aq. MeOH layer was concentrated under reduced pressure. The residue was dissolved in AcOEt (300 ml), washed with H_2O (100 ml), and concentrated under reduced pressure to yield a deep-yellow amorphous solid (2.2 g). This crude extract was subjected to CC (*Sephadex LH-20* column; 20% CH_2Cl_2/MeOH; and then silica gel; 0–5% MeOH/CH_2Cl_2) to provide a mixture of cyclodepsipeptides (1.99 g) [6]. This mixture was separated by repeated prep. HPLC (MeCN/H_2O 70:30; flow rate. 20 ml/min) to yield enniatin L (**9**), enniatins M_1, M_2, B (**10**/**11**/**13**, resp.), enniatin B_4, beauvenniatin B (**17**/**8**, resp.), enniatins N, H (**12**/**14**, resp.), beauvenniatins H_1, H_2, H_3 (**5**/**6**/**7**, resp.), enniatin I (**15**), beauvenniatins G_1, G_2, G_3 (**2**/**3**/**4**, resp.) MK 1688 (**16**), and beauvenniatin F (**1**) in the order with respect to the retention times. Each fraction was subjected to further HPLC separation with MeOH/H_2O 80:20 to obtain pure compounds: enniatin L (**9**; 21 mg), enniatins M_1/M_2 (**10**/**11**; 63 mg), enniatin B (**13**; 126 mg), enniatin B_4 (**17**; 27 mg), beauvenniatin B (**8**; 2 mg), enniatin N (**12**; 25 mg), enniatin H (**14**; 246 mg), beauvenniatins H_1/H_2/H_3 (**5**/**6**/**7**; 45 mg), enniatin I (**15**; 224 mg), beauvenniatins G_1/G_2/G_3 (**2**/**3**/**4**; 40 mg), MK 1688 (**16**; 173 mg), and beauvenniatin F (**1**; 17 mg).

Beauvenniatin F (=(*3S,6R,9S,12R,15S,18R*)*-3-Benzyl-4,10,16-trimethyl-9,15-bis(1-methylethyl)-6,12,18-tris[(2S)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **1**). Colorless powder. M.p. 60–63°. $[\alpha]_D^{25} = -110$ ($c = 0.20$, $CHCl_3$). UV (MeOH): 206 (4.56). IR (KBr): 2969, 2936, 1742, 1658, 1467, 1191, 1008, 746, 700. ^{1}H- and ^{13}C-NMR (CD_3OD): see *Table 1*. HR-MS (ESI-TOF): 752.4469 ($[M + Na]^+$, $C_{40}H_{63}N_3NaO_9^+$; calc. 752.4462).

Beauvenniatins G_1 (=(*3S,6R,9S,12R,15S,18R*)*-3-Benzyl-4,10,16-trimethyl-9,15,18-tris(1-methylethyl)-6,12-bis[(2S)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **2**)/G_2 (=(*3S,6R,9S,12R,15S,18R*)*-3-Benzyl-4,10,16-trimethyl-9,12,15-tris(1-methylethyl)-6,18-bis[(2S)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **3**)/G_3 (=(*3S,6R,9S,12R,15S,18R*)*-3-Benzyl]-4,10,16-trimethyl-6,9,15-tris(1-methylethyl)-12,18-bis[(2S)-2-methylpropyl-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **4**). Colorless powder. M.p. 67–71°. $[\alpha]_D^{25} = -109$ ($c = 0.20$, $CHCl_3$). UV (MeOH): 206 (4.56). IR (KBr): 2968, 2936, 1741, 1666, 1469, 1189, 1009, 749, 700. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 2*. HR-MS (ESI-TOF): 738.4306 ($[M + Na]^+$, $C_{39}H_{61}N_3NaO_9^+$; calc. 738.4306).

Beauvenniatins H_1 (=(*3S,6R,9S,12R,15S,18R*)*-3-Benzyl-4,10,16-trimethyl-9,12,15,18-tetrakis(1-methylethyl)-6-[(2S)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*;

5)/H_2 (=(*3*S,*6*R,*9*S,*12*R,*15*S,*18*R)*-3-Benzyl-4,10,16-trimethyl-6,9,15,18-tetrakis(1-methylethyl)-12-[(2*S*)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **6**)/H_3 (=(*3*S,*6*R,*9*S,*12*R,*15*S,*18*R)*-3-Benzyl-4,10,16-trimethyl-6,9,12,15-tetrakis(1-methylethyl)-18-[(2*S*)-2-methylpropyl]-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone*; **7**). Colorless powder. M.p. 70–73°. $[\alpha]_D^{25} = -110$ ($c = 0.20$, $CHCl_3$). UV (MeOH): 206 (4.59). IR (KBr): 2968, 2936, 1741, 1663, 1470, 1189, 1011, 750, 701. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 3*. HR-MS (ESI-TOF): 702.4333 ($[M+H]^+$, $C_{38}H_{60}N_3O_9^+$; calc. 702.4329).

Hydrolysis of Beauvenniatins and HPLC Analysis [9][10]. A mixture of compounds **2**/**3**/**4** (1 mg) was hydrolyzed in 6N HCl (0.5 ml) at 110° for 15 h, then concentrated *in vacuo*. The hydrolysate was dissolved in MeOH (100 µl) and then analyzed by HPLC with a chiral column (*SUMICHIRAL OA-5000* (4.6 × 150 mm); flow rate, 1 ml/min; injection volume, 20 µl). A mixture of compounds **5**/**6**/**7** and pure **1** were also subjected to hydrolysis and HPLC determination by the method as described for **2**/**3**/**4**. Standard compounds, DL-*N*-methylvaline and DL-2-hydroxyisovaleric acid, were purchased from *Aldrich*. *N*-Methyl-L-phenylalanine and (*2R*,*3S*)-2-hydroxy-3-methylpentanoic acid (D-Hmp) were obtained by acid hydrolysis of beauvericin [11] and MK 1688, resp.

Retention times of standard amino acids and 2-hydroxycarboxylic acids: *1*) DL-*N*-methylvaline (*N*-Me-L-Val, t_R 8.0 min; and *N*-Me-D-Val, t_R 11.9 min; solvent, 5% MeOH in 2 mM aq. $CuSO_4$). *2*) 2-hydroxyisovaleric acid (L-Hiv, t_R 30.9 min; and D-Hiv, t_R 57.2 min; solvent, 30% MeOH in 2 mM aq. $CuSO_4$). *3*) *N*-methyl-L-phenylalanine (*N*-Me-L-Phe, t_R 23.4 min; solvent, 30% MeOH in 2 mM aq. $CuSO_4$). *4*) D-2-hydroxy-3-methylpentanoic acid (D-Hmp, t_R 39.6 min; solvent, 15% ^{i}PrOH in 2 mM aq. $CuSO_4$; and t_R 114.4 min; solvent, 30% MeOH in 2 mM aq. $CuSO_4$).

Biological Assays. Antimalarial activity against *Plasmodium falciparum* K1 was evaluated by using microculture radioisotope technique, as described by *Desjardins et al.* [12]. Growth inhibition against *Mycobacterium tuberculosis* H37Ra was determined by using *Microplate Alamar-Blue* assay [13]. Cytotoxicity assays against oral human epidermal carcinoma (KB) cells, human breast cancer (BC) cells, human small-cell lung cancer (NCI-H187) cells, and African green monkey kidney fibroblast (*Vero* cells) were carried out using a colorimetric method [11].

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