NEW CYCLODEPSIPEPTIDES, ENNIATINS D, E AND F PRODUCED BY *Fusarium* sp. FO-1305

HIROSHI TOMODA, HIROYUKI NISHIDA†, XIN-HUI HUANG, ROKURO MASUMA, YOUNG KOOK KIM and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

(Received for publication February 20, 1992)

New cyclodepsipeptides named enniatins D, E and F were isolated from the culture broth of *Fusarium* sp. FO-1305 as inhibitors of acyl-CoA : cholesterol acyltransferase (ACAT). The respective structures of enniatins D, E and F were determined to be cyclo[D-α-hydroxyisovaleryl(D-Hiv)-L-*N*-methylleucinyl(L-Me-Leu)-D-Hiv-L-*N*-methylvalinyl(L-Me-Val)-D-Hiv-L-Me-Val], a mixture of cyclo-[D-Hiv-L-Me-Leu-D-Hiv-L-*N*-methylisoleucinyl(L-Me-Ile)-D-Hiv-L-Me-Val] and cyclo(D-Hiv-L-Me-Ile-D-Hiv-L-Me-Leu-D-Hiv-L-Me-Val), and cyclo(D-Hiv-L-Me-Leu-D-Hiv-L-Me-Ile-D-Hiv-L-Me-Ile) by spectral analyses and chemical degradation. The IC_{50} values of enniatins D, E and F for ACAT activity in an enzyme assay using rat liver microsomes were calculated to be 87, 57 and 40 μM, respectively.

Acyl-CoA : cholesterol acyltransferase (ACAT) [EC 2.3.1.26] is believed to be one of the promising inhibition sites for the treatment of atherosclerosis and hypercholesterolemia. We have been interested in ACAT inhibitors of microbial origin and screening work has been carried out. Purpactins produced by *Penicillium purpurogenum* were isolated and evaluated as ACAT inhibitors[1~3]. In the course of our continuous screening, a series of cyclodepsipeptides produced by a fungal strain FO-1305 was found to inhibit ACAT activity. Seven cyclodepsipeptides, enniatins A, A1, B, B1 and new compounds named enniatins D, E and F, were isolated from the culture broth of the strain. In this paper, the taxonomy of the producing strain, fermentation, the structure determination and biological activity of enniatins D, E and F are described.

Taxonomy of Producing Organism

Strain FO-1305 was isolated from a soil sample collected at Jingu Gaien, Shinjuku-ku, Tokyo, Japan. The organism grew well on potato-dextrose agar, corn meal agar, malt extract agar, CZAPEK's agar and yeast extract-soluble starch (YpSs) agar for 6 days at 25°C to form colonies with a diameter of 25~36 mm. The colony surface was tomentose or floccose to felty with a color of white to pale brown. The reverse color of the colonies was white to yellowish brown. The growth was nil for 14 days at 5°C and 37°C. No teleomorph was observed. Macroconidia having 3 to 5 transverse septa are hyaline in color, slightly curved in shape and 25~40×3~6 μm in size. Chlamydospores formed abundantly like mycelia and were subglobose in shape and 7~10 μm in diameter. From these morphological characteristics, the strain FO-1305 was determined to belong to the genus *Fusarium*.

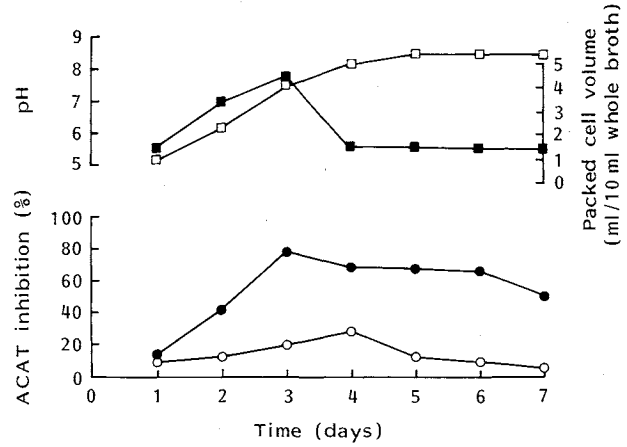
Fermentation and Isolation

A slant culture of strain FO-1305 grown on YpSs agar was inoculated into 500-ml Erlenmyer flasks

† Present address: Pfizer Central Research, Nagoya, Pfizer Pharmaceuticals Inc., 5-2 Taketoyo, Aichi 470-23, Japan.

Fig. 1. A typical time course of enniatin complex production as ACAT inhibitory activity.

○ Supernatant, ● mycelia, □ pH, ■ packed cell volume.



Ten ml of the culture broth of *Fusarium* sp. FO-1305 was removed and centrifuged and the mycelial part was extracted with 10 ml of ethyl acetate and the mycelial part was treated with 10 ml of acetone. Each extract (10 μl), transferred into ACAT assay tubes, was evaporated and dissolved in 10 μl of ethanol. ACAT activity was assayed according to the method reported previously[1].

containing 100 ml of a seed medium (glucose 2.0%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton 0.5%, KH_2PO_4 0.1%, agar 0.1%, pH 6.0). The flasks were shaken on a rotary shaker for 2 days at 27°C. Two hundred milliliters of the seed culture was transferred into 20 liters of a production medium (glucose 1%, Tryptone 0.5%, yeast extract 0.3%, malt extract 0.3%, agar 0.1%, pH 6.0) in a 30-liter jar fermenter. The fermentation was carried out at 27°C. A typical time course of the fermentation is shown in Fig. 1. Potent inhibitory activity against ACAT was detected mostly in the mycelial part and reached a maximum at 72 hours, then decreased gradually. Four-day cultured broth (15 liters) was centrifuged to obtain the mycelial part, which was extracted with 10 liters of acetone. After filtration, the acetone extracts, concentrated to an aqueous solution, were extracted with 3 liters of ethyl acetate. The extracts were concentrated *in vacuo* to dryness to yield a brown oily material (4.2 g). The material was applied to a silica gel column (E. Merck, Kieselgel 60, 100 g). After washing with 1 liter of chloroform, the active compounds were eluted with 1 liter of chloroform - methanol (99 : 1), and each 200 ml was successively collected. The 4th fraction was concentrated *in vacuo* to give a pale brown oily material (1.4 g). Then, the material was purified through a Sephadex LH-20 column (column size: 35 × 320 mm, solvent: methanol) to yield a colorless oily material (1.1 g). Final purification of enniatins was carried out by HPLC (column: Yamamura Chemical Laboratory, YMC pack ODS 10 × 250 mm, solvent: 75% aq CH_3CN, detection: UV at 225 nm, flow rate: 4.0 ml/minute). Seven peaks showing ACAT inhibitory activity (Fig. 2) were collected to obtain enniatins B (4.6 mg, peak 1), D (2.1 mg, peak 2), B1 (44.8 mg, peak 3), E (23.7 mg, peak 4), A1 (212.7 mg, peak 5), F (120 mg, peak 6) and A (450 mg, peak 7).

Structure of Enniatins D, E and F

Enniatins D (**1**), E (**2**) and F (**3**) were obtained as white powders, and their respective molecular formulas were determined to be $C_{34}H_{59}N_3O_9$, $C_{35}H_{61}N_3O_9$ and $C_{36}H_{63}N_3O_9$ by high resolution electron impact mass spectrometry (HREI-MS). The IR spectra of them showed the presence of ester carbonyl (1735 cm^{-1}) and amide (1655 cm^{-1}) groups. The ^{13}C NMR spectra of **1**, **2** and **3** are summarized in Table 1.

Fig. 2. HPLC chromatogram of enniatin complex purified through silica gel and Sephadex LH-20 columns.

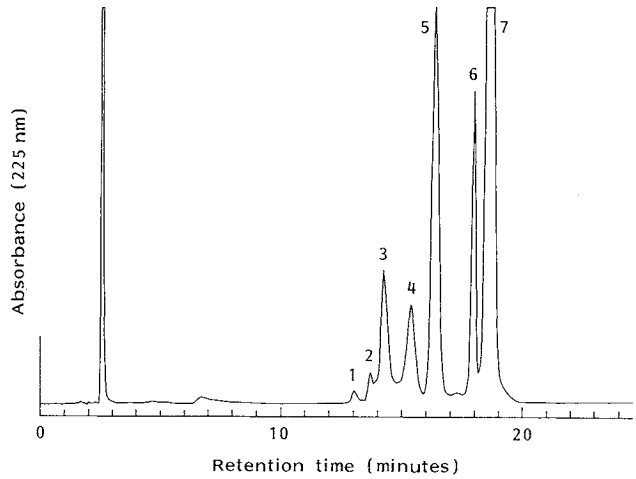


Table 1. Assignments of the ^{13}C NMR spectra of enniatins D (**1**), E (**2**) and F (**3**).

Carbon type		**1**	**2**	**3**
Val	α-CH	61.4, 63.1	61.2, 63.1	
	β-CH	27.6, 27.8	27.7, 27.9	
	γ-CH_3	19.9, 20.3	19.2, 19.6, 19.9, 20.3	
Leu	α-CH	57.0	57.7	57.3
	β-CH_2	37.9	37.9	37.9
	γ-CH	25.3	25.3	25.3
	δ-CH_3	21.5, 23.3	21.5, 23.3	21.4, 23.3
Ile	α-CH		59.9, 61.2	59.9, 61.2
	β-CH		33.5, 33.7	33.8
	γ-CH_2		25.1, 25.4	25.1, 25.5
	γ-CH_3		15.8, 16.1	15.8, 16.1
	δ-CH_3		10.5, 10.8	10.7, 10.8
Hiv	α-CH	75.0, 75.3, 77.2	74.9, 75.1, 75.3	75.0, 75.1, 77.2
	β-CH	29.7, 29.8, 30.0	29.7, 29.8, 30.0	29.7, 29.8, 30.3
	γ-CH_3	18.28, 18.33, 18.4, 18.6, 18.7, 18.9	18.26, 18.34, 18.38, 18.42, 18.5, 18.6, 18.7, 18.94, 18.97	18.28, 18.33, 18.4, 18.7, 19.0
	N-CH_3	31.7, 32.8, 33.6	31.6, 31.7, 32.4, 32.9	31.6, 32.4, 33.7
	CO-N	169.4	169.4, 169.7	169.3, 169.4, 169.8
	CO-O	170.4, 170.5, 170.8	170.4, 170.5, 170.72, 170.75	170.5, 170.7

To determine their *N*-methyl amino acid and hydroxy acid constituents including their stereochemistry, **1**, **2** and **3** were hydrolyzed with 6 N HCl at 115°C for 16 hours. The hydrolysates were diluted with water and extracted with diethyl ether. Both the phases and authentic samples were analyzed by HPLC using a chiral column (Fig. 3). As shown in Table 2, only D-α-hydroxyisovaleic acid (D-Hiv) was detected in the organic phases and L-*N*-methylleucine (L-Me-Leu) and/or L-*N*-methylisoleucine (L-Me-Ile) and L-*N*-methylvaline (L-Me-Val) were detected in the aqueous phases. In comparison with peaks of the hydrolysates and the authentic samples (L-*N*-methyl amino acids and D-Hiv), **1** was composed of one mole of L-Me-Leu, two moles of L-Me-Val and three moles of D-Hiv, **2** of one mole each of L-Me-Leu, L-Me-Ile

Table 2. The amino acid and hydroxy acid compositions of the acid hydrolysates of enniatins D (**1**), E (**2**), F (**3**), A, A1, B and B1.

Amino acid and hydroxy acid	Retention time (minutes)	Composition						
		1	**2**	**3**	A	A1	B	B1
N-Methyl-L-valine	5.3	2	1	0	0	1	3	2
N-Methyl-D-valine	6.2	0	0	0	0	0	0	0
N-Methyl-L-isoleucine	9.3	0	1	2	3	2	0	1
N-Methyl-D-isoleucine	11.7	0	0	0	0	0	0	0
N-Methyl-L-leucine	13.3	1	1	1	0	0	0	0
N-Methyl-D-leucine	17.4	0	0	0	0	0	0	0
α-Hydroxy-L-isovaleic acid	32.2	0	0	0	0	0	0	0
α-Hydroxy-D-isovaleic acid	56.0	3	3	3	3	3	3	3

Fig. 3. Separation of authentic chiral Me-Val, Me-Ile, Me-Leu and Hiv by HPLC.

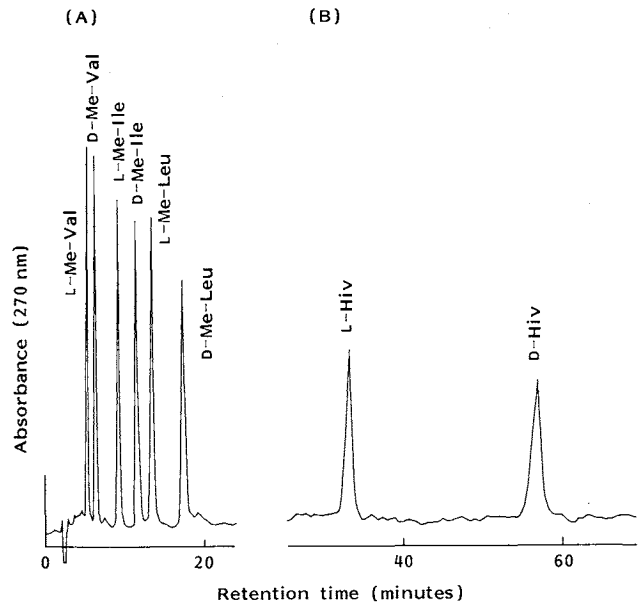


Table 3. The compositions of the reductive degradation compounds of enniatins D (**1**), E (**2**), F (**3**), A, A1, B and B1.

Compound	Retention time (minutes)	Composition						
		1	**2**	**3**	A	A1	B	B1
4	12.0	2	1	0	0	1	3	2
5	21.0	1	1	1	0	0	0	0
6	19.5	0	1	2	3	2	0	1

and L-Me-Val and three moles of D-Hiv and **3** of one mole of L-Me-Leu, two moles of L-Me-Ile and three moles of D-Hiv.

To determine the sequences between L-*N*-methyl amino acids and D-Hiv, **1**, **2** and **3** were reductively cleaved with $LiBH_4$ in THF and then acetylated with acetic anhydride in pyridine. Each of the products

was isolated by HPLC using an ODS column (Table 3) to give **4**, **5** and **6**, respectively. The structures of **4**, **5** and **6** were established as shown in Fig. 4 by their EI-MS and ^{1}H NMR spectra.

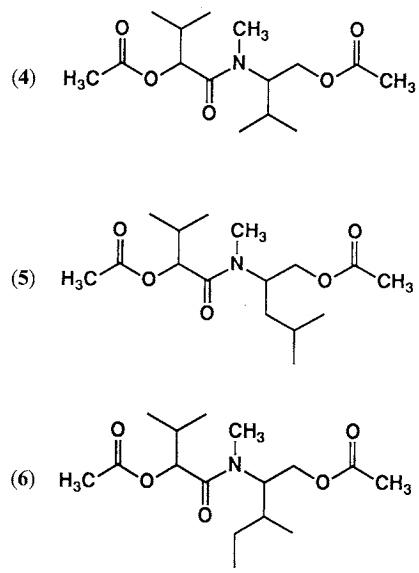
Accordingly, the structures of enniatins D, E and F were deduced to be cyclo(D-Hiv-L-Me-Leu-D-Hiv-L-Me-Val-D-Hiv-L-Me-Val), a mixture of cyclo(D-Hiv-L-Me-Leu-D-Hiv-L-Me-Ile-D-Hiv-L-Me-Val) and cyclo(D-Hiv-L-Me-Ile-D-Hiv-L-Me-Leu-D-Hiv-L-Me-Val), and cyclo(D-Hiv-L-Me-Leu-D-Hiv-L-Me-Ile-D-Hiv-L-Me-Ile) as shown in Fig. 5.

Biological Activity

ACAT inhibitory activity of cyclodepsipeptides including enniatins and beauvericin are reported in detail elsewhere[4]. In brief, all the enniatins described here inhibited ACAT activity in an enzyme assay using rat liver microsomes[1] with IC_{50} values of 22 to 110 μM (Fig. 6). More hydrophobic enniatins appears to show more potent ACAT inhibition in this assay. Among the cyclodepsipeptides tested, beauvericin showed the most potent inhibitory activity with an IC_{50} value of 3.0 μM under the same conditions.

The antimicrobial activity of enniatins A, A1, B, B1, E and F and beauvericin are summarized in Table 4. New enniatins E and F showed weak activity against Gram-possitive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* and very weak activity against *Candida albicans* and *Pyricularia oryzae*. None of the cyclodepsipeptides tested here showed potent antimicrobial activity.

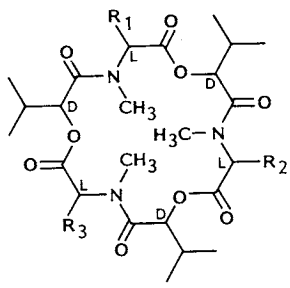
Fig. 4. Structures of reductive compounds **4**, **5** and **6**.



Discussion

Three new cyclodepsipeptides named enniatins D, E and F were isolated from *Fusarium* sp. in our

Fig. 5. Structures of enniatins.



Compound	R_1	R_2	R_3
Enniatin D (**1**)	I	I	II
Enniatin E (**2**)	I	II	III
	I	III	II
Enniatin F (**3**)	II	III	III
Enniatin A	III	III	III
Enniatin A1	I	III	III
Enniatin B	I	I	I
Enniatin B1	I	I	III
Enniatin C	II	II	II

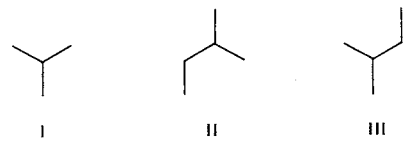


Fig. 6. ACAT inhibition by enniatins in an enzyme assay using rat liver microsomes.

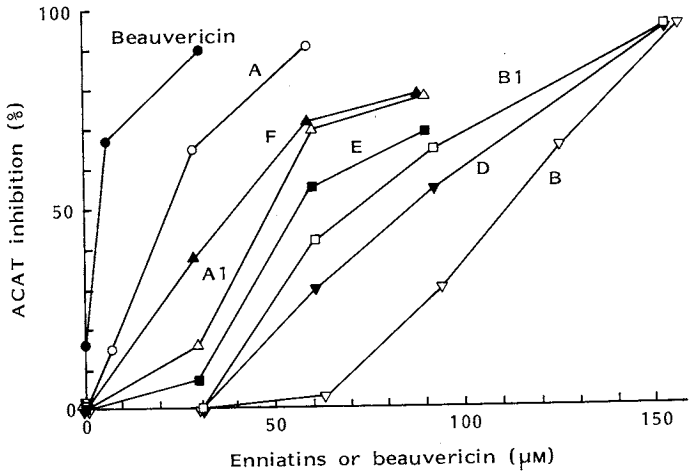


Table 4. Antimicrobial spectra of enniatins and beauvericin.

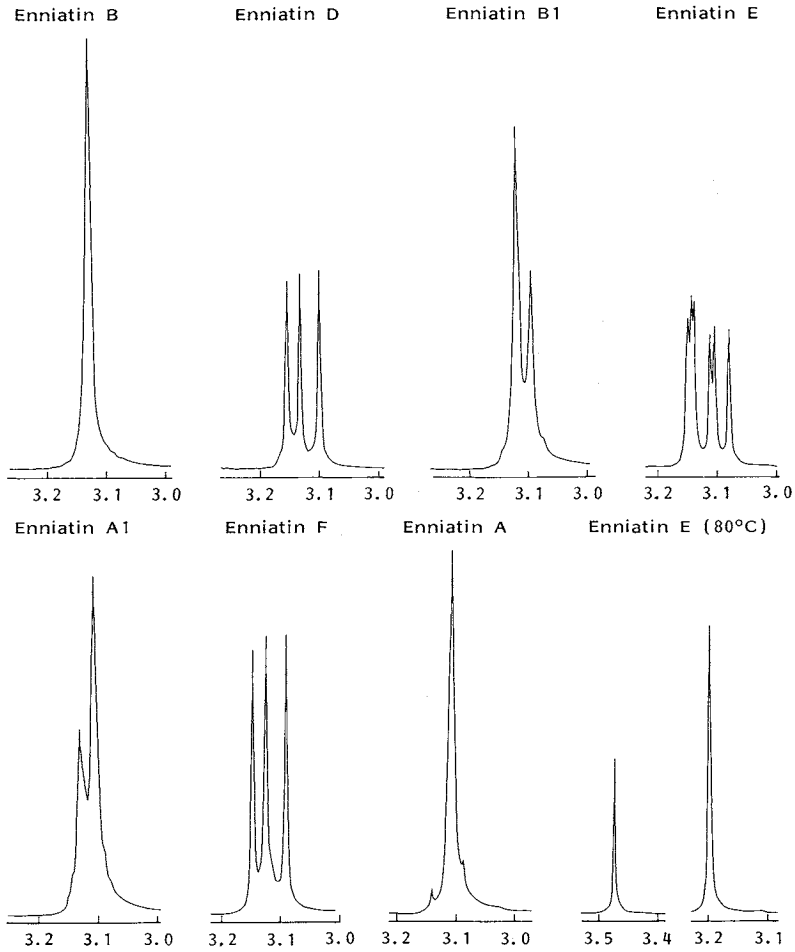
Test organism	MIC (μg/ml)					
	Enniatins					Beauvericin
	A	A1	B1	E	F	
Staphylococcus aureus ATCC 6538p KB 210	12.5	12.5	25	25	50	50
Bacillus subtilis ATCC 6633 KB 211	12.5	25	50	25	50	50
Bacillus cereus IFO 3001 KB 143	25	25	50	50	50	50
Micrococcus luteus ATCC 9341 KB 212	1.56	1.56	6.25	6.25	12.5	50
Mycobacterium smegmatis ATCC 607 KB 42	>50	>50	>50	>50	>50	>50
Escherichia coli NIHJ JC-2 KB 174	>50	>50	>50	>50	>50	>50
Klebsiella pneumoniae ATCC 10031 KB 214	>50	>50	>50	>50	>50	>50
Pseudomonas aeruginosa IFO 3080 KB 115	>50	>50	>50	>50	>50	>50
Xanthomonas oryzae KB 88	50	50	>50	>50	50	>50
Candida albicans KF 1	>50	>50	>50	>50	>50	>50
Saccharomyces sake KF 26	>50	>50	>50	>50	>50	>50
Pyricularia oryzae KF 180	>50	>50	>50	>50	50	>50
Trichphyton interdigitale KF 62	>50	50	>50	>50	>50	>50
Mucor racemosus IFO 4581 KF 223	>50	>50	>50	>50	>50	>50
Aspergillus niger KF 105	>50	>50	>50	>50	>50	>50

Bacteria: Mueller-Hinton agar (pH 7.3) (Difco); 37°C, 20 hours.
Fungi: Potato-dextrose agar (pH 5.6) (Difco); 27°C, 48 hours.

ACAT screening system. Up to now, a total of five enniatins (A, A1, B, B1 and C) have been reported[5]. Enniatins A, A1, B, and B1 have in common D-Hiv and L-Me-Ile and/or L-Me-Val and enniatin C was formed with L-Me-Leu and D-Hiv (Fig. 5). On the other hand, novel enniatins D (**1**), E (**2**) and F (**3**) consist of both D-Hiv and L-Me-Leu in common and L-Me-Ile and/or L-Me-Val. This time, we could determine their structures including the stereochemistry easily by using a chiral column-HPLC system without the chemical synthesis. The sensitivity of the detection of *N*-methyl amino acids and α-hydroxy-isovaleic acid on the chiral column-HPLC is less than 0.01 μg.

In the ^{1}H and ^{13}C NMR spectra of enniatin E (**2**), six *N*-methyl protons (Fig. 7) and nine γ-methyl

Fig. 7. *N*-methyl signals of enniatins by ^{1}H NMR analyses (400 MHz, in $CDCl_3$).



carbons in D-Hiv residue and four γ-methyl carbons in L-Me-Val residue (Table 1) were observed. These spectral data suggest that **2** is a mixture of two nonequivalent isomers or exists as two conformers [cyclo(D-Hiv-L-Me-Leu-D-Hiv-L-Me-Ile-D-Hiv-L-Me-Val) and/or cyclo(D-Hiv-L-Me-Ile-D-Hiv-L-Me-Leu-D-Hiv-L-Me-Val)]. A similar phenomenon was observed in the *N*-methyl proton signals of bassianolide[6], an insecticidal cyclodepsipeptide, and upon raising the temperature to 80°C, the signals were simplified. Bassianolide exists as two conformers in $CDCl_3$. It separated clearly into two components on a silica gel TLC and each component is interconverted together at room temperature. Concerning enniatin E (**2**), six *N*-methyl proton signals observed at room temperature merged into two signals upon raising the temperature to 80°C (Fig. 7). But in the case of **2**, the complexity of the ^{13}C and ^{1}H NMR spectra could be due to a mixture of two nonequivalent isomers rather than two conformers because of the following points: 1) Enniatin E (**2**) showed no interconversible spots on a silica gel TLC at various temperature such as bassianolide does, and 2) enniatins D (**1**) and F (**3**) have L-Me-Leu in common with **2**, but they showed no complicated signals (conformer signals) in the ^{13}C and ^{1}H NMR spectra such as **2** does.

Experimental

^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian XL-400 spectrometer, and MS spectra were obtained on a Jeol model DX-300 spectrometer. UV and IR spectra were recorded on a Shimadzu model UV-200S spectrophotometer and a Jasco model A-102 infrared spectrophotometer, respectively.

Enniatins D (**1**), E (**2**) and F (**3**)

1, **2** and **3** were purified from the cultured broth of *Fusarium* sp. FO-1305 by the method described above. Physico-chemical properties of **1**: $[\alpha]_D^{18}$ −63.0° (*c* 0.2, $CHCl_3$); IR ν_{max} (CCl_4) cm^{-1} 1735 (CO–O), 1655 (CO–N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.86~1.05 (36H), 1.53 (1H, m), 1.78 (2H, m), 2.25 (5H, m), 3.10 (3H, s), 3.13 (3H, s), 3.15 (3H, s), 4.46 (1H, d, J=9.5 Hz), 4.70 (1H, br s), 4.92 (1H, br d, J=9.5 Hz), 4.99 (1H, d, J=8.5 Hz), 5.08 (1H, d, J=8.5 Hz), 5.19 (1H, d, J=8.5 Hz). The ^{13}C NMR spectral data (100 MHz, $CDCl_3$) is shown in Table 1. HREI-MS *m/z* 653.4227 (calcd 653.4225 for $C_{34}H_{59}N_3O_9$); FAB-MS *m/z* 654 $(M+H)^+$, 676 $(M+Na)^+$, **2**: $[\alpha]_D^{18}$ −77.0° (*c* 1.0, $CHCl_3$); IR ν_{max} (CCl_4) cm^{-1} 1735 (CO–O), 1655 (CO–N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.84~1.04 (36H), 1.08 (1H, m), 1.39 (1H, m), 1.52 (1H, m), 1.73 (1H, m), 1.83 (1H, m), 2.01 (1H, m), 2.24 (5H, m), 3.07 (1.5H, s), 3.09 (1.5H, s), 3.10 (1.5H, s), 3.12 (1.5H, s), 3.13 (1.5H, s), 3.14 (1.5H, s), 4.44 (0.5H, d, J=10.0 Hz), 4.65 (0.5H, d, J=10.0 Hz), 4.70 (1H, br s), 4.91 (0.5H, d, J=10.0 Hz), 4.95 (0.5H, d, J=9.0 Hz), 4.97 (0.5H, d, J=9.0 Hz), 5.03 (0.5H, d, J=8.5 Hz), 5.04 (0.5H, d, J=10.0 Hz), 5.07 (0.5H, d, J=8.5 Hz), 5.16 (0.5H, d, J=8.5 Hz), 5.20 (0.5H, d, J=8.5 Hz). The ^{13}C NMR spectral data (100 MHz, $CDCl_3$) is shown in Table 1. HREI-MS *m/z* 667.4403 (calcd 667.4406 for $C_{35}H_{61}N_3O_9$); FAB-MS *m/z* 668 $(M+H)^+$, 690 $(M+Na)^+$, **3**: $[\alpha]_D^{18}$ −70.0° (*c* 1, $CHCl_3$); IR ν_{max} (CCl_4) cm^{-1} 1735 (CO–O), 1655 (CO–N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.83~1.01 (36H), 1.08 (2H, m), 1.39 (2H, m), 1.53 (1H, m), 1.74 (1H, m), 1.83 (1H, m), 2.02 (2H, m), 2.25 (3H, m), 3.08 (3H, s), 3.11 (3H, s), 3.13 (3H, s), 4.65 (2H, d, J=10.0 Hz), 4.94 (1H, d, J=9.0 Hz), 5.03 (1H, d, J=8.5 Hz), 5.05 (1H, d, J=10 Hz), 5.19 (1H, d, J=8.5 Hz). The ^{13}C NMR spectral data (100 MHz, $CDCl_3$) is shown in Table 1. HREI-MS *m/z* 681.4575 (calcd 681.4564 for $C_{36}H_{63}N_3O_9$); FAB-MS *m/z* 682 $(M+H)^+$, 704 $(M+Na)^+$.

Acid Hydrolysis

One mg of each of **1**, **2** and **3** was hydrolyzed with 6 N HCl (1 ml) at 115°C in a sealed tube for 16 hours. Each hydrolysate was diluted with water and extracted with diethyl ether. Both the diethyl ether and water layers were concentrated to dryness and subjected to HPLC (column: Sumika Chemical Analysis Service Ltd., Sumichiral OA 5100, 4.6×120 mm; detection: UV 270 nm; flow rate: 0.8 ml/minute; mobile phase: 5 mM $CuSO_4$ in water-methanol, 85:15 for the isolation of *N*-methyl amino acids (Fig. 3A) or 72:28 for the hydroxy acid (Fig. 3B)).

Reduction with $LiBH_4$ and Acetylation with Acetic Anhydride

Ten mg of each of **1**, **2** and **3** was dissolved in THF (1 ml), and $LiBH_4$ (5 mg) was added. After stirring for 6 hours in an ice-bath, the reaction mixture was poured into water (10 ml) and extracted with diethyl ether (10 ml). The ether layer was concentrated to dryness, the residue was dissolved in pyridine (100 μl), and acetic anhydride (100 μl) was added. After stirring for 16 hours at room temperature, the reaction mixture was poured into water and extracted with EtOAc. The EtOAc layer was concentrated to dryness and subjected to HPLC (column: Chemco, Chemcosorb 5ODS-UH (4.6×150 mm), detection: UV 220 nm, flow rate: 0.8 ml/minute, mobile phase: acetonitrile-water (60:40)) to give **4**, **5** and **6**. Physico-chemical properties of **4**: EI-MS *m/z* 301 $(M)^+$, 258, 241; FAB-MS *m/z* 302 $(M+H)^+$, 324 $(M+Na)^+$, ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (3H, d, J=6.5 Hz), 0.99 (3H, d, J=6.6 Hz), 1.01 (6H, d, J=6.5 Hz), 1.86 (1H, m), 2.02 (3H, s), 2.11 (3H, s), 2.17 (1H, m), 2.98 (3H, s), 4.21 (2H, d, J=5.5 Hz), 4.40 (1H, m), 5.01 (1H, d, J=6.5 Hz), **5**: EI-MS *m/z* 315 $(M)^+$, 242; FAB-MS *m/z* 316 $(M+H)^+$, 338 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, J=6.5 hz), 0.93 (3H, d, J=6.5 Hz), 0.99 (3H, d, J=6.5 Hz), 1.00 (3H, d, J=6.5 Hz), 1.17 (1H, m), 1.43 (1H, m), 1.46 (1H, m), 2.02 (3H, s), 2.11 (3H, s), 2.15 (1H, m), 2.92 (3H, s), 4.00 (1H, dd, J=11.0, 4.7 Hz), 4.11 (1H, dd, J=11.0, 9.0 Hz), 4.97 (1H, m), 4.99 (1H, d, J=6.3 Hz), **6**: EI-MS *m/z* 315 $(M)^+$, 258, 242; FAB-MS *m/z* 315 $(M+H)^+$, 324 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (3H, t, J=7.0 Hz), 0.95 (3H, d, J=6.5 Hz), 1.01 (3H, d, J=6.5 Hz), 1.08 (1H, m), 1.36 (1H, m), 1.69 (1H, m), 2.02 (3H, s), 2.11 (3H, s), 2.16 (1H, m), 2.95 (3H, s), 4.21 (1H, d, J=6.5 Hz), 4.54 (1H, m), 5.01 (1H, d, J=6.3 Hz).

Acknowledgment

We express our thanks to Ms. N. SATO, School of Pharmaceutical Sciences, Kitasato University, for measuring

NMR spectra. This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and from Japan Keirin Association.

References

1) TOMODA, H.; H. NISHIDA, R. MASUMA, J. CAO, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Penicillium purpurogenum*. I. Production, isolation and physico-chemical and biological properties. J. Antibiotics 44: 136～143, 1991
2) NISHIDA, H.; H. TOMODA, J. CAO, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Penicillium purpurogenum*. II. Structure elucidation of purpactins A, B and C. J. Antibiotics 44: 144～151, 1991
3) NISHIDA, H.; H. TOMODA, J. CAO, S. ARAKI, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Penicillium purpurogenum*. III. Chemical modification of purpactin A. J. Antibiotics 44: 152～159, 1991
4) TOMODA, H.; X.-H. HUANG, J. CAO, H. NISHIDA, R. NAGAO, S. OKUDA, H. TANAKA, S. ŌMURA, H. ARAI & K. INOUE: Inhibition of acyl-CoA : cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J. Antibiotics, 45 (10): 1992, in press
5) DEOL, B. S.; D. D. RIDLEY & P. SINGH: Isolation of cyclodepsipeptides from plant pathogenic fungi. Aust. J. Chem. 31: 1397～1399, 1978
6) KANAOKA, M.; A. ISOGAI, S. MURAKOSHI, M. ICHINOE, A. SUZUKI & S. TAMURA: Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. Agric. Biol. Chem. 42: 629～635, 1978