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New Beauvericins, Potentiators of Antifungal Miconazole Activity, Produced by *Beauveria* sp. FKI-1366

II. Structure Elucidation

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The structures of beauvericins D, E and F, novel potentiators of miconazole activity against *Candida albicans* produced by *Beauveria* sp. FKI 1366, were elucidated by various spectroscopic analyses including UV, NMR, and MS and degradation experiments. They have the common skeleton of the 18-membered cyclodepsipeptides.

During the course of screening for potentiators of antifungal miconazole activity, new beauvericins D, E and F (Fig. 1) were isolated along with known beauvericin and beauvericin A from the culture broth of *Beauveria* sp. FKI-1366[1]. The fermentation, isolation and their biological properties are described in the preceding paper[1]. We report herein the structure elucidation of beauvericins D, E and F.

Materials and Methods

Materials

Beauvericin and beauvericins A, D, E and F were purified from the culture broth of *Beauveria* sp. FKI 1366 as described in the preceding paper[1]. (*S*)-2-*N*-methylamino-3-phenylpropionic acid (L-*N*-methylphenylalanine) and (*R*)-2-*N*-methylamino-3-phenylpropionic acid (D-*N*-methylphenylalanine) were purchased from Watanabe Chemical Co. (Hiroshima, Japan). (*S*)-2-amino-3-phenylpropionic acid (L-phenylalanine) and (*R*)-2-amino-3-phenylpropionic acid (D-phenylalanine) and (*S*)-2-amino-4-methylpentanoic acid (L-leucine) and (*R*)-2-amino-4-methylpentanoic acid (D-leucine) were purchased from Kanto Chemical Co. (Tokyo, Japan). (*R*) and (*S*)-2-hydroxy-3-methylbutanoic acids were obtained from Aldrich Chemical Co. (Missouri, USA) and (*R*) and (*S*)-2-hydroxy-4-methylpentanoic acids were synthesized from D- and L-leucines by the established method[2], respectively.

General Experimental Procedures

Optical rotations were recorded with a DIP-370 digital polarimeter (JASCO, Tokyo, Japan). Melting points were measured with a micro melting apparatus (Yanaco, Kyoto, Japan). FAB-MS spectrometry was conducted on a JMS-AX505H spectrometer (JEOL, Tokyo, Japan). UV and IR spectra were measured with a DU640 spectrophotometer (Beckman, California, USA) and an FT-210 Fourier transform infrared spectrometer (Horiba, Kyoto, Japan), respectively. The various NMR spectra were measured with an XL-400 spectrometer (Varian, California, USA).

Acid Hydrolysis

Beauvericins D, E and F (500 μg) were degraded in a gas phase of 6 N HCl (990 μl) at 115°C for 2 hours using the PICO·TAG work station (Waters, Massachusetts, USA). The degradation products were dissolved in MeOH (200 μl), and used for determining amino acid and hydroxy

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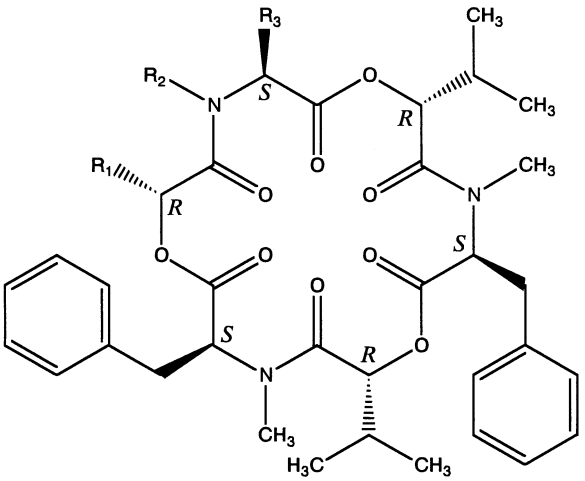
Fig. 1. Structures of beauvericin, beauvericins A, D, E and F.



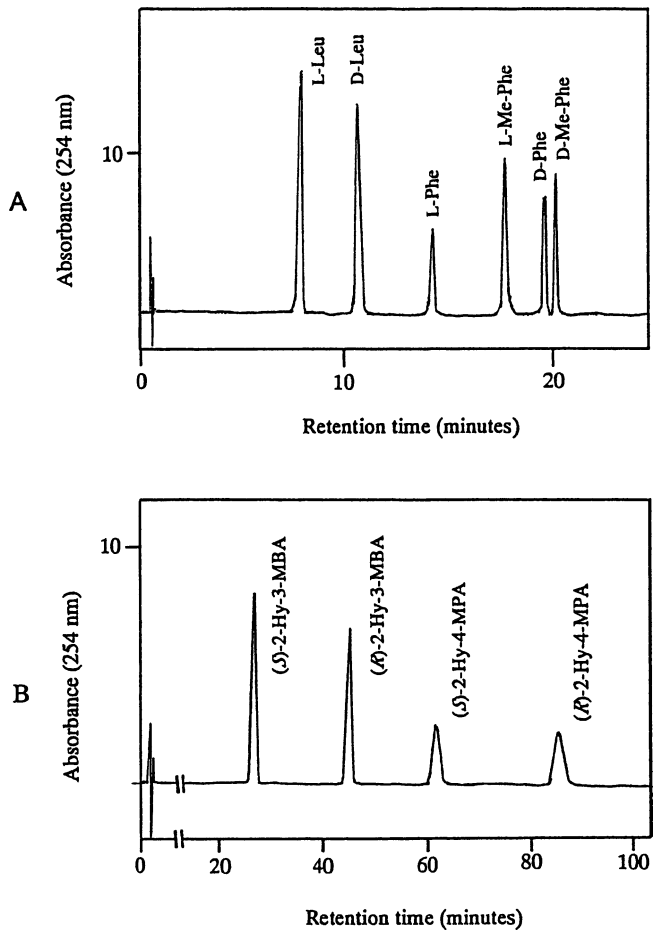
Compound	R_1	R_2	R_3
Beauvericin	$CH(CH_3)_2$	CH_3	$CH_2C_6H_5$
Beauvericin A	$CH(CH_3)CH_2CH_3$	CH_3	$CH_2C_6H_5$
Beauvericin D	$CH(CH_3)_2$	H	$CH_2C_6H_5$
Beauvericin E	$CH(CH_3)_2$	H	$CH_2CH(CH_3)_2$
Beauvericin F	$CH_2CH(CH_3)_2$	CH_3	$CH_2C_6H_5$

acid constituents.

Analysis of Stereochemistries of Amino Acids and Hydroxy Acids by HPLC

To determine the stereochemistry of the amino acid contents, a part of the MeOH solution (20 μl) was analyzed on a HP1100 system (Hewlett Packard Inc., Germany) under the following conditions: column, SUMICHIRAL OA-5000 (Sumika Chemical Analysis Service, Ltd., Osaka, Japan), 4.6 i.d.×150 mm; flow rate, 1.0 ml/minute; detection, UV at 254 nm). Using 30% MeOH in 2 mM aq $CuSO_4$ as a solvent for HPLC, D- and L-*N*-methylphenylalanines, D- and L-phenylalanines and D- and L-leucines were eluted as peaks with retention times of 20.8, 18.5, 19.9, 14.4, 11.0, 7.9, respectively (Fig. 2A). Under the same conditions, (*R*) and (*S*)-2-hydroxy-4-methylpentanoic acids and (*R*) and (*S*)-2-hydroxy-3-methylbutanoic acids were eluted as peaks with retention times of 85.4, 61.4, 44.1 and 25.5 minutes, respectively (Fig. 2B).

Fig. 2. Separation of D- and L-amino acids (A) and (*R*)-2-hydroxy-3-methylbutanoic acid ((*R*)-2-Hy-3-MBA), (*S*)-2-hydroxy-3-methylbutanoic acid ((*S*)-2-Hy-3-MBA), (*R*)-2-hydroxy-4-methylpentanoic acid ((*R*)-2-Hy-4-MPA), and (*S*)-2-hydroxy-4-methylpentanoic acid ((*S*)-2-Hy-4-MPA) (B) by HPLC.



Column, SUMICHIRAL OA-5000, 4.6×150 mm; flow rate, 1.0 ml/minute; detection, UV at 254 nm; a solvent, 30% MeOH in 2 mM aq $CuSO_4$. Each amino acid and hydroxy acid (20 μg) were injected.

Results

Physico-chemical Properties of Beauvericins D, E and F

Physico-chemical properties of beauvericins D, E and F are summarized in Table 1. Similarity in their data indicated that they are structurally related. Beauvericins D, E and F showed the same end absorption in the UV spectra. Their IR spectra showed absorptions at 1743, 1662～1664

Table 1. Physico-chemical properties of beauvericins D, E and F.

	Beauvericin D	Beauvericin E	Beauvericin F
Appearance	white powder	white powder	white powder
Melting point	84~85℃	73~75℃	63~65℃
$[\alpha]_D^{26}$	+9.0 (*c* 0.1, CH_3OH)	+18.4 (*c* 0.1, CH_3OH)	+31.4 (*c* 0.1, CH_3OH)
Molecular formula	$C_{44}H_{55}N_3O_9$	$C_{41}H_{57}N_3O_9$	$C_{46}H_{59}N_3O_9$
Molecular weight	769	735	797
HR-FAB-MS *m/z* $(M+Na)^+$			
Calcd	792.3835	758.3992	820.4148
Found	792.3829	758.3970	820.4186
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	213 (26,000)	208 (30,500)	208 (11,200)
IR ν_{max}^{KBr} cm^{-1}	2966, 2875, 1743, 1662 1456, 1261, 1203, 1105	2964, 2873, 1743, 1664 1456, 1261, 1201, 1101	2962, 2873, 1743, 1662 1484, 1261, 1203, 1101
Solubility			
Soluble	DMSO, CH_3OH $CHCl_3$, ethyl acetate	DMSO, CH_3OH $CHCl_3$, ethyl acetate	DMSO, CH_3OH $CHCl_3$, ethyl acetate
Insoluble	*n*-Hexane, H_2O	*n*-Hexane, H_2O	*n*-Hexane, H_2O

and 1456~1484 cm^{-1}, suggesting the presence of carbonyl and phenyl groups.

Structure Elucidation of Beauvericin D

The molecular formula of beauvericin D was determined to be $C_{44}H_{55}N_3O_9$ on the basis of HRFAB-MS measurement (Table 1). The ^{13}C NMR spectrum (in $CDCl_3$) showed 44 resolved signals, which were classified into eight methyl carbons, three methylene carbons, three *O*-methine carbons, three *N*-methine carbons, three methine carbons, fifteen sp^2 methine carbons, three quaternary carbons and six carbonyl carbons by analysis of DEPT spectra. The ^1H NMR spectrum (in $CDCl_3$) showed six methyl signals, two *N*-methyl signals, three methylene signals, three *O*-methine signals, three *N*-methine signals, three methine signals and fifteen aromatic signals. The connectivity of proton and carbon atoms was established by the ^{13}C-^1H HMQC spectrum as shown in Table 2. Analysis of the ^1H-^1H COSY and ^{13}C-^1H HMBC spectra revealed the three partial structures I, II and III (Fig. 3).

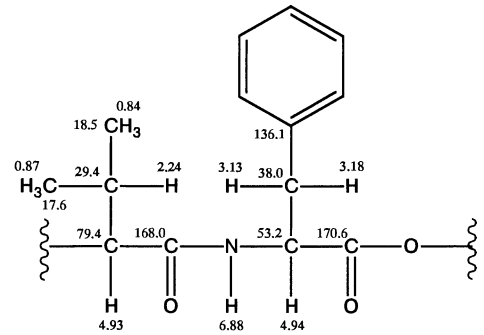
The ^{13}C-^1H long range couplings of ^2J and ^3J observed in the ^{13}C-^1H HMBC experiments (Fig. 4) gave the following evidence. 1) The cross peaks from 1-H (δ 4.93) to C-3 (δ 29.4), C-4 (δ 17.6), C-5 (δ 18.5) and C-6 (δ 168.0), from 3-H (δ 2.24) to C-1 (δ 79.4), C-4, C-5 and C-6, from 4-H_3 (δ 0.87) to C-1, C-3 and C-5, from 5-H_3 (δ 0.84) to C-1, C-3 and C-4, from 8-H (δ 4.94) to C-6, C-9 (δ 38.0), C-10 (δ 136.1) and C-16 (δ 170.6) and from 9-H_2 (δ 3.13, 3.18) to C-8 (δ 53.2), C-10 and C-16 supported the partial structure I. 2) The cross peaks from 17-H (δ 4.81) to C-18 (δ 29.8), C-19 (δ 17.6), C-20 (δ 18.0) and C-21 (δ 169.3), from 18-H (δ 1.90) to C-17 , C-19, C-20 and C-21, from 19-H_3 (δ 0.81) to C-17, C-18 and C-20, from 20-H_3 (δ 0.55) to C-17, C-18 and C-19, from 22-H_3 (δ 2.80) to C-21 and C-23 (δ 60.3), from 23-H (δ 4.94) to C-21, C-22, C-24 (δ 34.6), C-25 (δ 136.3) and C-31 (δ 170.6) and from 24-H_2 (δ 3.01, 3.42) to C-23, C-25 and C-31 supported the partial structure II. 3) The cross peaks from 32-H (δ 5.02) to C-33 (δ 29.1), C-34 (δ 17.3), C-35 (δ 18.0) and C-36 (δ 169.5),

Table 2. ^{1}H and ^{13}C NMR chemical shifts of beauvericins D, E and F.

	Beauvericin D		Beauvericin E		Beauvericin F	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	79.4	4.93 (1H, d, J =7.0 Hz)	80.0	4.97 (1H, d, J =7.0 Hz)	69.8	5.20 (1H, dd, J =9.5, 4.0 Hz)
C-2					38.5	1.28 (1H, m)
						1.64 (1H, m)
C-3	29.4	2.24 (1H, m)	30.0	2.18 (1H, m)	24.3	1.46 (1H, m)
C-4	17.6	0.87 (3H, d, J =6.5 Hz)	17.9	0.93 (3H, d, J =7.0 Hz)	21.6	0.80 (3H, d, J =6.5 Hz)
C-5	18.5	0.84 (3H, d, J =6.5 Hz)	18.5	0.92 (3H, d, J =7.0 Hz)	22.9	0.86 (3H, d, J =6.5 Hz)
C-6	168.0		168.4		169.2	
C-7					34.2	2.76 (3H, s)
NH		6.88 (1H, d, J =8.0 Hz)		6.78 (1H, d, J =9.5 Hz)		
C-8	53.2	4.94 (1H, m)	50.0	4.84 (1H, td, J =9.5, 5.5 Hz)	60.7	4.63 (1H, m)
C-9	38.0	3.13 (1H, dd, J =14.5, 9.0 Hz)	42.0	1.58 (1H, dd, J =15.0, 9.5 Hz)	34.5	3.12 (1H, dd, J =12.0, 4.5 Hz)
		3.18 (1H, dd, J =14.5, 7.0 Hz)		1.78 (1H, dd, J =15.0, 4.0 Hz)		3.30 (1H, dd, J =12.5, 12.0 Hz)
C-10	136.1				137.1	
C-11	126.0-129.0	7.15-7.35 (1H, m)			126.7-129.1	7.18-7.32 (1H, m)
C-12	126.0-129.0	7.15-7.35 (1H, m)			126.7-129.1	7.18-7.32 (1H, m)
C-13	126.0-129.0	7.15-7.35 (1H, m)			126.7-129.1	7.18-7.32 (1H, m)
C-14	126.0-129.0	7.15-7.35 (1H, m)			126.7-129.1	7.18-7.32 (1H, m)
C-15	126.0-129.0	7.15-7.35 (1H, m)			126.7-129.1	7.18-7.32 (1H, m)
C-16	170.6		171.7		169.8	
C-17	75.6	4.81 (1H, d, J =6.2 Hz)	75.2	5.15 (1H, d, J =9.5 Hz)	74.7	5.06 (1H,d, J =9.0 Hz)
C-18	29.8	1.90 (1H, m)	28.7	2.23 (1H,m)	29.7	2.00 (1H, m)
C-19	17.6	0.81 (3H, d, J =6.5 Hz)	17.6	0.55 (3H, d, J =7.0 Hz)	17.3	0.39 (3H, d, J =6.5 Hz)
C-20	18.0	0.55 (3H, d, J =6.5 Hz)	17.9	0.84 (3H, d, J =7.0 Hz)	18.1	0.77 (3H, d, J =6.5 Hz)
C-21	169.3		169.5		169.2	
C-22	33.8	2.80 (3H, s)	33.1	2.90 (3H, s)	31.6	3.04 (3H, s)
C-23	60.3	4.94 (1H, m)	59.4	5.18 (1H, m)	56.4	5.65 (1H, m)
C-24	34.6	3.01 (1H, dd, J =14.5, 11.5 Hz)	33.1	3.05 (1H, dd, J =14.5, 12.0 Hz)	34.0	2.99 (1H, dd, J =15.0, 11.5 Hz)
		3.42 (1H, dd, J =14.5, 4.5 Hz)		3.47 (1H, dd, J =14.5, 5.0 Hz)		3.31 (1H, dd, J =15.0, 5.5 Hz)
C-25	136.3		135.9		136.3	
C-26	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-27	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-28	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-29	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-30	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-31	170.6		169.7		170.1	
C-32	75.6	5.02 (1H, d, J =9.0 Hz)	76.0	4.77 (1H, d, J =7.0 Hz)	74.9	5.06 (1H, d, J =9.0 Hz)
C-33	29.1	2.08 (1H, m)	29.8	1.90 (1H, m)	29.6	2.10 (1H, m)
C-34	17.3	0.44 (3H, d, J =6.5 Hz)	17.7	0.51 (3H, d, J =7.0 Hz)	17.3	0.37 (3H, d, J =6.5 Hz)
C-35	18.0	0.70 (3H, d, J =6.5 Hz)	17.8	0.83 (3H, d, J =7.0 Hz)	18.2	0.75 (3H, d, J =6.5 Hz)
C-36	169.5		169.4		169.2	
C-37	31.6	2.90 (3H, s)	30.7	2.85 (3H, s)	32.0	3.06 (3H, s)
C-38	56.0	5.74 (1H, m)	55.8	5.88 (1H, m)	56.8	5.54 (1H, m)
C-39	33.5	3.01 (1H, dd, J =14.5, 11.5 Hz)	34.5	3.04 (1H, dd, J =14.5, 12.0 Hz)	35.2	2.94 (1H, dd, J =14.0, 11.5 Hz)
		3.37 (1H, dd, J =14.5, 5.5 Hz)		3.44 (1H, dd, J =14.5, 5.0 Hz)		3.34 (1H, dd, J =14.0, 5.5 Hz)
C-40	136.8		136.6		136.5	
C-41	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-42	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-43	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-44	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-45	126.0-129.0	7.15-7.35 (1H, m)	126.8-129.0	7.15-7.32 (1H, m)	126.7-129.1	7.18-7.32 (1H, m)
C-46	170.0		171.0		169.9	
C-47			24.7	1.59 (1H, m)		
C-48			21.6	0.95 (3H, d, J =7.0 Hz)		
C-49			23.1	0.92 (3H, d, J =7.0 Hz)		

a) Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm. b) Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.

Fig. 3. Partial structures I, II and III of beauvericin D.



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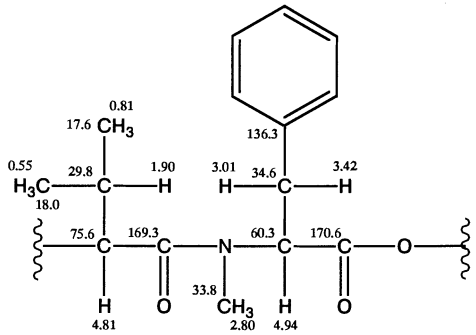

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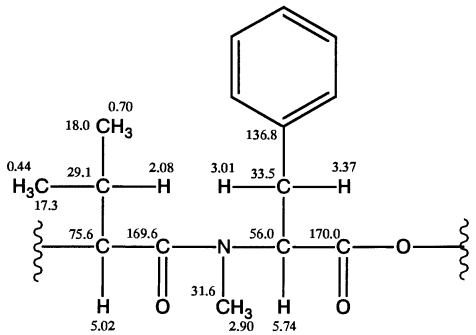

III

from 33-H (δ 2.08) to C-32 (δ 75.6), C-34 and C-35, from 34-H_3 (δ 0.44) to C-32, C-33 and C-35, from 35-H_3 (δ 0.70) to C-32, C-33 and C-34, from 37-H_3 (δ 2.90) to C-36 and C-38 (δ 56.0) and from 39-H_2 (δ 3.01, 3.37) to C-38, C-40 (δ 163.8) and C-46 (δ 170.0) supported the partial structure III. 4) The cross peaks from 1-H to C-46, from 17-H to C-16 and from 32-H to C-31 indicated that the partial structures I, II and III are linked to give a cyclic structure as shown in Fig. 4. The structure is in good agreement with the molecular formula.

Taken together, the structure of beauvericin D was elucidated as shown in Fig 1. All the data assigned here is very reasonable in comparison with the data of beauvericin reported previously[1].

Structure Elucidation of Beauvericin E

The molecular formula ($C_{41}H_{57}N_3O_9$) of beauvericin E is C_3 smaller and H_2 bigger than that of beauvericin D. The different point between beauvericins D and E is that the partial structure IV (Fig. 5) for beauvericin E, in place of the partial structure I for beauvericin D, was elucidated by the ^{1}H-^{1}H COSY experiment. The cross peaks from 1-H (δ 4.97) to C-3 (δ 30.0), C-4 (δ 17.9), C-5 (δ 18.5) and C-6 (δ 168.4), from 3-H (δ 2.18) to C-1 (δ 80.0), C-4, C-5 and C-6, from 4-H_3 (δ 0.93) to C-1, C-3 and C-5, from 5-H_3 (δ 0.92) to C-1, C-3 and C-4, 8-H (δ 4.84) to C-6, C-9 (δ 42.0), C-47 (δ 24.7) and C-16 (δ 171.7), from 9-H_2 (δ 1.58, 1.78) to C-8 (δ 50.0), C-47 and C-16, from 47-H (δ 1.59) to C-9, C-48 (δ 21.6) and C-49 (δ 23.1), 48-H_3 (δ 0.95) to C-47 and C-49 and from 49-H_3 (δ 0.92) to C-47 and C-48 were observed in the ^{13}C-^{1}H HMBC experiments to give the partial structure IV (Fig. 5). The cross peaks from 1-H to C-46 (δ 171.0) and from 17-H (δ 5.15) to C-16 indicated that the partial structures IV, II and III are linked to give a cyclic structure as shown in Fig. 1. The molecular formula also supported this structure (Table 1). Thus, the structure of beauvericin E was elucidated as shown in Fig. 1.

Structure Elucidation of Beauvericin F

The molecular formula ($C_{46}H_{59}N_3O_9$) of beauvericin F is C_2H_4 bigger than that of beauvericin D. The partial structure V (Fig. 6) in place of the partial structure I for beauvericin D was defined by the ^{1}H-^{1}H COSY experiments (Fig. 6). The cross peaks from 1-H (δ 5.20) to C-2 (δ 38.5), C-3 (δ 24.3) and C-6 (δ 169.2), from 2-H_2 (δ 1.28, 1.64) to C-1 (δ 69.8), C-3, C-4 (δ 21.6), C-5 (δ 22.9) and C-6, from 3-H (δ 1.46) to C-1, C-2, C-4 and C-5, from 4-H_3 (δ 0.80) to C-2, C-3 and C-5, from 5-H_3 (δ 0.86) to C-2, C-3 and C-4, from 7-H_3 (δ 2.76) to C-6, 8-H (δ 4.63) to C-6, C-9 (δ 34.5), C-10 (δ 137.0) and C-16 (δ 169.8) and from 9-H_2 (δ 3.12, 3.30) to C-8 (δ 60.7), C-10 and C-16 in the ^{13}C-^{1}H HMBC experiments supported the partial structure V. The cross peaks from 1-H to C-46 (δ 169.9) and from 17-H (δ 5.06) to C-16 indicated that the partial structures V, II and III are linked to give a cyclic structure as shown in Fig. 1. The molecular formula is also

Fig. 4. ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of beauvericin D.

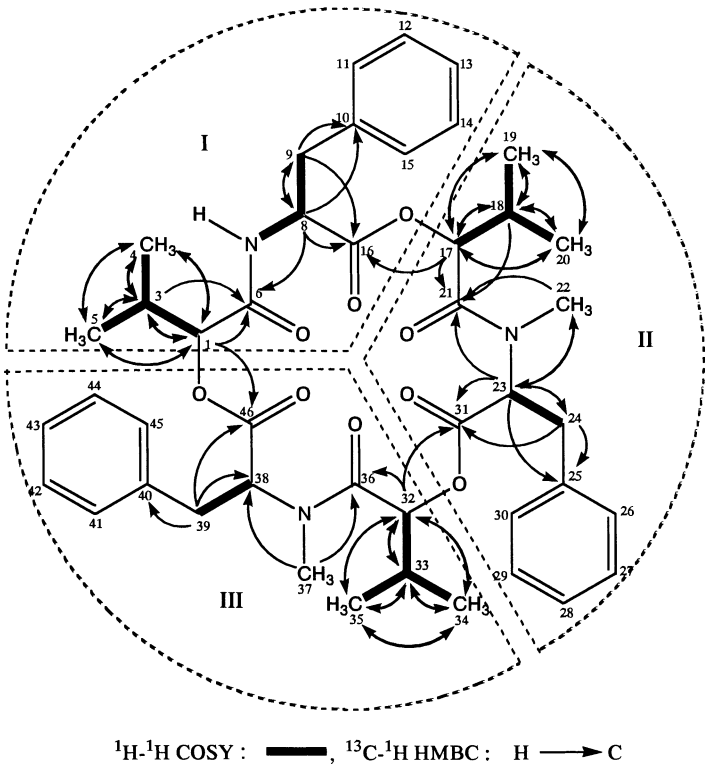

Fig. 5. Partial structure IV of beauvericin E.

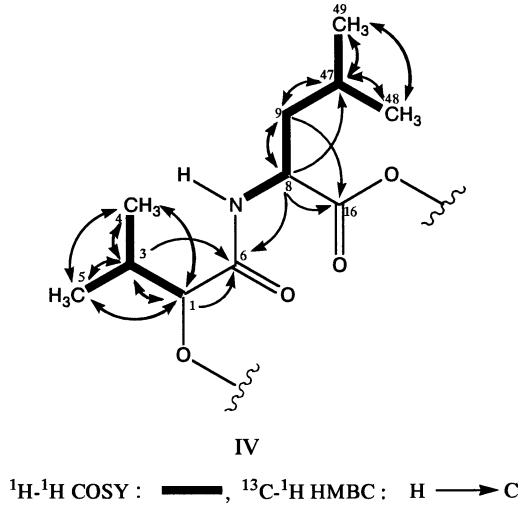

Fig. 6. Partial structure V of beauvericin F.

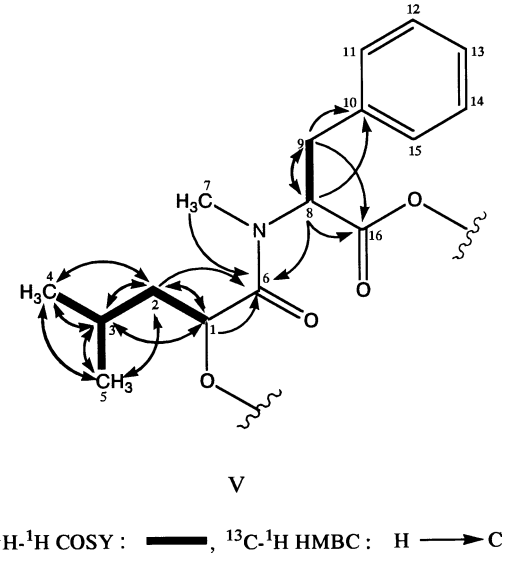

Table 3. Determination of stereochemistries of amino acid and hydroxy acid compositions in beauvericin hydrolysates by HPLC.

Amino acid and hydroxy acid	Retention time* (minutes)	Beauvericin D	Beauvericin E	Beauvericin F
		Composition (mole ratio)		
L-Leucine	7.9	0	1	0
D-Leucine	11.0	0	0	0
L-Phenylalanine	14.4	1	0	0
L-*N* -Methylphenylalanine	18.5	2	2	3
D-Phenylalanine	19.9	0	0	0
D-*N* -Methylphenylalanine	20.8	0	0	0
(*S*)-2-Hydroxy-3-methylbutanoic acid	25.5	0	0	0
(*R*)-2-Hydroxy-3-methylbutanoic acid	44.1	3	3	2
(*S*)-2-Hydroxy-4-methylpentanoic acid	61.4	0	0	0
(*R*)-2-Hydroxy-4-methylpentanoic acid	85.4	0	0	1

* Separation by HPLC column, SUMICHIRAL OA-5000 (4.6 i.d. x 150 mm); UV at 254nm; solvent 30% MeOH in 2 mM aq $CuSO_4$ 1ml/minute.

compatible with this structure (Table 1). Thus, the structure of beauvericin F was elucidated as shown in Fig. 1.

Stereochemistries of Beauvericins

The stereochemistries of amino acid and hydroxy acid constituents were determined by HPLC using a chiral column. In comparison with the authentic amino acids (Fig. 2A) and hydroxy acids (Fig. 2B), beauvericin D was found to consist of two L-*N*-methylphenylalanines, one L-phenylalanine, and three (*R*)-2-hydroxy-3-methylbutanoic acids. Similarly, beauvericin E consists of two L-*N*-methylphenylalanines, one L-leucine, and three (*R*)-2-hydroxy-3-methylbutanoic acids, and beauvericin F consists of three L-*N*-methylphenylalanines, and two (*R*)-2-hydroxy-3-methylbutanoic acids and one (*R*)-2-hydroxy-4-methylpentanoic acid (Table 3).

Taken together, the structures of beauvericins D, E and F including the absolute stereochemistries were elucidated as shown in Fig. 1.

Discussion

Beauvericin, a cyclohexadepsipeptide antibiotic consisting of three L-*N*-methylphenylalanine units connected alternatively with three (*R*)-2-hydroxy-3-methylbutanoic acid residues. Seven beauvericin members of 18-membered cyclodepsipeptides family have been reported so far: namely, beauvericin, beauvericins A to C[3~5] and allobeauvericins A to C[6]. They have the following structural characteristics; 1) three *N*-methylphenylalanines and three hydroxylic acids are condensed mutually to form a cyclic structure, and 2) hydroxylic acids are 2-hydroxy-3-methylpentanoic acid or 2-hydroxy-3-methylbutanoic acid.

As elucidated in this paper, however, beauvericins D and E are the first member of beauvericins containing one *N*-demethyl-amino acid. Furthermore, beauvericin E has one *N*-demethylleucine instead of *N*-methylphenylalanine for most beauvericins. Beauvericin F is also the first member containing 2-hydroxy-4-methylpentanoic acid as a hydroxylic acid. Thus, beauvericins D to F described in this paper are structurally unique members of beauvericins.

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