Fusaricidins B, C and D, New Depsipeptide Antibiotics Produced by *Bacillus polymyxa* KT-8: Isolation, Structure Elucidation and Biological Activity

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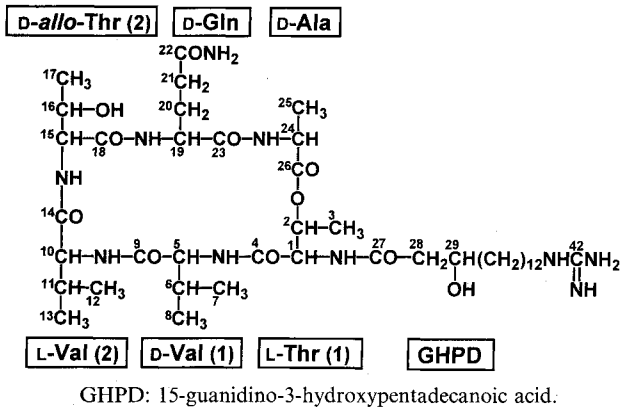
Fusaricidins B, C and D, new depsipeptide antibiotics, have been isolated as minor components from the culture broth of *Bacillus polymyxa* KT-8 which was obtained from the rhizosphere of garlic suffering from the basal rot caused by *Fusarium oxysporum*. The structure of fusaricidin B has been elucidated mainly by various NMR experiments coupled with amino acid analysis in relation to fusaricidin A, the main component of the complex, whose structure was reported previously. The fraction consisting of fusaricidins C and D was unsuccessfully separated, giving roughly a 4:1 mixture of the two, respectively. The structures of fusaricidins C and D have been determined within the mixture by detailed analyses of the 2D NMR spectra. Fusaricidins B, C and D are active against fungi and Gram-positive bacteria almost as well as fusaricidin A.

In the course of our screening for new antifungal antibiotics, we have isolated bacillopeptins A, B and C[1] from *Bacillus subtilis* FR-2 and fusaricidin A[2] from *Bacillus polymyxa* KT-8. These bacterial strains were obtained from the rhizosphere of garlic[3,4] suffering from the basal rot caused by *Fusarium oxysporum* and identified by taxonomic studies[1,2]. The structures of bacillopeptins[1] were determined to be cyclic lipopeptides and that of fusaricidin A was elucidated to be a cyclic depsipeptide containing a unique fatty acid, 15-guanidino-3-hydroxypentadecanoic acid[2]. The fusaricidin complex isolated from *Bacillus polymyxa* KT-8 was found to contain other components related to fusaricidin A, named fusaricidins B (**1**), C (**2**) and D (**3**). This paper

Fig. 1. Structure of fusaricidin B (**1**).



GHPD: 15-guanidino-3-hydroxypentadecanoic acid.

Fig. 2. Structure of fusaricidin C (**2**).

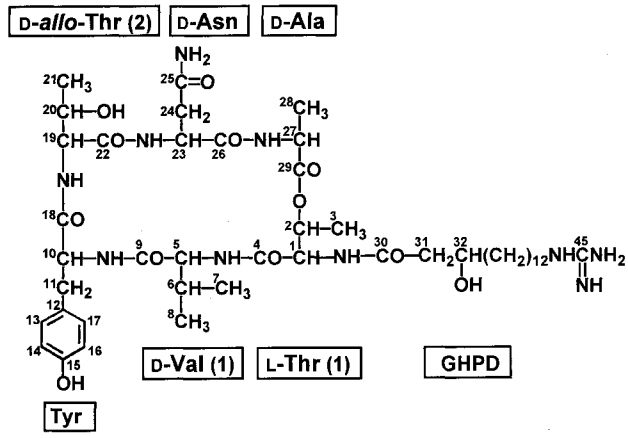
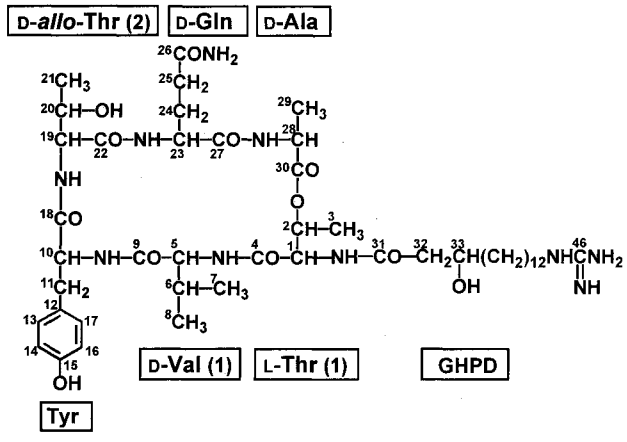


Fig. 3. Structure of fusaricidin D (**3**).



describes the isolation, structure elucidation and biological activities of fusaricidins B, C and D.

Results

Production, Isolation and Purification

The fermentation of the producing strain was carried out as described in our preceding paper[2]. The isolation procedure for fusaricidins B, C and D is outlined in Fig. 4. Activity against *F. oxysporum* HF 8801 was monitored during the isolation. The cultured broth was centrifuged and the supernatant was extracted with *n*-BuOH. The *n*-BuOH extract was purified in the same way as described previously[2] up to the HPLC isolation. The reverse phase preparative HPLC using a Capcell Pak C18 SG-120 column (4.6×250 mm, Shiseido Co., Ltd.) was carried out with 1% CH_3CN in 0.1% TFA as mobile phase A and 80% CH_3CN in 0.1% TFA as mobile phase B with a flow rate of 1 ml/minute. A gradient was run from 30% to 70% phase B in 10 minutes with UV detection at 215 nm to give two peaks, namely the peak 1 (8.8 minutes) and the peak 2 (9.7 minutes). The peak 2 was further separated by repeated preparative HPLC using the same column in turn under an isocratic condition with 32% CH_3CN in 0.1% TFA to yield fusaricidin A (20 mg) and fusaricidin B (**1**) (8 mg). The peak 1 also proved to be a mixture of two components and its further separation was unsuccessfully tried out under various HPLC conditions, giving roughly a 4:1 mixture (4 mg) of the two components named fusaricidins C (**2**) and D (**3**), respectively.

Physico-chemical Properties

The physico-chemical properties of fusaricidin B and the mixture of fusaricidins C and D are summarized in Table 1. They are easily soluble in MeOH and DMSO, slightly soluble in water, and insoluble in $CHCl_3$ and *n*-hexane. They gave positive reaction with Biuret reagent and Sakaguchi reagent, but were negative in ninhydrin test. Their color reactions and ^1H and ^{13}C NMR data were very similar to fusaricidin A, suggesting that they should be lipopeptides similar to fusaricidin A.

Fig. 4. Isolation procedure for fusaricidins A, B (**1**), C (**2**) and D (**3**).

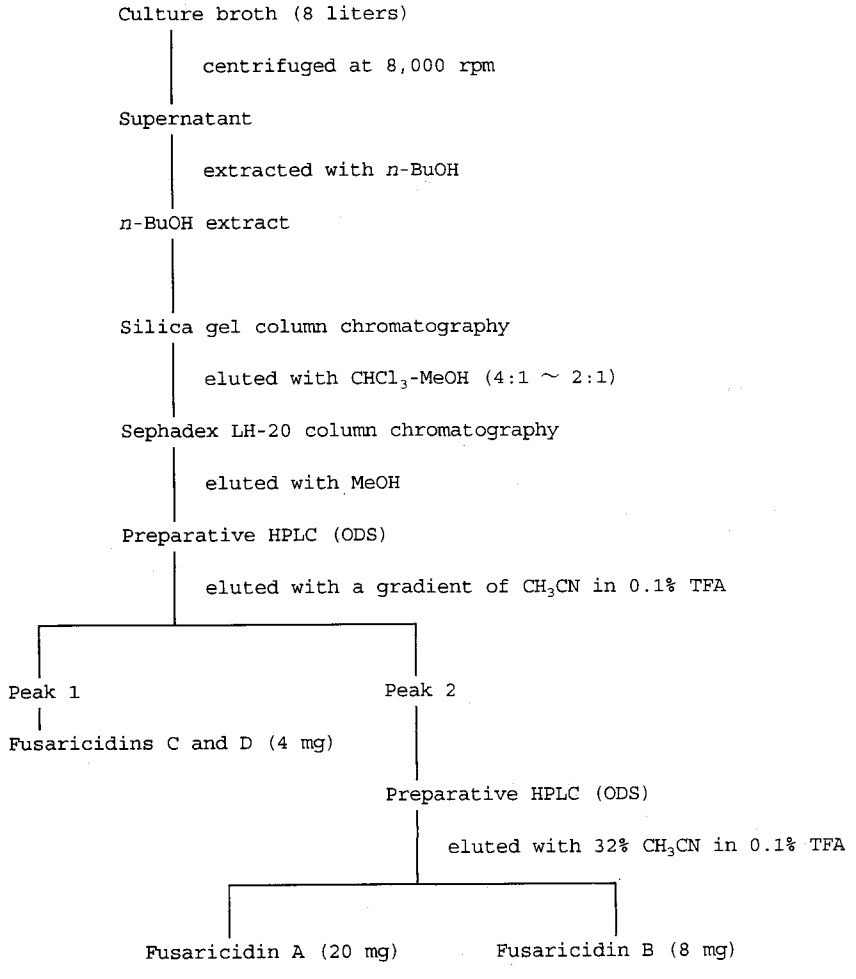


Table 1. Physico-chemical properties of fusaricidins B (**1**), C (**2**) and D (**3**).

	B	C	D
		(Mixture)	
Appearance	White powder	White powder	
Molecular formula	$C_{42}H_{76}N_{10}O_{11}$	$C_{45}H_{74}N_{10}O_{12}$	$C_{46}H_{76}N_{10}O_{12}$
Molecular weight	896	946	960
FAB-MS (m/z)	897 $(M+H)^+$	947 $(M+H)^+$	961 $(M+H)^+$
HRFAB-MS (m/z)			
Found:	897.5712 $(M+H)^+$	947.5622 $(M+H)^+$	961.5747 $(M+H)^+$
Calcd:	897.5774	947.5566	961.5723
	(as $C_{42}H_{77}N_{10}O_{11}$)	(as $C_{45}H_{75}N_{10}O_{12}$)	(as $C_{46}H_{77}N_{10}O_{12}$)
$[\alpha]_D^{25}$(MeOH)	+10.6° (*c* 0.1)	Not tested.	
UV λ_{max}^{MeOH} nm	End absorption	224, 277, 284 (sh)	
Color reaction			
Positive:	Biuret, Sakaguchi	Biuret, Sakaguchi	
Negative:	Ninhydrin	Ninhydrin	
Solubility			
Soluble:	MeOH, DMSO	MeOH, DMSO	
Insoluble:	$CHCl_3$, *n*-hexane	$CHCl_3$, *n*-hexane	

Structure of Fusaricidin B (1)

The molecular formula of fusaricidin B (**1**) was determined to be $C_{42}H_{76}N_{10}O_{11}$ from HRFAB-MS and ^{13}C NMR data. This is larger than that of fusaricidin A[2] by one CH_2 unit. Complete acid hydrolysis of **1** was performed and the resulting hydrolysate was extracted with $CHCl_3$. Amino acid analysis of the water layer of the hydrolysate showed the presence of two moles each of threonine (Thr) and valine (Val), and one mole each of alanine (Ala) and glutamic acid (Glu). The Glu was revealed to have come from glutamine (Gln) residue in **1** as described below. The stereochemistries of the amino acids in the hydrolysate were examined by chiral HPLC using a SUMICHIRAL OA-5000 column in the same manner as the case of fusaricidin A[2]. The results proved the presence of L-Val, D-Val, L-Thr, D-*allo*-Thr, D-Gln and D-Ala residues in **1**. A lipophilic compound contained in the $CHCl_3$ layer of the hydrolysate gave the identical positive FAB-MS spectrum ($(M+H)^+$, *m/z* 298) and the same behaviors on the TLC ($CHCl_3$-MeOH, 3:1; Rf 0.51) as the compound obtained from 15-guanidino-3-hydroxypentadecanoic acid (GHPD) residue in fusaricidin A. As described in our preceding paper[2], the GHPD residue might undergo dehydration during complete acid hydrolysis of **1** and give an $\alpha\beta$-unsaturated fatty acid possessing a molecular weight of 297. Fusaricidin B itself showed a negative ninhydrin test and did not react with diazomethane. These facts suggest that **1** is a cyclic lipopeptide, related to fusaricidin A, composed of the above six amino acid residues and GHPD residue.

The ^1H and ^{13}C NMR spectrum of **1** measured in DMSO-d_6 were very similar to those of fusaricidin A and are shown in Figs. 5 and 6, respectively. The DEPT, ^1H-^1H COSY, HSQC, NOESY and HMBC spectra of **1** were also measured in DMSO-d_6. One guanidino carbon at δ_C 156.6, eight carbonyl carbons at δ_C 174.2, 172.2, 171.9, 171.0, 170.5, 170.4, 170.3, 168.1, and no olefinic carbons, were observed in the ^{13}C NMR spectrum of **1**, indicating that **1** possesses one ring in the molecule since ten degrees of unsaturation are required from its molecular formula. Among nine proton signals observed in the low-field region of the ^1H NMR spectrum of **1**, six protons at δ_H 8.40, 8.36, 7.87, 7.50, 7.39, 7.30 could be assigned to the α amide protons of the six amino acid residues by the analysis of ^1H-^1H COSY data. The two singlet protons at δ_H 7.28 and 6.84 which are correlating to each other in the ^1H-^1H COSY spectrum were attributed to the terminal amide protons ($CONH_2$) of the Gln residue, establishing that the Glu in the

Fig. 5. ^{1}H NMR spectrum of fusaricidin B (**1**) (400 MHz, DMSO-d_6).

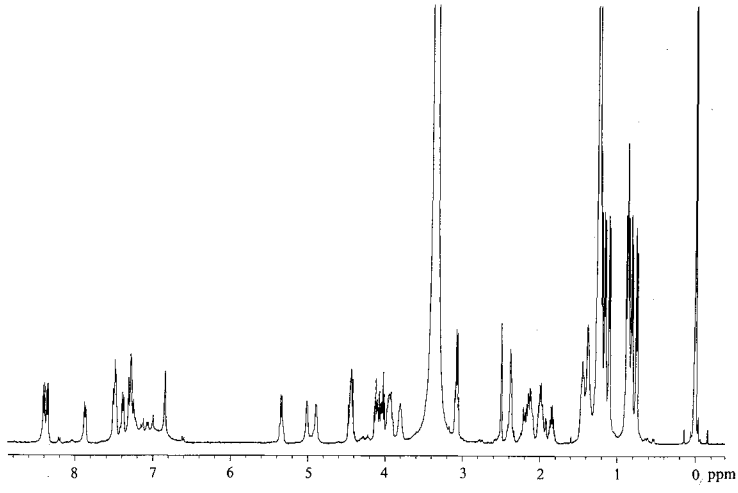
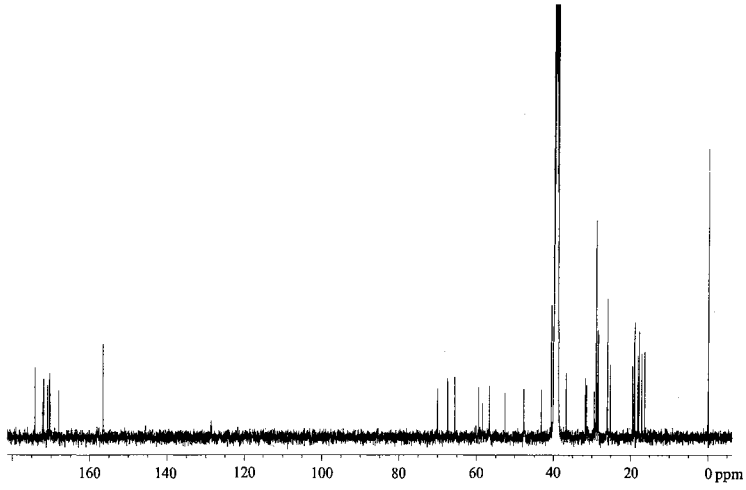


Fig. 6. ^{13}C NMR spectrum of fusaricidin B (**1**) (100 MHz, DMSO-d_6).



hydrolysate derived from the Gln residue in **1**. One remaining triplet-like signal at δ_H 7.48 was ascribed to the guanidino (–N*H*C(=NH)NH$_2$) proton. Seven methyl signals in the ^{1}H NMR spectrum of **1** were assigned as follows based on the above 2D NMR spectra. That is, the two doublet methyl signals at δ_H 0.81 and 0.75 were assigned to the methyls of one Val residue, which was temporarily named Val (1). The two doublet methyls at δ_H 0.88 and 0.86 were assigned to those of the other Val residue named Val (2), and the doublet methyl signal at δ_H 1.10 was ascribed to that of one Thr (or *allo*-Thr) residue named Thr (2). The doublet signal at δ_H 1.16 was attributed to that of the other Thr (or *allo*-Thr) residue named Thr (1) and the remaining doublet signal at δ_H 1.21 was assigned to Ala residue. The full assignments of all protons and carbons of the all amino acids and GHPD residues in **1** have thus been obtained as displayed in Table 2 by detailed analyses of the DEPT, ^{1}H-^{1}H COSY, HSQC, NOESY and HMBC data.

The sequence of the six amino acid residues in **1** was determined as follows by the NOESY and HMBC experiments as shown in Fig. 7. The NOESY correlations were observed between the α proton of Thr (1) (δ_H 4.43) and the NH of Val (1) (δ_H 7.30), between the α-H of Val (1) (δ_H 4.45) and the NH of Val (2) (δ_H 8.40), between the α-H of Val (2) (δ_H 4.12) and the NH of Thr (2) (δ_H 8.36), between the α-H of Thr (2) (δ_H 4.03) and the NH of Gln (δ_H 7.87) as well as between this NH of Gln (δ_H 7.87) and the NH of Ala (δ_H 7.39). Furthermore, an HMBC correlation was observed between the β proton of Thr (1) at δ_H 5.34 and the carbonyl carbon of Ala at δ_C 170.5 (Fig. 7). This HMBC correlation indicates the presence

of an ester linkage between the hydroxyl group of Thr (1) and the carbonyl group of Ala resulting in a depsipeptide ring as well as fusaricidin A. In consequence of this acylation of the OH group, Thr (1) had a remarkable downfield shift in the ^{13}C NMR spectrum at the β-CH carbon (C-2) to δ_C 70.1 and upfield shifts at the α-CH (C-1) and the γ-CH_3 (C-3) to δ_C 56.7 and 16.4, respectively. We have proposed the name "Threonine Shifts"[2] for these characteristic carbon chemical shift changes of the Thr residue in the situation like this. Based on these findings, the sequence of the amino acids in the depsipeptide ring of **1** was determined as cyclic [Thr (1)→Val (1)→Val (2)→Thr (2)→D-Gln→D-Ala] with an ester linkage between Thr (1) and D-Ala.

The fatty acid side chain was also determined to attach to the Thr (1) residue through an amide bond by the analyses of the HMBC and NOESY experiments for **1** (Fig. 7).

In order to assign the stereochemistries of Thr (1), Thr (2), Val (1) and Val (2) residues, partial acid hydrolysis of **1** was carried out under the same condition[2] as for fusaricidin A. From the resulting hydrolysate, one peptide was obtained together with several amino acids. This peptide was examined in the same way as for fusaricidin A[2] and determined to be L-Thr→D-Val. Therefore, Thr (1) is L-Thr, Val (1) is D-Val, Val (2) is L-Val, and Thr (2) is D-*allo*-Thr.

Based on the results described above, the total structure of fusaricidin B (**1**) has thus been elucidated to be a hexadepsipeptide having a D-Gln residue in place of D-Asn of fusaricidin A as shown in Fig. 1.

Structures of Fusaricidins C (**2**) and D (**3**)

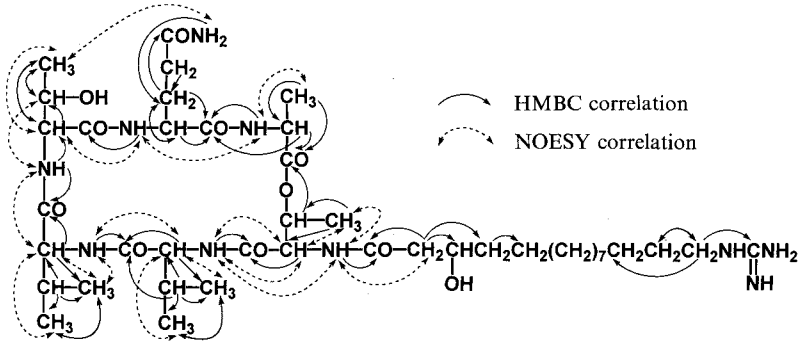
As described above, separation of fusaricidin C (**2**) and fusaricidin D (**3**) was very difficult and they could not be obtained as pure form but as roughly a 4:1 mixture, respectively. As shown in Table 1, the positive

Table 2. ^1H and ^{13}C NMR chemical shifts of fusaricidin B (**1**) in DMSO-d_6.

Moiety	position	δ_C	δ_H (J in Hz)
L-Thr (1)	1	56.7	4.43 br d (8.5)
	2	70.1	5.34 m
	3	16.4	1.16 d (5.6)
	4	168.1	
	1-NH		7.50 d (8.5)
D-Val (1)	5	56.7	4.45 m
	6	31.5	1.85 m
	7	17.9	0.75 d (6.4)
	8	19.0	0.81 d (6.4)
	9	171.0	
	5-NH		7.30 d (9.5)
L-Val (2)	10	58.4	4.12 dd (7.2, 7.2)
	11	29.5	2.00 m
	12	18.2	0.86 d (7.1)
	13	19.2	0.88 d (7.1)
	14	172.2	
	10-NH		8.40 d (7.1)
D-*allo*-Thr (2)	15	59.4	4.03 dd (6.8, 6.8)
	16	65.6	3.96 m
	17	19.6	1.10 d (6.1)
	18	170.3	
	15-NH		8.36 d (7.1)
	16-OH		5.01 br s
D-Gln	19	52.7	3.92 m
	20	26.1	1.99 m
			2.13 m
	21	31.8	2.09 ~ 2.25 m
	22	174.2	
	23	170.4	
	19-NH		7.87 d (8.1)
	22-NH_2		6.84 s
			7.28 s
D-Ala	24	47.8	4.07 dd (7.3, 7.3)
	25	17.1	1.21 d (7.3)
	26	170.5	
	24-NH		7.39 d (7.3)
GHPD	27	171.9	
	28	43.2	2.38 m
	29	67.4	3.80 br s
	30	36.7	1.38 br s
	31	25.2	~ 1.24 br
	32~37	28.9	
		28.9	
	38	28.5	
	39	25.9	
	40	28.3	1.45 t-like
	41	40.6	3.07 dd (12.6, 6.3)
	42	156.6	
	41-NH		7.48 t-like
	29-OH		4.89 br s

GHPD: 15-guanidino-3-hydroxypentadecanoic acid.

Fig. 7. HMBC and NOESY correlations for fusaricidin B (**1**).



FAB-MS spectrum of the mixture gave two strong peaks, *m/z* 947 and *m/z* 961, the intensity of which are roughly 4:1, in the molecular ion peak region and they could be assigned to the molecular ion ($(M+H)^+$) of **2** and **3**, respectively. The HRFAB-MS of these molecular ion peaks and the ^1H and ^{13}C NMR data revealed their molecular formula of $C_{45}H_{74}N_{10}O_{12}$ for **2** and $C_{46}H_{76}N_{10}O_{12}$ for **3**. Complete acid hydrolysis of the mixture was performed, and the resulting hydrolysate was extracted with $CHCl_3$. Amino acid analysis of the water layer of the hydrolysate showed the presence of Thr (2.37 mol), Ala (1.24), Val (1.21), tyrosine (Tyr) (1.16), aspartic acid (Asp) (1.00) and Glu (0.25). The $CHCl_3$ layer of the hydrolysate was proved to contain only one and the same lipophilic compound derived from GHPD during acid hydrolysis as in the case of fusaricidin B (**1**). Judging from these findings and by comparison with the data of **1** and fusaricidin A, **2** could be composed of Thr, Val, Tyr, *allo*-Thr, Asn, Ala and GHPD, and **3** could consist of Thr, Val, Tyr, *allo*-Thr, Gln, Ala and GHPD. In the ^1H and ^{13}C NMR spectra of the mixture measured in DMSO-d_6, the signals of **2** were accompanied in close vicinity with smaller correspondent signals of **3** and sometimes they were overlapped as shown in Figs. 8 and 9, respectively. The DEPT, ^1H-^1H COSY, HSQC, NOESY, HMBC and HOHAHA spectra of the mixture of **2** and **3** were also measured in DMSO-d_6 and carefully examined. From these 1D and 2D NMR data, all protons and carbons of the six amino acids and GHPD residues in **2** and **3** were assigned as listed in Table 3. A very striking feature found in the ^1H NMR spectra of **2** and **3** is the large upfield shifts of the methyl signals of each Val residue observed at δ_H 0.57 and 0.39, and δ_H 0.57 and 0.44, respectively. These remarkable

Fig. 8. ^1H NMR spectrum of fusaricidins C (**2**) and D (**3**) (400 MHz, DMSO-d_6).

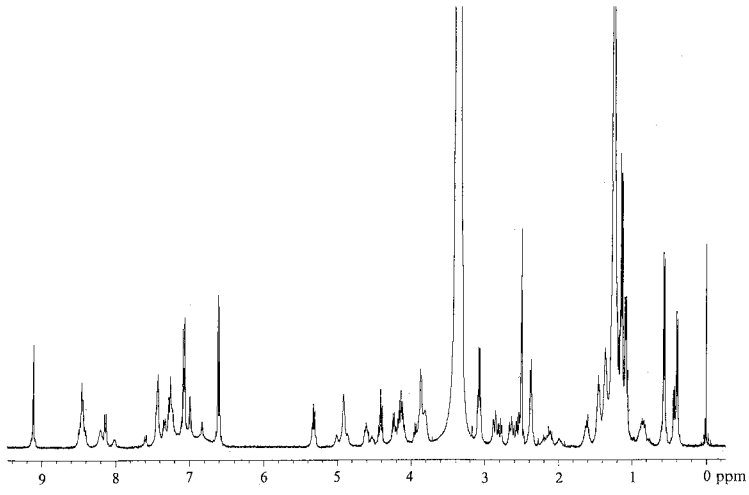


Fig. 9. ^{13}C NMR spectrum of fusaricidins C (**2**) and D (**3**) (100 MHz, DMSO-d_6).

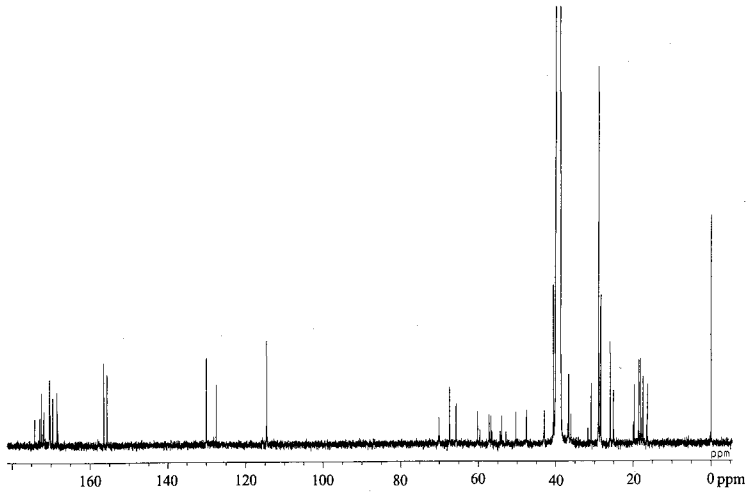
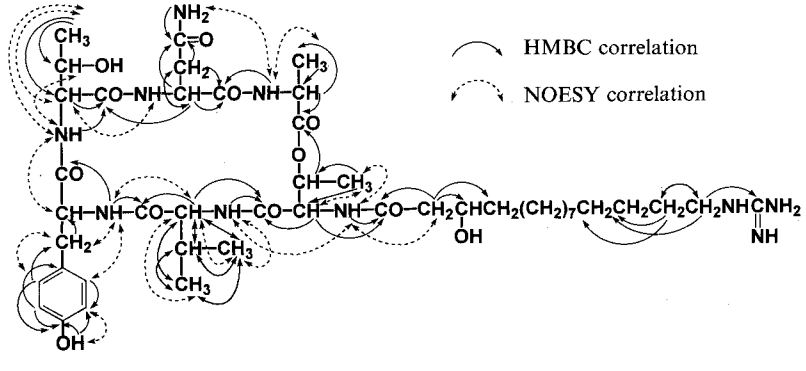


Table 3. ^{1}H and ^{13}C NMR chemical shifts of fusaricidins C (**2**) and D (**3**) in DMSO-d_6.

Fusaricidin C				Fusaricidin D			
Moiety	position	δ_C	δ_H (J in Hz)	Moiety	position	δ_C	δ_H (J in Hz)
Thr	1	56.8	4.40 dd(8.8, 2.0)	Thr	1	56.6	4.42 dd(8.8, 2.0)
	2	70.1	5.31 dd(6.6, 2.0)		2	70.0	5.33 dd(6.6, 2.0)
	3	16.3	1.14 d(6.3)		3	16.5	1.15 d(5.9)
	4	168.4			4	168.2	
	1-NH		8.13 d(8.5)		1-NH		7.59 d(8.3)
Val	5	57.3	4.13 dd(8.5, 8.5)	Val	5	57.2	4.16 m
	6	30.9	1.60		6	30.9	1.62 m
	7	18.1	0.57 d(6.8)		7	17.9	0.57 d(6.8)
	8	18.5	0.39 d(6.6)		8	18.6	0.44 d(6.8)
	9	170.1			9	170.2	
	5-NH		7.27 d(8.9)		5-NH		7.33 d(8.8)
Tyr	10	54.0	4.60 m	Tyr	10	54.4	4.52 m
	11	36.9	2.63 dd(13.5, 11.1)		11	36.9	2.65 m
			2.86 dd(13.4, 3.9)				2.86 m
	12	127.5			12	127.5	
	13, 17	130.0	7.07 d(8.5)		13, 17	130.0	7.06 d(8.3)
	14, 16	114.6	6.60 d(8.5)		14, 16	114.6	6.60 d(8.5)
	15	155.7			15	155.9	
	18	172.9			18	172.9	
	10-NH		8.44 d(8.1)		10-NH		8.49 d(7.6)
	15-OH		9.11 s		15-OH		9.11 s
allo-Thr	19	60.3	3.86 m	*allo*-Thr	19	59.7	3.94 dd(7.0, 7.0)
	20	65.7	3.86 m		20	65.7	3.89 m
	21	19.7	1.09 d(5.9)		21	19.9	1.07 d(6.0)
	22	170.4			22	170.4	
	19-NH		8.45 br s		19-NH		8.41 d(6.8)
	20-OH		4.91 br s		20-OH		5.01 br s
Asn	23	50.4	4.23 dd(13.6, 6.6)	Gln	23	52.9	3.87 m
	24	36.1	2.55 dd(15.6, 6.6)		24	25.9	1.98 m
			2.79 dd(15.4, 6.1)				2.11 m
	25	172.4			25	31.7	2.07 ~ 2.23 m
	26	169.5			26	174.2	
	23-NH		8.20 br s		27	172.3	
	25-NH_2		6.99 s		23-NH		8.01 d(7.1)
			7.42 s		26-NH_2		6.83 s
Ala	27	47.6	4.11 m				7.25 s
	28	17.5	1.14 d(7.5)	Ala	28	47.7	4.18 m
	29	170.3			29	17.5	1.19 d(7.3)
	27-NH		7.23 d(7.8)		30	170.4	
GHPD	30	171.8			28-NH		7.34 d(7.9)
	31	43.0	2.38 d(5.6)	GHPD	31	171.8	
	32	67.4	3.81 br s		32	43.1	2.36 d(6.1)
	33	36.7	1.36 br s		33	67.4	3.81 br s
	34	25.1			34	36.7	1.36 br s
	35~40	28.9			35	25.1	
		28.9	~ 1.24 br		36~41	28.9	
	41	28.5				28.9	~ 1.24 br
	42	25.9			42	28.5	
	43	28.3	1.45 t-like		43	25.9	
	44	40.6	3.07 dd(12.8, 6.7)		44	28.3	1.45 t-like
	45	156.5			45	40.6	3.07 dd(12.8, 6.7)
	44-NH		7.43 t-like		46	156.5	
	32-OH		4.91 br s		45-NH		7.43 t-like
					33-OH		4.85 br s

GHPD: 15-guanidino-3-hydroxypentadecanoic acid.

Fig. 10. HMBC and NOESY correlations for fusaricidin C (**2**).



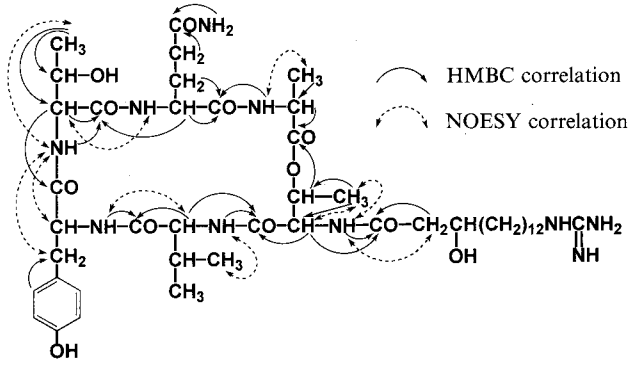
shielding effects could be due to the magnetic anisotropy caused by π-electron currents of the benzene ring in each Tyr residue, which should be located adjacent to each Val residue.

The sequence of the six amino acids and GHPD residues in **2** and **3** was determined as shown in Figs. 10 and 11 respectively, by HMBC and NOESY correlations for **2** and **3**.

Though the stereochemistry of each amino acid residue in **2** and **3** could not be determined because of the shortage of the materials, they should be related biosynthetically to **1** or fusaricidin A and therefore the positional relation between Thr and *allo*-Thr and the chirality of each amino acid could be all the same. The difficulty of separation between fusaricidin A and **1** and between **2** and **3** might support this suggestion.

Based on these results described above, the structures of fusaricidins C (**2**) and D (**3**) have thus been proposed as shown in Figs. 2 and 3, respectively; **2** being a hexadepsipeptide having a Tyr residue in place of L-Val (2) of fusaricidin A, and **3** possessing a Tyr residue instead of L-Val (2) of fusaricidin B (**1**).

Fig. 11. HMBC and NOESY correlations for fusaricidin D (**3**).



Biological Activities

Antimicrobial activities of fusaricidin B (**1**) and the mixture of fusaricidins C (**2**) and D (**3**) were determined in the same way as reported previously[2] and the resulting MICs are shown in Table 4 together with those of fusaricidin A. As was expected, **1** and the mixture of **2** and **3** showed strong activity against a wide variety of fungi as well as fusaricidin A. They also exhibited remarkably strong activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Micrococcus luteus*. Furthermore, **1** showed medium activity against yeasts such as *Candida albicans* IFO 1594 and *Saccha-*

Table 4. Antimicrobial activities of fusaricidins A, B (**1**), C (**2**) and D (**3**).

Test organisms	MIC (μg/ml)		
	A	B	C and D (mixture)
Staphylococcus aureus FDA 209P	<0.78	1.56	<0.78
S. aureus Smith	<0.78	1.56	<0.78
Micrococcus luteus IFO 3333	<0.78	1.56	<0.78
Bacillus subtilis ATCC 6633	3.12	6.25	3.12
Escherichia coli NIHJ	>100	>100	>100
Klebsiella pneumoniae KC-1	>100	>100	>100
Pseudomonas aeruginosa IFO 3445	>100	>100	>100
Serratia marcescens IFO 3736	>100	>100	>100
Candida albicans IFO 1594	>100	12.5	>100
Saccharomyces cerevisiae HUT 7099	>100	12.5	>100
Fusarium oxysporum HF 8801 (pathogenic to garlic)	1.56	1.56	1.56
F. oxysporum HF 8835 (nonpathogenic to garlic)	1.56	1.56	1.56
Aspergillus niger HUT 2016	3.12	3.12	3.12
A. oryzae IFO 4214	3.12	3.12	3.12
Penicillium thomii	3.12	3.12	3.12

romyces cerevisiae HUT 7099, whereas **2** and **3** showed no activity even at 100 μg/ml against these yeasts.

Discussion

In the present study, we have isolated new antifungal and antibacterial antibiotics, fusaricidins B (**1**), C (**2**) and D (**3**), as minor components from the culture broth of a bacterial strain *Bacillus polymyxa* KT-8. Their structures have been elucidated to be cyclic hexadepsipeptides, very similar to that of fusaricidin A, the main component of the complex, and containing a unique 15-guanidino-3-hydroxypentadecanoic acid side chain (Figs. 1, 2 and 3).

Among the known peptide antibiotics, fusaricidins A and C (**2**) structurally resemble KT-6291A and KT-6291B[5] respectively, which were isolated from an unidentified strain *Bacillus* sp. KB-291. However, as described previously[2], the distinctions of the positions of Thr and *allo*-Thr in KT-6291A and KT-6291B were not made, nor were reported the absolute configurations of the amino acid residues. Furthermore, KT-6291A and KT-6291B were not active even at 100 μg/ml against *Fusarium oxysporum* f. sp. cucumerinum, whereas fusaricidins A and C both showed activity at 1.56 μg/ml against *Fusarium oxysporum* HF 8801 or *F. oxysporum* HF 8835.

Fusaricidins C and D displayed strong activity against Gram-positive bacteria especially *Staphylococcus aureus* FDA 209P, *S. aureus* Smith and *Micrococcus luteus* IFO 3333 as did fusaricidin A, but fusaricidin B showed weaker activity against those microbes than the fusaricidins C and D mixture (Table 4).

Experimental

General

TLC was carried out on Merck Kieselgel 60 F-254 plates (Art. No. 5715). Silica gel column chromatography was performed using Wakogel C-200. Spectral data were recorded on the following instruments: ^{1}H and ^{13}C NMR, JEOL JNM-A400 spectrometer; low and high resolution FAB-MS, JEOL JMS-SX102 spectrometer; UV-vis, JASCO V-520 spectrophotometer; optical rotation, Union PM-101 automatic digital polarimeter.

Analysis of the Amino Acids

Each antibiotic was completely hydrolyzed with 6 N HCl at 105°C for 24 hours in a sealed tube and the reaction mixture was extracted with $CHCl_3$. The water layer of the hydrolysate was analyzed on a Hitachi L-8500 amino acid autoanalyzer.

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