Structure of New Cyclic Depsipeptides, Bu-2841-08 and -10

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The structures of new antibiotics, Bu-2841-08 and -10, have been determined. They are cyclic depsipeptides and the sequence of amino acid residues was established by mass spectral analysis of the hydrolyzed linear peptide and NMR spectral analysis of the parent cyclic peptides.

Results and Discussion

An unidentified Gram-negative bacteria, K-341-B7, was found to produce a new antibiotic complex, Bu-2841. The antibiotic complex was isolated from whole broth by chromatography on silica gel and Sephadex LH-20. HPLC analysis showed that the complex was composed of at least ten components. Four components, Bu-2841-02, -04, -08, and -10, were isolated from the complex and purified by reversed phase HPLC. Chemical characterization of the components indicated that they were peptides. Although the four antibiotics isolated here behaved as a single entity under all conditions tested for HPLC, their NMR spectra indicated that Bu-2840-08 and -10 were a mixture of three or four homologous components, and the others were mixtures of more than four peptides. In this paper, we will describe the isolation, structures and biological properties of Bu-2841-08 and -10.

Isolation and Purification

A flow diagram for the isolation procedure is given in Fig. 1. Activities against *Staphylococcus aureus* FDA 209P and *Bacillus subtilis* (PCI 219 (medium pHs were 6 and 8), H17, and M45) were employed to monitor the isolation of antibiotic Bu-2841 complex. The whole broth (39 liters) was extracted with *n*-butanol and the extract was concentrated *in vacuo*. It was then diluted with ethyl acetate until a precipitate appeared. The precipitate was extracted three times with aqueous acetonitrile. The acetonitrile was evaporated and then lyophilized to yield

Fig. 1. *Isolation procedure of Bu-2841-02, -04, -08, and -10.*

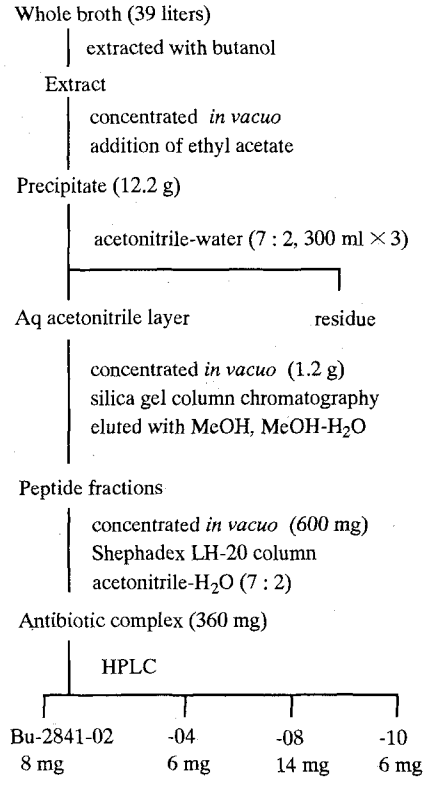

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Table 1. Consistent amino acids[a] and optical rotations of Bu-2841 complex.

Consistent amino acid	$[\alpha]_D$ (5N HCl)	Assigned chirality
Asp (3.2)	-5° (*c* 1.0)	2D + 1L
Thr (1.0)	-12° (*c* 0.4)	L
Ser (1.0)	-10° (*c* 0.5)	D
Gly (2.2)		
Phe (1.2)	-5° (*c* 1.0)	L
Dab[b] (2.1)	0° (*c* 1.5)	D + L
Ile (0.9)	+35° (*c* 1.0)	L
Leu (0.3)		
Val (0.2)	+19° (*c* 0.3)	L

[a] The molecular ratios. [b] 2,4-diaminobutyric acid.

Table 2. Constituent amino asid[a] and FAB-MS of Bu-2841-02, -04, -08, -10.

	Antibiotics			
	Bu-2841-02	-04	-08	-10
Asp	(3.2)	(3.2)	(3.1)	(3.1)
Thr	(1.0)	(1.0)	(1.0)	(1.0)
Ser	(0.9)	(1.0)	(0.9)	(0.9)
Gly	(2.2)	(2.2)	(2.1)	(2.1)
Phe	(1.1)	(1.4)	(1.2)	(1.0)
Dab	(1.8)	(1.8)	(1.8)	(1.8)
Ile	(0.6)	(1.0)	(0.8)	(0.9)
Leu	(0.02)	(0.3)		
Val	(0.4)	(0.3)	(0.2)	(0.1)
Trp	(0.4)	(0.4)	(0.5)	(0.3)
FAB-MS $(M+H)^+$	1435	1449	1463	1477

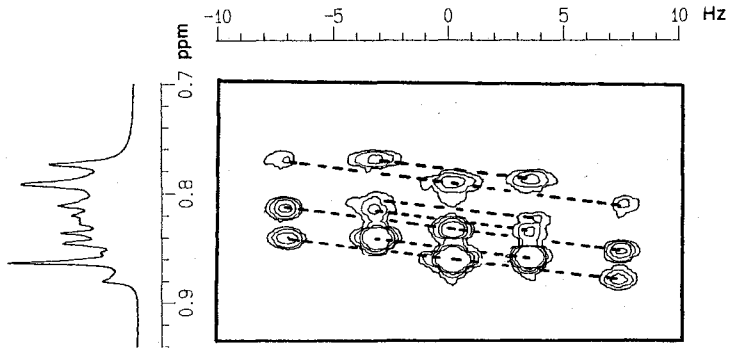
[a] The molecular ratios.

a residue. The residue was dissolved in a small amount of methanol and subjected to a silica gel column subsequently eluted with methanol and aqueous methanol. Active fractions were further purified by a Sephadex LH-20 column eluted with aqueous acetonitrile. The antibiotic complex was obtained as a colorless powder. An analytical HPLC chromatogram of the complex showed that it consisted of at least ten components designated as Bu-2841-01 ~ Bu-2841-10. Amino acid analysis of the complex after hydrolysis with 6N HCl and optical rotations of these amino acids (Table 1) suggested that the main peptides contained D-aspartic acid (2 eq.), L-aspartic acid (1 eq.), L-threonine (1 eq.), D-serine (1 eq.), glycine (2 eq.), L-isoleucine (1 eq.), L-phenylalanine (1 eq.), D-2,4-diaminobutyric acid (1 eq.), and L-2,4-diaminobutyric acid (1 eq.)[1]. UV spectrum of the complex also indicated the presence of a tryptophan residue in the antibiotics (UV λ_{max}: 205, 240 (sh), 274, 281, 290 nm). GC-MS of methyl esters of the fatty acids which were obtained by hydrolysis of the antibiotic complex suggested that the acyl group consisted of more than ten β-hydroxylfatty acids ($C_8 \sim C_{12}$) and normal fatty acids. In order to isolate each component, the complex was subjected to a reversed phase HPLC under the condition as follows: Packing; Capcell Pak C_{18} (Shiseido, 10 × 250 mm), mobile phase; isopropanol-acetonitrile-0.1% trifluoroacetic acid (3:1:6), flow rate; 1.2 ml/minute, detection; UV at 230 nm. By this procedure, the four components, Bu-2841-02 (8 mg), -04 (6 mg), -08 (14 mg), and -10 (6 mg), were isolated. The results of FAB-MS and amino acid analyses of these antibiotics are shown in Table 2.

Structures of Bu-2841-08 (**1**) and -10 (**2**)

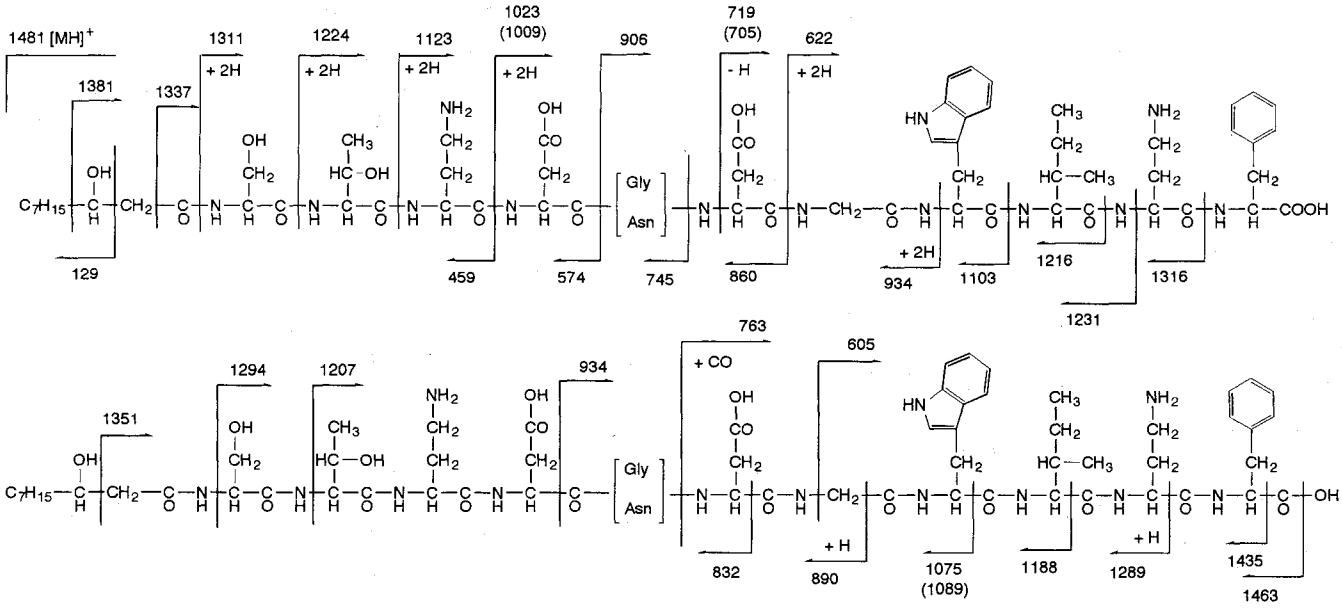
Amino acid analysis of Bu-2841-08 (**1**) gave a composition: aspartic acid (3 eq.), serine (1 eq.), glycine (2 eq.), phenylalanine (1 eq.), 2,4-diaminobutiric acid (2 eq.), tryptophan (1 eq.), threonine (1 eq.), isoleucine (0.8 eq.), and valine (0.2 eq.). High resolution FAB-MS of **1** gave a MW, *m/z* 1463.7170 $(M+H)^+$, which fits the molecular formula, $C_{67}H_{98}O_{21}N_{16}$. These data indicated that the peptide contains one asparagine and two aspartic acids and consists of two kinds of cyclic peptide moieties having isoleucine ($C_{57}H_{79}O_{19}N_{16}$) and valine ($C_{56}H_{77}O_{19}N_{16}$) in a ratio of 8 : 2. Furthermore, the acyl groups of the peptides were formulated as $C_{10}H_{19}O_2$ and $C_{11}H_{21}O_2$, respectively. ^{1}H NMR studies of **1** (COSY and 2D-homonuclear Hartmann-Hahn spectroscopy) confirmed the presence of all aforementioned amino acids[2] (Table 3) and β-hydroxylcarboxylic acid residues (δ 0.75 ~ 0.9 (Me), 1.10 (m), 1.23 (br s), 1.35 (m, γ-H), 3.80 (m, β-H), 2.23 (dd, J = 8 and 14 Hz, α-H), 2.30 (dd, J = 5 and 14 Hz, α-H)). The methyl signals of the complex appeared between δ 0.75 ~ 0.9 and overlapped each other. The presence of anteiso and straight-chain carboxylic acid residues was suggested with *J*-resolved 2D spectrum of **1** (Fig. 2). The spectrum indicated the presence of seven methyl signals in the region: δ 0.78 (d, Ile), 0.79 (t, Ile), 0.83, 0.84 (each d, Val), 0.82 (t, straight-chain carboxylic acid), 0.85 (d, anteiso carboxylic acid), and 0.86 (t, anteiso carboxylic acid). By a peak height comparison of the triplet signals of the anteiso carboxylic acid and the methyl signal of the isoleucine, the ratio of anteiso carboxylic acid to straight-chain isomer was calculated to be about 4 : 1. The structures of the β-hydroxylcarboxylic acid residues were confirmed

Fig. 2. *J*-Resolved 2D spectrum of Bu-2841-08 (**1**) in DMSO-d_6.



The region between δ 0.7~0.92 (400 MHz, 60°C).

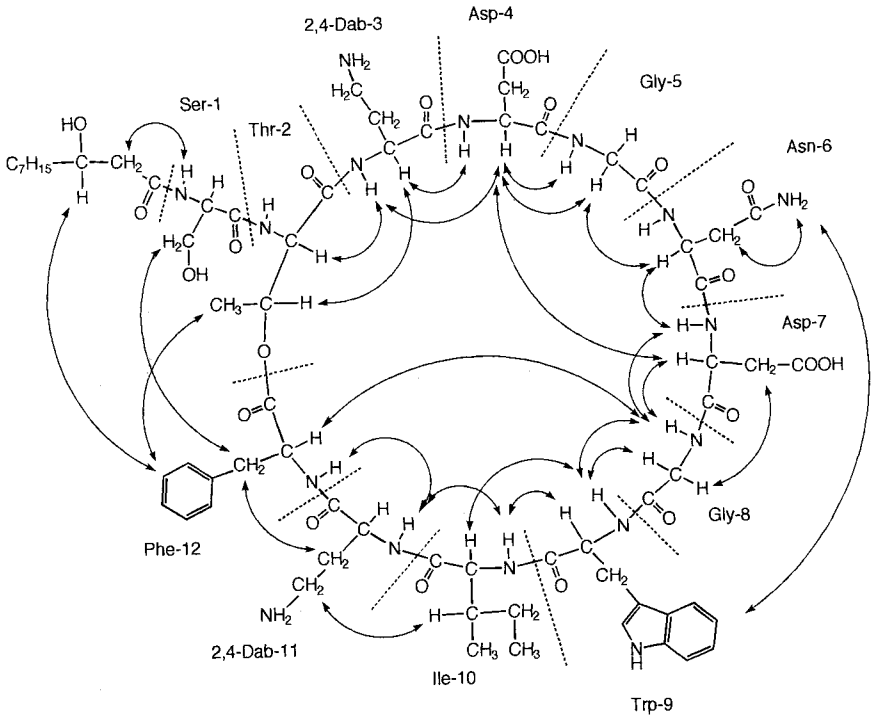
Fig. 3. Fragment ion peaks observed in the positive ion FAB-MS/MS of $(M+H)^+$ ion of **1a**.



The mass numbers shown in parentheses were fragment ions derived from minor peptide(s) (containing valine residue instead of isoleucine).

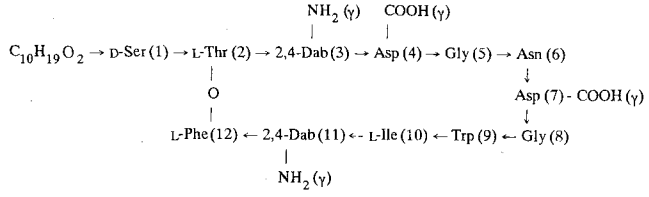
with the ^{13}C NMR spectrum: β-hydroxyanteisodecanoic acid, δ 10.6 (C9, Me), 21.8 (C10, Me), 24.7 (C5), 31.0 (C8), 36.0 (C6), 36.7 (C4), 40.4 (C7), 43.2 (C2), 67.5 (C3), and β-hydroxydecanoic acid, δ 13.6 (C10), 24.3 (C9), 26.3 (C5), 28.4 (C7), 28.8 (C6), 33.5 (C8), 36.7 (C4), 43.2 (C2), 67.5 (C3)[3]. The minor acyl group (C_{11}) was not detected in the spectrum of **1**. On the other hand, the ratio of an anteiso carboxylic acid to straight-chain isomer (C_{11}) of Bu-2841-10 (**2**) was about 4:1 as described in next subsection, from which the ratio of the minor carboxylic acid residues (C_{11}) of **1** may be same as that of main acyl groups of **2**. The presence of an intramolecular ester linkage was found by hydrolysis of **1** with tris-buffer (pH 8.5) at 40°C. In the ^1H NMR spectrum of the hydrolysate (**1a**, *m/z* 1481 $(M+H)^+$), β-proton signal of threonine (δ 4.07 (m)) appeared at more upfield than that of **1**, while those of serine and the acyl group were observed at almost same region of those of **1**. No information for the structure was obtained from the FAB-MS of **1** except the molecular weight. Positive ion FAB-MS/MS of *quasi*-molecular ion $(M+H)^+$ of **1** as a precursor ion, however, gave the fragment ions derived from cleavage of the side chain, *m/z* 1293 $(MH-C_{10}H_{18}O_2)^+$, 1206 $(MH-C_{10}H_{19}O_2-Ser+H)^+$, (1279 $(MH-C_{11}H_{20}O_2)^+$: minor peptide), *etc.*[4], and also from cleavage between α- and β-carbons of amino acid residue(s) accompanied by elimination of an amide proton, *m/z* 1418 $(MH-C_2H_7N$ (2,4-Dab))$^+$, 1403 $(MH-C_2H_4O_2$ (Asp))$^+$, 1343 $(MH-C_4H_8O_4$ (2 × Asp))$^+$, *etc.* However, the spectrum did not give any

Fig. 4. NOE data of **1** observed with ROESY spectrum.



500 MHz, at 60°C, mixing time: 0.1 second.

ion peaks from cleavage of the cyclic peptide chain (cleavages of peptide bond or ester bond and between α-carbon and amino or carbonyl group). The above data indicated that the *C*-terminal amino acid is linked by an ester bond to the threonine residue and that the γ-amino group of 2,4-diaminobutyric acids and the γ-carbonyl group of aspartic acids did not form a peptide bond. The amino acid sequence of **1a** was determined by the positive ion FAB-MS/MS of its *quasi*-molecular ion ($(M+H)^+$) except for the sequence of glycine and asparagine (Fig. 3)[4]. The fifth and sixth residues were determined with rotating frame NOE spectroscopy (ROESY) spectrum as glycine and asparagine, respectively, as shown in Fig. 4. Thus, the structure of the main component of Bu-2841-08 is as follows:



FAB-MS of Bu-2841-10 (**2**) gave a protonated molecular ion peak at *m/z* 1477; this peak was 14 daltons larger than that of Bu-2841-08 (**1**). Positive ion FAB-MS/MS of the *quasi*-molecular ion ($(M+H)^+$) of **2** gave fragment ions at *m/z* 1293 $(MH-C_{11}H_{20}O_2)^+$ and 1206 $(MH-C_{11}H_{21}O_2-Ser+H)^+$. Amino acid analysis of **2** gave the same result as **1** except a ratio of isoleucine (0.9 eq.) to valine (0.1 eq.). In ^1H NMR spectrum of **2**, the chemical shifts and coupling patterns of the amino acid residues were similar to those of **1** (Table 3). These data suggested that the structure of cyclic peptide moiety is identical with that of **1**, and the sequence of amino acids and acyl group was confirmed with NOESY spectrum of **2**. The structures of acyl groups ($C_{11}H_{21}O_2$) were determined by *J*-resolved 2D spectrum as 3-hydroxy-8-methyldecanoic acid and 3-hydroxyundecanoic acid (the ratio was about 4:1). In the ^{13}C NMR spectrum of **2**, the carbon signals of 3-hydroxy-8-methyldecanoic acid moiety were observed at δ 10.6 (C10, Me), 18.9 (C11, Me), 26.4 (C5), 28.7 (C6), 33.5 (C9), 35.8 (C7), 36.8 (C4), 42.4 (C8), 43.2 (C2), 67.5 (C3), but those of 3-hydroxyundecanoic moiety were not detected. From these data, **2** was determined to have the same core peptide as **1** but different acyl groups.

The stereochemistry of amino acid residues (residue-3, -4, -6, -7, -9, and -11) was determined by the NOE data

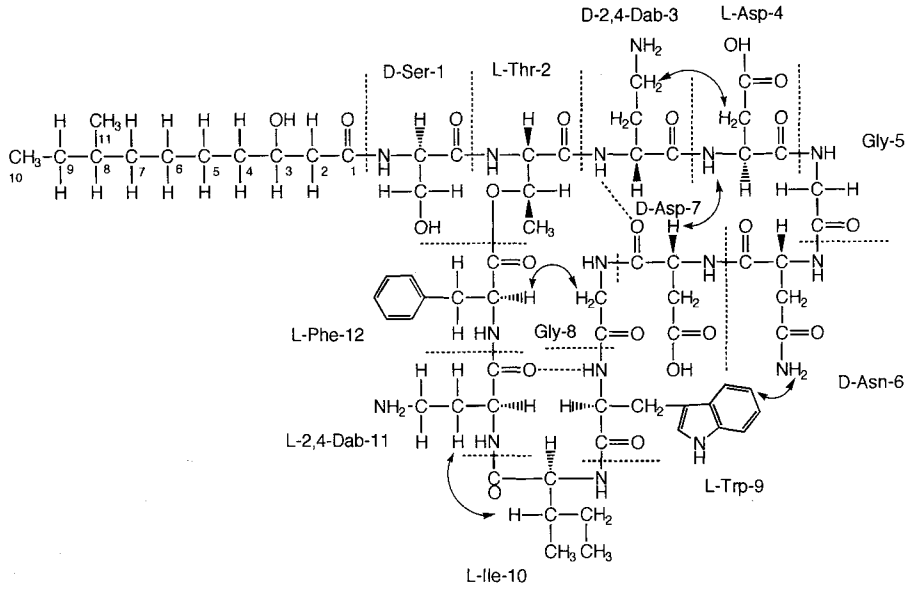
Table 3. ^{1}H and ^{13}C NMR spectral data[a] of amino acid residues of Bu-2841-08 (**1**) and -10 (**2**) in DMSO-d_6 at 60°C (500 MHz).

Position	1 δ_H (multiplicity)	1 δ_C	2 δ_H
Ser-1			
α	4.40 (m)	55.2	4.40
β	3.61 (dd, 5 and 10[b])	61.4	3.63
	3.66 (dd, 5 and 10)		3.68
NH	7.91 (d, 6)		7.94
Thr-2			
α	4.52 (m)	55.6	4.52
β	5.18 (m)	70.2	5.20
γ	1.02 (d, 6)	15.5	1.03
NH	8.00 (d)		8.02
Dab-3[c]			
α	4.43 (m)	36.7*	4.42
β	1.87 (m)	29.9	1.88
	2.00 (m)		2.01
γ	2.81 (m)	49.9	2.82
NH	7.97 (d, 8)		7.99
Asp-4			
α	4.68 (m)	50.5	4.65
β	2.59 (dd, 8 and 16)	35.7	2.60
	2.71 (dd, 5 and 16)		2.70
NH	8.33 (d, 7)		8.34
Gly-5			
α	3.80 (d, 6)	42.4*	3.80
NH	8.02 (t, 6)		8.01
Asn-6			
α	4.60 (m)	49.6	4.60
β	2.65 (br s)	35.2	2.66
NH_2	7.39 (br)		
NH	8.10 (d, 8)		8.09
Asp-7			
α	4.51 (m)	50.5	4.52
β	2.63 (dd, 2 and 16)	35.9	2.66
	2.73 (dd, 5 and 16)		2.72
NH	8.27 (d, 8)		8.26
Gly-8			
α	3.52 (dd, 6 and 16)	42.3*	3.53
	3.77 (dd, 6 and 16)		3.77
NH	8.18 (t, 6)		8.16
Trp-9			
α	4.50 (m)	53.1	4.51
β	3.03 (dd, 8 and 15)	27.3	3.04
	3.15 (dd, 4 and 15)		3.15
2'	7.20 (br s)	123.6	7.18
3'		109.8	
4'	7.56 (br d, 8)	118.0	7.56
5'	6.96 (dt, 1 and 8)	120.6	6.95
6'	7.04 (dt, 1 and 8)	118.1	7.05
7'	7.31 (br d, 8)	111.1	7.32
8'		136.0	
9'		127.0	
1'-NH	10.65 (br s)		10.66
NH	7.74 (d, 8)		7.77
Ile-10			
α	4.18 (t, 8)	57.2	4.17
β	1.73 (m)	35.9	1.74
γ	0.78 (d, 6)	15.1	0.78
	1.06 (m)	25.1	1.06
	1.40 m		1.40
δ	0.79 (t, 7)	10.9	0.79
NH	7.78 (d, 8)		7.77
Val-10'			
α	4.15		
β	2.00		
γ	0.83 (d, 7)		
	0.84 (d, 7)		
Dab-11			
α	4.43 (m)	36.8*	4.42
β	1.87 (m)	29.9	1.88
	2.00 (m)		2.01
γ	2.81 (m)	49.9	2.82
NH	7.94 (d, 8)		7.94
Phe-12			
α	4.60 (m)	53.9	4.62
β	3.00 (dd, 8 and 15)	35.9	2.99
	3.11 (dd, 6 and 15)		3.10
2'–6'	7.15–7.25 (m)		7.15–7.25
NH	8.10 (d, 8)		8.11
	(aromatic carbon)		
	1'	136.3	
	2', 6'	127.9	
	3', 5'	128.8	
	4'	126.3	

[a] Carbonyl groups: δ 168.6, 168.7, 168.8, 170.1, 170.6, 170.7, 170.8, 171.1, 171.3 (2C), 171.4, 171.5 (3C), 171.8.
[b] Coupling constants in Hz.
[c] 2,4-diaminobutyric acid.
* The assignments may be exchanged with corresponding carbons of same amino acid residue.

(Fig. 5) and an examination of molecular models considered from observed intramolecular hydrogen-bonds. The hydrogen-bonds were found for the amide NH protons of 2,4-diaminobutyric acid-3 and tryptophan-9 with ^{1}H NMR measurements of **2** at various temperatures (Table 4)[5]. By comparison of the spectra measured at 40°C and 60°C, the shifts of these amide NH protons were negligible (0~0.03 ppm), while those of other amide NH protons were 0.08~0.13 ppm. On the other hand, NOEs were observed between the amide NH proton of aspartic acid-4 and α-proton of the other aspartic acid-7 and between the α-protons of glycine-8 and phenylalanine-12 in the NOESY spectrum of **2** (Fig. 5). These data indicated that the hydrogen-bonds exist between the amide NH proton of 2,4-diaminobutyric acid-3 and the carbonyl oxygen of aspartic acid-7 and between the amide NH proton of tryptophan-9 and the carbonyl oxygen of 2,4-diaminobutyric acid-11. NOE was also observed between the β-proton of L-isoleucine-10 and β-protons (δ 1.87) of 2,4-diaminobutyric acid-11. A nearest distance between these β-protons is calculated to be 3.4 Å when the 2,4-diaminobutyric acid-11 is L-form and 6.1 Å when it is D-form in the molecular models. Therefore, 2,4-diaminobutyric acid-11 was determined as L-form, and consequently the other 2,4-diaminobutyric acid-3 is D-form. The assignment was confirmed by the NOE observed between the α-proton of 2,4-diaminobutyric acid-3 and the β-proton of L-phenylalanine-12 (nearest distance between the protons of L-phenylalanine-12 and D-2,4-diaminobutyric acid-3 is 1.9 Å, and that between L-phenylalanine-12 and L-2,4-diaminobutyric acid-3 is 4.8 Å). The NOE between

Fig. 5. NOE data of **2** observed with NOESY spectrum.



400 MHz, at 60°C, mixing time: 0.4 second.

Table 4. Chemical shifts of amide protons of Bu-2841-10 (**2**) in DMSO-d_6 at 40, 50, and 60°C (400 MHz).[a]

Temperature	40° C	50° C	60° C	Δ (40° C - 60° C)
Ser-1	8.03	7.98	7.94	0.09
Thr-2	8.13	8.07	8.02	0.11
Dab-3	8.02	8.00	7.99	0.03
Asp-4	8.44	8.38	8.34	0.10
Gly-5	8.10	8.05	8.01	0.09
Asn-6	8.17	8.13	8.09	0.08
Asp-7	8.36	8.31	8.26	0.10
Gly-8	8.26	8.21	8.16	0.10
Trp-9	7.77	7.76	7.77	0.00
(1'-NH)	(10.78)	(10.68)	(10.61)	(0.17)
Ile-10	7.90	7.84	7.77	0.13
Dab-11	8.02	7.99	7.94	0.08
Phe-12	8.19	8.15	8.11	0.08

[a] These protons were assigned with COSY spectra.

γ-proton(s) of D-2,4-diaminobutyric acid-3 and the β-proton of aspartic acid-4 (δ 2.59) was also observed. The nearest distance between the two protons is 2.6 Å (the residue-4 is L-form) and 3.8 Å (the residue-4 is D-form). Therefore, the residue -4 is L-aspartic acid and consequently the other aspartic acid-7 and asparagine-6 are D-forms. The observed NOE between an aromatic proton of tryptophan residue (C-5'-H) and the γ-amide NH_2 protons of the D-asparagine-6 indicated that tryptophan-9 is L-form (the nearest distance between these protons is 1.5 Å (D-asparagine-6 and L-tryptophan-9) and 10.2 Å (D-asparagine-6 and D-tryptophan-9)). To summarize these data, the structure of Bu-2841-08 (**1**) and -10 (**2**) were determined as shown in Fig. 6.

Previously, SHOJI and KATO reported the structure of brevistin (**3**)[6], a cyclic depsipeptide. The structure closely resembled those of the Bu-2841-08 (**1**) and -10 (**2**) reported here. However, significant differences reside in the amino acid contents and the acyl groups of brevistin and Bu-2841s.

Antimicrobial Activity

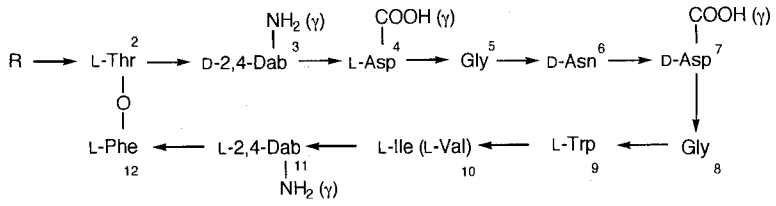
MICs of the antibiotics were determined by means of a two-fold serial agar dilution method in Mueller-Hinton media for the bacteria, in Sabouraud media for fungi, and in yeast morphology media for yeast. The antibacterial and antifungal spectra are given in Table 5. These antibiotics are similarly active against Gram-positive bacteria, but practically inactive against Gram-negative bacteria and fungi.

Experimental

Instruments

FAB-MS and FAB-MS/MS spectra were obtained with a Jeol JMS-SX102/102A tandem mass spectrometer. Xenon was used to provide the preliminary beam of atoms (5 Kw). The liquid matrix used for FAB ionization was 3-nitrobenzyl alcohol. MS/MS spectra were obtained

Fig. 6. Structures of Bu-2841-08 (**1**), -10 (**2**), and brevistin (**3**).



1: R = $CH_3CH_2CH(CH_3)(CH_2)_3CH(OH)CH_2CO$ → D-Ser[1]—,
$CH_3(CH_2)_6CH(OH)CH_2CO$ → D-Ser[1]—,
($CH_3CH_2CH(CH_3)(CH_2)_4CH(OH)CH_2CO$ → D-Ser[1]—, and/or
$CH_3(CH_2)_7CH(OH)CH_2CO$ → D-Ser[1]—)

2: R = $CH_3CH_2CH(CH_3)(CH_2)_4CH(OH)CH_2CO$ → D-Ser[1]—,
$CH_3(CH_2)_7CH(OH)CH_2CO$ → D-Ser[1]—,
($CH_3CH_2CH(CH_3)(CH_2)_5CH(OH)CH_2CO$ → D-Ser[1]—, and/or
$CH_3(CH_2)_8CH(OH)CH_2CO$ → D-Ser[1]—)

3: R = $CH_3CH_2CH(CH_3)(CH_2)_4CO$-

by activating the ions in the third field free region by collision with argon gas. GC-MS was recorded on a Shimadzu LKB-9000 apparatus using an OV-17 column with temperature programing of 5°C/minute from 100°C. NMR spectra were recorded on a Jeol JNM-EX400 (400 MHz for ^1H and 100 MHz for ^{13}C) or a Jeol JNM-α-500 (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometer. As ^1H NMR chemical shift reference, the center signal of DMSO-d_6 at δ 2.50, and as ^{13}C NMR reference, the methyl signal of DMSO-d_6 at δ 39.5 ppm were used. Amino acid analyses were performed with a Hitachi amino acid autoanalyzer model L-8500. UV spectra were recorded on a Shimadzu UV-265 spectrophotometer. Optical rotations were determined with a Jasco model DIP 140. For the examination with molecular models, Maruzen Biochemistry Molecular Model (peptide model) was used.

Acid Hydrolysis of Antibiotics

A solution of Bu-2841 complex (1.2 mg) in 0.5 ml of 6 N HCl was heated at 110°C for 24 hours in a vacuum-sealed tube. The hydrolysate, after concentration to dryness *in vacuo* on a water-bath at 60°C, was dissolved in 0.02 N HCl to 1.2 ml and 10 μl of the solution was analyzed with the amino acid analyzer (Table 1).

A mixture of each isolated peptides (0.2~0.6 mg), 6 N HCl (0.5 ml), and thioglycolic acid (50 μl) was heated at 110°C for 24 hours in a vacuum-sealed tube. The hydrolysate, after treatment of the usual manner, was analyzed with the amino acid analyzer. The results were given in Table 2.

In order to determine the constituent fatty acids and the chirality of amino acids, a mixture of Bu-2841 complex (1.0 g), 6 N HCl (42 ml), and thioglycolic acid (1.8 ml) was heated at 110°C for 17 hours in a vacuum-sealed tube. The reaction mixture was extracted with ethyl ether. Evaporation of the ether extract afforded an oily residue. The aqueous layer was concentrated *in vacuo* to a residue which was chromatographed on a column of Dowex 50W × 4 (H^+ form, 2 × 52 cm) de-

Table 5. Antibacterial and antifungal spectra of Bu-2841-08 (**1**) and -10 (**2**).

Organisms tested	MIC (μg/ml)	
	Bu-2841-08	Bu-2841-10
Bacillus cereus IFM 2058	25	50
B. megaterium IFM 2059	50	50
B. subtilis PCI 219 IFM 2060	25	25
Corynebacterium diphtheriae IFM 2056	12.5	25
C. xerosis IFM 2057	3.13	1.56
Micrococcus luteus IFM 2066	6.25	6.25
Mycobacterium smegmatis IFM 2051	100	100
Staphylococcus aureus FDA 209P IFM 2014	1.56	0.78
S. aureus IFM 2013	12.5	25
S. aureus IFM 2018	25	25
Streptococcus faecalis IFM 2001	25	25
S. salivarius IFM 2010	25	25
Escherichia coli IFM 3002	>100	>100
Pseudomonas aeruginosa IFM 3011	>100	>100
Aspergillus fumigatus IFM 25	>100	>100
Penicillium expansum IFM 40619	>100	>100
Trichophyton mentagrophytes IFM 40734	>100.00	>100
Candida albicans IFM 40009	>100.00	>100
Cryptococcus neoformans IFM 40038	>100.00	>100

veloping with an increasing concentration of HCl (0.05-2.0 N). Appropriate fractions (Asp (120 mg); eluted with H_2O, Asp, Ser, Thr (331 mg) with 0.2 N HCl, Val (24 mg) with 0.3 N HCl, Ile (77 mg) with 0.4 N HCl, Phe (137 mg) with 0.5 N HCl, 2,4-Dab (229 mg) with 0.7 N HCl)) were pooled and concentrated under reduced pressure. The amino acids were further purified with column chromatography on Amberlite IRA-400 (Cl^- form) and XAD-2, eluted with H_2O, to give aspartic acid (203 mg), glycine (115 mg), valine (22 mg), isoleucine (72 mg), phenylalanine (117 mg), and 2,4-diaminobutyric acid (218 mg) as hydrochlorides. Threonine hydrochloride (72 mg) and serine hydrochloride (52 mg) were isolated by preparative TLC (Avicel, developer; BuOH - AcOH - H_2O (8 : 1 : 1)) and column chromatography on Amberlite IRA-400 (Cl^- form, H_2O). A part of the oily residue obtained from the above ethereal extract was treated with diazomethane in ethereal solution. After evaporation of the solvent, the residue was analyzed by the GC-MS spectrometer.

Bu-2842-08 (1)

Bu-2841-08 (**1**) was obtained as a colorless powder. HRFAB-MS: *m/z* 1463.7170 $(M+H)^+$, $\Delta -0.1$ mmu for $C_{67}H_{99}O_{21}N_{16}$. Positive ion FAB-MS: *m/z* 1523 $(M-H+Na+K)^+$, 1501 $(M+K)^+$, 1485 $(M+Na)^+$, 1463 $(M+H)^+$, 732 $(M+2H)^{2+}/2$. Negative ion FAB-MS: *m/z* 1461 $(M-H)^-$. Positive ion FAB-MS/MS of $(M+K)^+$ at *m/z* 1501; *m/z* 1483, 1456, 1443, 1441, 1411, 1383, 1372, 1371, 1331, 1315, 1272. Positive ion FAB-MS/MS of $(M+Na)^+$ at *m/z* 1485: *m/z* 1467, 1455, 1441, 1427, 1368, 1367, 1355, 1315, 1228, 129. Positive ion FAB-MS/MS of $(M+H)^+$ at *m/z* 1463: *m/z* 1445, 1433, 1419, 1418 $(MH-C_2H_7N$ (cleavage between α- and β-bond of 2,4-Dab))$^+$, 1405 $(MH-C_4H_{10}$ (Ile))$^+$, 1404 $(MH-C_2H_5ON$ (Asn))$^+$, 1403 $(MH-C_2H_4O_2$ (Asp))$^+$, 1372 $(MH-C_7H_7$ (Phe))$^+$, 1345 $(MH-C_4H_{10}$ (Ile)$-C_2H_4O_2$ (Asp))$^+$, 1344 $[MH-C_2H_4O_2$ (Asp)$-C_2H_5ON$ (Asn))$^+$, 1343 $(MH-2C_2H_4O_2$ (2Asp))$^+$, 1332 $(MH-C_9H_9N))^+$, 1319, 1293, 1279, 1276, 1233, 1206. Negative ion FAB-MS/MS of $(M-H)^-$ at *m/z* 1461: *m/z* 1445, 1443, 1332, 1275, 700.

Bu-2841-10 (2)

Bu-2841-10 (**2**) was obtained as a colorless powder. Positive ion FAB-MS: *m/z* 1537 $(M-H+Na+K)^+$, 1515 $(M+K)^+$, 1499 $(M+Na)^+$, 1477 $(M+H)^+$, $(M+2H)^{2+}/2$. Negative ion FAB-MS: *m/z* 1475 $(M-H)^-$, 700. Positive ion FAB-MS/MS of $(M+H)^+$ at *m/z* 1477: *m/z* 1459, 1447, 1433, 1432, 1419, 1418, 2386, 2359, 1358, 1293, 1276, 1262, 1234, 1206, 1189, 272, 143. Positive ion FAB-MS/MS of $(M+Na)^+$ at *m/z* 1499: *m/z* 1481, 1469, 1455, 1441, 1440, 1439, 1398, 1368, 1315. Positive ion FAB-MS/MS of $(M+K)^+$ at *m/z* 1515: *m/z* 1497, 1485, 1471, 1456, 1386, 1331, 1329, 1286. Negative ion FAB-MS/MS of $(M-H)^-$ at *m/z* 1476: *m/z* 1457, 1346, 1232, 700. ^{13}C NMR (100 MHz, DMSO-d_6): amino acid, Ser δ 55.3, 61.4; Thr δ 15.6, 55.7, 70.2; 2,4-Dab δ 29.5, 29.9, 36.1 (2C), 49.9, 50.0; Asp δ 36.3, 35.8, 50.5 (2C); Gly δ 42.4, 42.5; Asn δ 36.0, 49.7; Trp δ 26.7, 54.1, 109.8, 111.1, 118.1, 120.7, 127.1, 123.6, 136.0; Ile δ 10.9, 15.1, 25.1, 36.0, 57.3; Phe δ 36.1, 53.7, 126.4, 128.0, 128.9, 136.4, carbonyl carbons δ 168.8, 168.9, 169.0, 169.8, 170.3, 170.8, 170.9 (2C), 171.2 (2C), 171.3, 171.6, 171.7 (2C), 171.8 (2C).

Mild Alkaline Hydrolysis of Bu-2841-08 (1)

A mixture of Bu-2841-08 (5 mg), acetonitrile (3 ml), H_2O (1 ml), and 0.2 M tris-HCl buffer (pH 8.5, 5 ml) was kept at 40°C for 17 hours. The reaction mixture was subjected on a ODS column and eluted sequentially with H_2O and MeOH. The MeOH eluate, after evaporated under reduced pressure, was purified by reversed phase HPLC (column; Senshu-Pak ODS-4251-W, 10 × 250 mm, mobile phase; *iso*-PrOH - acetonitrile - 0.1% TFA (3 : 1 : 6), flow rate; 1 ml/minute, detection: UV 230 nm) to yield **1a** (1 mg) as a colorless powder. Positive ion FAB-MS: *m/z* 1522 $(M+K)^+$, 1503 $(M+Na)^+$, 1481 $(M+H)^+$, 1463, 1435, 1075, 934, 741 $(M+2H)^{2+}/2$. Negative ion FAB-MS: *m/z* 1501 $(M+Na-2H)^-$, 1479 $(M-H)^-$, 1021, 1461, 858, 647, 572. Positive ion FAB-MS/MS of $(M+Na)^+$ at *m/z* 1503: *m/z* 1485, 1473, 1459, 1338, 1210, 1186, 1079, 1045, 882, 624, 496. Negative ion FAB-MS/MS of $(M-H)^-$ at *m/z* 1479: *m/z* 1461, 1449, 1435, 1314, 1214, 801, 700, 358. 185. Positive ion FAB-MS/MS of ion at *m/z* 1075: *m/z* 1057, 817, 889, 860, 832, 745, 717, 617, 547, 546, 488. Positive ion FAB-MS/MS of ion at *m/z* 934: *m/z* 917, 916, 890, 860, 778, 576, 566, 460, 371. ^1H NMR (400 MHz, DMSO-d_6, 60°C): amino acid, Ser δ 3.62 (2H, m), 4.32 (1H, m), 7.99 (br); Thr δ 1.60 (3H, d, $J=6$ Hz), 4.07, 4.15 (each 1H, m), 7.72 (d, $J=9$ Hz), 2,4-Dab 1.87, 2.00 (each 2H, m), 2.82 (4H, m), 4.43 (2H, m), 7.97 (br), 8.00 (br); Asp δ 2.51, 2.58, 2.64, 2.69 (each 1H, m), 4.58 (2H, m), 8.10 (br); Gly δ 3.37, 3.60 (each 2H, m), 8.09 (br); Asn δ 2.60 (2H, m), 4.52 (1H, m), 8.15 (br); Trp δ 3.08, 3.15 (each 1H, dd, $J=5$ and 14 Hz), 4.50 (1H, m), 6.97, 7.05 (1H, dt, $J=1$ and 8 Hz), 7.17 (1H, br s), 7.32, 7.57 (each 1H, br d, $J=8$ Hz), 7.88 (br), 10.68 (1H, br s); Ile δ 0.78 (2.7H, d, $J=6$ Hz), 0.80 (2.7H, t, $J=7$ Hz), 1.10, 1.39, 1.75, 4.18 (each 0.9H, m), 7.76 (d, $J=8$ Hz); Phe δ 3.00 (1H, dd, $J=8$ and 14 Hz), 3.15 (1H, dd, $J=4$ and 14 Hz), 4.52 (1H, m), 7.88 (br); acyl group δ 0.83 (*ca.* 0.5H, d, $J=7$ Hz), 0.85 (*ca.* 2.5H, d, $J=7$ Hz), 0.86 (*ca.* 2.5H, t, $J=7$ Hz), 1.25 (*ca.* 9H, br), 1.37 (2H, m), 2.27 (2H, br d, $J=7$ Hz), 3.80 (1H, m), the amide protons were observed as broad signals. These signals were assigned by the NOESY spectrum.

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