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Rhodopeptins, Novel Cyclic Tetrapeptides with Antifungal Activities from *Rhodococcus* sp.

II. Structure Elucidation

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The structures of rhodopeptins, novel antifungal peptides, were determined on the basis of physico-chemical analyses of the intact molecules and their acid hydrolysates. The structures of rhodopeptins C1, C2, C3, C4 and B5 were determined to be *cyclo* (-Gly-L-Orn-L-Val-3-amino-10-methyldodecanoyl-), *cyclo* (-Gly-L-Orn-L-Ile-3-amino-10-methyldodecanoyl-), cyclo (-Gly-L-Orn-L-Val-3-amino-12-methyltridecanoyl-), *cyclo* (-Gly-L-Orn-L-Val-3-amino-12-methyltetradecanoyl-) and *cyclo* (-Gly-L-Lys-L-Val-3-amino-13-methyltetradecanoyl-), respectively. They are novel cyclic tetrapeptides containing a lipophilic β-amino acid.

In the preceding paper, we described, the taxonomy and fermentation of the producing strain, isolation, physico-chemical properties and biological activities of rhodopeptins.[1] The aim of this paper is to describe the structure elucidation of rhodopeptins.

Results

Structure of Rhodopeptin C1

Rhodopeptin C1 (**1**, Fig. 1) was obtained as a white powder, mp over 240°C; $[\alpha]_D^{26}$ −15.6° (*c* 0.14, methanol). The UV spectrum showed end absorption. The IR spectrum showed intense C=O (ν=1680 and 1649 cm^{-1}) and N–H (ν=3434, 1553 cm^{-1}) signals, which are typical of peptide compounds. In the positive-ion FAB-MS spectrum, the $(M+H)^+$ peak appeared at *m/z* 482, and in the negative-ion FAB-MS spectrum, the $(M-H)^-$ peak appeared at *m/z* 480. The molecular formula was established to be $C_{25}H_{47}N_5O_4$ by HRFAB-MS (*m/z* 482.3708 $(M+H)^+$; calcd for $C_{25}H_{48}N_5O_4$ 482.3706) and detailed NMR analyses.

The ^1H and ^{13}C NMR spectra of **1** are shown in Fig. 2 and 3, respectively. The analysis of the NMR spectra (^1H, ^{13}C, DEPT, ^1H-^1H COSY, HMQC, HMBC) of **1** in CD_3OD suggested the presence of the four substructures shown in Fig. 4. One of them was a β-amino acid unit, and the others were α-amino acid units.

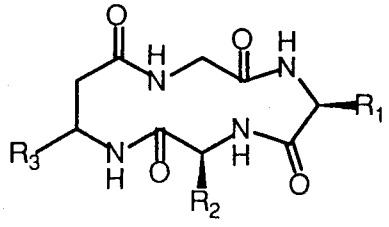
I. Valine: The presence of an isopropyl group was indicated by ^1H-^1H coupling between two methyl groups (δ 0.92) and a β-methine proton (δ 2.24). This methine proton was also coupled with the α-methine proton (δ 3.97, J=8.1 Hz) which displayed a long-range ^1H-^{13}C coupling to an amide carbonyl carbon (δ 172.36).

II. Glycine: The α-methylene *gem*-pair (δ 3.33, 4.18, J=13.9 Hz) showed a long-range ^1H-^{13}C coupling to an amide carbonyl carbon (δ 173.21).

III. Ornithine: In the COSY spectrum, an α-methine proton (δ 4.19), β,γ,δ-methylenes (δ 1.84, 1.80, 2.99) were coupled in order with each other. The α-methine proton showed a long-range ^1H-^{13}C coupling to an amide carbonyl carbon (δ 174.15).

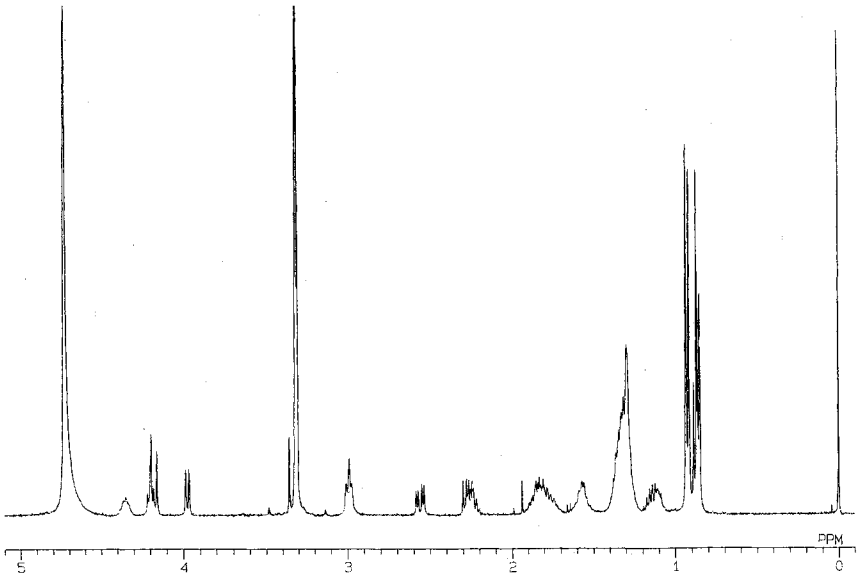
IV. 3-Amino-10-methyldodecanoic acid (abbreviated as Amdo): In the COSY spectrum, the proton signals at δ 2.27 (dd) and 2.56 (dd) showed a geminal coupling (J=13.9 Hz)

Fig. 1. Structures of rhodopeptins.



	R_1	R_2	R_3
Rhodopeptin C1 (1)	$-(CH_2)_3NH_2$	$-CH(CH_3)-CH_3$	$-(CH_2)_6CH(CH_3)-CH_2CH_3$
Rhodopeptin C2 (2)	$-(CH_2)_3NH_2$	$-CH(CH_3)-CH_2CH_3$	$-(CH_2)_6CH(CH_3)-CH_2CH_3$
Rhodopeptin C3 (3)	$-(CH_2)_3NH_2$	$-CH(CH_3)-CH_3$	$-(CH_2)_8CH(CH_3)-CH_3$
Rhodopeptin C4 (4)	$-(CH_2)_3NH_2$	$-CH(CH_3)-CH_3$	$-(CH_2)_8CH(CH_3)-CH_2CH_3$
Rhodopeptin B5 (5)	$-(CH_2)_4NH_2$	$-CH(CH_3)-CH_3$	$-(CH_2)_9CH(CH_3)-CH_3$

Fig. 2. ^1H NMR spectrum of rhodopeptin C1 (**1**) (400 MHz, CD_3OD).



with each other and couplings to the methine proton (δ 4.35, m), respectively. The analysis of the HMQC spectrum indicated that this *gem*-pair was coupled to the methylene carbon (δ 41.74) and the proton (δ 4.35) was coupled to the methine carbon (δ 48.13). These signals are characteristic of a β-amino acid with a long side chain and could be as-

Fig. 3. ^{13}C NMR spectrum of rhodopeptin C1 (**1**) (100 MHz, CD_3OD).

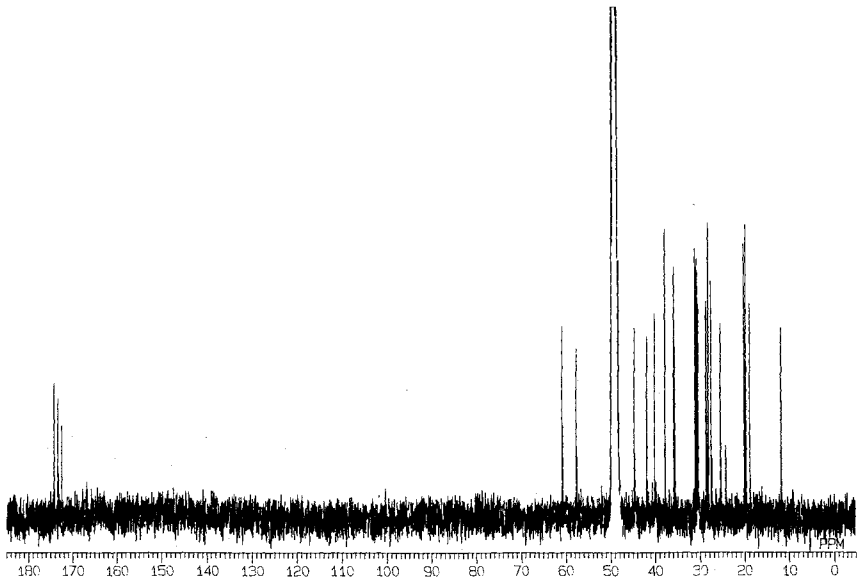
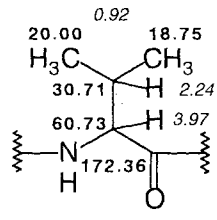
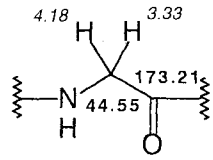


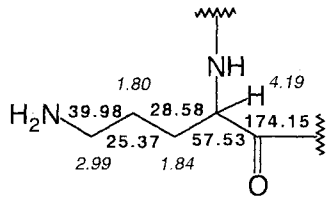
Fig. 4. Partial structures of rhodopeptin C1 (**1**) with ^1H (italics) and ^{13}C (boldface) NMR assignments (400 MHz, CD_3OD).



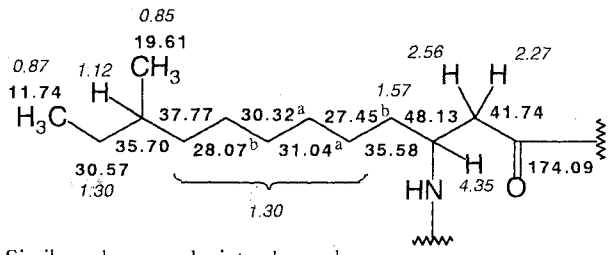
I. Valine



II. Glycine



III. Ornithine



a,b Similar values may be interchanged.

IV. 3-Amino-10-methyldodecanoic acid (Amdo)

signed to the α-methylene and the β-methine of the long-chain β-amino acid.[2] The α-methylene proton showed a long-range ^{1}H-^{13}C coupling to an amide carbonyl carbon (δ 174.09). Furthermore, the β-methine proton was coupled to the methylene protons (δ 1.57), which were also coupled to the large signal at δ 1.30. On the other hand, the methyl protons (δ 0.87 (t) and 0.85 (d)) were coupled to the protons (δ 1.30 (m) and 1.12 (m)), respectively. The analysis of the HMQC spectrum indicated that these methyl protons were coupled to the carbons (δ 11.74 and 19.61), respectively. It is known that ^{1}H NMR spectra of aliphatic long chain compounds having different terminal branch structures of the chains differ from each other in their methyl regions. ^{1}H NMR spectra indicate a 3H distorted triplet for a *normal*-type compound, a 6H doublet for an *iso*-type compound, and a 6H multiplet due to triplet plus doublet for an

Table 1. ^{1}H NMR data for rhodopeptins (400 MHz, CD_3OD).

Assignment	δ_H (mult, J (Hz))				
	C1(1)	C2(2)	C3(3)	C4(4)	B5(5)
Val (Ile)					
Cα	3.97 (d, 8.1)	4.03 (d, 8.4)	3.97 (d, 8.1)	3.97 (d, 8.1)	3.97 (d, 7.7)
Cβ	2.24 (m)	1.99 (m)	2.24 (m)	2.24 (m)	2.23 (m)
Cγ	0.92 (6H, d, 6.6)	a) 1.12 (m)	0.92 (6H, d, 6.6)	0.92 (6H, d, 6.6)	0.92 (6H, d, 7.0)
		b) 1.54 (m)			
(βMe)	———	0.908 (3H, d, 6.6)	———	———	———
(γMe)	———	0.913 (3H, t, 7.3)	———	———	———
Gly					
Cα	a) 3.33 (d, 13.9)	a) 3.33 (d, 13.6)	a) ca.3.3 (obscured)	a) 3.32 (d, 13.6)	a) ca.3.3 (obscured)
	b) 4.18 (d, 13.9)	b) 4.18 (d, 13.6)	b) 4.18 (d, 13.9)	b) 4.18 (d, 13.6)	b) 4.18 (d, 13.6)
Orn (Lys)					
Cα	4.19 (t, 7.3)	4.19 (m)	4.19 (m)	4.19 (m)	4.19 (m)
Cβ	1.84 (2H, m)	1.65-1.90 (4H, m)	1.70-1.90 (4H, m)	1.65-1.90 (4H, m)	1.80 (2H, m)
Cγ	1.80 (2H, m)				1.29 (2H, m)
Cδ	2.99 (2H, br t, 7.3)	2.98 (2H, br t, 8.1)	2.98 (2H, m)	2.98 (2H, br t, 6.6)	a) 1.29 (m)
					b) 1.71 (m)
Cε	———	———	———	———	2.98 (2H, m)
β-Amino acid					
C2	a) 2.27 (dd, 13.9, 8.4)	a) 2.25 (dd, 14.3, 8.4)	a) 2.26 (dd, 14.3, 8.8)	a) 2.26 (dd, 14.3, 8.8)	a) 2.26 (dd, 14.3, 8.4)
	b) 2.56 (dd, 13.9, 4.8)	b) 2.56 (dd, 14.3, 5.1)	b) 2.56 (dd, 14.7, 5.1)	b) 2.56 (dd, 14.3, 5.5)	b) 2.56 (dd, 13.9, 5.1)
C3	4.35 (m)	4.36 (m)	4.35 (m)	4.35 (m)	4.35 (m)
C4	1.57 (2H, m)	1.54 (2H, m)	1.56 (2H, m)	1.56 (2H, m)	1.55 (2H, m)
C5	1.30 (10H, m)	1.29 (10H, m)	1.29 (12H, m)	1.29 (14H, m)	1.29 (14H, m)
C6					
C7					
C8					
C9					
C10	1.12 (m)	1.12 (m)			
C11	1.30 (2H, m)	1.29 (2H, m)	1.16 (2H, m)		
C12	0.87 (3H, t, 7.0)	0.87 (3H, t, 7.0)	1.53 (m)	1.12 (m)	1.16 (2H, m)
C13	0.85 (3H, d, 6.2)	0.85 (3H, d, 6.6)	0.87 (6H, d, 7.0)	1.29 (2H, m)	1.50 (m)
C14	———	———		0.87 (3H, t, 7.0)	0.88 (3H, d, 6.6)
C15	———	———	———	0.86 (3H, d, 6.6)	0.88 (3H, d, 6.6)

Table 2. ^{13}C NMR data for rhodopeptins (100 MHz, CD_3OD).

Assignment	δ_C		
	C1(1)	C2(2)	C4(4)
Val (Ile)			
CO	172.36	172.40	171.04
Cα	60.73	60.06	60.79
Cβ	30.71	37.1	30.77
Cγ	1) 18.75	25.99	1) 18.79
	2) 20.00		2) 20.07
(βMe)	—	16.14	—
(γMe)	—	11.86	—
Gly			
CO	173.21	173.19	172.42
Cα	44.55	44.66	44.63
Orn			
CO	174.15	174.18	174.23
Cα	57.53	57.53	57.59
Cβ	28.58	28.57	28.66
Cγ	25.37	25.41	25.43
Cδ	39.98	40.04	40.04
β-Amino acid			
CO	174.09	174.04	174.15
C2	41.74	41.89	41.79
C3	48.13	48.09	48.10
C4	35.58	35.67	35.63
C5	27.45 [a]	27.49 [c]	27.49 [e]
C6	30.32 [b]	30.37 [d]	30.33 [f]
C7	31.04 [b]	31.10 [d]	30.65 [f]
C8	28.07 [a]	28.13 [c]	30.74 [f]
C9	37.77	37.83	31.12 [f]
C10	35.70	35.76	28.25 [e]
C11	30.57	30.63	37.84
C12	11.74	11.79	35.76
C13	19.61	19.67	30.71 [f]
C14	—	—	11.79
C15	—	—	19.67

[a~f] Similar values may be interchanged.

anteiso-type compound.[3] Additionally, in ^{13}C NMR spectra, methyl carbons of aliphatic long chain compounds usually resonate at approximate δ 14 for *normal*-type compounds, δ 23 for *iso*-type compounds, and δ 11 and 19 for *anteiso*-type compounds. Therefore, the data suggested that this residue was the *anteiso*-type β-amino acid. The large signal at δ 1.30 was assigned to the methylene protons at C-5~C-9 and the overlapping methylene protons at C-11. Long-range couplings were observed between the methyl proton (δ 0.87) and the methylene carbon (δ 30.57), and the methyl proton (δ 0.85) and the methylene carbon (δ 37.77), respectively. Thus these carbons at δ 30.57 and 37.77 were assigned to the methylenes at C-11 and C-9. The assignments of ^1H and ^{13}C NMR spectra of **1** are shown in Tables 1 and 2.

Rhodopeptin C1 (**1**) was hydrolyzed with 6 N HCl at 120°C for 16 hours. Water-soluble components and a lipophilic one were obtained. The polar components were identified as two α-amino acids in comparison with standard α-amino acids (Gly and Orn) by an amino acid analyser and TLC using silica gel with solvents A~E (see experimental section) and cellulose powder with solvent A. Quantitative analysis by an amino acid analyser indicated that the molar amounts of Gly and Orn were equal. The lipophilic moiety was extracted with *n*-butanol and gave one product. This compound was also analyzed qualitatively by TLC as described above and detected with ninhydrin reagent. The Rf value of this compound was higher than those of normal α-amino acids. The spectral data for this compound implied that this was the dipeptide, Val→Amdo (**6**). The amino acid sequence of this dipeptide (**6**) was determined by analysis of the collision-induced dissociation (abbreviated as CID) FAB-MS spectrum as shown in Fig. 5 and 6. A fragment ion peak at *m/z* 230 was derived from the elimination of Amdo from *m/z* 329 (the parent peak). This result suggested that the dipeptide (**6**) was Val→Amdo because the fragment ion peaks derived from the parent peak of Amdo→Val should require *m/z* 118 or 212. This result was similar to that reported for the iturin group of antibiotics, cyclic octapeptides containing a β-amino acid.[4]

This dipeptide (**6**) was submitted to a further acid hydrolysis (6 N HCl, 150°C, 8 hours) according to the procedure of BESSON *et al.*,[4] to give two products, a water-soluble one and a lipophilic one. The water-soluble product was identified as Val by TLC using silica gel in comparison with an authentic sample. On silica gel TLC, the lipophilic product gave one characteristic spot which had a higher Rf value than that of the dipeptide (**6**) and that gave an orange color with ninhydrin reagent. The non-polar substance was extracted with *n*-butanol, and the structure was elucidated as an *anteiso*-type C_{13}-β-amino acid, 3-amino-10-methyldodecanoic acid (**7**), on the basis of ^1H NMR, IR and FAB-MS spectra.

Fig. 5. CID FAB-MS spectrum of the dipeptide (**6**).

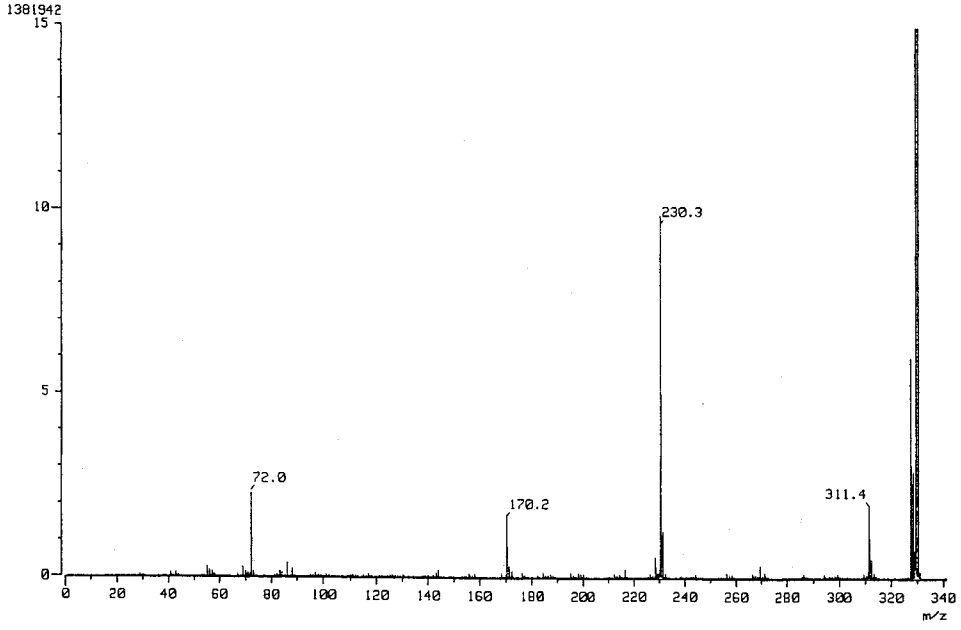
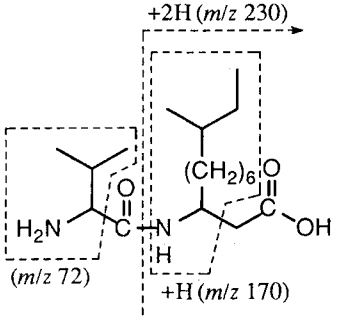


Fig. 6. CID FAB-MS fragmentations of the dipeptide (**6**).



The aqueous hydrolysate containing Gly and Orn was chromatographed by preparative silica gel TLC to isolate Orn. The absolute configuration of Orn (retention time of 5.2 minutes) was determined to be L by a chiral HPLC analysis: column: Crownpack CR(+), 4×150 mm, Daicel; mobile phase: $HClO_4$ (pH 1.5); flow rate: 0.4 ml/minute; detection: UV 210 nm; temperature: room temperature. The retention times of authentic D- and L-Orn were 4.7 and 5.2 minutes. Val (Rf value of 0.62) was identified as the L-enantiomer by TLC using a chiral pre-coated HPTLC plate in comparison with authentic D- and L-Val, which gave Rf values of 0.50 and 0.62. The absolute configuration of C-3 of Amdo (**7**) will be described later.[5]

Confirmed partial structures required four degrees of unsaturation by four carbonyl functions, but five degrees of unsaturation were required by its molecular formula. Thus the overall structure of rhodopeptin C1 (**1**) was determined to be cyclic.

Rhodopeptin C1 (**1**) was acetylated to give *N*-Ac rhodopeptin C1 (**8**), which had a molecular weight of 523 and a molecular formula of $C_{27}H_{49}N_5O_5$. In the ^1H NMR spectrum (CD_3OD), the observed chemical shift of the α-methine proton of Orn in **1** was δ 4.19, and that in **8** was δ 4.17. In comparison, the chemical shift of the δ-methylene protone of Orn in **1** was δ 2.99, and that in **8** was δ 3.15. This low field chemical shift in **8** indicated that the δ-amino group was acetylated in **8**, indicating that the δ-NH_2 in **1** was free and the α-NH was connected with an amide bond.

The cyclic amino acid sequence of rhodopeptin C1 (**1**) was determined by analysis of the CID FAB-MS spectrum as shown in Fig. 7 and 8. A series of fragment ion peaks at *m/z* 383, 172 and 115 was derived from the successive elimination of Val, Amdo and Gly from *m/z* 482 (the parent peak). Thus the structure of rhodopeptin C1 (**1**) was determined to be *cyclo* (-Gly-L-Orn-L-Val-3-amino-10-methyldodecanoyl-) (Fig. 1).

Fig. 7. CID FAB-MS spectrum of rhodopeptin C1 (**1**).

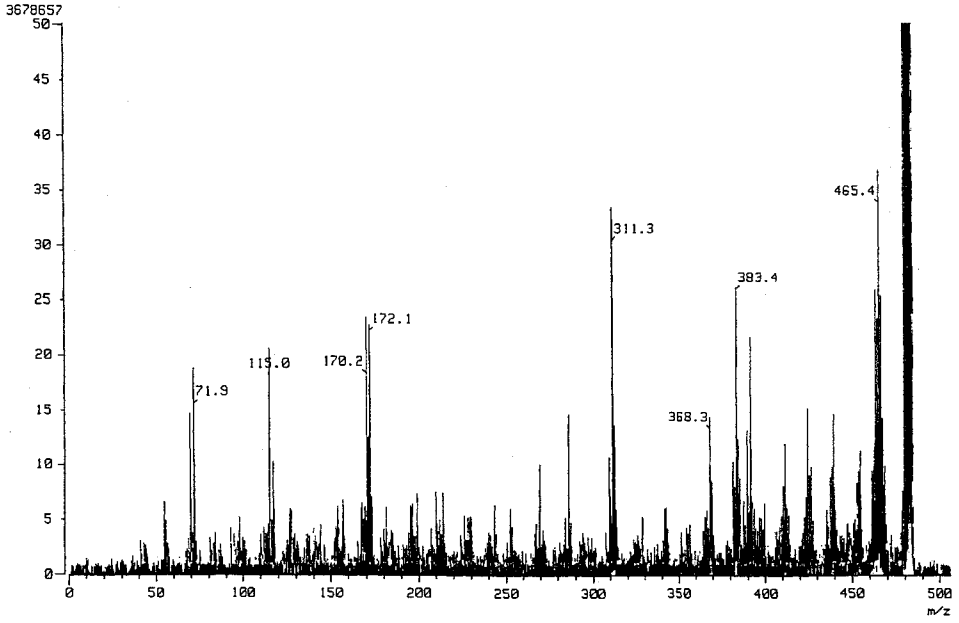
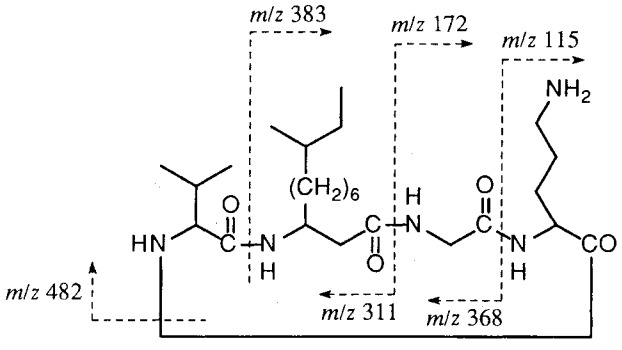


Fig. 8. CID FAB-MS fragmentations of rhodopeptin C1 (**1**).



Structures of Rhodopeptin C2, C3, C4 and B5

Preliminary physico-chemical analyses showed that rhodopeptin C1 (**1**), C2 (**2**), C3 (**3**), C4 (**4**) and B5 (**5**) were structurally related. The ^{1}H and ^{13}C NMR data for rhodopeptins are shown in Tables 1 and 2, respectively.

Structure of Rhodopeptin C2 (**2**)

The molecular formula of **2** was determined to be $C_{26}H_{49}N_5O_4$ on the basis of HRFAB-MS and ^{13}C NMR data. The formula was larger than that of **1** by one methylene unit. Comparison of the spectral data between **1** and **2** indicated that Ile in **2** substitutes for Val in **1**.

In the ^{1}H NMR spectrum of **2**, two multiplet methyl signals (δ 0.913, 0.908) were observed instead of the 6H doublet signals at δ 0.92 in that of **1**. The methyl proton at δ 0.913 was coupled to the methylene protons (δ 1.54, 1.12), and the other methyl proton at δ 0.908 was coupled to the methine proton (δ 1.99). This methine proton was coupled to those methylene protons and the α-methine proton (δ 4.03).

The amino acid cyclic sequence of **2** was determined by the CID FAB-MS spectrometric analysis. A series of fragment ion peaks at *m/z* 383, 172 and 115 was derived from the successive elimination of Ile, β-amino acid and Gly from *m/z* 496 (the parent peak), respectively. Another fragment ion peak at *m/z* 325 was detected strongly, suggesting the presence of an Ile→β-amino acid dipeptide unit.

After acid hydrolysis of **2**, Ile, Rf 0.62, was identified as the L-enantiomer by TLC using a chiral pre-coated TLC plate in comparison with authentic D- and L-Ile, which gave Rf values of 0.49 and 0.62.

Therefore, the structure of rhodopeptin C2 (**2**) was determined to be *cyclo* (-Gly-L-Orn-L-Ile-3-amino-10-methyldodecanoyl-) (Fig. 1).

Structure of Rhodopeptin C3 (**3**)

The molecular formula of **3** was determined to be

$C_{26}H_{49}N_5O_4$ on the basis of MS and NMR data, which was equal to **2**. Comparison of the spectral data between **1** and **3** indicated that an *anteiso*-type C_{13}-β-amino acid in the structure of **1** changes to an *iso*-type C_{14}-β-amino acid in the structure of **3**. In the ^1H NMR spectrum of **3**, the 6H doublet signals at δ 0.87 were observed instead of the 3H multiplet signals at δ 0.85 and 0.87 in that of **1**.

The amino acid cyclic sequence of **3** was determined by the CID FAB-MS spectrometric analysis as in the case of **1**. A series of fragment ion peaks at *m/z* 397, 172 and 115 was derived from the successive elimination of Val, β-amino acid and Gly from *m/z* 496 (the parent peak), respectively. Another fragment ion peak at *m/z* 325 was strongly detected, suggesting the presence of a Val→β-amino acid dipeptide unit. In this case, the β-amino acid is 3-amino-12-methyltridecanoic acid (**9**), which is identical with one of the iturinic acids that are components of iturins.[3,6]

Therefore, the structure of rhodopeptin C3 (**3**) was determined to be *cyclo* (-Gly-L-Orn-L-Val-3-amino-12-methyltridecanoyl-) (Fig. 1).

Structure of Rhodopeptin C4 (**4**)

The molecular formula of **4** was determined to be $C_{27}H_{51}N_5O_4$ on the basis of HRFAB-MS and ^{13}C NMR data, which is larger than that of **1** by C_2H_4. Comparison of the spectral data between **1** and **4** indicated that the side chain of a β-amino acid in the structure of **4** is larger than that in the structure of **1** by two methylene units.

The amino acid cyclic sequence of **4** was determined by the CID FAB-MS spectrometric analysis as in the case of **1**. A series of fragment ion peaks at *m/z* 411, 172 and 115 was derived from the successive elimination of Val, β-amino acid and Gly from *m/z* 510 (the parent peak), respectively. Another fragment ion peak at *m/z* 339 was strongly detected, suggesting the presence of Val→β-amino acid dipeptide unit. In this case, the β-amino acid is 3-amino-12-methyltetradecanoic acid (**10**), and is also identical with one of the iturinic acids.[3,6]

Therefore, the structure of rhodopeptin C4 (**4**) was determined to be *cyclo* (-Gly-L-Orn-L-Val-3-amino-12-methyltetradecanoyl-) (Fig. 1).

Structure of Rhodopeptin B5 (**5**)

The molecular formula of **5** was determined to be $C_{28}H_{53}N_5O_4$ on the basis of MS and NMR data, which is larger than that of **1** by C_3H_6. Comparison of the spectral data between **3** and **5** indicated that Orn in the structure of **3** is replaced by Lys in the structure of **5**. The side chain of a β-amino acid in the structure of **5** is larger than that in the structure of **3** by one methylene unit.

The cyclic amino acid sequence of **5** was determined by the CID FAB-MS spectrometric analysis as in the case of **1**. A series of fragment ion peaks at *m/z* 425, 186 and 129 was derived from the successive elimination of Val, β-amino acid and Gly from *m/z* 524 (the parent peak), respectively, and the latter two fragment peaks suggested the presence of Lys instead of Orn. Additionally, another fragment ion peak at *m/z* 339 was strongly detected, suggesting the presence of Val→β-amino acid dipeptide unit. In this case, the β-amino acid is 3-amino-13-methyltetradecanoic acid (**11**), and is also identical with one of the iturinic acids.[3,6]

After acid hydrolysis of **5**, the absolute configuration of Lys (retention time of 6.2 minutes), was determined to L by HPLC on a chiral column in comparison with authentic D- and L-Lys, which gave the retention times 5.6 minutes and 6.2 minutes.

Therefore, the structure of rhodopeptin B5 (**5**) was determined to be *cyclo* (-Gly-L-Lys-L-Val-3-amino-13-methyltetradecanoyl-) (Fig. 1).

Discussion

Various cyclic tetrapeptides are known as microbial metabolites, but none of them contains a β-amino acid with a long alkyl side chain. It is known that the iturin group of antibiotics are cyclic peptides containing unusual β-amino acids,[6~10] but they are heptapeptides. Thus it is unique that cyclic tetrapeptides have lipophilic β-amino acids like the rhodopeptins. The β-amino acid residue in rhodopeptins C1 (**1**) and C2 (**2**), 3-amino-10-methyldodecanoic acid (**7**), is smaller by one methylene unit than the smallest iturinic acid, 3-amino-12-methyltridecanoic acid (**9**).[3,6] β-Amino acids similar to **7** are sometimes found in peptides produced by blue-green algae, *e.g.* laxaphycins,[2] but they are smaller than **7**. This is the first time that **7** has been identified as a component of a natural product.

In general, cyclic peptides give complex spectra on CID FAB-MS measurements since they have many possible fragmentation pathways.[11] However, in the case of the rhodopeptins, the spectra are relatively simple. In the spectrum of rhodopeptin C1 (**1**), a series of fragment ions arising from the successive elimination of Val, β-amino acid and Gly from the parent ion was clearly detected. This parent ion was the acylium ion derived from the cleavage of the peptide bond connecting Orn with Val. Therefore the CID FAB-MS measurement was an effective method for the structure determination of rhodopeptins.

Experimental

General

Melting points were measured with a Yanagimoto micro-mp apparatus and are uncorrected. FAB-MS and HRFAB-MS were obtained on a Jeol JMS-SX102A spectrometer. In the FAB-MS measurement, rhodopeptins were dissolved in TFA, and derivatives of them were dissolved in MeOH. Each 1 μl of solution was mixed with 1 μl of matrix on the target. Glycerin was used as a matrix except in the case of rhodopeptin C1 acetate (**8**). The CID FAB-MS spectra were obtained by B/E linked scan using nitrogen as a collision gas. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-GSX400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are given on the δ scale (ppm). TMS was used as an internal standard at δ 0.0. In the ^{13}C NMR spectrum of rhodopeptin C1 (**1**) chemical shifts were referenced to the solvent (CD_3OD) peak at δ 49.0. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Coupling constants (*J*-values) are given in Hz. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a Jasco FT/IR-5300 spectrometer, respectively. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. Amino acid analysis was carried out with a Shimadzu LC-6A Amino Acid Analyzer. TLC was carried out on silica gel 60 F_{254} (Merck) or cellulose powder (Avicel). Solvent systems used were: *n*-BuOH - AcOH - H_2O (4 : 1 : 2, by vol)=solvent A; EtOH - H_2O (7 : 3, by vol)=solvent B; *n*-PrOH - H_2O (7 : 3, by vol)=solvent C; Pyridine - Me_2CO - NH_4OH - H_2O (10 : 6 : 1 : 4, by vol)=solvent D; 2-Butanone - Pyridine - H_2O - AcOH (70 : 15 : 15 : 2, by vol)=solvent E; $CHCl_3$ - MeOH - H_2O (65 : 25 : 4, by vol)=solvent F. The products were detected by ninhydrin reagent with heating at 120°C. Chiral TLC was performed on CHIR pre-coated TLC plates (Merck) with the solvent system MeOH - H_2O - acetonitrile (1 : 1 : 4, by vol)=solvent G. Preparative silica gel TLC was performed on Empore TLC sheets (Analitichem Int.) with solvent A.

Acid Hydrolysis of Rhodopeptin C1 (1)

Rhodopeptin C1 (**1**) (9.7 mg) was hydrolyzed with 6 N HCl (0.8 ml) at 120°C for 16 hours in a sealed tube. The reaction mixture was diluted with water and concentrated under reduced pressure. This procedure was repeated. The residue was eluted with water and extracted twice with *n*-BuOH. Combined alcoholic extracts were concentrated to dryness under reduced pressure to yield a dipeptide (**6**) (5.9 mg). The aqeous layer was evaporated *in vacuo* to give a solid.

Properties of **6**: $C_{18}H_{36}N_2O_3$; TLC Rf 0.29 (silica gel, solvent F), 0.62 (silica gel, solvent A); FAB-MS, positive *m/z* 329 $(M+H)^+$, negative *m/z* 327 $(M-H)^-$; HRFAB-MS, *m/z* 329.2815 $(M+H)^+$, calcd 329.2804; IR ν_{max} (KBr) 3207, 3067, 2961, 2926, 2857, 1718, 1669, 1562, 1503, 1464, 1404, 1302, 1249, 1194, 905 cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.87 (3H, t, *J*=7.3 Hz, *n*-Me), 0.88 (3H, d, *J*=6.6 Hz, *anteiso*-Me), 1.04 (3H, d, *J*=7.0 Hz, Val-Me), 1.07 (3H, d, *J*=7.0 Hz, Val-Me), 1.14 (1H, m, *anteiso*-CH), 1.30 (12H, m), 1.60 (2H, m, βAA-γH), 2.18 (1H, m, Val-βH), 2.49 (1H, dd, *J*=15.8, 6.6 Hz, βAA-αHa), 2.55 (1H, dd, *J*=15.8, 6.6 Hz, βAA-αHb), 3.60 (1H, br d, *J*=5.5 Hz, Val-αH), 4.20 (1H, m, βAA-βH); ^{13}C NMR (CD_3OD): δ 175.02 s (βAA-CO), 168.96 s (Val-CO), 60.00 d (Val-α), 48.28 d (βAA-β), 40.12 t (βAA-α), 35.74 d (*anteiso*-CH), 31.58 t (Val-β), 19.66 q (*anteiso*-Me), 19.06 q (Val-Me), 17.89 q (Val-Me), 11.78 q (*n*-Me).

Further Hydrolysis of Dipeptide (6)

Dipeptide (**6**) (5.9 mg) was hydrolyzed with 6 N HCl (1.0 ml) at 150°C for 8 hours in a sealed tube. The reaction mixture was diluted with water and concentrated under reduced pressure. The residue was eluted with water and extracted with *n*-BuOH. The alcoholic extract was concentrated under reduced pressure to yield a residue (Amdo) (**7**) (3.8 mg). The aqeous layer was evaporated *in vacuo* to give a solid. This was identified as Val by TLC in comparison with an authentic sample: Rf 0.36 (silica gel, solvent A), 0.11 (silica gel, solvent F).

Properties of **7**: $C_{13}H_{27}NO_2$; TLC Rf 0.38 (silica gel, solvent F), 0.62 (silica gel, solvent A); FAB-MS, positive *m/z* 230 $(M+H)^+$, negative *m/z* 228 $(M-H)^-$; IR ν_{max} (KBr) 3453, 2963, 2926, 2855, 1719, 1564, 1468, 1395, 1262, 1098, 801 cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.87 (6H, m, C(12)H_3, C(13)H_3), 1.13 (1H, m, C(10)H), 1.33 (12H, m, C(5)H_2~C(9)H_2, C(11)H_2), 1.61 (2H, m, C(4)H_2), 2.34 (1H, dd, *J*=16.9, 8.8 Hz, C(2)Ha), 2.54 (1H, dd, *J*=16.9, 4.0 Hz, C(2)Hb), 3.35 (1H, m, C(3)H).

Acetylation of Rhodopeptin C1 (1)

Rhodopeptin C1 (**1**, 15 mg) was acetylated with acetic anhydride (2.5 ml) in AcOH (5 ml) at room temperature for 25 hours. The reaction mixture was concentrated to dryness. The residue was suspended in *n*-BuOH and evaporated *in vacuo* to give *N*-Ac rhodopeptin C1 (17 mg) (**8**): $C_{27}H_{49}N_5O_5$; TLC Rf 0.6 (silica gel, solvent A, not detected by ninhydrin); FAB-MS (Matrix, positive: Glycerin - Thioglycerin=1 : 1; negative: Magic Bullet) positive *m/z* 524 $(M+H)^+$, negative *m/z* 522 $(M-H)^-$; IR ν_{max} (KBr) 3445,

3289, 3086, 2959, 2926, 2857, 1649, 1551, 1462, 1439, 1379, 1298, 1119, 711, 621 cm^{-1}; ^1H NMR (CD_3CO_2D): δ 0.85 (3H, d, J=6.6 Hz), 0.86 (3H, t, J=6.6 Hz), 0.90 (6H, d, J=6.6 Hz), 1.11 (1H, m), 1.31 (12H, m), 1.53 (2H, m), 1.59 (2H, t-like, J=7.3 Hz), 1.89 (1H, m), 2.14 (2H, dd-like, J=14.7, 7.0 Hz), 2.36 (1H, dd, J=14.7, 10.3 Hz), 2.68 (1H, dd, J=14.7, 4.0 Hz), 3.25 (2H, br t, J=6.2 Hz), 3.49 (1H, d, J=13.9 Hz), 4.03 (1H, d, J=9.5 Hz), 4.33 (1H, d, J=13.9 Hz), 4.37 (1H, t-like), 4.50 (1H, m).

^1H NMR (CD_3OD - $CDCl_3$, 8 : 1): δ 0.86 (3H, d, J=7.3 Hz, *anteiso*-Me), 0.87 (3H, t, J=7.0 Hz, *n*-Me), 0.91 (6H, d, J=7.0 Hz, Val-Me_2), 1.10 (1H, m, *anteiso*-CH), 1.28 (12H, m), 1.55 (2H, m, Amdo-γH), 1.77 (4H, m, Orn-βH_2, γH_2), 1.93 (3H, s), 2.25 (1H, m, Val-βH), 2.26 (1H, dd, J=13.9, 7.7 Hz, Amdo-αHa), 2.54 (1H, dd, J=13.9, 7.0 Hz, Amdo-αHb), 3.15 (2H, m, Orn-δH_2), 3.33 (1H, d, J=13.9 Hz, Gly-Ha), 4.03 (1H, d, J=7.7 Hz, Val-αH), 4.17 (2H, m, Gly-Hb, Orn-αH), 4.32 (1H, m, Amdo-βH).

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