Cyclic Peptides from Higher Plants. 33.† Delavayins A−C, Three New Cyclic Peptides from *Stellaria delavayi*

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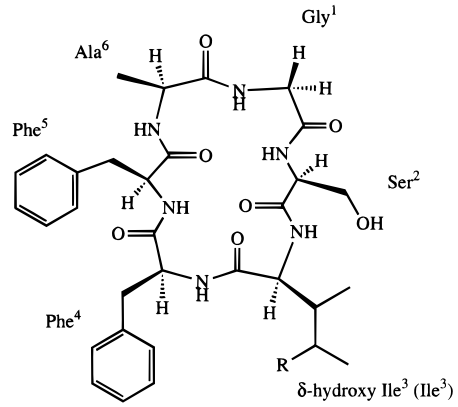
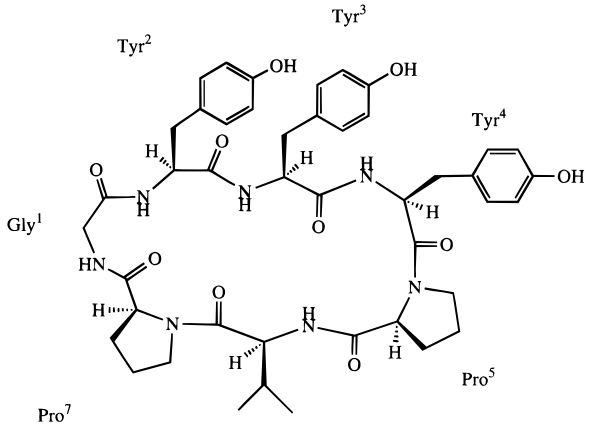
Three new cyclic peptides, delavayins A−C (**1**−**3**), were isolated from the roots of *Stellaria delavayi*. The structures of **1**−**3**, *cyclo*(-Gly-Ser-γ-hydroxy Ile-Phe-Phe-Ala-) (**1**), *cyclo*(-Gly-Ser-Ile-Phe-Phe-Ala-) (**2**), *cyclo*(-Gly-Tyr-Tyr-Tyr-Pro-Val-Pro-) (**3**), were elucidated from spectroscopic evidence and by chemical degradation.

As part of a continuing investigation on new biologically active cyclic peptides from higher plants, we have previously isolated several cyclic peptides; yunnanins from the roots of *Stellaria yunnanensis*,[2−4] pseudostellarins from the roots of *Pseudostellaria heterophylla*,[5−8] dichotomins from the roots of *Stellaria dichotoma* var. *lanceolata*,[9,10] and segetalins from the roots of *Vaccaria segetalis*.[11−13] All of these plants belong to the family Caryophyllaceae. As a result of the investigation of *Stellaria delavayi* Franch., which also belongs to the same family, we have isolated three new cyclic peptides, delavayins A−C (**1**−**3**). In this paper, we describe the isolation and structure elucidation of **1**−**3**.



1: R=OH
2: R=H



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Results and Discussion

A MeOH extract (1.217 kg) of the roots of *S. delavayi* (10.0 kg) was partitioned between *n*-BuOH and H_2O, and the *n*-BuOH-soluble fraction (179.1 g) was subjected to Diaion HP-20 column chromatography (H_2O−MeOH gradient system). The 80% MeOH-eluted fraction was chromatographed on a Si gel column ($CHCl_3$−MeOH gradient system) followed by repeated HPLC on ODS columns with 28% CH_3CN or 55% MeOH to yield delavayins A−C (**1**, 0.0001%; **2**, 0.0004%; **3**, 0.00004%).

Delavayin A (**1**) was obtained as a colorless powder, $[\alpha]_D$ +17.2° (*c* 0.19, MeOH). The IR absorptions at 3427 and 1650 cm^{-1} were attributed to amino and amide carbonyl groups, respectively. The FABMS spectrum of **1** showed a pseudomolecular ion at *m*/*z* 661 $[M + Na]^+$, and the molecular formula was shown to be $C_{32}H_{42}N_6O_8$ by HRFABMS analysis (found *m*/*z* 639.3168 $[M + H]^+$, calcd 639.3142). The amino acid composition of **1** was determined to be 1 mol each of serine (Ser), glycine (Gly), alanine (Ala), and two mol of phenylalanine (Phe) residues, and their absolute configuration was determined to be L by using Marfey's method.[14] In spite of the presence of five amino acid residues, six amino acids were suggested by the observations of six amide protons (δ 8.26, 8.70, 9.20, 9.31, 9.48, 10.20) in the ^{1}H-NMR spectrum and six amide carbonyl carbons (δ 170.08, 171.00, 171.17, 171.60, 172.65, 173.61) in the ^{13}C-NMR spectrum. Therefore, one of the amino-acid constituents of **1** must be unusual. Detailed analysis of the ^{1}H−^{1}H COSY, HOHAHA,[15] and HMQC[16] NMR spectra led to the complete assignment of the ^{1}H- and ^{13}C-NMR chemical shifts of each amino acid residue of **1** (Table 1). The unusual amino acid was elucidated as γ-hydroxyisoleucine by the following proton-coupling sequence. In the ^{1}H−^{1}H COSY spectrum, a coupling sequence ascribable to NH (δ 9.20)/Hα (δ 4.98)/Hβ (δ 2.25) was observed. The Hβ proton was coupled to both the doublet methyl group at δ 1.01 and a methine proton at δ 3.85, which was also coupled with a methyl proton at δ 1.08. The Hγ proton resonated at lower field than that of isoleucine attached to a hydroxyl-bearing carbon at δ 67.02. From these results, all of the six constituent amino acids were determined in **1**. Their sequencing

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† For Part 32 of this series, see Morita *et al.*[1]

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Table 1. ^{1}H- and ^{13}C-NMR Assignments for Delavayin A (**1**) in Pyridine-d_5

position	$^\delta$H [int, mult, *J* (Hz)]	$^\delta$C
Gly^1		
α	3.97 (1H, dd, 4.4, 16.7)	43.86
	4.90 (1H, dd, 8.0, 16.7)	
NH	10.20 (1H, dd, 4.4, 7.7)	
C=O		170.08
Ser^2		
α	5.25 (1H, ddd, 3.9, 4.6, 8.4)	55.45
β	4.09 (1H, dd, 5.0, 11.6)	63.12
	4.40 (1H, dd, 3.9, 11.6)	
NH	9.48 (1H, d, 8.1)	
C=O		171.00
γ-hydroxy Ile^3		
α	4.98 (1H, m)	60.39
β	2.25 (1H, m)	39.61
γ	3.85 (1H, br dd, 6.0, 12.3)	67.02
γ-CH$_3$	1.01 (3H, d, 7.1)	15.26
δ	1.08 (3H, d, 6.4)	21.75
NH	9.20 (1H, d, 7.2)	
C=O		172.65
Phe^4		
α	5.32 (1H, ddd, 3.9, 8.5, 11.1)	56.24
β	3.14 (1H, dd, 11.1, 14.1)	39.32
	3.63 (1H, dd, 3.9, 14.1)	
γ		138.43
δ	7.39 (2H, d, 7.2)	129.63
ϵ	7.25−7.29 (2H, m)	128.75^a
ζ	7.17−7.23 (1H, m)	127.02^b
NH	8.70 (1H, d, 8.5)	
C=O		171.17
Phe^5		
α	4.69 (1H, br dd, 6.2, 12.3)	54.76
β	3.44 (2H, br d, 6.3)	38.43
γ		137.65
δ	7.47 (2H, d, 7.4)	130.54
ϵ	7.25−7.29 (2H, m)	128.61^a
ζ	7.17−7.23 (1H, m)	126.90^b
NH	8.26 (1H, d, 5.9)	
C=O		171.60
Ala^6		
α	4.54 (1H, dq, 3.9, 6.9)	52.17
β	1.53 (3H, d, 6.9)	16.57
NH	9.31 (1H, d, 3.6)	
C=O		173.61

^{a,b} Assignments with the same superscripts may be interchanged.

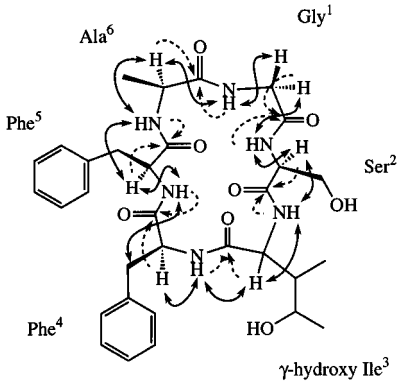

Figure 1. HMBC correlations (dashed arrows) and ROE correlations (arrows) for delavayin A (**1**) in pyridine-d_5.

was established as *cyclo*(-Gly-Ser-*γ*-hydroxyIle-Phe-Phe-Ala-) by the HMBC[17] and ROE correlations in a phase-sensitive ROESY spectrum,[18] as shown in Figure 1. To date, only a few peptides containing a *γ*-hydroxyIle unit, such as *γ*-amanitin have been reported,[19] and we have isolated yunnanins B and E containing a *δ*-hydroxyisoleucine unit as an unusual amino acid.[2,4]

Delavayin B (**2**) was obtained as a colorless powder, with $[\alpha]_D$ +6.0° (*c* 0.20, MeOH). The FABMS spectrum

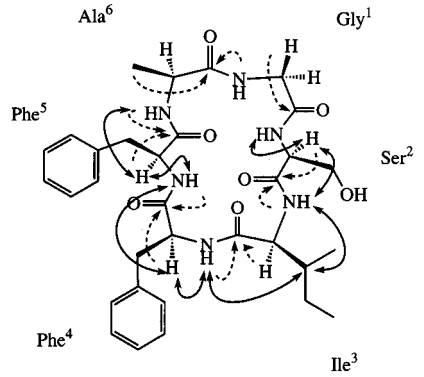

Figure 2. HMBC correlations (dashed arrows) and ROE correlations (arrows) for delavayin B (**2**) in DMSO-d_6.

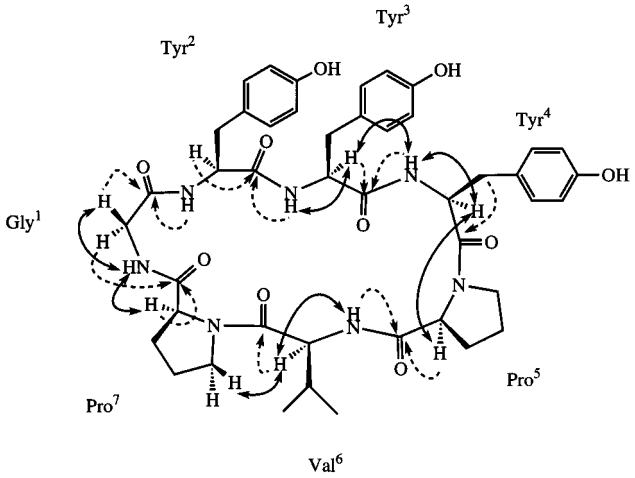

Figure 3. HMBC correlations (dashed arrows) and ROE correlations (arrows) for delavayin C (**3**) in pyridine-d_5.

of **2** gave an $[M + Na]^+$ ion at *m/z* 645, and the molecular formula, $C_{32}H_{42}N_6O_7$, was established by HRFABMS. Because the IR absorption bands (3397 and 1670 cm^{-1}) characteristic of amino and amide carbonyl groups indicated **2** to be a peptide, it was subjected to a standard amino acid analysis, which implied the presence of 1 mol each of Gly, Ser, Ala, and Ile and 2 mol of Phe. The ^{1}H- and ^{13}C-NMR spectra revealed six amide proton and six carbonyl signals, which were involved in amide linkages. Detailed 2D NMR analysis enabled us to complete the ^{1}H and ^{13}C assignments for **2** as shown in Table 2, which closely resembled those of delavayin A (**1**). The stereochemistry of all of the amino acid residues was confirmed to be in the L-configuration by Marfey's derivatization, followed by HPLC analysis. The HMBC and ROESY NMR data suggested the six-amino-acid sequence presented in the structure of **2**. The sequence, Gly-Ser-Ile-Phe-Phe-Ala, was assigned by two-bond ^{1}H−^{13}C correlations between NH and CO, and between Hα and CO, and ROE correlations between neighboring amino acids, as shown in Figure 2.

Delavayin C (**3**), a colorless powder with $[\alpha]_D$ −52.4° (*c* 0.08, MeOH), exhibited a HRFABMS quasimolecular ion $[M + H]^+$ peak at *m/z* 840.3955, corresponding to a molecular formula of $C_{44}H_{53}N_7O_{10}$. Amino acid analysis of the acid hydrolysate showed the presence of the following residues: three Tyr, two Pro, one Gly, and one Val. The stereochemistry of these amino acids was confirmed to be all L by Marfey's derivatization, followed by HPLC analysis. In the NMR spectra of **3**, ^{1}H- and

Table 2. ^{1}H-and ^{13}C-NMR Assignments for Delavayin B (**2**) in DMSO-d_6

position	$^\delta$H [int, mult, *J* (Hz)]	$^\delta$C
Gly[1]		
α	3.43 (1H, dd, 4.9, 16.8)	42.41
	3.92 (1H, dd, 4.9, 16.8)	
NH	8.62 (1H, m)	
C=O		168.57
Ser[2]		
α	4.35 (1H, m)	53.60
β	3.48 (1H, br s)	60.14
	3.59 (1H, br m)	
NH	8.09 (1H, d, 8.8)	
C=O		169.32
Ile[3]		
α	3.90 (1H, m)	59.04
β	1.62 (1H, m)	35.42
γ	0.96 (1H, m)	24.40
	1.31 (1H, m)	
γ-CH_3	0.36 (3H, d, 6.7)	14.81
δ	0.71 (3H, t, 7.3)	10.29
NH	7.88 (1H, d, 8.4)	
C=O		170.68
Phe[4]		
α	4.30 (1H, m)	54.95
β	2.66 (1H, dd, 11.2, 13.8)	39.32
	2.94 (1H, dd, 4.3, 13.8)	
γ		137.73
δ	7.21 (2H, m)	128.81
ϵ	7.12 (2H, m)	128.10[a]
ζ	7.16 (1H, m)	126.35[b]
NH	8.21 (1H, d, 8.8)	
C=O		170.38
Phe[5]		
α	4.47 (1H, ddd, 4.2, 8.0, 9.2)	52.83
β	2.78 (1H, dd, 9.5, 13.9)	37.98
	3.11 (1H, dd, 4.2, 13.9)	
γ		137.21
δ	7.29 (2H, m)	129.37
ϵ	7.33 (2H, m)	127.97[a]
ζ	7.23 (1H, m)	126.23[b]
NH	7.46 (1H, d, 7.7)	
C=O		171.00
Ala[6]		
α	3.92 (1H, m)	50.12
β	1.22 (3H, d, 6.9)	16.13
NH	8.63 (1H, br d, 3.9)	
C=O		172.20

[a–b] Assignments with the same superscripts may be interchanged.

Table 3. ^{1}H and ^{13}C NMR Assignments for Delavayin C (**3**) in Pyridine-d_5

position	$^\delta$H [int, mult, *J* (Hz)]	$^\delta$C
Gly[1]		
α	3.70 (1H, dd, 4.6, 17.1)	43.71
	4.74 (1H, dd, 7.8, 17.1)	
NH	9.84 (1H, dd, 4.6, 7.7)	
C=O		169.05
Tyr[2]		
α	5.56 (1H, br dd, 9.1, 9.2)	56.29
β	2.84 (1H, dd, 4.0, 13.5)	39.30
	3.41 (1H, m)	
γ		128.28[a]
δ	7.58 (2H, d, 8.4)	131.01
ϵ	7.17 (2H, d, 8.4)	116.66
ζ		158.01
NH	8.62 (1H, d, 9.8)	
C=O		170.79
Tyr[3]		
α	4.97 (1H, m)	56.73
β	3.36 (1H, dd, 7.2, 13.7)	38.24
	3.18 (1H, m)	
γ		128.16[a]
δ	7.33 (2H, d, 8.4)	131.65
ϵ	7.08 (2H, d, 8.4)	116.10
ζ		157.68
NH	7.79 (1H, d, 6.1)	
C=O		172.55
Tyr[4]		
α	4.48 (1H, br dd, 4.0, 11.7)	55.50
β	2.85 (1H, dd, 4.5, 12.5)	37.31
	3.16 (1H, m)	
γ		125.96
δ	7.04 (2H, d, 8.4)	131.10
ϵ	6.90 (2H, d, 8.4)	116.44
ζ		158.25
NH	10.06 (1H, br d, 4.9)	
C=O		170.14
Pro[5]		
α	3.86 (1H, d, 7.5)	61.71
β	1.23 (1H, m)	30.82
	2.52 (1H, m)	
γ	1.56 (2H, m)	22.49
δ	3.65 (1H, m)	46.64
	3.75 (1H, m)	
C=O		171.46
Val[6]		
α	4.65 (1H, dd, 8.5, 10.7)	58.54
β	3.05 (1H, m)	30.65
γ	1.16 (3H, d, 6.5)	19.62
	1.32 (3H, d, 6.6)	19.31
NH	10.07 (1H, br s)	
C=O		171.76
Pro[7]		
α	4.42 (1H, t, 7.4)	61.71
β	1.99 (2H, m)	29.87
γ	1.66 (1H, m)	25.59
	1.95 (1H, m)	
δ	3.78 (1H, m)	48.85
	4.85 (1H, m)	
C=O		173.16

[a] Assignments with the same superscript may be interchanged.

^{13}C-signals based on five amide protons and seven amide carbonyl carbons were observed, indicating the heptapeptide nature of **3** with two Pro residues. The peptide sequence was determined by 2D NMR analyses including of ^{1}H−^{1}H COSY, HOHAHA, HMQC, HMBC, and ROESY as follows. Individual ^{1}H- and ^{13}C-NMR assignments of the above seven amino acids were conducted by combination of ^{1}H−^{1}H COSY, HOHAHA, and HMQC spectra. Two segments, Pro-Gly-Tyr-Tyr-Tyr and Pro-Val were assigned by two-bond ^{1}H−^{13}C correlations of NH/CO, and Hα/CO (Figure 3). Two structural units analyzed by the HMBC correlations could be linked by ROE enhancements between Tyr[4]-Hα and Pro[5]-Hα, and between Val[6]-Hα and Pro[7]-H*δ* in a phase-sensitive ROESY spectrum. The whole structure of **1** was determined to be *cyclo* (-Gly-Tyr-Tyr-Tyr-Pro-Val-Pro-). A through-space interaction between Tyr[4]-Hα and Pro[5]-Hα is diagnostic of a *cis*-peptide bond between Tyr[4] and Pro[5], which is also supported by the ^{13}C-NMR resonances (*δ* 30.82 and 22.49) of *β* and *γ* in Pro,[5,20] and the occurrence of a doublet signal of Hα in Pro[5] has also been correlated in the *cis*-peptide bond.[21]

An investigation of the delavayins in biological assays is ongoing.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a JASCO DIP-4 polarimeter. The IR spectra (KBr) were obtained on a Perkin-Elmer 1710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were run in pyridine-d_5 or DMSO-d_6 using a Bruker AM-500 and Varian Unity 400 instruments, with chemical shifts (*δ*) reported in ppm. The spectra were recorded at 303 °K. Phase-sensitive ROESY NMR

experiments were acquired with mixing times of 100 ms. The value of the delay to optimize one-bond correlations in the HMQC spectrum and suppress them in the HMBC spectrum was 3.2 Hz, and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 ms. MS were recorded on a VG Autospec instrument. HPLC was performed on an Inertsil PREP-ODS packed with 10 μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck), and the spots were detected by spraying with Dragendorff's reagent.

Plant Material. The roots of *S. delavayi* were collected in Kun Ming, Yunnan, People's Republic of China, in August 1995. The botanical identification was made by Dr. Zhi-Sheng Qiao, Department of Pharmacognosy, College of Pharmacy, Second Military Medical University, Shanghai, China. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy & Life Science.

Extraction and Isolation. The roots of *S. delavayi* (10.0 kg) were extracted with hot MeOH four times to give a MeOH extract (1.217 kg) that was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction (179.1 g) was subjected to Diaion HP-20 column chromatography using a H_2O−MeOH gradient system (1:0−0:1). The fraction eluted with 80% MeOH was further subjected to Si gel column chromatography using a $CHCl_3$−MeOH gradient system (1:0−0:1). The fraction eluted with 10% MeOH was subjected to ODS HPLC with a 28% CH_3CN solvent system to give delavayin A (**1**, 11.0 mg) and delavayin C (**3**, 4 mg). The fraction eluted with 20% MeOH was subjected to ODS HPLC with a 55% MeOH solvent system to give delavayin B (**2**, 35.0 mg).

Delavayin A (1): colorless powder; $[\alpha]_D$ +17.2° (*c* 0.19, MeOH); IR (KBr) ν max 3427 and 1650 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; FABMS *m/z* [M + Na]$^+$ 661; HRFABMS *m/z* found 639.3168, calcd for $C_{32}H_{43}N_6O_8$ 639.3142.

Delavayin B (2): colorless powder; $[\alpha]_D$ +6.0° (*c* 0.20, MeOH); IR (KBr) ν max 3397 and 1670 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 2; FABMS *m/z* [M + Na]$^+$ 645; HRFABMS *m/z* found 645.3036, calcd for $C_{32}H_{42}N_6O_7Na$ 645.3013.

Delavayin C (3): colorless powder; $[\alpha]_D$ −52.4° (*c* 0.08, MeOH); IR (KBr) ν max 3426 and 1634 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 3; FABMS *m/z* [M + Na]$^+$ 862; HRFABMS *m/z* found 840.3955, calcd for $C_{44}H_{54}N_7O_{10}$ 840.3932; UV (MeOH) λ max 278 (ϵ 4027).

Absolute Configuration of Amino Acids. Each solution of **1**−**3** (1 mg) in 6 N HCl was heated at 110 °C for 12 h. The solution was concentrated to dryness. The residue was dissolved in H_2O and treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) and 1 M $NaHCO_3$ at 35 °C for 1 h. After cooling, 2 M HCl was added and then concentrated to dryness. This residue was subjected to HPLC [Lichrospher 100, RP-18 (10 μm), Merck], flow rate 1 mL/min, detection 340 nm, solvent 10−50% CH_3CN−50 mM triethylamine phosphate (TEAP) buffer. The t_R values (min) were L-Ser 19.69, L-Ala 25.63, L-Pro 28.66, L-Tyr 32.09, L-Phe 40.02, and L-Ile 40.63, respectively.

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