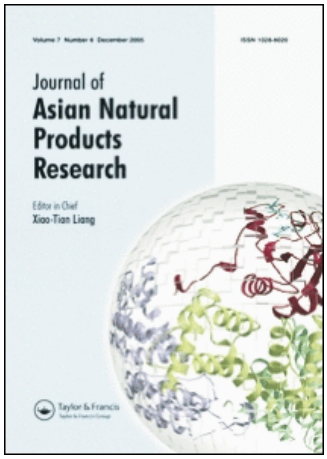

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New cyclopeptides in *Arenaria oreophila* (Caryophyllaceae)

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Two new cyclopeptides, arenariphilin G (**1**) and arenariphilin H (**2**), were isolated from the whole plants of *Arenaria oreophila* Hook. Their structures were determined as cyclo(Pro-Ile-Ser^{2}-Gly-Ala^{1}-Ala^{2}-Val-Ser^{1}) (**1**) and cyclo(Pro-Gly- Phe-Ser-Leu) (**2**), respectively, by detailed spectroscopic analysis.

Keywords: *Arenaria oreophila*; Caryophyllaceae; Cyclopeptide; Arenariphilin G; Arenariphilin H

1. Introduction

Arenaria oreophila Hook belongs to the family Caryophyllaceae, many species of which are Chinese folk medicines, such as *A. serpyllifolie* L., *A. prezewalskii*, *A. melanadra* [1], and grows as a perennial herb in Yunnan, Sichuan, Tibet and Qinghai provinces. The components of only a few species of genus *Arenaria* such as *A. kansuensis* have been reported containing the terpenoids, flavonoids, and carboline alkaloids [2,3], and one cyclopeptide from *A. juncea* [4]. As a series of investigation of cyclopeptides in this species [5], two new cyclopeptides, named arenariphilin G (**1**) and arenariphilin H (**2**) (figure 1), were further isolated. This paper describes the isolation and structural elucidation of the two new compounds.

2. Results and discussion

Arenariphilin G (**1**) was white amorphous, and showed as negative to ninhydrin reagent but positive after hydrolysis with 6 mol/L HCl [6]. Its IR bands at 3427 and 1642 cm^{-1} were characteristic of amino and amide carbonyl groups. The FAB-MS gave a quasi-molecular ion peak $[M + 1]^+$ at *m*/*z* 683, and the molecular formula $C_{30}H_{50}N_8O_{10}$ was derived from the HRFAB-MS $[M]^+$ at *m*/*z* 682.3668, indicating the presence of 10 degrees of unsaturation.

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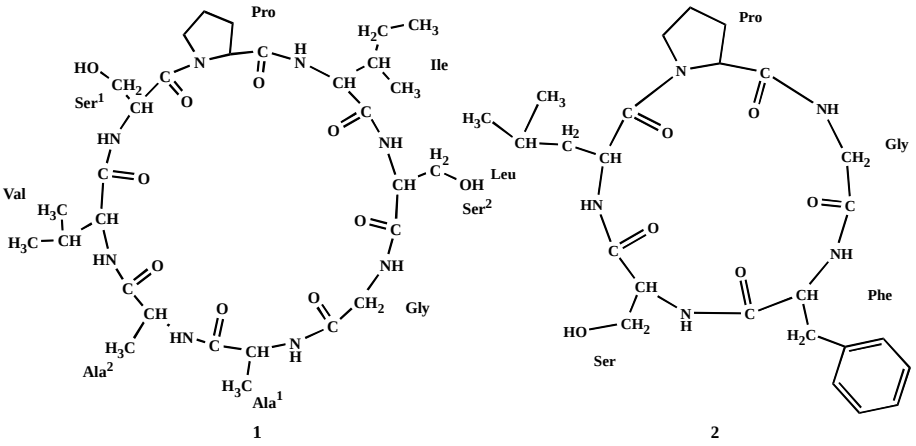


Figure 1. Structures of arenariphilin G (**1**) and arenariphilin H (**2**).

The ^{13}C NMR and ^{1}H NMR spectra (table 1) showed the presence of eight amide carbonyl signals and seven amide NH signals, respectively, suggesting that **1** was a cyclopeptide.

The structure elucidation began with identification of the amino acid residues. By extensive analysis of the $^{1}H-^{1}H$ COSY, HMQC, TOCSY and HMBC spectra, the amino acid residues were shown to be one proline, one isoleucine, one valine, two serines, one glycine, and two alanines respectively. These amino acid residues accounted for the molecular weight observed in the FAB-MS. The sequence of these amino acid residues was elucidated on the basis of HMBC correlations. The HMBC correlations are summarized in figure 2. From the information obtained in the FAB-MS and the correlations shown in figure 2, the structure of **1** was elucidated as cyclo(Gly-Ala^{1}-Ala^{2}-Val-Ser^{1}- Pro-Ile-Ser^{2}) (**1**). In addition, FAB-MS also showed some important ion peaks as follows:

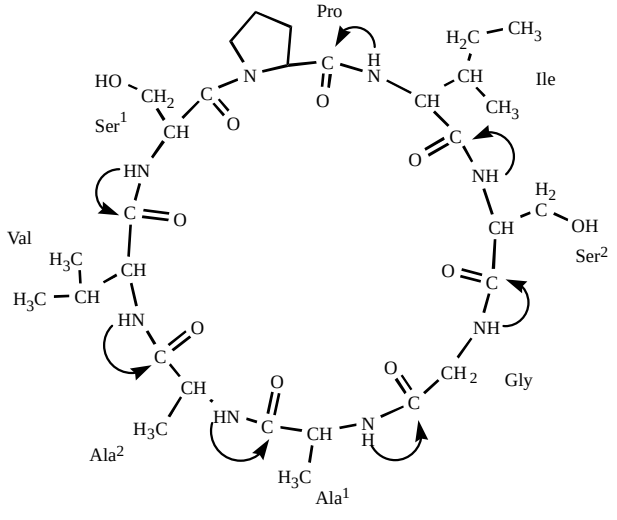


Figure 2. The key HMBC correlations of **1**.

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of **1** (pyridine-d_5).

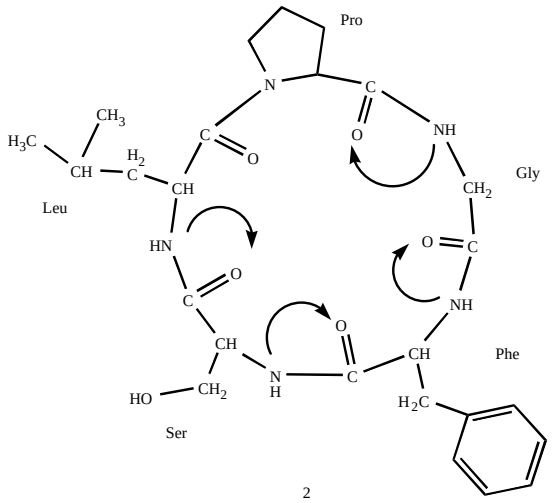
		$\delta^{1}H$ (ppm) J in Hz mult.	$\delta\ ^{13}C$ (ppm) mult.
Gly	CO		171.7 (*s*)
	NH	8.77 (*br. s*, 1H)	
	CH_2 (α)	4.18 (*m*, 1H),	41.7 (*t*)
		3.73 (*m*, 1H)	
Ala[1]	CO		172.4 (*s*)
	NH	10.63 (*br. s*, 1H)	
	CH (α)	5.18 (*m*, 1H)	51.6 (*d*)
	CH_3 (β)	1.10 (*m*, 3H)	18.6 (*q*)
Ala[2]	CO		170.3 (*s*)
	NH	9.86 (*br. s*, 1H)	
	CH (α)	5.07 (*m*, 1H)	50.8 (*d*)
	CH_3 (β)	1.09 (*m*, 3H	17.3 (*q*)
Val	CO		174.4 (*s*)
	NH	8.97 (*d*, $J = 8.80$, 1H)	
	CH (α)	5.16 (*m*, 1H)	60.9 (*d*)
	CH (β)	2.39 (*m*, 1H)	31.5 (*d*)
	CH_3 (γ)	1.01 (*m*, 3H)	20.4 (*q*)
	CH_3 (γ)	1.10 (*m*, 3H)	19.1 (*q*)
Ser[1]	CO		172.8 (*s*)
	NH	9.37 (*br. s*, 1H)	
	CH (α)	4.49 (*m*, 1H)	53.6(*d*)
	CH_2 (β)	4.67(*m*, 1H),	63.7 (*t*)
		4.41 (*m*, 1H)	
Pro	CO		173.5 (*s*)
	CH (α)	5.13 (*m*, 1H)	61.6 (*d*)
	CH_2 (β)	2.57 (*br. s*, 1H),	30.5 (*t*)
		2.40 (*m*, 1H)	
	CH_2 (γ)	2.11 (*m*, 1H),	23.9 (*t*)
		2.07 (*m*, 1H)	
	CH_2 (δ)	4.59 (*m*, 1H),	48.0 (*t*)
		4.18 (*m*, 1H)	
Ile	CO		172.1 (*s*)
	NH	8.35 (*d*, $J = 9.04$, 1H)	
	CH (α)	5.08 (*m*, 1H)	56.4 (*d*)
	CH (β)	2.11 (*br. s*, 1H)	37.7 (*d*)
	CH_2 (γ)	1.28 (*m*, 1H),	23.5 (*t*)
		1.22 (*m*, 1H)	
	CH_3 (γ)	1.24 (*m*, 3H)	16.0 (*q*)
	CH_3 (δ)	0.97 (*m*, 3H)	10.7 (*q*)
Ser[2]	CO		171.2 (*s*)
	NH	8.43 (*br. s*, 1H)	
	CH (α)	4.74 (*m*, 1H)	55.4 (*d*)
	CH_2 (β)	4.70 (*m*, 1H),	65.5 (*t*)
		4.22 (*m*, 1H)	

I. *m/z* 683[Gly-Ala[1]-Ala[2]-Val-Ser[1]-Pro-Ile-Ser[2] + H]$^+$
II. *m/z* 626[Ala[1]-Ala[2]-Val-Ser[1]-Pro-Ile-Ser[2] + H]$^+$
III. *m/z* 484[Val-Ser[1]- Pro-Ile-Ser[2] + H]$^+$
IV. *m/z* 297[Pro-Ile-Ser[2]]$^+$
V. *m/z* 211[Pro-Ile + H]$^+$

Arenariphilin H (**2**) was white amorphous, and showed as negative to ninhydrin reagent but positive after hydrolysis with 6 mol/L HCl [6]. Its IR bands at 3446, and 1638 cm^{-1} were characteristic of amino and amide carbonyl groups. The FAB-MS gave a quasi-molecular ion peak $[M + 1]^+$ at *m/z* 502, and the molecular formula $C_{25}H_{35}N_5O_6$ was derived from the HRFAB-MS $[M]^+$ at *m/z* 501.2619, indicating the presence of 11 degrees of unsaturation.

Table 2. ^{1}H NMR and ^{13}C NMR spectral data of **2** (pyridine-d_5).

		δ^{1}H (ppm) J in Hz mult.	*δ ^{13}C (ppm) mult.*
Pro	CO		171.6 (*s*)
	CH (α)	4.50 (*m*, 1H)	60.6 (*d*)
	CH_2 (β)	2.01 (*m*, 1H),	30.1 (*t*)
		1.86 (*m*, 1H)	
	CH_2 (γ)	1.99 (*m*, 1H),	24.8 (*t*)
		1.59 (*m*, 1H)	
	CH_2 (δ)	4.07 (*m*, 1H),	47.5 (*t*)
		3.70(*m*, 1H)	
Gly	CO		172.0 (*s*)
	NH	10.19 (*br. s*, 1H)	
	CH_2 (α)	4.75 (*m*, 1H),	44.0 (*t*)
		3.93 (*m*, 1H)	
Phe	CO		173.2 (*s*)
	NH	9.10 (*d*, $J = 7.80$, 1H)	
	CH (α)	4.96 (*m*, 1H)	55.4 (*d*)
	CH_2 (β)	3.75 (*m*, 1H)	37.1 (*t*)
		3.36 (*m*, 1H)	
	ArH (δ)	7.00–7.50 (*m*, 5H)	1′ 138.9 (*s*),
			3′,5′ 130.7 (*d*),
			2′,6′ 128.7 (*d*),
			4′ 126.9 (*d*)
Ser	CO		170.0 (*s*)
	NH	9.75 (*br. s*, 1H)	
	CH (α)	4.71 (*m*, 1H)	50.8 (*d*)
	CH_2 (β)	4.48 (*m*, 1H),	61.8 (*t*)
		4.41 (*m*, 1H)	
Leu	CO		171.2 (*s*)
	NH	8.62 (*d*, $J = 6.04$, 1H)	
	CH (α)	4.52 (*m*, 1H)	52.7 (*d*)
	CH_2 (β)	2.01 (*m*, 1H),	41.0 (*t*)
		1.46 (*m*, 1H)	
	CH (γ)	1.30 (*m*, 1H)	25.4 (*d*)
	CH_3 (δ)	0.75 (*d*, $J = 6.80$, 3H)	23.2 (*q*)
	CH_3 (δ′)	0.70 (*d*, $J = 6.80$, 3H)	21.5 (*q*)



2

Figure 3. The key HMBC correlations of **2**.

The ^{13}C NMR and ^{1}H NMR spectra (table 2) showed the presence of five amide carbonyl signals and four amide NH signals respectively, suggesting that **2** was a cyclopeptide.

The structure elucidation began with identification of the amino acid residues. By extensive analysis of the $^{1}H-^{1}H$ COSY, HMQC, TOCSY and HMBC spectra, the amino acid residues were shown to be one proline, one glycine, one phenylalanine, one serine, and one leucine, respectively. These amino acid residues accounted for the molecular weight observed in FAB-MS. The sequence of these amino acid residues was elucidated on the basis of HMBC correlations. The key HMBC correlations are summarized in figure 3. From the information obtained in the FAB-MS and the correlations shown in figure 3, the structure of **2** was elucidated as cyclo(Pro-Gly-Phe-Ser-Leu) (**2**). In addition, FAB-MS also showed some important ion peaks as follows:

I. *m/z* 502[Pro-Gly-Phe-Ser-Leu + H]$^{+}$
II. *m/z* 445[Phe-Ser-Leu-Pro + H]$^{+}$
III. *m/z* 298[Ser-Leu-Pro + H]$^{+}$

3. Experimental

3.1 General experimental procedures

Optical rotation values were recorded on a Jasco 20C polarimeter; ^{1}H NMR (400 and 500 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Chemical shifts (δ ppm) are relative to internal $SiMe_4$, coupling constants (J) are in Hz; ^{1}H NMR and ^{13}C NMR assignments were supported by $^{1}H-^{1}H$ COSY, HMQC, HMBC, and TOCSY experiments; The FAB-MS spectra were recorded on a VG-Auto-Spec-3000 mass spectrometer; IR spectra were recorded on a Bio-Rad FTS-135 spectrometer λ_{max} (cm^{-1}); Commercial silica gel plates (Qingdao Haiyang Chemical Group Co.) were used for TLC; 200–300 mesh, 300–400 mesh silica gel (Qingdao Haiyang Chemical Group Co.), and Diaion HP-20 (Pharmacia Chemical Co.) were used for column chromatography.

3.2 Plant material

The whole plants of *Arenaria oreophila* were collected in Deqing county of Yunnan province, China, in September 2001, and identified by Professor Zhe Kun Zhou. A voucher specimen is preserved in the Herbarium of Kunming Institute of Botany, *The Chinese Academy of Sciences*.

3.3 Extraction and isolation

The dried whole plants of *Arenaria oreophila* (26.0 kg) were extracted 3 times with 95% EtOH under reflux (3 × 1000 L) for 4, 2 and 1 h, respectively. After evaporation of the combined extracts, the residue was suspended in H_2O and then extracted with petroleum ether (60–90°C), EtOAc, and BuOH. The EtOAc extract (700.0 g) was decoloured on Diaion HP 20 eluting with a gradient H_2O/MeOH 0:1 → 1:0. The 70% MeOH elute (200.0 g) was subsequently subjected to column chromatography (silica gel, $CHCl_3$/MeOH 50:1 → 5:1),

and purified with column chromatography to give arenariphilin G (**1**, 6.9 mg), and arenariphilin H (**2**, 10.5 mg), respectively.

3.3.1 Arenariphilin G (1). $C_{30}H_{50}N_8O_{10}$; amorphous white powder; $[\alpha]_D^{26.7} = 0$ (*c* 0.070, MeOH); UV λ_{max}^{MeOH} nm (log ε): 203 (1.53); IR: 3427, 1642 cm^{-1}; ^1H NMR and ^{13}C NMR: table 1; FAB-MS *m/z*: 683(M + 1, 90), 626, 484, 406, 297, 86 (100); HRFAB-MS $[M]^+$*m/z*: 682.3668 (calcd for $C_{30}H_{50}N_8O_{10}$, 682.3647).

3.3.2 Arenariphilin H (2). $C_{25}H_{35}N_5O_6$; amorphous white powder; $[\alpha]_D^{26.7} = -49.28$ (*c* 0.115, MeOH); UV λ_{max}^{MeOH} nm (log ε): 209 (2.17), 259; IR: 3446, 2926, 1638, 1517; ^1H NMR and ^{13}C NMR: table 2; FAB-MS *m/z*: 502 ($[M + 1]^+$, 55), 445, 298, 113, 98 (100); HRFAB-MS $[M]^+$*m/z*: 501.2619 (calcd for $C_{40}H_{66}N_{10}O_{11}$, 501.2585).

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