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New Minor Cyclopeptides from *Arenaria oreophila* (Caryophyllaceae)

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Abstract: New minor cyclic peptides, named arenariphilin C **1** and arenariphilin D **2**, were isolated from the whole plants of *Arenaria oreophila* (Caryophyllaceae). Their structures were determined as cyclo(Pro[1]-Pro[2]-Leu[1]-Leu[2]-Phe-Ser-Gly-Thr) and cyclo (Ser-Cys) on the basis of spectroscopic data, especially by two-dimension NMR (2D NMR) technologies.

Keywords: *Arenaria oreophila*, Caryophyllaceae, cyclopeptide, arenariphilin C, arenariphilin D.

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, Sichun, Tibet and Qinghai Province. As a series investigation of cyclopeptides in this species[2], two new cyclopeptides named arenariphilin C **1** and arenariphilin D **2** were further isolated.

The dried whole plants of *A. oreophila* (26.0 kg) were extracted 3 times with 95% EtOH under reflux (3×100 L) for 4, 2 and 1 h, respectively. After concentration of the combined extracts, the residue was suspended in H_2O and then extracted with petroleum ether (60-90℃), AcOEt, and BuOH. The AcOEt extract (700.0 g) was decolored 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 CC (silica gel, $CHCl_3$/MeOH 50:1→5:1), and resubmitted to CC (silica gel, $CHCl_3$/MeOH 20:1→9:1) to give arenariphilin C **1**, (9.6 mg, 0.000037 %) and arenariphilin D **2**, (16.4 mg, 0.000063 %), respectively.

Arenariphilin C **1**, white amorphous, $[\alpha]_D^{25.9}$ -45(c 0.200, MeOH), UV λ_{max}^{MeOH} nm (logε): 207 (4.17), was negative to ninhydrin reagent but positive after hydrolysis with 6 mol/L HCl[3]. The molecular formula $C_{40}H_{60}N_8O_{10}$ was derived from the HRFAB$^+$MS ($[M]^+$ at *m/z* 812.4437, calcd. 812.4432). IR (KBr) bands at 3428, 1716, 1635 cm^{-1} were characteristic of amino and amide carbonyl groups, respectively. The ^{13}C NMR and ^1H NMR spectra (**Table 1**) exhibited the presence of eight amide carbonyl signals, seven methine signals and six amide NH signals, respectively.

The above facts suggested **1** was a cyclopeptides. Using ^1H-^1H COSY, TOCSY, HMQC and HMBC spectra, the amino acid residues were identified as two prolines, two leucines, one phenylalanine, one serine, one glycine, and one threonine, respectively.

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Figure 1 Structure of **1** (H→C HMBC; H↔H REOSY)

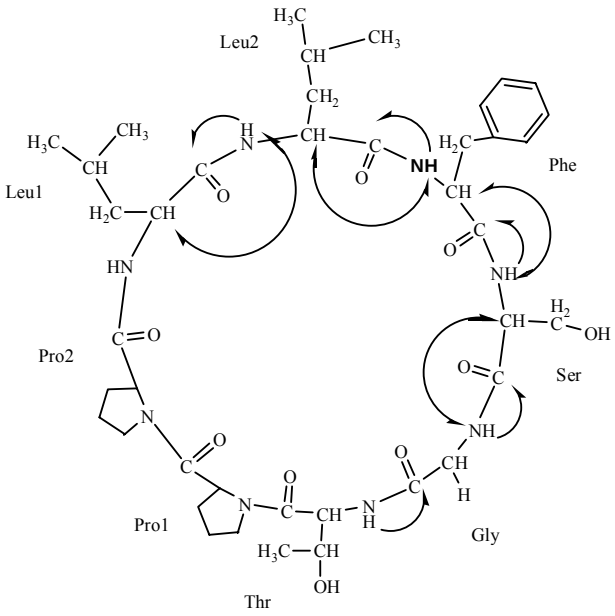
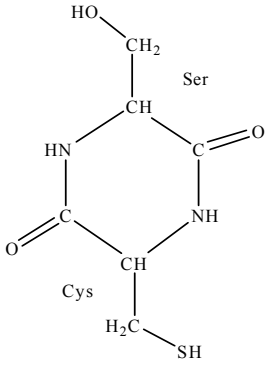


Figure 2 Structure of arenariphilin D **2**



The sequence of these amino acid residues was determined by HMBC and NOESY as summarized in **Figure 1**. By analysis correlations between each amide proton (NH) and carbonyl carbon in HMBC, and the correlations of amino acid residue α-H or β-H with the amide proton (NH) of the next amino acid residue in REOSY, one peptide residue was found to be -N-Leu^1-Leu^2-Phe-Ser-Gly-Thr-CO-. Besides, this peptide contained two prolines. In this case, only one linkage is reasonable. Hence the structure of **1** was established as cyclo(Pro^1-Pro^2-Leu^1-Leu^2-Phe-Ser-Gly-Thr).

Arenariphilin D **2**, white amorphous, $[\alpha]_D^{18.7}$ -111.11 (c 0.84, MeOH), UV λ_{max}^{MeOH} nm (logε): 205 (2.01), was negative to ninhydrin reagent but positive after hydrolysis with 6 mol/L HCl[3]. The molecular formula $C_6H_{10}N_2O_3S$ was derived from the HRFAB$^+$MS ($[M]^+$ at *m/z* 190.0406, calcd. 190.0411). Bands at 3427, 1641 cm^{-1} in IR (KBr) were

characteristic of amino and amide carbonyl groups, respectively. The ^{13}C NMR and ^{1}H NMR spectra (**Table 2**) exhibited the presence of two amide carbonyl signals, two methine signals and two amide NH signals, respectively. The above results suggested **2** was a cyclopeptides. The amino acid residues were identified by ^{1}H NMR and ^{13}C NMR spectra as one cysteine and one serine. In this case, the structure of **2** was established as cyclo (Ser-Cys).

Table 1 ^{1}H and ^{13}C NMR spectral data of **1** (500MHz, δ ppm, J Hz, pyridine-d_5)

No.	δ_H	δ_C	No.	δ_H	δ_C
1. Pro-1			5. Phe		
CO		171.56 (s)	CO		176.86 (s)
CH (α)	4.87(m, 1H)	63.03(d)	NH	8.25 (br s, 1H)	
CH_2 (β)	2.85(m, 1H), 2.58(m, 1H)	30.86 (t)	CH (α)	4.71(m, 1H)	56.90 (d)
CH_2 (γ)	1.71 (m, 1H),1.61(m, 1H)	24.27 (t)	CH_2 (β)	3.35 (m, 1H),	35.00 (t)
CH_2 (δ)	3.97(br s, 1H),	48.82(t)		2.97(m, 1H)	
	3.55(br s, 1H)		ArH (δ)	7.01-7.80(m, 5H)	1' 139.38(s),
2. Pro-2					2', 6' 129.38(d),
CO		171.11 (s)			3',5' 128.78(d),
CH (α)	4.97 (m, 1H)	60.32(d)			4' 126.71(d)
CH_2 (β)	2.31(m, 1H), 2.04(m, 1H)	30.51 (t)	6. Ser		
CH_2 (γ)	1.77(m, 1H), 1.61(m, 1H)	23.39 (t)	CO		172.00 (s)
CH_2 (δ)	3.85(br s, 1H),	48.33 (t)	NH	8.46 (overlapped,	
	3.64(br s, 1H)			1, H)	
3. Leu-1			CH (α)	4.54 (br s, 1H)	56.00 (d)
CO		173.83 (s)	CH_2 (β)	3.88 (br s, 1H),	63.28 (t)
NH	9.44 (br s, 1H)			3.64 (br s, 1H)	
CH (α)	4.29 (br. s, 1H)	52.16(d)	7. Gly		
CH_2 (β)	2.33 (m, 2H),	41.12 (t)	CO		170.50(s)
	2.04(br s, 1H)		NH	9.63(d, J=7.25,	43.92(t)
CH (γ)	1.61(br s, 1H)	26.70(d)	CH_2 (α)	1H)	
CH_3 (δ)	1.53 (d, 3H, J=8.50)	23.24(q)		4.83(m, 1H),	
CH_3(δ')	0.63(d, 3H, J=6.90)	20.47(q)		3.83(br s, 1H)	
4. Leu-2			8. Thr		
CO		170.88 (s)	CO		172.29(s)
NH	8.92 (br s, 1H)		NH	7.99(d, J=11.94,	59.12(d)
CH (α)	4.54(br s, 1H)	50.63 (d)		1H)	
CH_2 (β)	2.23 (m, 1H),	39.13 (t)	H (α)	5.08(br s, 1H)	
	2.04 (br s, 1H)		CCH (β)	4.89(m, 1H)	69.68(d)
CH (γ)	1.71 (br s, 2H)	25.63 (d)	CH_3 (γ)	1.34(br s, 3H)	21.21(q)
CH_3 (δ)	1.27 (d, 3H, J=7.85)	23.14 (q)			
CH_3(δ')	0.79 (d, 3H, J=7.25)	19.54 (q)			

Table 2 ^{1}H and ^{13}C NMR spectral data of **2** (400MHz, δ ppm, J Hz, pyridine-d_5)

No.	δ_H	δ_C	No.	δ_H	δ_C
1. Ser			2. Cys		
CO		176.00 (s)*	CO		170.81 (s)*
NH	8.60 (d, J=9.08, 1H)		NH	7.64(br. s,1H)	
CH (α)	4.62 (br. s, 1H)	53.66 (d)	CH (α)	8.52 (br. s, 1H)	50.34 (d)
CH_2 (β)	4.44 (m, 1H)	65.69 (t)	CH_2 (β)	4.34 (br. s, 1H)	62.68(t)
	4.28(br. s, 1H)			4.28 (br. s, 1H)	

* The chemical shifts of ketones between Cys and Ser may be exchanged.

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