Two New Cyclopeptides from *Arenaria oreophila* (Caryophyllaceae)

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Two new cyclopeptides, named arenariphilin A (**1**) and arenariphilin B (**2**), were isolated from the whole plants of *Arenaria oreophila*. Their structures were determined as cyclo-(Thr-Gly) (**1**) and cyclo-(Ser_1-Gly -Ser_2-Ile -Phe_1-Phe_2) (**2**) on the basis of spectral data, especially by 2D-NMR.

1. Introduction. – *Arenaria oreophila* HOOK. *f.* (Caryophyllaceae) is a perennial herb, distributed in Yunnan, Sichuan, Tibet, and Qinghai Province of West China, chemical studies of which have not been reported. Some of *Arenaria* plants such as *A. serpyllifolie*, *A. prezewalskii*, *A. melanadra* [1] have been used as Chinese folk drugs. Only a few *Arenaria* plants have been chemically investigated leading to the isolation of terpenoids, flavonoids, and carboline alkaloids from *A. kansuensis* [2][3], and one cyclopeptide from *A. juncea* [4]. During our cyclopeptide investigations on Caryophyllaceae plants [5–11], two new cyclopeptides, named arenariphilin A (**1**) and arenariphilin B (**2**), were isolated from the whole plants of *A. oreophila*. Here, we describe their structural elucidation.

2. Results and Discussion. – Arenariphilin A (**1**) was isolated as a white amorphous powder; it tested negative to ninhydrin reagent but positive after hydrolysis with concentrated HCl, indicating that **1** might belong to cyclopeptides [5]. The molecular formula $C_6H_{10}N_2O_3$ was deduced from the HR-FAB-MS (M^+ at *m/z* 158.0682; calc. 158.0691), indicating three degrees of unsaturation. IR Bands at 3442 and 1683 cm^{-1} were characteristic of NH, OH, and CO groups. Data mentioned above indicated that **1** might belong to cyclodipeptides. The ^{13}C- and ^1H-NMR spectra (*Table 1*) showed the presence of two CO, two CH, one Me, and two NH signals, indicating that **1** was composed of two amino acid residues. The amino acid residues were identified by ^1H- and ^{13}C-NMR spectra as one glycine (Gly) and one threonine (Thr). Therefore, the structure of **1** was established as cyclo-(Thr-Gly).

Arenariphilin B (**2**) was also isolated as a white amorphous powder; it tested negative to ninhydrin reagent but positive after hydrolysis with concentrated HCl, indicating that **2** might also belong to cyclopeptides [5]. The molecular formula $C_{32}H_{42}N_6O_8$ was deduced from the HR-FAB-MS ($[M+H]^+$ at *m/z* 639.3164; calc. 639.3142), indicating 15 degrees of unsaturation. IR Bands at 3418 and 1652 cm^{-1} were characteristic of NH, OH, and CO groups. These data indicated that **2** might belong to cyclohexapeptides. The ^{13}C- and ^1H-NMR spectra (*Table 2*) showed the presence of six CO, six CH, six CH_2, two Me, and six NH signals, indicating that **2** was composed of six

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** ((D$_5$)pyridine; δ in ppm, J in Hz)

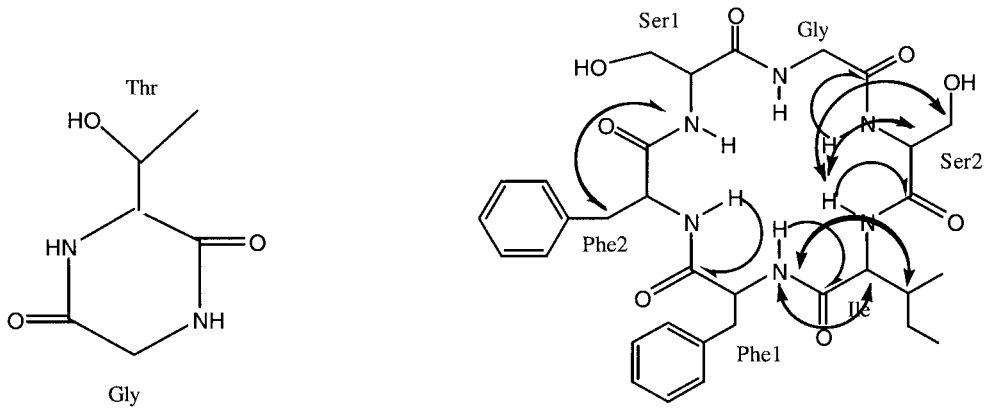
		^{1}H-NMR	^{13}C-NMR
Thr	CO		172.81 (*s*)[a]
	NH	7.58 (*d*, *J* = 7.64, 1 H)	
	CH(α)	4.52 (*d*, *J* = 8.12, 1 H)	58.80 (*d*)
	CH(β)	4.97 (*m*, 1 H)	69.30 (*d*)
	Me(γ)	1.51 (*d*, *J* = 6.32, 1 H)	24.83 (*q*)
Gly	CO		169.70 (*s*)[b]
	NH	8.51 (br. *s*, 1 H)	
	CH$_2$(α)	4.60 (*m*, 1 H)	42.80 (*t*)
		3.82 (*m*, 1 H)	

[a]) [b]) Signals may be interchanged

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2** ((D$_5$)pyridine; δ in ppm, J in Hz)

		^{1}H-NMR	^{13}C-NMR
Ser$_1$	CO		171.83 (*s*)
	NH	9.45 (br. *s*, 1 H)	
	CH(α)	4.84 (*m*, 1 H)	55.25 (*d*)
	CH$_2$(β)	4.50 (*m*, 1 H), 4.36 (*m*, 1 H)	61.49 (*t*)
Gly	CO		169.99 (*s*)
	NH	10.24 (br. *s*, 1 H)	
	CH$_2$(α)	4.88 (*m*, 1 H), 3.88 (*m*, 1 H)	43.63 (*t*)
Ser$_2$	CO		171.20 (*s*)
	NH	9.42 (*d*, *J* = 8.10, 1 H)	
	CH(α)	5.15 (*m*, 1 H)	55.42 (*d*)
	CH$_2$(β)	4.36 (*m*, 1 H), 3.93 (*m*, 1 H)	62.08 (*t*)
Ile	CO		172.37 (*s*)
	NH	8.59 (*d*, *J* = 7.70, 1 H)	
	CH(α)	4.71 (*t*, *J* = 7.70, 1 H)	59.38 (*d*)
	CH(β)	2.01 (*m*, 1 H)	36.49 (*d*)
	CH$_2$(γ)	1.40 (*m*, 1 H), 1.05 (*m*, 1 H)	24.90 (*t*)
	Me(γ')	0.70 (*d*, *J* = 6.80, 3 H)	15.34 (*q*)
	Me(δ)	0.65 (*t*, *J* = 7.25, 3 H)	10.82 (*q*)
Phe$_1$	CO		171.28 (*s*)
	NH	9.06 (*d*, *J* = 8.10, 1 H)	
	CH(α)	5.08 (*m*, 1 H)	56.65 (*d*)
	CH$_2$(β)	3.75 (*m*, 1 H), 3.31 (*t*, *J* = 12.6, 1 H)	37.47 (*t*)
	ArH(δ)	7.17–7.54 (*m*, 5 H)	137.74 (*s*), 129.35 (*d*), 128.40 (*d*), 126.59 (*d*)
Phe$_2$	CO		171.75 (*s*)
	NH	8.45 (*d*, *J* = 5.55, 1 H)	
	CH(α)	4.82 (*m*, 1 H)	58.28 (*d*)
	CH$_2$(β)	3.62 (*m*, 1 H), 3.52 (*m*, 1 H)	37.84 (*t*)
	ArH(δ)	7.17–7.54 (*m*, 5 H)	138.54 (*s*), 130.12 (*d*), 128.51 (*d*), 126.65 (*d*)

amino acid residues. The amino acid residues were identified by ^{1}H,^{1}H-COSY, TOCSY, HMQC, and HMBC spectra as two serines (Ser), two phenylalanines (Phe), one isoleucine (Ile) and one glycine (Gly). The sequence of these amino acid residues was achieved by HMBC and ROESY as depicted in the *Figure*. By analysis of HMBC



Arenariphilin A (**1**) Arenariphilin B (**2**)

Figure. *Structures of* **1** *and* **2** (→ : selected HMBC correlations; ↔ : selected REOSY correlations)

correlations between amino-acid-residue amide H-atom (NH_{i+1}) and carbonyl C-atom (CO_i), and by analysis of ROESY correlations between each amino acid residue α-H_{i+1} or β-H_{i+1} and amide H-atom (NH_i), two peptide residues are found to be -NH-Gly-Ser_2-Ile-Phe_1-Phe_2-CO- and -NH-Phe_2-Ser_1-CO-. In addition, FAB-MS also showed some important ion peaks as follows: 639 ([Phe-Ser-Gly-Ser-Ile-Phe + H]$^+$), 492 ([Phe-Ser-Gly-Ser-Ile + H]$^+$), 379 ([Phe-Ser-Gly-Ser + H]$^+$), 232 [Ser-Gly-Ser + H]$^+$), 145 [Ser-Gly + H]$^+$).

Therefore, the structure of **2** was established as cyclo-(Ser_1-Gly-Ser_2-Ile-Phe_1-Phe_2).

Experimental Part

General. Optical rotation: *Jasco 20C* polarimeter. UV Spectra: *UV 210A* spectrometer. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra: *Bruker AM-400* spectrometer; ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra: *Bruker AM-500* spectrometer; δ in ppm rel. to Me_4Si (= 0 ppm), *J* in Hz. MS: *VG-Auto-Spec-3000* mass spectrometer, *m/z* (rel. %).

Plant Material. The whole plants of *A. oreophila* were collected in Deqing county of Yunnan province (China) on September 2001. It was identified by Prof. *Z. K. Zhou*, and a voucher specimen was preserved in the Herbarium of Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and Isolation. 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, resp. After evaporation 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* with a gradient H_2O/MeOH 0:1 → 1:0. The 70%-MeOH eluate (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 A (**1**, 6.0 mg, 0.000023%) and arenariphilin B (**2**, 14.0 mg, 0.000054%).

Arenariphilin A (**1**). Amorphous powder. $[\alpha]_D^{25.6} = +3.33$ ($c = 0.15$, MeOH). UV: 257 (0.78), 205 (8.98). IR: 3442, 1739, 1683, 1652, 1213. ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS: 158 (100, M^+), 84 (14). HR-FAB-MS (pos.): 158.0682 ($C_6H_{10}N_2O_3^+$; calc. 158.0691).

Arenariphilin B (**2**). Amorphous powder. $[\alpha]_D^{25.4} = 0$ ($c = 0.10$, MeOH). UV: 205 (3.18). IR: 3418, 1652, 1539, 1521. ^{1}H- and ^{13}C-NMR: *Table 2*. FAB-MS (pos.): 639 (27, [M + H$^+$]), 492, 379, 232, 145, 120 (100). HR-FAB-MS (pos.): 639.3164 ([$C_{32}H_{43}N_6O_8$ + H]$^+$; calc. 639.3142).

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