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Cyclopeptidic Constituents from *Arenaria oreophila* J. D. Hooker (Caryophyllaceae)

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Abstract

To further investigate the cyclopeptides of the Caryophyllaceae family, two new cyclopeptides, named Arenariphilin E (compound 1) and Arenariphilin F (compound 2), were obtained from *Arenaria oreophila* J. D. Hooker using some isolation methods, e. g. normal and reverse silica gel. By detailed spectroscopic analysis, such as FAB$^+$-MS, 1D NMR, 2D NMR, the structures of Arenariphilin E (compound 1) and Arenariphilin F (compound 2) were determined as cyclo(Ile^1-Gly-Val^1-Ala-Leu-Ile^3-Ile^2-Val^2-Pro) and cyclo(Pro^2-Pro^1-Gly^2-Ile-Val-Leu-Gly^1-Ala-Thr- Gly^3), respectively.

Key words: *Arenaria oreophila*; Arenariphilin E; Arenariphilin F; Caryophyllaceae; Cyclopeptide.

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Arenaria oreophila J. D. Hooker belongs to the family Caryophyllaceae, many species of which are Chinese folk medicines, such as *A. serpyllifolie* Linnaeus, *A. przewalskii* Maximowicz, and *A. melandryoides* Edgeworth (Tang et al. 1996), and grows as a perennial herb in Yunnan, Sichun, Tibet and Qinghai Province. The components of only a few species of *Arenaria* such as *A. kansuensis* have been reported and the plants contain terpenoids, flavonoids and carboline alkaloids (Wu et al. 1989, 1990), as well as one cyclopeptide isolated from *A. juncea* (Zhao et al. 1994). As part of a series investigation of the cyclopeptides in this species (Jia et al. 2003), two new cyclopeptides named Arenariphilin E (compound **1**) and Arenariphilin F (compound **2**) have been isolated and are described herein.

Results and Discussion

Arenariphilin E (compound **1**) was obtained as a white amorphous, and was negative to ninhydrin reagent but was positive after hydrolysis with 6 mol/L HCl (Zhou and Tan 2000). The IR bands for Arenariphilin E at 3 431 and 1 651 cm^{-1} were characteristic of amino and amide carbonyl groups. The FAB$^+$-MS gave an $[M+2]^+$ ion at *m/z* 877, and the molecular formula $C_{44}H_{77}N_9O_9$ was derived from the HR-FAB$^+$-MS ($[M+2]^+$ at *m/z* 876.589 0, calc. for *m/z* 876.592 3), indicating the presence of 11 degrees of unsaturation. The ^{13}C-NMR and ^{1}H-NMR spectra (Table 1) showed the presence of nine amide carbonyl signals and eight amide NH signals respectively, suggesting that compound **1** was a cyclopeptide.

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 leucine, three isoleucines, two valines, one glycine, and one alanine, respectively (Jia et al. 2003). These amino acid residues accounted for the molecular weight observed in the FAB$^+$-MS. The sequence of these amino acid residues is elucidated on the basis of HMBC and REOSY correlations. The HMBC and REOSY correlations are summarized in Figure 1. From the information obtained in the FAB$^+$-MS and the correlations shown in Figure 1, the structure of compound **1** was elucidated as cyclo(Ile^1-Gly-Val^1-Ala-Leu-Ile^3-Ile^2-Val^2-Pro). In addition, FAB$^+$-MS also showed some important ion peaks as follows (Jia et al. 2003, 2004):

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

		^{1}H-NMR	^{13}C-NMR
Ile-1	CO		174.01 (s)
	NH	8.32 (d, *J* = 9.40, 1H)	
	CH (α)	4.87 (m, 1H)	60.16 (d)
	CH (β)	2.54 (d, *J* = 6.80, 1H)	36.61 (d)
	CH_2 (γ)	1.89 (m, 1H), 1.79 (m, 1H)	25.87 (t)
	CH_3 (γ')	1.16 (m, 3H)	16.56 (q)
	CH_3 (δ)	0.85 (overlapped, 3H)	11.80 (q)
Gly	CO		168.91 (s)
	NH	9.77 (overlapped, 1H)	
	CH_2 (α)	4.43 (dd, *J* = 3.85, 3.00, 1H)	42.97 (t)
		3.90 (br s, 1H)	
Val-1	CO		171.75 (s)
	NH	9.40 (br s, 1H)	
	CH (α)	4.22 (br s, 1H)	58.30 (d)
	CH (β)	2.74 (br s, 1H)	30.97 (d)
	CH_3 (γ)	1.30 (d, *J* = 6.45, 3H)	20.08 (q)
	CH_3 (γ')	1.10 (overlapped, 3H)	19.29 (q)
Ala	CO		172.52 (s)
	NH	8.85 (d, *J* = 6.45, 1H)	
	CH (α)	5.15 (br s, 1H)	50.46 (d)
	CH_3 (β)	1.76 (d, *J* = 6.40, 3H)	17.36 (q)
Leu	CO		173.74 (s)
	NH	9.25 (d, *J* = 8.10, 1H)	
	CH (α)	5.16 (m, 1H)	52.05 (d)
	CH_2 (β)	2.07 (t, *J* = 6.85, 6.40, 1H),	41.94 (t)
		1.89 (m, 1H)	
	CH (γ)	1.80 (br s, 1H)	25.32 (d)
	CH_3 (δ)	0.99 (overlapped, 3H)	23.12 (q)
	CH_3 (γ')	0.97 (overlapped, 3H)	22.92 (q)
Ile-3	CO		172.87 (s)
	NH	9.51 (br s, 1H)	
	CH (α)	5.00 (br s, 1H)	58.47 (d)
	CH (β)	2.36 (m, 1H)	34.96 (d)
	CH_2 (γ)	1.90 (m, 1H), 1.80 (m, 1H)	24.59 (t)
	CH_3 (γ')	1.14 (overlapped, 3H)	16.09 (q)
	CH_3 (δ)	0.86 (overlapped, 3H)	10.57 (q)
Ile-2	CO		173.74 (s)
	NH	8.53 (br s, 1H)	
	CH (α)	5.09 (br s, 1H)	59.78 (d)
	CH (β)	2.39 (m, 1H)	38.14 (d)
	CH_2 (γ)	1.93 (m, 1H), 1.80 (m, 1H)	25.00 (t)
	CH_3 (γ')	1.13 (overlapped, 3H)	16.24 (q)
	CH_3 (δ)	0.88 (overlapped, 3H)	10.82 (q)
Val-2	CO		172.16 (s)
	NH	9.80 (br s, 1H)	
	CH (α)	4.85 (m, 1H)	58.30 (d)
	CH (β)	2.33 (m, 1H)	30.70 (d)
	CH_3 (γ)	1.24 (overlapped, 3H)	19.90 (q)
	CH_3 (γ')	1.09 (overlapped, 3H)	19.62 (q)
Pro	CO		172.39 (s)
	CH (α)	4.89 (m, 1H)	61.30 (d)
	CH_2 (β)	2.36 (m, 1H), 1.93 (m, 1H)	30.03 (t)
	CH_2 (γ)	1.35 (m, 2H)	26.33 (t)
	CH_2 (δ)	4.03 (br s, 1H),	47.19 (t)
		3.49 (d, *J* = 8.55, 1H)	

m/z 877 [Ile^1-Gly-Val^1-Ala-Leu-Ile^3-Ile^2-Val^2-Pro+2H]$^+$;
m/z 763 [Gly-Val^1-Ala-Leu-Ile^3-Ile^2-Val^2-Pro+H]$^+$;
m/z 536 [Leu-Ile^3-Ile^2-Val^2-Pro+H]$^+$;
m/z 326 [Ile^3-Ile^2-Val^2+H]$^+$;
m/z 268 [Pro-Ile^1-Gly +H]$^+$; and
m/z 171 [Ile^1-Gly+H]$^+$.

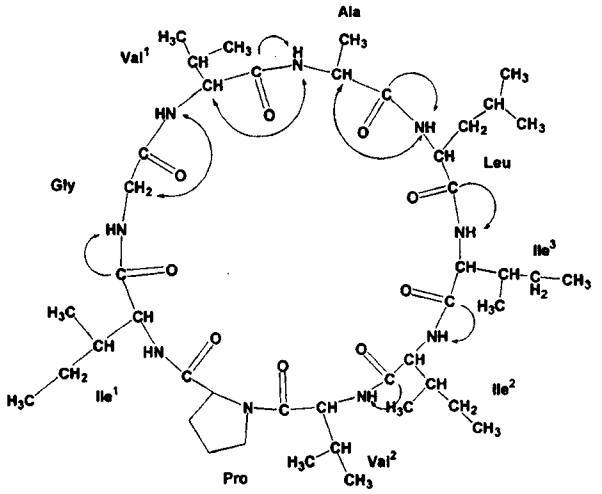

Figure 1. Structure of Arenariphilin E (compound **1**), in which → shows selected HMBC correlations; and ↔ shows selected REOSY correlations.

m/z 862 [Pro^2-Pro^1-Gly^2-Ile-Val-Leu-Gly^1-Ala-Thr-Gly^3]$^+$;
m/z 749 [Val-Leu-Gly^1-Ala-Thr-Gly^3-Pro^2-Pro^1-Gly^2]$^+$;
m/z 650 [Leu-Gly^1-Ala-Thr-Gly^3-Pro^2-Pro^1-Gly^2]$^+$;
m/z 537 [Gly^1-Ala-Thr-Gly^3- Pro^2-Pro^1-Gly^2]$^+$;
m/z 480 [Ala-Thr-Gly^3- Pro^2-Pro^1-Gly^2]$^+$;
m/z 326 [Ala-Thr-Gly^3-Pro^2]$^+$; and
m/z 154 [Gly^3-Pro^2]$^+$.

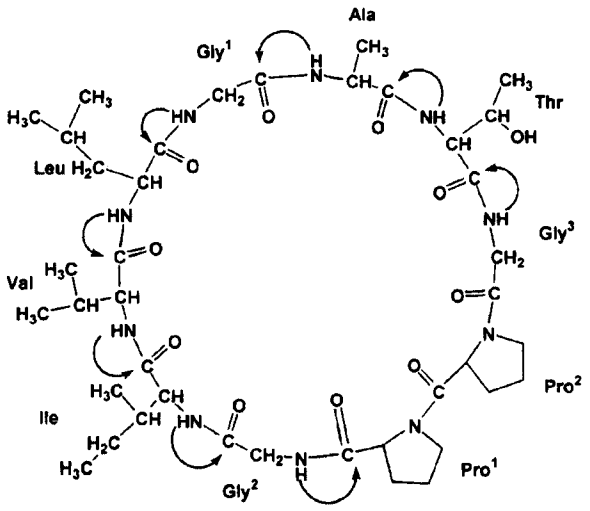

Figure 2. Structure of Arenariphilin F (compound **2**), in which → shows selected HMBC correlations.

Arenariphilin F (compound **2**) was obtained as a white amorphous, and was negative to ninhydrin reagent but positive after hydrolysis with 6 mol/L HCl (Zhou and Tan 2000). Its IR bands at 3 441 and 1 652 cm^{-1} were characteristic of amino and amide carbonyl groups. The FAB$^+$-MS gave an [M]$^+$ ion at *m/z* 862, and the molecular formula $C_{40}H_{66}N_{10}O_{11}$ was derived from the HR-FAB$^+$-MS ([M]$^+$ at *m/z* 862.491 3, calc. for *m/z* 862.491 3), indicating the presence of 13 degrees of unsaturation. The ^{13}C-NMR and ^{1}H-NMR spectra (Table 2) showed the presence of 10 amide carbonyl signals, and eight amide NH signals, respectively, suggesting that compound **2** was a cyclopeptide.

The structure of compound **2** was also elucidated by 1D and 2D NMR as for compound **1**. The amino acid residues were shown to be two prolines, one leucine, one isoleucine, one alanine, three glycines, one valine, and one threonine, respectively (Jia et al. 2003). 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 compound **2** was elucidated as cyclo(Pro^2-Pro^1- Gly^2-Ile-Val-Leu-Gly^1-Ala-Thr-Gly^3) (compound **2**). The FAB$^+$-MS also showed some useful peaks as follows (Jia et al. 2003, 2004):

Experimental

General experimental procedures

Optical rotation was measured with a Jasco 20C polarimeter (Jasco, Japan); for ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.6 MHz) spectra, Bruker AM-400 and DDRX-500 spectrometers (Bruker, Switzerland) were used, δ in ppm rel. to $SiMe_4$ (=0 ppm), *J* in Hz; for MS, a VG-Auto-Spec-3000 mass spectrometer (Micromass, England) was used, *m/z* (rel. %); IR spectra was measured with a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA, USA) was used; for column chromatography, 200–300 mesh and 300–400 mesh silica gels (Qingdao Marine Chemical Factory, Qingdao, China) and Diaion HP-20 (Pharmacia, Japan) were used.

Plant materials

Whole plants of *Arenaria oreophila* J. D. Hooker were collected in Deqing County, Yunnan Province, China, in September 2001. Plants were identified by Professor Zhe-Kun Zhou (Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, Yunnan, China), and a voucher specimen was preserved in the Herbarium of Kunming Institute of Botany, the

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

		^{1}H-NMR	^{13}C-NMR
Pro-2	CO		170.81 (s)
	CH (α)	5.08 (m, 1H)	59.84 (d)
	CH (β)	2.50 (m, 1H), 2.22 (m, 1H)	27.83 (t)
	CH_2 (γ)	1.88 (m, 2H)	22.53 (t)
	CH_2 (δ)	3.62 (br s, 1H),	46.51 (t)
		3.46 (m, 1H)	
Gly	CO		168.22 (s)
	NH	9.40 (br s, 1H)	
	CH_2 (α)	5.00 (m, 1H),	45.03 (t)
		3.90 (br s, 1H)	
Thr	CO		169.18 (s)
	NH	9.77 (br s, 1H)	
	CH (α)	5.01 (m, 1H)	57.92 (d)
	CH (β)	4.56 (m, 1H)	68.20 (d)
	CH_3 (γ)	1.12 (m, 3H)	21.17 (q)
Ala	CO		174.33 (s)
	NH	8.53 (br s, 1H)	
	CH (α)	5.10 (m, 1H)	52.14 (d)
	CH_3 (β)	1.75 (d, *J* = 6.95, 3H)	17.10 (q)
Gly-3	CO		171.55 (s)
	NH	8.32 (d, *J* = 9.40, 1H)	
	CH (α)	5.03 (m, 1H),	44.09 (t)
		4.05 (br s, 1H)	
Pro-1	CO		173.29 (s)
	CH (α)	5.00 (br s, 1H)	62.70 (d)
	CH (β)	2.50 (m, 1H), 2.40 (m, 1H)	30.28 (t)
	CH_2 (γ)	1.92 (m, 2H)	23.02 (t)
	CH_2 (δ)	3.97 (br s, 1H),	47.39 (t)
		3.46 (m, 1H)	
Gly-2	CO		170.30 (s)
	NH	9.10 (d, *J* = 7.50, 1H)	
	CH (α)	4.89 (m, 1H),	44.62 (t)
		3.46 (br s, 1H)	
Ile	CO		176.10 (s)
	NH	9.51 (br s, 1H)	
	CH (α)	4.99 (m, 1H)	56.97 (d)
	CH (β)	2.70 (br s, 1H)	37.20 (d)
	CH_2 (γ)	1.80 (m, 1H), 1.67 (m, 1H)	24.32 (t)
	CH_3 (γ')	1.01 (overlapped, 3H)	15.82 (q)
	CH_3 (δ)	1.03 (overlapped, 3H)	10.82 (q)
Val	CO		169.38 (s)
	NH	8.84 (d, *J* = 6.40, 1H)	
	CH (α)	4.40 (d, *J* = 14.10, 1H)	58.65 (d)
	CH (β)	2.49 (m, 1H)	31.14 (d)
	CH_3 (γ)	1.33 (m, 3H)	19.57 (q)
	CH_3 (γ')	1.18 (m, 3H)	18.33 (q)
Leu	CO		175.90 (s)
	NH	9.24 (d, *J* = 8.15, 1H)	
	CH (α)	5.32 (br s, 1H)	50.14 (d)
	CH_2 (β)	2.08 (m, 1H), 1.87 (m, 1H)	42.37 (t)
	CH (γ)	1.87 (m, 1H)	26.67 (d)
	CH_3 (δ)	1.00 (m, 3H)	21.62 (q)
	CH_3 (δ')	0.99 (overlapped, 3H)	20.15 (q)

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Extraction and isolation

Dried whole plants of *A. oreophila* (26.0 kg) were extracted three times with 95% EtOH under reflux (3 × 100 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), 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 E (compound **1**, 7.3 mg, 0.000 028%) and Arenariphilin F (compound **2**, 11.2 mg, 0.000 043%), respectively.

Identification

Arenariphilin E (compound 1)

$C_{44}H_{77}N_9O_9$, amorphous power, $[\alpha]_D^{26.3}$ 0° (*c* 0.050, MeOH); UV λ_{max}^{MeOH} nm (log ε): 203 (3.88); IR ν_{max}^{KBr} cm^{-1}: 3 431, 1 651; ^{1}H- and ^{13}C-NMR, see Table 1; FAB$^+$-MS *m/z*: 877 (M+2, 35), 763, 536, 480, 326, 268, 86 (100); HRFAB$^+$-MS *m/z*: 876.589 0 ($C_{44}H_{77}N_9O_9$+2H, calc. 876.592 3).

Arenariphilin F (compound 2)

$C_{40}H_{66}N_{10}O_{11}$, amorphous power, $[\alpha]_D^{26.2}$ –36.7° (*c* 0.150, MeOH); UV λ_{max}^{MeOH} nm (logε): 205 (2.27), 259; IR ν_{max}^{KBr} cm^{-1}: 3 441, 2 928, 1 652; ^{1}H- and ^{13}C-NMR, see Table 2; FAB$^+$-MS *m/z*: 862 (M$^+$, 95), 749, 650, 537, 480, 384, 254, 154, 86 (100); HRFAB$^+$-MS *m/z*: 862.491 3 ($C_{40}H_{66}N_{10}O_{11}$, calc. 862.491 3).

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