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Two new minor cyclopeptides from *Sagina japonica* (Caryophyllaceae)

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ABSTRACT

Two new minor cyclopeptides, named japonicin A (1), japonicin B (2), were isolated from the whole plants of *Sagina japonica* (Caryophyllaceae). Their structures were determined as cyclo-(Pro[1]-Pro[2]-Leu[2]-Leu[1]-Phe[2]-Pro[3]-Gly-Ser-Phe[1]) (1) and cyclo-(Pro[1]-Ile-Tyr-Asp-Pro[2]-Phe[2]-Pro[3]-Phe[1]) (2) on the basis of spectroscopic data, especially by two-dimension NMR technologies.

1. Introduction

S. Japonica Ohwi (Caryophyllaceae) is a Chinese folk herb used for clearing up toxic heat, curing laccol, drawing out pus [1], and it's named in Chinese (Qi Gu Cao) because of its curing laccol (Qichuang in Chinese) [2]. It is distributed in Yunnan Province, Changjiang River, and Huanghe River valley area in China. As a continued study of Caryophyllaceae cyclopeptides, and to confirm that cyclopeptide is one characteristic component of Caryophyllaceae [3–9], two minor cyclopeptides were isolated from the EtOAc portion of whole plants. Their structures were characterized as cyclo-(Pro[1]-Pro[2]-Leu[2]-Leu[1]-Phe[2]-Pro[3]-Gly-Ser-Phe[1]) (**1**) named japonicin A and cyclo-(Pro[1]-Ile-Tyr-Asp-Pro[2]-Phe[2]-Pro[3]-Phe[1]) (**2**) named japonicin B respectively by means of spectroscopic method, especially by 2D-NMR, and both of which are new cyclopeptides.

2. Results and discussion

Japonicin A (**1**): white amorphous, was negative to ninhydrin but positive after hydrolysis with concentrated hydrochloric acid [10]. The molecular formula $C_{50}H_{69}N_9O_{10}$ was deduced from the HRESI-MS ([M+Na]+ at *m/z* 978.5041 ($C_{50}H_{69}N_9O_{10}Na$+; calc. *m/z* 978.5065), indicating 21 degrees of unsaturation. IR bands at 3410 cm^{-1} and 1657 cm^{-1} were characteristics of NH, OH and CO respectively. The ^{13}C NMR and ^{1}H NMR spectra (Table 1) showed the presence of nine amide carbonyls, twenty methines, four methyl, fifteen methylenes, and six amide NH, respectively. The overall facts suggested that **1** was a cyclopeptide. The amino acid residues were identified by two-dimension NMR technologies such as REOSY, TOCSY, HMQC and HMBC as three prolines, a serine, two phenylalanines, two leucines and one glycine, and the residues were also confirmed by amino acid analysis method. The sequence of these amino acid residues was achieved by HMBC and REOSY as summarized in Fig. 1(1). By analysis of HMBC correlations between each amino acid residue amide proton (NH) and next amino acid residue carbonyl carbon, and by analysis of REOSY correlations between each amino acid residue α-H or β-H and next amino acid residue amide proton (NH), or by other analysis of REOSY correlations as shown in Fig. 1 (1),

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Table 1
^{1}H and ^{13}C NMR spectral data of **1** (Pyridine-d$_5$), δ in ppm, J in Hz.

		^{1}H-NMR	^{13}C-NMR
Pro-1			
	CO		173.99 (*s*)
	CH (α)	4.13 (*m*, 1H)	61.74 (*d*)
	CH$_2$ (β)	2.75 (*br s*, 1H)	30.22 (*t*)
		1.80 (*br d*, *J* 8.56, 1H)	
	CH$_2$ (γ)	1.44 (*br d*, *J* 8.80, 2H)	22.55 (*t*)
	CH$_2$ (δ)	1.47 (*dd*, *J* 8.56, 11.08, 1H)	47.53 (*t*)
		3.29 (*dd*, *J* 9.28, 1H)	
Pro-2			
	CO		171.81 (*s*)
	CH (α)	5.09 (*m*, 1H)	61.41 (*d*)
	CH$_2$ (β)	2.23 (*br., s*, 1H)	29.78 (*t*)
		1.95 (*d*, *J* 7.40, 1H)	
	CH$_2$ (γ)	1.80 (*d*, *J* 8.50, 1H)	25.01 (*t*)
		1.68 (*d*, *J* 12.08, 1H)	
	CH$_2$ (δ)	4.14 (*m*, 1H)	47.75 (*t*)
		4.05 (*m*, 1H)	
Leu-2			
	CO		172.02 (*s*)
	NH	8.38 (*d*, *J* 8.56, 1H)	
	CH (α)	5.11 (*d*, *J* 8.50, 1H)	49.01 (*d*)
	CH$_2$ (β)	1.80 (*d*, *J* 8.56, 1H)	40.28 (*t*)
		1.68 (*d*, *J* 12.08, 1H)	
	CH (γ)	1.80 (*d*, *J* 8.56, 1H)	25.00 (*d*)
	CH$_3$ (δ)	0.88 (*d*, *J* 5.76, 3H)	23.26 (*t*)
	CH$_3$ (δ')	0.72 (*d*, *J* 6.04, 3H)	21.56 (*t*)
Leu-1			
	CO		171.51 (*s*)
	NH	7.61 (*br s*, 1H)	
	CH (α)	4.56 (*dd*, *J* 5.00, 5.80, 1H)	53.53 (*d*)
	CH$_2$ (β)	1.65 (*d*, *J* 12.08, 1H)	36.49 (*t*)
		1.43 (*br d*, *J* 8.80, 1H)	
	CH (γ)	1.62 (*m*, 1H)	25.09 (*d*)
	CH$_3$ (δ)	0.76 (*d*, *J* 6.56, 3H)	23.05 (*q*)
	CH$_3$ (δ')	0.58 (*d*, *J* 6.32, 3H)	21.07 (*q*)
Phe-2			
	CO		175.87 (*s*)
	NH	10.67 (*br s*, 1H)	
	CH (α)	4.24 (*br., s*, 1H)	57.48 (*d*)
	CH$_2$ (β)	3.79 (*m*, 1H)	35.32 (*t*)
		3.71 (*dd*, *J* 12.36, 13.56, 1H)	
	ArH (δ)	7.27–7.82 (*m*, 5H)	140.09 (*s*)
			128.71 (*d*)
			130.09 (*d*)
			126.33 (*d*)
Pro-3			
	CO		170.85 (*s*)
	CH (α)	4.97 (*br s*, 1H)	58.41 (*d*)
	CH$_2$ (β)	1.80 (*br d*, *J* 8.56, 2H)	28.60 (*t*)
	CH$_2$ (γ)	1.93 (*d*, *J* 7.04, 1H)	25.39 (*t*)
		1.80 (*d*, *J* 8.56, 1H)	
	CH$_2$ (δ)	1.83 (*m*, 1H)	46.96 (*t*)
		3.61 (*m*, 1H)	
Gly			
	CO		170.61 (*s*)
	NH	8.78 (*br s*, 1H)	
	CH$_2$ (α)	1.72 (*d*, *J* 7.80, 1H)	
		3.71 (*m*, 1H)	
Ser			
	CO		171.70 (*s*)
	NH	7.97 (*br s*, 1H)	
	CH$_2$ (α)	4.83 (*m*, 1H)	53.97 (*d*)
	CH$_2$ (β)	4.62 (*m*, 2H)	63.21 (*t*)
Phe-2			
	CO		172.32 (*s*)
	NH	9.88 (*br s*, 1H)	
	CH (α)	5.69 (*br s*, 1H)	56.27 (*d*)
	CH$_2$ (β)	4.07 (*m*, 2H)	34.11 (*t*)
	ArH (δ)	7.20–7.60 (*m*, 5H)	140.33 (*s*)

Table 1 (*continued*)

	^{1}H-NMR	^{13}C-NMR
Phe-2		
		130.86 (*d*)
		128.86 (*d*)
		126.80 (*d*)

only one peptide residues are found to be -N-Pro^1-Pro^2-Leu^2-Leu^1-Phe^2-Pro^3-Gly-Ser-Phe^1-CO-. In addition, FAB+-MS also showed some important ion peaks as follows:

m/z 956[Pro^1-Pro^2-Leu^2-Leu^1-Phe^2-Pro^3-Gly-Ser-Phe^1+H]$^+$

m/z 756[Leu^1-Phe^2-Pro^3-Gly-Ser-Phe^1-Pro^1+H]$^+$

m/z 472[Leu^1-Phe^2-Pro^3-Gly-Ser+H]$^+$

m/z 359[Phe^2-Pro^3-Gly-Ser+H]$^+$

m/z 314[Phe^2-Pro^3]$^+$

m/z 234[Ser-Phe^2]+

In this case, the structure of **1** was established as cyclo (Pro^1-Pro^2-Leu^2-Leu^1-Phe^2-Pro^3-Gly-Ser-Phe^1).

Japonicin B (**2**): white amorphous, was negative to ninhydrin but positive after hydrolysis with concentrated hydrochloric acid [10]. The molecular formula $C_{52}H_{64}N_8O_{11}$ was deduced from the HRESI-MS: 998.4495 ([M+Na]$^+$; calc. 998.4514), indicating 25 degrees of unsaturation. IR bands at 3441 cm^{-1} and 1641 cm^{-1} were characteristics of amino and amide carbonyl groups, respectively. The ^{13}C NMR and ^{1}H NMR spectra (Table 2) showed the presence of eight amide carbonyls and one ester carbonyl, nine methines, two methyl, fourteen methylenes, and five amide NH, respectively. The overall facts suggested that **2** was a cyclopeptide. The amino acid residues were first identified by amino acid analysis as three proline, two phenylalanines, one isoleucine, one aspartic acid and one tyrosine, and then these residues were also confirmed by two-dimension NMR technologies such as REOSY, TOCSY, HMQC and HMBC. The sequence of these amino acid residues was achieved by HMBC and REOSY as summarized in Fig. 1 (1). By analysis of HMBC correlations between each amino acid residue amide proton (NH) and next amino acid residue carbonyl carbon, and by analysis of REOSY correlations between each amino acid residue α-H or β-H and next amino acid residue amide proton (NH) or other ROESY correlation as shown in Fig. 1 (2), one peptide residues are found to be N-Pro^1-Ile-Tyr-Asp-Pro^2-Phe^2-Pro^3-Phe^1-CO.

In addition, FAB$^+$-MS also showed some important ion peaks as follows:

m/z 976[Pro^1-Ile-Tyr-Asp-Pro^2-Phe^2-Pro^3-Phe^1]$^+$

m/z 699[Pro^2-Phe^2-Pro^3-Phe^1-Pro^1-Ile+H]$^+$

m/z 603[Phe^2-Pro^3-Phe^1-Pro^1-Ile+2H]$^+$

m/z 210[Pro^1-Ile]$^+$

m/z 97[Pro^1]$^+$

Therefore, the structure of **2** was established as cyclo(Pro^1-Ile-Tyr-Asp-Pro^2-Phe^2-Pro^3-Phe^1).

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

		^{1}H-NMR	^{13}C-NMR
Pro-2			
	CO		171.79 (*s*)
	CH (α)	4.71 (*d*, *J* 7.65, 1H)	61.86 (*d*)
	CH_2 (β)	2.44 (*m*, 1H),	32.49 (*t*)
		2.15 (*m*, 1H)	
	CH_2 (γ)	2.25 (*m*, 1H),	22.40 (*t*)
		1.72 (*m*, 1H)	
	CH_2 (δ)	3.80 (*m*, 2H)	48.07 (*t*)
Tyr			
	CO		171.35 (*s*)
	NH	9.94 (*br*., *s*, 1H)	
	CH (α)	4.67 (*m*, 1H)	55.00 (*d*)
	CH_2 (β)	3.59 (*m*, 1H)	38.17 (*t*)
		3.53 (*m*, 1H)	
	ArH (δ)	7.50 (*d*, *J* 8.25, 2H)	157.62 (*s*), 131.37 (*d*)
		7.15 (*d*, *J* 8.25, 2H)	128.55 (*s*), 116.31 (*d*)
Pro-1			
	CO		171.71 (*s*)
	CH (α)	4.15 (*d*, *J* 7.65, 1H)	61.91 (*d*)
	CH_2 (β)	2.19 (*m*, 1H),	31.79 (*t*)
		1.64 (*m*, 1H)	
	CH_2 (γ)	1.62 (*m*, 1H),	22.19 (*t*)
		1.59 (*m*, 1H)	
	CH_2 (δ)	3.61 (*m*, 2H)	47.05 (*t*)
Ile			
	CO		171.23 (*s*)
	NH	7.85 (*m*, 1H)	
	CH (α)	4.61 (*dd*, *J* 7.95, 8.25, 1H)	61.12 (*d*)
	CH_2 (β)	2.16 (*m*, 1H)	36.84 (*d*)
	CH_3 (γ)	1.62 (*m*, 2H)	25.83 (*t*)
	CH_3 (γ')	0.90 (*dd*, *J* 6.60, 9.303H)	16.08 (*q*)
	CH_3 (δ)	0.80 (*t*, *J* 7.10, 7.15, 3H)	10.76 (*q*)
Asp			
	CO		170.89 (*s*)
	NH	8.40 (*d*, *J* 8.75, 1H)	
	CH (α)	5.68 (*m*, 1H)	52.62 (*d*)
	CH_2 (β)	3.35 (*m*, 2H)	38.34 (*t*)
	CO (γ)		170.89 (*s*)
Pro-3			
	CO		173.63 (*s*)
	CH (α)	4.66 (*m*, 1H)	59.76 (*d*)
	CH_2 (β)	1.88 (*m*, 1H),	29.08 (*t*)
		1.73 (*m*, 1H)	
	CH_2 (γ)	2.03 (*m*, 1H),	24.91 (*t*)
		1.72 (*m*, 1H)	
	CH_2 (δ)	3.65 (*m*, 2H)	47.63 (*t*)
Phe-2			
	CO		174.21 (*s*)
	NH	8.60 (*d*, *J* 8.75, 1H)	
	CH (α)	5.60 (*m*, 1H)	53.44 (*d*)
	CH_2 (β)	3.64 (*m*, 1H)	37.66 (*d*)
		3.47 (*m*, 1H)	
	ArH (δ)	7.00–7.60 (*m*, 5H)	138.48 (*s*)
			127.75 (*d*)
			129.94 (*d*)
			129.39 (*d*)
Phe-1			
	CO		172.89 (*s*)
	NH	9.02 (*d*, *J* 6.05, 1H))	
	CH (α)	5.41 (*m*, 1H)	54.51 (*d*)
	CH_2 (β)	3.36 (*m*, 1H)	37.78 (*t*)
		2.95 (*dd*, *J* 12.1, 2.7, 1H)	
	ArH (δ)	7.00–7.60 (*m*, 5H)	136.55 (s)
			129.86 (*d*)
			126.64 (*d*)
			128.60 (*d*)

3. Experimental part

3.1. General

Optical rotations were measured on a *Jasco 20C* polarimeter. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-400 spectrometer, the chemical shifts δ were given in ppm relative to TMS as a internal standard and coupling constants were given in Hz. The multiplicity of ^{13}C NMR was determined as DEPT. Two-dimensional (2D) spectra in Bruker DRX-500. Fast-atom bombardment mass spectrometry (FAB-MS) was recorded on a VG AutoSpec 3000 mass spectrometer. Infrared (IR) spectra were obtained with a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets.

Column chromatography (CC) was performed over silica gel (200–300 and 230–400 mesh), LiChroprep RP-8 gel (40–63 μm) and Sephadex LH-20 gel (25–100, Pharmacia). Thin Layer chromatography (TLC) was carried out on plates precoated with Merck RP-18 and silica gel (Qingdao Marine Chemical Ltd., People's Republic of China).

3.2. Plant material

The whole plants of *S. Japonica* were collected in Songming county of Yunnan province, China, in September 2002. 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.

3.3. Extraction and isolation

The dried whole plants of *S. Japonica* (21.0 kg) were extracted 3 times with 95% EtOH under reflux (3 × 100 L) for 3, 1 and 1 h respectively. After evaporation of the combined extracts, the residue was suspended in H2O and then extracted with petroleum ether (60–90 °C), AcOEt, and BuOH. The AcOEt extract (620.0 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 japonicin A (**1**, 12.0 mg, 0.000057%) and japonicin B (**2**, 8.0 mg, 0.000038%).

Japonicin A (**1**). $C_{50}H_{69}N_9O_{10}$, Amorphous power, $[\alpha]_D^{26.7} = -142.31$ (c = 0.130, MeOH). UV λ_{max}^{MeOH} nm (logε): 206 (1.91). IR: 3410, 2957, 1657, 1529, 1453. ^{1}H- and ^{13}C-NMR: Table 1. FAB^{+}-MS: 956 ($M+H^+$, 27), 865, 746, 472, 359, 217, 70 (100). HRESI-MS: 978.5041 ($C_{50}H_{69}N_9O_{10}Na^+$; calc. 978.5065).

Japonicin B (**2**). $C_{52}H_{64}N_8O_{11}$, Amorphous power, $[\alpha]_D^{18.4} = -104.92$ (c = 0.278, MeOH). UV λ_{max}^{MeOH} nm (logε): 209 (2.03). IR: 3441, 2927, 1641, and 1450. ^{1}H- and ^{13}C-NMR: Table 2. FAB-MS: 976 (M^+, 30), 812, 699, 603, 506, 428, 202, 70 (100). HRESI-MS: 998.4495 ($C_{52}H_{64}N_8O_{11}Na^+$; calc. 998.4514).

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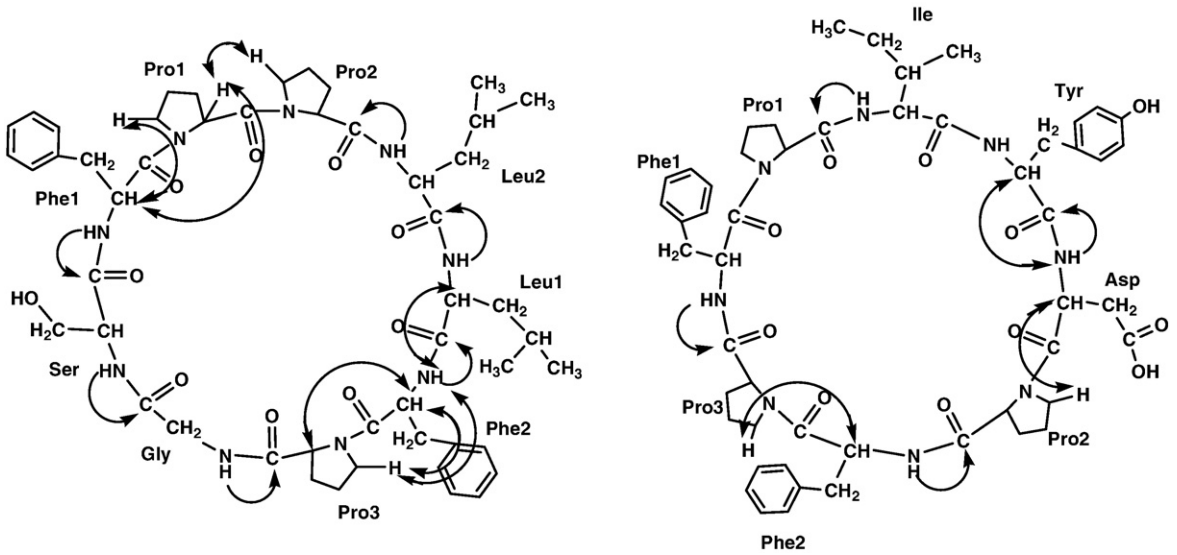

Fig. 1. Structures of **1** and **2** (in which → showed selected HMBC correlations; ↔ showed selected REOSY correlations).

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