Cyclopeptides from *Sagina japonica* (Caryophyllaceae)

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Two new cyclic peptides, named sajaponicin C (**1**) and sajaponicin D (**2**), were isolated from the whole plants of *Sagina japonica* (Caryophyllaceae). Their structures were determined as cyclo(Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-Pro^1) (**1**) and cyclo(Pro^1-Pro^2-Pro^3-Pro^4-Phe^1-Gly-Thr-Ser-Phe^2-Ile-Tyr) (**2**) on the basis of spectroscopic data, especially by two-dimensional (2D) NMR techniques.

1. Introduction. – *Sagina japonica* Ohwi (Caryophyllaceae) is a Chinese folk herb used for clearing up toxic heat, curing laccol, and drawing out pus [1], and it is named in Chinese '*Qi Gu Cao*' because of its bioactivity in curing laccol (*Qichuang* in Chinese) [2]. It is distributed in Yunnan Province, Changjiang River and Huanghe River valley area, in China. The chemical investigation of this plant has just focused on few flavonoids [3]. As a continued study of Caryophyllaceae cyclopeptides [4], two cyclopeptides were isolated from the AcOEt portion of whole plants. Their structures were characterized as cyclo(Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-Pro^1) (**1**) named sajaponicin C, and cyclo(Pro^1-Pro^2-Pro^3-Pro^4-Phe^1-Gly-Thr-Ser-Phe^2-Ile-Tyr) (**2**) named sajaponicin D, by means of spectroscopic methods, especially by 2D-NMR. Both compounds are new cyclopeptides.

2. Results and Discussion. – Sajaponicin C (**1**), obtained as white amorphous powder, was negative to ninhydrin but positive after hydrolysis with concentrated HCl [5]. The molecular formula $C_{54}H_{70}N_8O_9$ was deduced from the HR-ESI-MS ($[M+Na]^+$ at *m/z* 997.5174 ($C_{54}H_{70}N_8NaO_9^+$; calc. 997.5163)), indicating 24 degrees of unsaturation. IR Bands at 3431 and 1640 cm^{-1} were characteristic of NH and amide CO, respectively. The ^{13}C- and ^1H-NMR spectra (*Table 1*) showed the presence of eight amide CO groups, 24 CH, four Me, and 14 CH_2, and five amide NH groups. The overall data suggested that **1** was a cyclopeptide. The amino acid residues were identified by two-dimensional (2D) NMR techniques, such as ^1H,^1H-COSY, TOCSY, HMQC, and HMBC, as three prolines, two leucines, two phenylalanines, and one tyrosine, and the residues were also confirmed by the amino acid analysis method. The sequence of these amino acid residues was elucidated by HMBC and ROESY as shown in the *Figure*. By analysis of HMBC correlations between each amino acid residue amide proton (NH) and the next amino acid residue carbonyl C-atom, and by analysis of ROESY

correlations between each amino acid residue α-H or β-H, and the next amino acid residue amide proton (NH), or by other analysis of ROESY correlations as shown in the *Figure*, a peptide segment was found to be -N-Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-CO-, and there was one proline left. In this case, only one sequence was reasonable, *i.e.*, -N-Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-Pro^1-CO. In addition, the FAB$^+$-MS displayed the following relevant ion peaks:

I. *m/z* 975 [Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-Pro^1 + H]$^+$
II. *m/z* 715 [Pro^3-Phe^2-Pro^1-Pro^2-Leu^2-Tyr + H]$^+$
III. *m/z* 618 [Phe^2-Pro^1-Pro^2-Leu^2-Tyr + H]$^+$
IV. *m/z* 472 [Pro^1-Pro^2-Leu^2-Tyr + 2 H]$^+$
V. *m/z* 358 [Leu^1-Phe^1-Pro^3 + H]$^+$
VI. *m/z* 262 [Leu^1-Phe^1 + 2 H]$^+$

On the basis of these data, the structure of **1** was established as cyclo(Pro^2-Leu^2-Tyr-Leu^1-Phe^1-Pro^3-Phe^2-Pro^1).

Sajaponicin D (**2**), obtained as white amorphous powder, was negative to ninhydrin but positive after hydrolysis with concentrated HCl [5]. The molecular formula $C_{62}H_{81}N_{11}O_{14}$ was deduced from the HR-ESI-MS: 1226.4903 ([M+Na]$^+$, $C_{62}H_{81}N_{11}NaO_{14}^+$; calc. 1226.4890), indicating 28 degrees of unsaturation. IR Bands at 3416 and 1640 cm^{-1} were characteristic of NH and amide CO groups, respectively. The ^{13}C- and ^{1}H-NMR spectra (*Table 2*) showed the presence of eleven amide CO, 26 CH, three Me, 18 CH_2, and seven amide NH groups. These data suggested that **2** was a cyclopeptide. The amino acid residues were first identified by amino acid analysis as four prolines, two phenylalanines, one isoleucine, one glycine, one threonine, one tyrosine, and one serine, and then these residues were also confirmed by 2D-NMR techniques such as ^{1}H,^{1}H-COSY, TOCSY, HMQC, and HMBC. The sequence of these amino acid residues was established by HMBC and ROESY as depicted in the *Figure*. By analysis of HMBC correlations between each amino acid residue NH and the next amino acid carbonyl C-atom, and by analysis of ROESY correlations between each amino acid residue α-H or β-H, and the next amino acid residue amide proton (NH), or other ROESY correlation as shown in the *Figure*, one peptide segment was found to be -N-Pro^4-Phe^1-Gly-Thr-Ser-Phe^2-Ile-Tyr-CO-, and three prolines were left. Therefore, the structure of **2** was established as cyclo(Pro^1-Pro^2-Pro^3-Pro^4-Phe^1-Gly-Thr-Ser-Phe^2-Ile-Tyr).

Experimental Part

General. Column chromatography (CC): silica gel (200–300 and 230–400 mesh), *LiChroprep RP-8* gel (40–63 μm) and *Sephadex LH-20* (25–100 μm, *Pharmacia*). TLC: plates precoated with *Merck RP-18* and silica gel (*Qingdao Marine Chemical Ltd.*, People's Republic of China).Optical rotations: *Jasco 20C* polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrophotometer with KBr pellets. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer, the chemical shifts δ are given in ppm relative to TMS as a internal standard, and coupling constants *J* are given in Hz. The multiplicity of C-atoms was determined as DEPT. 2D Spectra on a *DRX-500* instrument. Fast-atom bombardment mass spectrometry (FAB-MS): *VG AutoSpec 3000* mass spectrometer.

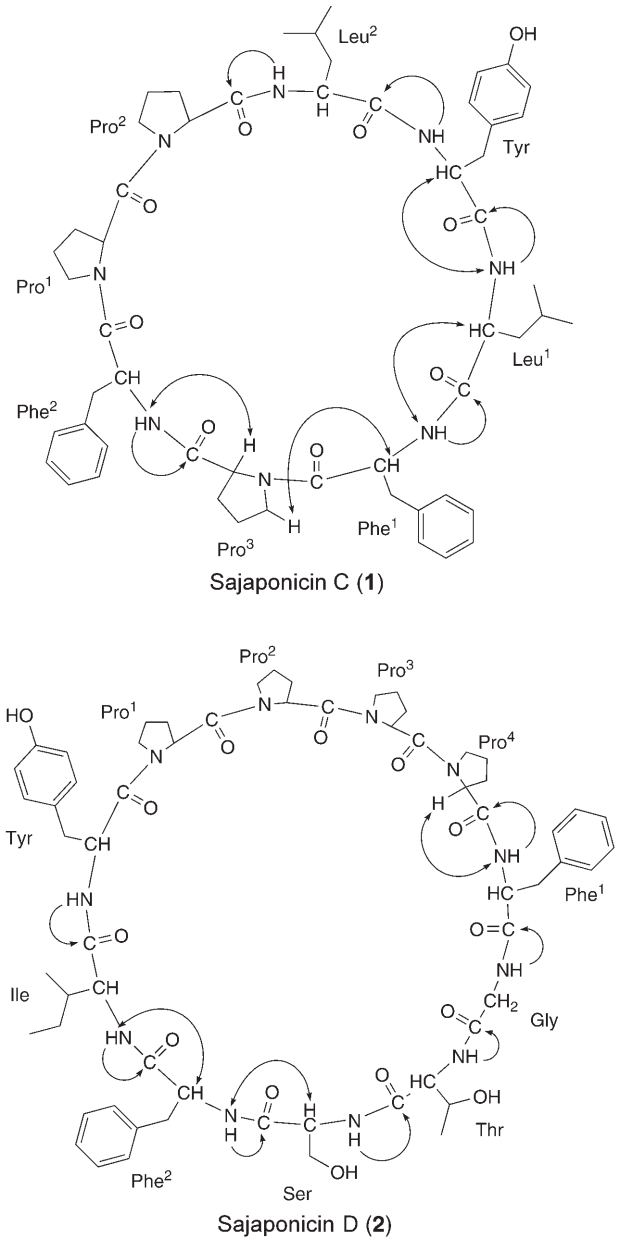

Figure. *Structures of* **1** *and* **2** (→: selected HMBC correlations; ↔: selected ROESY correlations)

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.

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** (in (D_5)Pyridine; δ in ppm, J in Hz)

		^{1}H-NMR	^{13}C-NMR
Pro[2]	CO		172.64 (*s*)
	H–C(α)	3.43 (*d*, J = 7.70, 1 H)	61.07 (*d*)
	CH_2(β)	2.37 (*m*, 2 H)	31.33 (*t*)
	CH_2(γ)	1.14 (*m*, 2 H)	22.03 (*t*)
	CH_2(δ)	3.69 (*m*, 2 H)	47.40 (*t*)
Leu[2]	CO		171.85 (*s*)
	NH	9.66 (*d*, J = 8.15, 1 H)	
	H–C(α)	4.98 (*m*, 1 H)	52.54 (*d*)
	CH_2(β)	2.74 (*m*, 2 H)	39.163 (*t*)
	H–C(γ)	2.00 (*m*, 1 H)	26.58 (*d*)
	Me(δ)	0.93 (*d*, J = 6.85, 3 H)	22.66 (*q*)
	Me(δ')	0.39 (*d*, J = 6.40, 3 H)	20.99 (*q*)
Tyr	CO		170.68 (*s*)
	NH	9.61 (*d*, J = 9.00, 1 H)	
	H–C(α)	5.23 (*m*, 1 H)	55.77 (*d*)
	CH_2(β)	3.81 (*d*, J = 5.89, 1 H), 3.00 (*m*, 1 H)	38.37 (*t*)
	arom. H(δ)	7.61 (*d*, J = 8.15, 2 H), 7.04 (*d*, J = 8.15, 2 H)	158.22 (*s*), 126.92 (*s*), 116.64 (*d*), 128.44 (*d*)
Leu[1]	CO		174.48 (*s*)
	NH	9.01 (*d*, J = 6.40, 1 H)	
	H–C(α)	4.95 (*m*, 1 H)	52.18 (*d*)
	CH_2(β)	1.85 (*d*, J = 7.70, 11.95, 2 H)	40.90 (*t*)
	H–C(γ)	1.80 (*m*, 1 H)	24.70 (*d*)
	Me(δ)	1.02 (*d*, J = 6.40, 3 H)	23.57 (*q*)
	Me(δ')	0.50 (*t*, J = 6.40, 3 H)	21.23 (*q*)
Phe[1]	CO		171.68 (*s*)
	NH	9.72 (br. *s*, 1 H)	
	H–C(α)	5.38 (*d*, J = 5.38, 1 H)	55.47 (*d*)
	CH_2(β)	3.79 (*d*, J = 5.90, 1 H), 3.25 (*d*, J = 12.35, 1 H)	38.31 (*t*)
	arom. H (δ)	7.50–7.17 (*m*, 5 H)	137.08 (*s*), 126.43 (*d*), 130.07 (*d*), 129.16 (*d*)
Pro[3]	CO		173.52 (*s*)
	H–C(α)	4.83 (*d*, J = 5.15, 1 H)	59.68 (*d*)
	CH_2(β)	2.35 (*m*, 1 H), 1.95 (*m*, 1 H)	29.49 (*t*)
	CH_2(γ)	1.94 (*m*, 1 H), 1.56 (*m*, 1 H)	25.63 (*t*)
	CH_2(δ)	3.47 (*m*, 1 H), 3.66 (*m*, 1 H)	47.05 (*t*)
Phe[2]	CO		171.00 (*s*)
	NH	9.50 (br. *s*, 1 H)	
	H–C(α)	5.27 (*m*, 1 H)	55.77 (*d*)
	CH_2(β)	3.11 (*dd*, J = 11.95, 12.40, 1 H), 2.88 (*dd*, J = 11.95, 12.40, 1 H)	37.57 (*t*)
	arom. H(δ)	7.50–7.17 (*m*, 5 H)	140.28 (*s*), 127.56 (*d*), 131.28 (*d*), 129.63 (*d*)

Table 1 (cont.)

		^{1}H-NMR	^{13}C-NMR
Pro[1]	CO		171.76 (*s*)
	H–C(α)	3.68 (*d*, *J* = 5.25, 1 H)	61.16 (*d*)
	CH_2(β)	1.20 (*m*, 1 H),	30.83 (*t*)
		2.34 (*m*, 1 H)	
	CH_2(γ)	1.16 (*m*, 2 H)	22.66 (*t*)
	CH_2(δ)	3.61 (*m*, 2 H)	47.40 (*t*)

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2** (in (D_5)Pyridine; δ in ppm, *J* in Hz)

		^{1}H-NMR	^{13}C-NMR
Pro[1]	CO		168.82 (*s*)
	H–C(α)	4.62 (*m*, 1 H)	62.54 (*d*)
	CH_2(β)	2.57 (br. *s*, 1 H),	28.63 (*t*)
		2.16 (*m*, 1 H)	
	CH_2(γ)	2.07 (*m*, 1 H),	25.38 (*t*)
		1.97 (*m*, 1 H)	
	CH_2(δ)	4.10 (*m*, 2 H)	47.65 (*t*)
Pro[2]	CO		170.40 (*s*)
	H–C(α)	4.47 (br. *s*, 1 H)	62.01 (*d*)
	CH_2(β)	2.57 (br. *s*, 1 H),	28.63 (*t*)
		2.14 (*m*, 1 H)	
	CH_2(γ)	2.05 (*m*, 1 H),	25.38 (*t*)
		1.95 (*m*, 1 H)	
	CH_2(δ)	4.12 (*m*, 1 H),	47.65 (*t*)
		3.85 (*m*, 1 H)	
Pro[3]	CO		172.03 (*s*)
	H–C(α)	5.07 (br. *s*, 1 H)	61.74 (*d*)
	CH_2(β)	2.94 (*m*, 2 H)	30.22 (*t*)
	CH_2(γ)	1.82 (*m*, 2 H)	26.14 (*t*)
	CH_2(δ)	3.56 (*m*, 1 H),	46.93 (*t*)
		3.38 (*m*, 1 H)	
Pro[4]	CO		173.84 (*s*)
	H–C(α)	4.16 (br. *s*, 1 H)	61.60 (*d*)
	CH_2(β)	2.78 (*m*, 2 H)	29.77 (*t*)
	CH_2(γ)	1.79 (*m*, 2 H)	25.87 (*t*)
	CH_2(δ)	3.56 (*m*, 1 H),	46.74 (*t*)
		3.39 (*m*, 1 H)	
Phe[1]	CO		169.73 (*s*)
	NH	9.37 (br. *s*, 1 H)	
	H–C(α)	4.96 (*m*, 1 H)	53.98 (*d*)
	CH_2(β)	3.40 (*m*, 1 H),	40.43 (*t*)
		2.78 (*m*, 1 H)	
	arom. H(δ)	7.83–7.10 (*m*, 5 H)	140.33 (*s*), 30.85 (*d*),
			128.85 (*d*), 26.78 (*d*)

Table 2 (cont.)

		^{1}H-NMR	^{13}C-NMR
Gly	CO		172.56 (*s*)
	NH	8.44 (br. *s*, 1 H)	
	$CH_2(\alpha)$	4.67 (*d*, *J* = 9.28, 1 H), 4.49 (*d*, *J* = 7.32, 1 H)	44.18 (*t*)
Thr	CO		175.81 (*s*)
	NH	8.35 (*d*, *J* = 9.08, 1 H)	
	H–C(α)	5.15 (*t*, *J* = 9.03, 7.47, 1 H)	59.29 (*d*)
	H–C(β)	4.72 (*m*, 1 H)	66.83 (*d*)
	Me(γ)	1.26 (*d*, *J* = 6.08, 3 H)	20.12 (*q*)
Ser	CO		172.95 (*s*)
	NH	10.63 (*d*, *J* = 6.28, 1 H)	
	H–C(α)	4.22 (*m*, 1 H)	57.49 (*d*)
	$CH_2(\beta)$	4.12 (*m*, 2 H)	63.23 (*t*)
Phe[2]	CO		171.17 (*s*)
	NH	8.76 (br. *s*, 1 H)	
	H–C(α)	4.56 (*m*, 1 H)	53.56 (*d*)
	$CH_2(\beta)$	3.31 (*m*, 1 H), 3.01 (*m*, 1 H)	40.32 (*t*)
	arom. H(δ)	7.83 – 7.10 (*m*, 5 H)	140.08 (*s*), 130.07 (*d*), 128.69 (*d*), 126.32 (*d*)
Ile	CO		173.99 (*s*)
	NH	8.97 (*d*, *J* = 8.84, 1 H)	
	H–C(α)	5.46 (*d*, *J* = 9.08, 1 H)	55.31 (*d*)
	H–C(β)	2.27 (*m*, 1 H)	37.86 (*d*)
	$CH_2(\gamma)$	1.80 (*m*, 2 H)	22.28 (*t*)
	Me(γ')	1.12 (*m*, 3 H)	16.26 (*q*)
	Me(δ)	0.89 (*t*, *J* = 7.32, 3 H)	11.76 (*q*)
Tyr	CO		171.70 (*s*)
	NH	9.85 (br. *s*, 1 H)	
	H–C(α)	5.68 (*m*, 1 H)	56.30 (*d*)
	$CH_2(\beta)$	3.70 (*m*, 1 H)	41.60 (*t*)
	arom. H(δ)	7.83–7.10 (*m*, 4 H)	157.15 (*s*), 129.09 (*s*), 115.71 (*d*), 131.72 (*d*)

Extraction and Isolation. The dried whole plants of *S. japonica* (21.0 kg) were extracted with 95% EtOH under reflux (3 × 100 l) for 3, 1, 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 (620.0 g) was decolored on *Diaion HP 20*, eluting with a gradient of H_2O/MeOH 0:1 → 1:0. The 70% MeOH extract (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 *sajaponicin A* (**1**, 13 mg) and *sajaponicin B* (**2**, 7.0 mg).

Sajaponicin C (**1**). $C_{54}H_{70}N_8O_9$. Amorphous powder. $[\alpha]_D^{18.4} = -112.16$ ($c = 0.159$, MeOH). UV (MeOH (log ε)): λ_{max} 203 (1.52). IR: 3431, 1640, 1448. ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 975 (27, $[M+H]^+$), 868, 800, 715, 618, 472, 358, 262, 70 (100). HR-ESI-MS: 997.5174 ($[M+Na]^+$, $C_{54}H_{70}N_8NaO_9^+$; calc. 997.5163).

Sajaponicin D (**2**). $C_{62}H_{81}N_{11}O_{14}$. Amorphous powder. $[\alpha]_D^{20.3} = -85.59$ ($c = 0.074$, MeOH). UV (MeOH (log ε)): λ_{max} 205 (1.90). IR: 3416, 2962, 1640, 1518. ^{1}H- and ^{13}C-NMR: *Table 2*. FAB-MS (pos.):

1204 (15, $[M+1]^+$), 993, 794, 698, 584, 311, 212, 70 (100). HR-ESI-MS: 1226.4903 ($[M+Na]^+$, $C_{62}H_{81}N_{11}NaO_{14}^+$; calc. 1226.4890).

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