

Cyclopeptides and Amides from *Pseudostellaria heterophylla* (Caryophyllaceae)

by Ya-bin Yang, Ning-hua Tan*, Fei Zhang, Ya-qing Lu, Min He, and Jun Zhou

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany,
Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China
(phone: +86-871-5223800; fax: +86-871-5223228; e-mail: nhtan@mail.kib.ac.cn)

From the roots of *Pseudostellaria heterophylla*, three cyclopeptides and three amides were isolated, besides heterophyllin A and B. Their structures were determined as cyclo (Ala-Gly-Pro-Val-Tyr-) (heterophyllin J; **1**), cyclo (Ala-Gly-Pro-Tyr-Leu-) (pseudostellarin A; **2**), cyclo (Gly-Gly-Gly-Pro-Pro-Phe-Gly-Ile-) (pseudostellarin B; **3**), methyl γ -hydroxypyroglutamate (**4**), methyl pyroglutamate (**5**), and pyroglutamic acid (**6**) on the basis of spectral data, especially 2D-NMR data. Among them, compounds **1** and **4** are new compounds.

1. Introduction. – The roots of *Pseudostellaria heterophylla* (MIQ.) PAX ex PAX et HOFFM. (Caryophyllaceae) are used as a traditional Chinese medicine named *tai-zi-shen*. The plant is distributed over East China, mainly Jiangsu, Shandong, and Anhui Province. In our previous paper, we reported two new cyclopeptides, heterophyllin A and B, obtained from the roots of *P. heterophylla* [1]. In subsequent investigations of *P. heterophylla*, three other cyclopeptides and three amides were isolated, *i.e.*, heterophyllin J (**1**), pseudostellarin A (**2**), pseudostellarin B (**3**), methyl γ -hydroxypyroglutamate (**4**), methyl pyroglutamate (**5**), and pyroglutamic acid (**6**), among which compounds **1** and **4** are new (see *Fig.*). In this paper, we describe the isolation and structure elucidation of compounds **1–6**.

2. Results and Discussion. – Compound **1** was isolated as a 1:2 mixture with compound **2**. The mixture did not react with ninhydrin reagent, but did so after hydrolysis with conc. HCl solution, indicating that **1** and **2** might be cyclopeptides or amides [2]. The ^{13}C - and ^1H -NMR spectra (*cf.* the *Table*) showed the presence of 5 CO, 9 CH, 5 CH₂, 3 Me, 2 C, and 4 NH signals for **1**, and 5 CO, 9 CH, 6 CH₂, 3 Me, 2 C, and 4 NH signals for **2**, indicating that **1** and **2** were composed of five amino acid residues. The molecular formulae C₂₄H₃₃N₅O₆ and C₂₅H₃₅N₅O₆ for **1** and **2**, respectively, were deduced from the FAB (neg.) and DEPT spectra, and indicated 11 degrees of unsaturation. These data suggested that **1** and **2** are cyclopentapeptides. The amino acid residues were identified by ^1H , ^1H COSY, HMQC, and HMBC experiments as 1 L-proline (Pro), 1 L-valine (Val), 1 L-tyrosine (Tyr), 1 L-alanine (Ala), and 1 glycine (Gly) residue for **1**, and 1 L-proline (Pro), 1 L-tyrosine (Tyr), 1 L-leucine (Leu), 1 L-alanine (Ala), and 1 glycine (Gly) for **2**. The sequences of these amino acid residues were determined by HMBC and ROESY, as summarized in the *Figure*. By analysis of the HMBC correlations between the amino acid residues amide proton NH(*i* + 1) and the carbonyl C-atom CO(*i*), and by analysis of the ROESY correlations between the

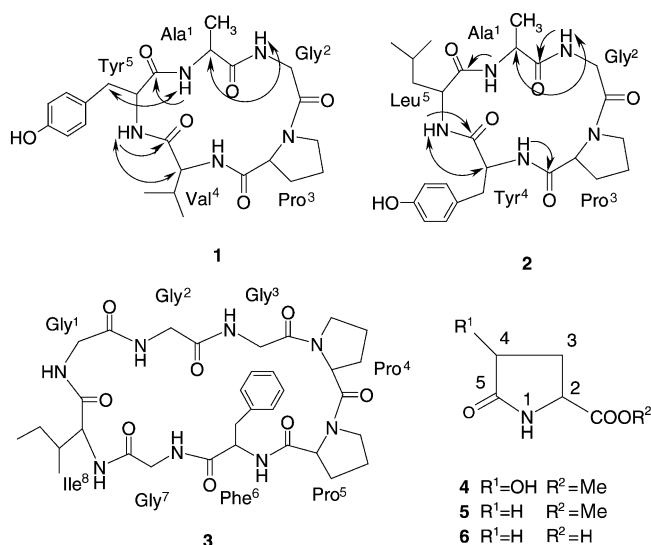


Figure. Structures of compounds **1**–**6**, with selected HMBC (\rightarrow) and selected ROESY (\leftrightarrow) correlations

protons H–C(α .i) or H–C(β .i) of each amino acid residue and the amide proton NH($i + 1$), the two sequences -Ala-Gly-Pro-Val-Tyr- and -Ala-Gly-Pro-Tyr-Leu- were found for **1** and **2**, respectively. In the FAB-MS (neg.) **1** and **2** gave $[M - H]^-$ at m/z 486 and 500, resp., in accordance with the sequences established above by HMBC and ROESY after cyclization. Therefore, the structures of **1** and **2** were determined as cyclo(-Ala-Gly-Pro-Val-Tyr-) and cyclo(-Ala-Gly-Pro-Tyr-Leu-), respectively, and named heterophyllin J (**1**) and pseudostellarin A (**2**). Compound **1** is a new compound, and **2** is identical with the known pseudostellarin A described in [3].

Compound **3** was elucidated as cyclo (-Gly-Gly-Gly-Pro-Pro-Phe-Gly-Ile-) by the same method and is identical with the known pseudostellarin B described in [3].

Compound **4** was isolated as colorless needles; reaction with ninhydrin reagent occurred only after hydrolysis with conc. HCl solution, indicating that **4** might be a cyclopeptide or amide [2]. The molecular formula $C_6H_9NO_4$ was deduced from the FAB-MS (neg.) and DEPT and indicated 3 degrees of unsaturation. IR Bands at 3332 (br.) and 1715 cm^{-1} were characteristic of NH and OH, and of CO groups, respectively. These data suggested that **4** is an amide. The ^{13}C -NMR spectra showed the presence of 2 CO, 2 CH, 1 CH_2 , and 1 Me signal, consistent with the structure of methyl γ -hydroxypyroglutamate, which was confirmed by $^1H, ^1H$ COSY, HMQC, and HMBC. Compound **4** is a new compound.

Compounds **5** and **6** were elucidated as methyl pyroglutamate and pyroglutamic acid by the same method, they are known compounds [4].

Table. ¹H- and ¹³C-NMR Data of Compounds **1–3**. In C₅D₂N; 500 MHz for δ(H), 125 MHz for δ(C), δ in ppm, J in Hz.

		Heterophyllin J (1)		Pseudostellarin A (2)				Pseudostellarin B (3)	
		δ(H)	δ(C)	δ(H)	δ(C)			δ(H)	δ(C)
Pro ³	H–C(α)	4.62 (<i>m</i>)	62.2	4.62 (<i>m</i>)	61.6	Gly ⁷	NH	8.51 (<i>br. s</i>)	–
	CH ₂ (β)	2.00 (<i>m</i>), 1.87 (<i>m</i>)	29.5	2.10 (<i>m</i>), 2.00 (<i>m</i>)	29.6		CH ₂ (α)	4.70 (<i>m</i>), 3.68 (<i>dd</i>)	44.2
	CH ₂ (γ)	1.87 (<i>m</i>), 1.65 (<i>m</i>)	24.7	1.87 (<i>m</i>), 1.65 (<i>m</i>)	24.4		CO	–	173.0
	CH ₂ (δ)	3.82 (<i>m</i>), 3.40 (<i>m</i>)	46.5	4.13 (<i>m</i>), 3.40 (<i>m</i>)	47.0	Ile ⁸	NH	8.40 (<i>br. s</i>)	–
	CO	–	172.3	–	171.9		H–C(α)	4.30 (<i>dd</i>)	60.8
Tyr ⁵ or ⁴	NH	9.46 (<i>d, J</i> = 7.99)	–	8.04 (<i>br. d, J</i> = 6.34)	–	H–C(β)	2.15 (<i>m</i>)	34.5	
	H–C(α)	4.62 (<i>m</i>)	59.0	5.10 (<i>m</i>)	56.9	CH ₂ (γ)	1.73 (<i>m</i>), 1.26 (<i>m</i>)	24.5	
	CH ₂ (β)	3.60 (<i>m</i>), 3.40 (<i>m</i>)	36.4	3.40 (<i>m</i>), 3.40 (<i>m</i>)	36.9	Me(γ')	0.92 (<i>d, J</i> = 6.55)	15.6	
	C(1')	–	128.2	–	128.2	Me(δ)	0.82 (<i>t, J</i> = 7.30)	10.5	
	H–C(2'), H–C(6')	7.29 (<i>d, J</i> = 8.24)	130.6	7.36 (<i>d, J</i> = 8.20)	130.7	CO	–	176.0	
	H–C(3'), H–C(5')	7.12 (<i>m</i>)	115.9	7.12 (<i>m</i>)	115.7	Gly ¹	NH	10.71 (<i>br. s</i>)	–
	C(4')	–	157.4	–	157.2		CH ₂ (α)	4.50 (<i>m</i>), 4.07 (<i>m</i>)	44.7
	CO	–	171.9	–	172.3		CO	–	170.3
Val ⁴	NH	7.91 (<i>br. s</i>)	–	–	–	Gly ²	NH	8.68 (<i>br. s</i>)	–
	H–C(α)	4.60 (<i>m</i>)	60.7	–	–		CH ₂ (α)	4.70 (<i>m</i>), 3.95 (<i>dd</i>)	43.6
	H–C(β)	2.42 (<i>m</i>)	30.6	–	–	CO	–	170.4	
	Me(γ), Me(γ')	1.00 (<i>d, J</i> = 6.72), 0.97 (<i>d, J</i> = 6.59)	19.5, 18.6	–	–	Gly ³	NH	7.80 (<i>br. s</i>)	–
CO	–	172.3	–	–	CH ₂ (α)		4.85 (<i>dd</i>), 3.50 (<i>m</i>)	41.7	
Leu ⁵	NH	–	–	9.69 (<i>d, J</i> = 6.54)	–	CO	–	169.1	
	H–C(α)	–	–	4.20 (<i>m</i>)	57.4	Pro ⁴	H–C(α)	4.65 (<i>d</i>)	61.5
	CH ₂ (β)	–	–	2.42 (<i>m</i>), 1.87 (<i>m</i>)	38.8		CH ₂ (β)	2.00 (<i>m</i>), 1.73 (<i>m</i>)	32.3
	H–C(γ)	–	–	1.55 (<i>m</i>)	24.8		CH ₂ (γ)	1.73 (<i>m</i>), 1.26 (<i>m</i>)	26.2
	Me(δ), Me(δ')	–	–	0.85 (<i>d, J</i> = 6.63), 0.75 (<i>d, J</i> = 6.50)	22.7, 21.1		CH ₂ (δ)	3.50 (<i>m</i>), 3.50 (<i>m</i>)	47.4
	CO	–	–	–	172.9	CO	–	173.7	
Ala ¹	NH	9.51 (<i>br. d, J</i> = 6.05)	–	9.17 (<i>d, J</i> = 8.44)	–	Pro ⁵	H–C(α)	4.58 (<i>d</i>)	59.5
	H–C(α)	4.75 (<i>m</i>)	49.6	5.00 (<i>m</i>)	49.0		CH ₂ (β)	2.00 (<i>m</i>), 1.73 (<i>m</i>)	28.7
	Me(β)	1.59 (<i>d, J</i> = 6.99)	16.4	1.56 (<i>d, J</i> = 6.89)	17.8		CH ₂ (γ)	1.26 (<i>m</i>), 0.93 (<i>m</i>)	21.7
	CO	–	173.6	–	174.5		CH ₂ (δ)	3.50 (<i>m</i>), 3.50 (<i>m</i>)	47.1
Gly ²	NH	8.97 (<i>br. s</i>)	–	9.83 (<i>br. s</i>)	–	CO	–	171.2	
	CH ₂ (α)	4.51 (<i>m</i>), 3.82 (<i>m</i>)	42.3	4.46 (<i>m</i>), 3.64 (<i>m</i>)	42.3	Phe ⁶	NH	8.95 (<i>br. s</i>)	–
	CO	–	168.5	–	168.8		H–C(α)	5.60 (<i>m</i>)	55.1
							CH ₂ (β)	4.07 (<i>m</i>), 3.50 (<i>m</i>)	39.0
					C(1')		–	138.8	
					H–C(2'), H–C(6')		7.26 (<i>m</i>)	129.6	
					H–C(3'), H–C(5')		7.30 (<i>m</i>)	129.0	
					H–C(4')		7.49 (<i>m</i>)	127.2	
					CO		–	172.5	

Experimental Part

General. CC = Column chromatography. M.p.: uncorrected. Optical rotation: *SEPA-300* polarimeter. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets, in cm^{-1} . ^1H - and ^{13}C -NMR Spectra: *Bruker-AM-400* spectrometer at 400 (^1H) and 100 MHz (^{13}C) and *Bruker-DRX-500* spectrometer at 500 (^1H) and 125 MHz (^{13}C); δ in ppm rel. to SiMe_4 (=0 ppm), J in Hz. MS: *VG-AutoSpec-3000* mass spectrometer in m/z .

Plant Material. The roots of *P. heterophylla* were bought from the Kunming Ju-Hua-Cun herb market in Yunnan Province of P.R. China in October 2000 and were identified by Prof. *Ning-Hua Tan*. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Dried roots of *P. heterophylla* (200 kg) were extracted 3 times with MeOH under reflux for 4, 2, and 1 h, resp. After evaporation of the combined extracts, the residue (32 kg) was suspended in MeOH, and the MeOH extract (18.5 kg) was subjected to CC (silica gel, $\text{CHCl}_3/\text{MeOH}$; *RP-18*, $\text{MeOH}/\text{H}_2\text{O}$): **1**/ **2** (35 mg), **3** (150 mg), **4** (45 mg), **5** (16 mg), and **6** (27 mg), besides heterophyllin A (6 mg) and B (765 mg).

Heterophyllin J (= *Cyclo(L-alanylglycyl-L-prolyl-L-valyl-L-tyrosyl)*; **1**): yellow amorphous powder. ^1H - and ^{13}C -NMR: *Table*. FAB-MS (neg.): 486 ($[M - \text{H}]^-$, $\text{C}_{24}\text{H}_{32}\text{N}_5\text{O}_6^-$).

Pseudostellarin A (= *Cyclo(L-alanylglycyl-L-prolyl-L-tyrosyl-L-leucyl)*; **2**): yellow amorphous powder. ^1H - and ^{13}C -NMR: *Table*. FAB-MS (neg.): 500 ($[M - \text{H}]^-$, $\text{C}_{25}\text{H}_{34}\text{N}_5\text{O}_6^-$).

Pseudostellarin B (= *Cyclo(glycylglycylglycyl-L-prolyl-L-prolyl-L-phenylalanylglycyl-L-isoleucyl)*; **3**): white needles. ^1H - and ^{13}C -NMR: *Table*. FAB-MS (neg.): 682 (M^- , $\text{C}_{33}\text{H}_{46}\text{N}_8\text{O}_8^-$, 652, 438, 396, 367, 325, 281, 255, 159, 129, 99, 80).

Methyl γ -Hydroxypyroglutamate (= *4-Hydroxy-5-oxo-L-proline Methyl Ester*; **4**): colorless needles. M.p. 114.5–115.0°. $[\alpha]_{\text{D}}^{22.8} = -44.08$ ($c = 0.1$, MeOH). IR: 3332, 2950, 2864, 1715, 1440, 1227, 1116, 1048, 1035, 746, 710, 660. ^1H -NMR (CD_3OD , 400 MHz): 4.30 (t , $J = 8.48$, H-C(4)); 4.21 (t , $J = 7.84$, H-C(2)); 3.76 (s , COOMe); 2.80 (m , 1 H-C(3)); 1.90 (m , 1 H-C(3)). ^{13}C -NMR (CD_3OD , 100 MHz): 178.9 (s , C(5)); 173.5 (s , COOMe); 69.9 (d , C(4)); 53.1 (d , C(2)); 52.9 (q , COOMe); 35.4 (t , C(3)). FAB-MS (neg.): 158 ($[M - \text{H}]^-$, $\text{C}_6\text{H}_8\text{NO}_4^-$), 144, 129, 98.

Methyl Pyroglutamate (= *5-Oxo-L-proline Methyl Ester*; **5**): colorless amorphous powder. ^1H -NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz): 4.38 (dd , $J = 4.04$, 6.84, H-C(2)); 3.60 (s , COOMe); 2.32 (m , 2 H-C(3), 2 H-C(4)). ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz): 178.1 (s , C(5)); 173.7 (s , COOMe); 56.0 (d , C(2)); 52.2 (q , COOMe); 29.9 (t , C(4)); 25.4 (t , C(3)). FAB-MS (neg.): 142 ($[M - \text{H}]^-$, $\text{C}_6\text{H}_8\text{NO}_3^-$).

Pyroglutamic Acid (= *5-Oxo-L-proline*; **6**): colorless needles. ^1H -NMR ($\text{C}_5\text{D}_5\text{N}$, 500 MHz): 4.53 (dd , $J = 4.86$, 8.29, H-C(2)); 2.40 (m , 2 H-C(3), 2 H-C(4)). ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$, 125 MHz): 178.0 (s , C(5)); 175.9 (s , COOH); 56.5 (d , C(2)); 30.2 (t , C(4)); 25.8 (t , C(3)). FAB-MS (neg.): 128 ($[M - \text{H}]^-$, $\text{C}_5\text{H}_6\text{NO}_3^-$).

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