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Cyclopentapeptides from *Dianthus chinensis*‡

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A new cyclopentapeptide dianthin I (1), together with two known ones pseudostellarin A (2) and heterophyllin J (3), was isolated from the aerial parts of *Dianthus chinensis*. The structure of 1 was elucidated as cyclo-(Gly[1]–L–Phe[2]–L–Pro[3]–L–Ser[4]–L–Phe[5]) on the basis of extensive spectroscopic analyses and chemical methods.

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Keywords: cyclopentapeptides; Caryophyllaceae; *Dianthus chinensis*; dianthin I

Introduction

Plant cyclopeptides, mainly formed with the peptide bonds of 2–37 protein or non-protein amino acids, have been divided into eight types based on their structures and distributions in plants. Among them, Caryophyllaceae-type cyclopeptides (CPs/type VI) were the largest type with about 200 CPs, which are homomonocyclopeptides with mainly five to twelve α-amino acid residues, isolated from over 40 higher plants [1].

The genus *Dianthus* plants (Caryophyllaceae) are widely used as garden plants in Europe and Asia [2]. The aerial parts of *D. chinensis* L., as well as *D. superbus* L., have been cited as a traditional Chinese medicine (TCM) Dianthi Herba (Chinese name 'Qu-Mai') in Chinese Pharmacopoeia (2010 Version), and are widely used for the treatment of diuresis and strangury [3]. CPs are one of the chemical markers of this family [1]. Some CPs isolated from *D. susperbus* have exhibited cytotoxic and proliferative activities [4–8]. Eight saponins [9–11] and one monosaccharide [12] have been reported from *D. chinensis*. Herein we report a new cyclopentapeptide dianthin I (**1**) and two known ones pseudosterllarin A (**2**) [13] and heterophyllin J (**3**) [14] isolated from the aerial parts of *D. chinensis* (Figure 1). All compounds were tested for cytotoxicity and antibacterial activities, but were found to be inactive. The structural elucidation showed as follows.

Materials and Methods

General Experimental Procedures

Melting points were obtained on an X-4 micromelting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were measured using a Chirascan spectrophotometer. IR spectra were obtained on a Tenor 27 spectrophotometer with KBr pellets. 1D and 2D NMR spectra were run on a Bruker AV-600 or Bruker DRX-500 spectrometer with TMS as internal standard. Mass spectra were recorded on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar TOF spectrometer. LC-MS spectra were run on a Waters Xevo TQ-S MS spectrometer or a Waters Xevo TQD mass spectrometer. Analytical or semi-preparative HPLC was performed on an Agilent 1100 with the Zorbax Eclipse-C_{18} (4.6 mm × 150 mm; 9.4 mm × 250 mm; 5 μm). Column chromatographies were performed on silica gel (200–300 mesh, Qingdao Yu-Ming-Yuan Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Uppsala, Sweden), or Lichroprep RP-18 (40–63 μM, Merck, Darmstadt, Germany). Fractions were monitored by TLC (GF254, Qingdao Yu-Ming-Yuan Chemical Co. Ltd., Qingdao, China), and spots were detected by spraying with ninhydrin reagent for cyclopeptides [15].

Plant Material

The aerial parts of *D. chinensis* were collected from Kunming Institute of Botany, Yunnan Province, PRC, in May, 2009. The material was identified by Prof. Zhe-Kun Zhou at Kunming Institute of Botany. The voucher specimen (No. 0270133) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered aerial parts of *D. chinensis* (75.0 kg) were extracted with 95% MeOH (4 × 100 L). After removal of the solvent under reduced pressure, the MeOH extract (21.0 kg) was suspended in H_2O and partitioned successively with EtOAc and

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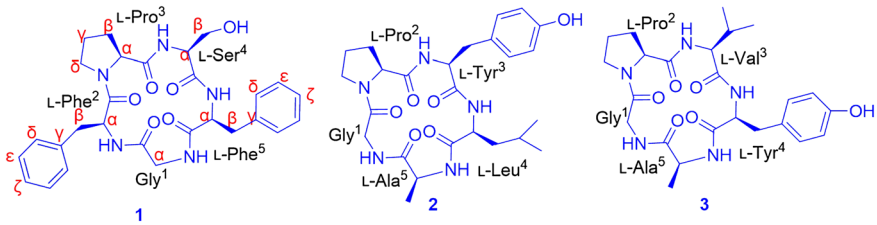

Figure 1. Structures of compounds **1–3**.

n-BuOH, giving an EtOAc-soluble portion (6.4 kg) and an *n*-BuOH-soluble portion (8.0 kg). The EtOAc fraction (6.4 kg) was subjected to silica gel column chromatography eluting with $CHCl_3$/MeOH (1:0, 95:5, 9:1, 8:2, 0:1) to afford a CP-containing fraction (1.2 kg, $CHCl_3$/MeOH, 95:5, 9:1, 8:2) and a CP-non-containing fraction (2.5 kg, $CHCl_3$/MeOH, 1:0, 0:1). The CP-containing fraction was loaded onto a silica gel column and eluted with gradient $CHCl_3/CH_3COOC_2H_5$ (30:1–1:1). Seven fractions (I–VII) were obtained, and fractions III contained CPs. Fraction III (100.3 g) was subjected to silica gel column ($CHCl_3/Me_2CO$ 10:1, 5:1), and sub-fraction III-3 (30.0 g) was subjected to Lichroprep RP-18 (MeOH/H_2O, 70:30–100:0), then Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), and further purified by HPLC (Eclipse XDB-C_{18}, 90 μM, 9.4 mm × 250 mm, 2.0 mL/min, UV detection at 205, 215, 230, 254, and 280 nm) eluting with 60–70% CH_3CN which contained 0.4‰ TFA to get **1** (21.1 mg). Sub-fraction III-4 (13.7 g) was subjected to CC on silica gel ($CHCl_3$/MeOH, 14:1) to get **2** (22.6 mg) and **3** (20.0 mg).

*Dianthin I (**1**)*

$C_{28}H_{33}N_5O_6$, colorless powder, mp. 157–160 °C; [α]21.4 D −153.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (1.85) nm; CD (MeOH) 205 (Δε −34.6), 214 (Δε −37.2); IR (KBr) ν_{max} 3423, 2927, 1644, 1531, 1452, 1204, 1134, 749, 702 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data see Table 1; positive ESI-MS *m/z* 558 $[M + Na]^+$; positive HREI-MS, *m/z* 535.2419 $[M]^+$ (calcd for $C_{28}H_{33}N_5O_6$, 535.2431).

*Pseudostellarin **A** (**2**)*

$C_{25}H_{35}N_5O_6$, colorless powder. mp. 151–152 °C; [α]21.4 D −201.0 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) 202 (1.91), 278 (0.88) nm; CD (MeOH) 200 (Δε −50.4), 206 (Δε −49.1); IR (KBr) ν_{max} 3428, 2959, 2930, 1664, 1517, 1452, 1203, 1139, 835, 802, 721 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data see Table 2; positive ESI-MS *m/z* 524 $[M + Na]^+$, 1025 $[2M + Na]^+$; positive HREI-MS, *m/z* 501.2582 $[M]^+$ (calcd for $C_{25}H_{35}N_5O_6$, 501.2587).

*Heterophyllin J (**3**)*

$C_{24}H_{33}N_5O_6$, colorless powder. mp. 147–151 °C; [α]21.6 D −328.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 202 (2.11), 222 (1.83), 278 (1.00) nm; CD (MeOH) 205 (Δε −77.3), 215 (Δε −63.9); IR (KBr) ν_{max} 3387, 2967, 1659, 1516, 1450, 1202, 1137, 834, 721 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data see Table 2; positive ESI-MS *m/z* 488 $[M + H]^+$; positive HREI-MS, *m/z* 487.2438 $[M]^+$ (calcd for $C_{25}H_{35}N_5O_6$, 487.2431).

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

Assignment		^1H NMR δ_H (int. mult, *J* (Hz))	^{13}C NMR δ_C
Gly[1]			
	α	3.69 (1H, d, 16.1)	43.9 (CH_2)
		4.84 (1H, dd, 16.1, 9.5)	
	NH	8.17 (1H, d, 9.5)	
	C=O		171.1 (C)
L-Phe[2]			
	α	5.40 (1H, dd,13.6, 8.8)	56.1 (CH)
	βa	3.45 (1H, dd, 13.6, 7.5)	40.3 (CH_2)
	βb	3.94 (1H, dd, 13.6, 7.5)	
	γ		138.7 (C)
	δ	7.57 (2H, overlap)	130.5 (CH)
	ε	7.42 (2H, t, 7.5)	129.2 (CH)
	ζ	7.25 (1H, overlap)	127.2 (CH)
	NH	8.68 (1H, d, 8.8)	
	C=O		171.6 (C)
L-Pro[3]			
	α	4.46 (1H, d, 7.6)	62.0 (CH)
	βa	1.75 (1H, overlap)	32.5 (CH_2)
	βb	2.05 (1H, m)	
	γa	1.50 (1H, m)	22.1 (CH_2)
	γb	1.75 (1H, overlap)	
	δa	3.41 (1H, overlap)	49.0 (CH_2)
	δb	3.61(1H, dd, 10.8, 7.6)	
	C=O		175.9 (C)
L-Ser[4]			
	α	5.09 (1H, dd, 13.0, 6.5)	58.0 (CH)
	β	4.25 (2H, t, 6.5)	61.9 (CH_2)
	NH	8.66 (1H, d, 6.5)	
	C=O		174.1 (C)
L-Phe[5]			
	α	4.30 (1H, overlap)	58.4 (CH)
	βa	3.76 (1H, d, 11.2)	35.6 (CH_2)
	βb	3.89 (1H, dd, 11.2, 3.7)	
	γ		140.4 (C)
	δ	7.45 (2H, d, 7.5)	130.4 (CH)
	ε	7.30 (2H, t, 7.5)	129.0 (CH)
	ζ	7.25 (1H, overlap)	126.9 (CH)
	NH	10.86 (1H, d, 6.9)	
	C=O		171.7 (C)

^1H NMR were recorded at 500 MHz, and ^{13}C NMR were recorded at 125 MHz.

Configuration Analysis of 1–3 (Advanced Marfey's Method [16])

Compounds **1–3** (1 mg each) were separately dissolved in 6 N HCl (1 mL) in a sealed glass tube and incubated at 115 °C for 24 h. The hydrolyzate was divided into two portions (0.5 mg each), then dried. Each hydrolyzate was dissolved in 100 μL of acetone, 1 M $NaHCO_3$ (20 μL), and 1% 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L- or D-FDLA, Marfey's reagent, Sigma Aldrich, 100 μL) were added.

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Table 2. ^{1}H-NMR and ^{13}C-NMR data for **2** and **3** in pyridine-*d*5 (δ in ppm, *J* in Hz)

		2				**3**	
Assignment		δ_H (int. mult, *J* (Hz)	δ_C	Assignment		δ_H (int. mult, *J* (Hz)	δ_C
Gly[1]				Gly[1]			
	α	4.06 (1H, brs)	42.6 (CH_2)		α	3.99 (1H, dd, 12.3, 3.9)	42.9 (CH_2)
		4.30 (1H, d, 13.4)				4.43 (1H, dd, 12.3, 3.9)	
	NH	9.44 (1H, overlap)			NH	8.84 (1H, brs)	
	C=O		170.0 (C)		C=O		169.6 (C)
L-Pro[2]				L-Pro[2]			
	α	4.52 (1H, brs)	62.7 (CH)		α	4.59 (1H, overlap)	63.1 (CH)
	β	1.26 (1H, m)	30.3 (CH_2)		β	2.04 (1H, m)	30.4 (CH_2)
		1.90 (1H, overlap)				2.12 (1H, m)	
	γ	1.60 (1H, overlap)	25.3 (CH_2)		γ	1.70 (1H, m)	25.6 (CH_2)
		1.93 (1H, overlap)				1.98 (1H, m)	
	δ	3.35 (1H, m)	48.2 (CH_2)		δ	3.34 (1H, dd, 13.6, 5.9)	47.7 (CH_2)
		4.23 (1H, brs)				3.93 (1H, m)	
	C=O		172.7 (C)		C=O		173.1 (C)
L-Tyr[3]				L-Val[3]			
	α	5.32 (1H, dd, 17.5, 9.8)	57.4 (CH)		α	4.59 (1H, overlap)	61.6 (CH)
	β	3.48 (2H, m)	37.8 (CH_2)		β	2.40 (1H, m)	31.4 (CH)
	γ		129.1 (C)		γ	0.95 (3H, d, 5.6)	19.6 (CH_3)
	δ	7.43 (2H, d, 8.0)	131.5 (CH)			1.03 (3H, d, 5.6)	20.4 (CH_3)
	ε	7.14 (2H, t, 8.0)	116.6 (CH)		NH	8.03 (1H, d, 7.0)	
	ζ		158.1 (C)		C=O		173.0 (C)
	NH	8.41 (1H, brs)		L-Tyr[4]			
	C=O		173.2 (C)		α	4.84 (1H, overlap)	59.5 (CH)
L-Leu[4]					β	3.47 (1H, dd, 11.3, 7.5)	37.2 (CH_2)
	α	4.65 (1H, brs)	56.2 (CH)			3.59 (1H, dd, 11.3, 7.5)	
	β	1.88 (1H, overlap)	40.4 (CH_2)		γ		129.0 (C)
		2.26 (1H, m)			δ	7.34 (2H, d, 7.0)	131.4 (CH)
	γ	1.70 (1H, overlap)	25.6 (CH)		ε	7.14 (2H, d, 7.0)	116.8 (CH)
	δ	0.83 (3H, d, 6.5)	22.3 (CH_3)		ζ		158.2 (C)
		0.85 (3H, d, 6.5)	23.5 (CH_3)		NH	9.49 (1H, overlap)	
	NH	9.47 (1H, overlap)			C=O		172.6 (C)
	C=O		173.6 (C)	L-Ala[5]			
					α	4.84 (1H, overlap)	50.7 (CH)
L-Ala[5]	α	4.95 (1H, d, 7.0)	51.1 (CH)		β	1.61 (3H, d, 5.9)	17.4 (CH_3)
	β	1.59 (3H, d, 7.0)	18.1 (CH_3)		NH	9.51 (1H, overlap)	
	NH	9.21 (1H, brs)			C=O		174.6 (C)
	C=O		175.5 (C)				

^{1}H NMR were recorded at 500 MHz, and ^{13}C NMR were recorded at 125 MHz.

The mixture was incubated at 45 °C for 2 h. The reaction was quenched by adding 2 N HCl (10 μL) after being cooled, and the dried mixture was dissolved in 50% aqueous CH_3CN (600 μL). Ten microliters of each solution of FDLA derivatives was analyzed by LC-MS.

The analysis of the L- and D,L-FDLA (mixture of D- and L-FDLA) derivatives of **1** was performed using an Agilent Eclipse XDB-C_{18} column (4.6 × 150 mm, 5 μm) maintained at 40 °C. Acetonitrile–1‰ HCOOH/H_2O was used as the mobile phase under a linear gradient eluent (acetonitrile, 10%–70%, 50 min) at a flow rate of 1 mL/min. A Waters Xevo TQ-S mass spectrometer was used for detection in ESI (negative) mode. The capillary voltage was kept at 2.5 KV and the ion source at 350 °C. Nitrogen gas was used as a sheath gas at 400 L/h. A mass range of *m/z* 100–1000 was scanned in 0.2 s. The LC-MS analysis of **2–3** was performed using a Waters SunFire^{TM}C_{18} 5 μm (4.6 × 150 mm, 5 μm) maintained at 35 °C. Acetonitrile–1‰ HCOOH/H_2O was used under a linear gradient eluent (acetonitrile, 20%–80%, 40 min) at a flow rate of 1 mL/min. A Waters Xevo TQD mass spectrometer was used for detection in ESI (negative) mode. The capillary voltage was kept at 3.5 KV and the ion source at 450 °C. Nitrogen gas was used as a sheath gas at 750 L/h. A mass range of *m/z* 200–1400 was scanned in 0.3 s.

Results and Discussion

Air-dried and powdered aerial parts of *D. chinensis* were extracted with 95% MeOH. The CP-containing fraction was fractionated on a silica gel column eluted with increasingly polar mixtures of $CHCl_3$/MeOH, and used the thin layer chromatography (TLC) detection method from the crude extracts. Further purification was achieved by chromatographies on Sephadex LH-20 ($CHCl_3$/MeOH), RP-18 (MeOH/H_2O), and HPLC (CH_3CN/H_2O containing 0.4‰ TFA or 1‰ HCOOH), yielding 3 cyclopeptides (**1–3**).

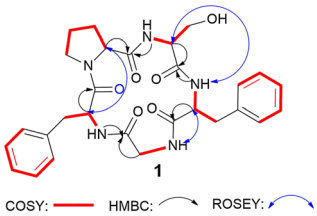

Figure 2. Key 2D NMR correlations of **1**.

Compound **1** was obtained as colorless powder, [α]21.4 D −153.0 (*c* 0.10, MeOH), which had the molecular formula $C_{28}H_{33}N_5O_6$ by HREI-MS (*m/z* 535.2419 $[M]^+$), indicating 15 degrees of unsaturation. The IR spectrum indicated the presence of amino (3423 cm^{-1}), amide (1644 cm^{-1}), and phenol ether (1204 and 1134 cm^{-1}) groups. The ^{13}C NMR spectrum revealed that there were five carbonyls (δ_C 171.1, 171.6, 171.7, 174.1, 175.9), twelve phenyl carbons (126.9, 129.0, 129.0, 130.4, 130.4, 140.4; 127.2, 129.2, 129.2, 130.5, 130.5, 138.7), four α-amino methines (δ_C 56.1, 58.0, 58.4, 62.0), and seven methylenes (δ_C 22.1, 32.5, 35.6, 40.3, 43.9, 49.0, 61.9). The ^1H NMR spectrum presented typical four NH (δ_H 8.17, 8.66, 8.68, 10.86), two mono-substitued phenyl groups (7.45, 7.45, 7.30, 7.30, 7.25; 7.57, 7.57, 7.42, 7.42, 7.25), and six α-H (δ_H 3.69, 4.84; 4.30, 4.46, 5.09, 5.40). These data suggested that **1** was a cyclic pentapeptide, which contained one proline, two phenylalanines, one serine, and one glycine. Correlations of Phe^2 NH to Gly^1 C=O, Ser^4 NH to Pro^3 C=O, and Phe^5 NH to Ser^4 C=O in the HMBC spectrum indicated the connections of Gly^1–Phe^2 and Pro^3–Ser^4–Phe^5. Interactions between Gly^1 NH and Phe^5 αH, and Phe^2 αH and Pro^3 αH observed in the ROESY spectrum indicated the linkages of Phe^5–Gly^1 and Phe^2–Pro^3 (Figure 2). Thus, the planar structure of **1** was determined as Cyclo-(Gly^1–Phe^2–Pro^3–Ser^4–Phe^5). Amino acid analysis of the hydrolysates of **1** showed L-Ser (11.96/398, Rt/ESI^--MS), L-Pro (16.34/408, Rt/ESI^--MS), and L-Phe (24.85/458) according to the advanced Marfey's method. Therefore **1** was elucidated as cyclo-(Gly^1-L-Phe^2-L-Pro^3-L-Ser^4-L-Phe^5) and named as dianchin I.

Conclusions

A new cyclopentapeptide dianthin I (**1**) was isolated from the aerial parts of *D. chinensis* L. Its structure was identified by extensive NMR and MS analysis, and the absolute configuration was determined by the advanced Marfey's method. This finding expands the cyclopeptide diversity in *D. chinensis*.

Acknowledgements

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Supporting Information

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