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CYCLOPEPTIDES FROM *DIANTHUS SUPERBUS*

YOU-CHU WANG, NING-HUA TAN, JUN ZHOU* and HOU-MING WU†

Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, P.R. China; †State Key Laboratory of Bio-Organic Chemistry and Natural Products, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, 200032, P.R. China

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Key Word Index—*Dianthus superbus*; Caryophyllaceae; cyclopeptides; dianthin A and B.

Abstract—Two new cyclic hexapeptides, dianthin A(**1**) and B(**2**), were isolated from the whole plants of *Dianthus superbus*. Their structures were established as cyclo-(Ala-Tyr-Asn-Phe-Gly-Leu) and cyclo-(Ile-Phe_2-Phe_1-Pro_2-Gly-Pro_1) by IR, UV 2D NMR and FABMS spectra. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Dithanus superbus L. (Caryophyllaceae), which is widely distributed in China, is commonly used as a traditional Chinese medicine for a diuretic and anti-inflammatory agent in the treatment of urinary infections, carbuncles and carcinoma of the oesophagus [1]. Since little chemical research has been published on it [2–4] and in a series of investigations on Caryophyllaceae cyclopeptides [5–7], in this paper we describe the isolation and structural determination of two new cyclopeptides named dianthin A(**1**) and dianthin B(**2**) from whole plants.

RESULTS AND DISCUSSION

Two new cycle hexapeptides, dianthin A(**1**) and dianthin B(**2**), were isolated from the ethyl acetate fraction of the ethanol extract of *Dianthus superbus* whole plants by column chromatography as described in the Experimental.

Dianthin A(**1**), cubic crystals, was negative to ninhydrin reagent but positive after hydrolysis with 6 M HCl. Its molecular formula was assigned as $C_{33}H_{43}N_7O_8$ (NMR and HR-FABMS) ($[M+1]^+$ at m/z 666.3223, ∇ 2.8 mDa). The IR spectrum exhibited intense N-H and C═O absorptions at 3300 and 1650 cm^{-1}, respectively. The ^{13}C NMR spectrum showed the presence of seven amide carbonyls (δ 174.1, 174.1, 173.0, 172.5, 172.2, 171.7, 170.4) and six methines (δ 58.1, 57.0, 53.0, 52.1, 49.9, 44.9) in the range δ 45–65 [8]. These results suggested that **1** was a cyclopeptide.

The six amino acid residues were identified by 2D NMR techniques. The 600 MHz ^1H NMR spectrum clearly showed the presence of eight amide protons at δ 9.84, 9.58, 9.51, 9.03, 8.77, 8.58, 7.83. Using TOCSY spectrum it was found that these amide protons constituted six independent spin systems [A_3X(NH), $3\times$AMX(NH), AX(NH), A_3B_3MPT(NH)], respectively. By following these spin systems of the eight amide protons using ^1H–^1H COSY, TOCSY, ^1H–^{13}C COSY, COLOC, these amino acid residues were deduced to be alanine, tyrosine, phenylalanine, asparagine, glycine and leucine units. The M_r of these amino acid residues was identical with those observed in the FABMS.

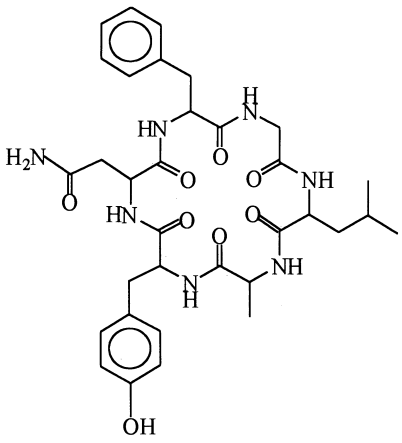
Evidence for the linkage of the amino acid residues was provided by the ROESY and COLOC correlations summarized in Fig. 1, and gave rise to the structure of **1** as cyclo-(Ala-Tyr-Asn-Phe-Gly-Leu). The proposed structure was further confirmed by FABMS.

Unambiguous assignments of the ^1H and ^{13}C NMR signals (Tables 1 and 2) of **1** were carried out by means of 2D NMR techniques including ^1H–^1H COSY, TOCSY, ROESY, ^1H–^{13}C COSY and COLOC.

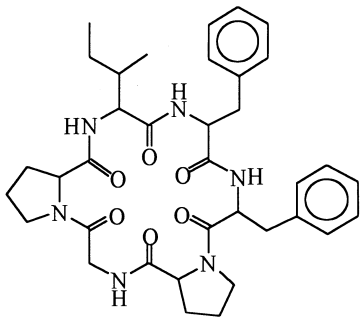
Dianthin B(**2**), amorphous powder, was negative to ninhydrin reagent but positive after hydrolysis with 6 M HCl. Its molecular formula was assigned as $C_{36}H_{46}N_6O_6$ (NMR and HR-FABMS) ($[M+1]^+$ at m/z 659.3494, ∇, 6.3 mDa). The IR spectrum exhibited intense N-H and C═O absorptions at 3300 and 1650 cm^{-1}, respectively. The ^{13}C NMR spectrum showed the presence of six amide carbonyls (δ 172.8, 172.8, 172.7, 171.4, 170.9, 168.6) and six methines (δ 62.4, 61.4, 60.4, 55.3, 53.8, 42.8) in the range δ 45–65 [8]. These results suggested that **2** was a cyclopeptide.

The six amino acid residues were identified by 2D NMR techniques. The 600 MHz ^1H NMR spectrum

* Author to whom correspondence should be addressed.



dianthin A(**1**)



dianthin B(**2**)

clearly showed the presence of four amide protons at δ 10.35, 9.79, 8.24, 8.22. Using TOCSY spectrum it was found that these amide protons constituted four independent spin systems including [$A_3MPT(B_3)X(NH)$, $2\times AMX(NH)$, $AX(NH)$]. By following these spin systems of the four amide protons using TOCSY, ROESY and HMQC, these amino acid residues were revealed to be one isoleucine, two phenylalanines and one glycine unit. Extensive analyses of 2D NMR spectra (TOCSY, ROESY and HMQC) led to the conclusion that the remaining 2D NMR signals constituted of two independent spin systems [$2\times A_2(T_2)MPX(NH)$] of the type (X-CH-CH_2-CH_2-CH_2-X) typical of proline. The M_r of these amino acid residues was identical with that observed in FABMS, so the cyclopeptide was composed of Ile (1 eq), Phe (2 eq), Gly (1 eq) and Pro (2 eq), also a cyclic hexapeptide.

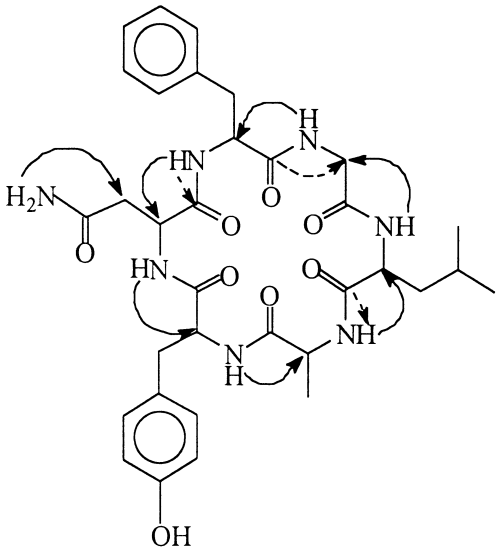


Fig. 1. Selected ROESY (→) and COLOC (--→) for dianthin A(**1**) in pyridine-d_5.

The sequence of these amino acid residues was determined by ROESY and FABMS. The compound gave several useful fragments at *m/z* 512 $[M\text{-Phe}]^+$, 365 $[M\text{-2Phe}]^+$, 252 $[M\text{-2Phe-Ile}]^+$, 155 $[M\text{-2Phe-Ile-Pro}]^+$ in FABMS. The ROESY correlations summarized in Fig. 2 suggested the structure of **2** as cyclo-(Ile-Phe_2-Phe_1-Pro_2-Gly-Pro_1).

Unambiguous assignments of the ^1H and ^{13}C NMR signals (Tables 1 and 2) of **2** were carried out by means of 2D NMR techniques including TOCSY, ROESY and HMQC.

EXPERIMENTAL

General

Mp (uncorr.) were determined on a Koffler apparatus; the optical rotations were measured with a Horiba Sepa-300 polarimeter; IR spectra were recorded on a Perkin-Elmer 577 instrument; UV spectra were recorded on a Shimadzu UV-210A spectrometer; NMR spectra were measured on Varian INOVA-600 and Bruker AM-400 instruments; FABMS spectra were recorded on a VG Auto Spec-3000 spectrometer.

Plant material

The whole plants of *Dianthus superbus* L. were bought from Yunnan Province Drug Company in April 1995.

Extraction and isolation

The whole plants of *Dianthus superbus* L. (15 kg) were extracted with 95% EtOH for 5 times. Removal of the solvent under red. pressure are yielded a syrup. The syrup was suspended in H_2O, extracted with EtOAc and *n*-butanol, respectively The EtOAc extracts were concentrated *in vacuo* to afford a residue (260 g). The residue was subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH

Table 1. ^{1}H NMR data of dianthin A and B (600 MHz, pyridine-d_5)

		H_N	α	β	γ	δ
A	Ala	8.77 (*d*, 7.2)	4.83 (*m*)	1.66 (*d*, 6.9)		
	Tyr	9.51 (*d*, 5.3)	4.53 (*m*)	3.54 (*m*)	ArH:7.21 (*d* 8.4)	
				3.43 (*m*)	7.09 (*d*, 8.4)	
	Phe	9.03 (*d* 6.5)	4.97 (*m*)	3.62 (*m*)	ArH:7.47 (*d* 6.9)	
				3.58 (*m*)	7.19 (*m*)	
					7.13 (*m*)	
	Asn	9.58 (*br s*)	5.02 (*m*)	3.51 (*m*)		8.58 (*br s*)
				3.38 (*m*)		7.83 (*br s*)
	Gly	9.84 (*br s*)	4.44 (*dd*, 5.5, 14.7)			
			3.74 (*dd*, 5.5, 14.7)			
	Leu	9.32 (*d*, 8.0)	4.93 (*m*)	2.01 (*m*)	1.82 (*m*)	0.85 (*d*, 4.2)
				1.90 (*m*)		0.84 (*d*, 4.2)
B	Ile	8.24 (*d*, 10.4)	4.77 (*t*, 10.2)	2.09 (*m*)	1.59 (*m*) 1.23 (*m*)	0.79 (*t*, 7.4)
					0.72 (*d*, 6.5)	
	Phe_2	8.22 (*d*, 10.0)	5.34 (*dd*, 8.3, 14.8)	3.39 (*m*)	ArH:7.24–7.23 (*m*)	
				3.14 (*m*)		
	Phe_1	10.35 (*br s*)	5.21 (*m*)	3.23 (*m*)	ArH:7.26–7.24 (*m*)	
				3.16 (*m*)		
	Pro_2		3.71 (*m*)	2.34 (*m*)	1.53 (*m*)	3.67 (*m*)
				1.55 (*m*)	1.17 (*m*)	
	Gly	9.79 (*dd*, 2.6, 8.7)	4.72 (*m*)			
			3.88 (*m*)			
	Pro_1		4.58 (*m*)	1.99 (*m*)	1.88 (*m*)	3.31 (*m*)
					1.63 (*m*)	3.12 (*m*)

Coupling constants (Hz) are given in parentheses.

Table 2. ^{13}C NMR data of dianthin A and B (150 MHz, pyridine-d_5)

	C═O	α	β	γ	β	δ
A	Ala	174.1	49.9	16.6		
	Tyr	172.5	58.1	35.8	128.8	131.0
						116.3
						157.7
	Phe	172.2	57.0	37.9	139.1	130.0
						128.7
						126.7
	Asn	171.7	52.1	37.3	174.1	
	Gly	170.4	44.9			
	Leu	173.0	53.0	41.2		
B	Ile	172.7*	60.4	36.3	15.6	10.2
					25.9	
	Phe_2	171.4*	53.8	38.8	137.9	130.0
						129.1
						127.5
	Phe_1	170.9*	55.3	38.1	136.5	129.9
						128.6
						126.7
	Pro_2	172.8*	61.4	22.4	31.0	47.2
	Gly	168.6*	42.8			
	Pro_1	172.8*	62.4	30.0	25.2	46.4

* Assignments might be interchangeable.

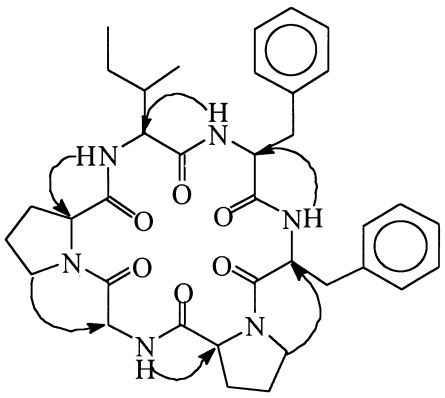


Fig. 2. Selected ROESY for dianthin B(**2**) in pyridine-d_5.

(0–30%) successively to give three fractions (Fr. I, Fr. II and Fr. III). Fr. II (175 g) was further purified by a combination of column chromatography on silica gel. Dianion HP-20, RP-18, RP-HPLC, etc. to give dianthin A (**1**, 35 mg) and dianthin B (**2**, 7 mg).

Dianthin A(**1**)

2.3×10^{-4}%, $C_{33}H_{43}N_7O_8$, cubic crystals (CH_3Cl–CH_3OH), ninhydrin reaction(−), mp 205–208°, $[\alpha]_D^{25}$ −38.6° (CH_3OH, *c* 0.290); UV λ_{max}^{MeOH} nm (log ε): 278 (2.76), 222 (3.07), 203 (3.51); IR ν_{max}^{KBr} cm^{-1}: 3300, 1650, 1500, 1230; FABMS *m/z* (rel. int.): 666 $[M+1]^+$ (base peak), 503 (4), 532 (3), 319 (5), 262 (8), 136 (32), 120 (42), 86 (60); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Dianthin B(**2**)

4.7×10^{-5}%, $C_{36}H_{46}N_6O_6$, amorphous powder, ninhydrin reaction(−), $[\alpha]_D^{25}$ −167.30 (CH_3OH, *c* 0.263), UV λ_{max}^{MeOH} nm (log ε): 206 (3.67); IR ν_{max}^{KBr} cm^{-1}: 3240, 1630; FABMS *m/z* (rel. int.): 659 $[M+1]^+$ (30), 512 (3), 365 (3), 252 (4), 155 (12), 120 (31), 70 (base peak); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

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