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多荚草中的新环肽

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摘要: 从石竹科植物多荚草(*Polycarpon prostratum* (Forssk.) Aschers. et Schwein. *ex* Aschers)中分离得到2个新的环肽化合物polycarponin B和C(**1**,**2**)。它们的结构通过波谱方法分别鉴定为:cyclo(-Gly$_1$-Ile-Val$_1$-Leu$_1$-Val$_2$-Gly$_2$-Leu$_2$-Pro)和cyclo(-Pro$_1$-Thr-Leu$_1$-Pro$_2$-Pro$_3$-Val-Leu$_2$-Phe)。

关键词: 多荚草; 石竹科; 环肽; 多荚草环肽
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Cyclopeptides from Polycarpon prostratum (Caryophyllaceae)

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Abstract: Two new cyclic peptides, polycarponin B and C (**1**, **2**), were isolated from the whole plants of *Polycarpon prostratum* (Forssk.) Aschers. *et* Schwein. *ex* Aschers. By detailed spectroscopic analysis, their structures were determined as cyclo(-Gly$_1$-Ile-Val$_1$-Leu$_1$-Val$_2$-Gly$_2$-Leu$_2$-Pro) and cyclo(-Pro$_1$-Thr-Leu$_1$-Pro$_2$-Pro$_3$-Val-Leu$_2$-Phe), respectively.

Key words: *Polycarpon prostratum*; Caryophyllaceae; cyclopeptide; polycarponin B and C

In a previous study of chemical constituents of *Polycarpon prostratum* (Forssk.) Aschers. *et* Schwein. *ex* Aschers[1-4], we have reported five new triterpenoid saponins (saikosaponin-like compounds) from n-BuOH soluble fraction and a new cyclopeptide (named polycarponin A) from EtOAc soluble fraction of this plant. As a part of our continuous studies on cyclopeptides from Caryophyllaceae[4-11], a further study on the EtOAc soluble fraction of this plant led to the isolation of two new cyclopeptides named polycarponin B and C. In this paper, we report the isolation and structure elucidation of these two compounds.

1 Results and Discussion

Polycarponin B (**1**) was obtained as colorless needles, and showed negative reaction to ninhydrin reagent, but positive after hydrolysis with 6 mol/L HCl. Its IR absorption bands at 3 290 and 1 640 cm^{-1} were characteristic of amino and amide carbonyl groups. The FABMS gave an $[M+H]^+$ ion at m/z 749 which together with the molecular formula of $C_{37}H_{64}N_8O_8$ derived from the HR-FABMS (found m/z 749.487 4 $[M+H]^+$, calcd m/z 749.492 5), indicated the presence of 10 degrees of unsaturation. The ^{13}C and ^1H-NMR spectra (Table 1) showed the presence of eight amide carbonyl signals and seven amide NH signals respectively, suggesting that **1** was a cyclopeptide.

The structure elucidation began with identification of the amino acid residues. By extensive analysis of the ^1H-^1H COSY, HMBC and HMQC spectra, the amino acid residues were shown to be two valines, two leucines, two glycines, one isoleucine and one proline, respectively. These amino acid residues accounted for the molecular weight observed in the FABMS. The sequence of these amino acids was elucidated on the basis of HMBC correlations. The HMBC correlations were summarized in Fig. 1. From the information obtained in the FABMS and the correlations between amide CO and NH in the HMBC spectrum, the structure of **1** was elucidated as cyclo(-Gly$_1$-Ile-Val$_1$-Leu$_1$-Val$_2$-Gly$_2$-Leu$_2$-Pro).

Polycarponin C (**2**) was obtained as colorless needles, and showed negative reaction to ninhydrin reagent, but positive after hydrolysis with 6 mol/L HCl. Its IR spectrum showed intense NH and CO absorptions at 3 300

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Table 1 ^{1}H and ^{13}C-NMR spectral data of polycarponin B (**1**) in pyridine-d_5

	CO	Cα	Cβ	Cγ	Cδ	H_N	Hα	Hβ	Hγ	Hδ
Gly_1	170.4	45.3				10.20 (br.s)	4.46 (dd) J = 15.8, 3.7 3.97 (dd) J = 15.5, 5.7			
Gly_2	169.5	44.9				9.53 (d) J = 7.0	4.79 (dd) J = 14.8, 7.0 3.81 (d) J = 14.5			
Pro	173.6	59.7	29.7	25.5	48.1		4.74 (ca.)	2.08 (m)	1.92 (m) 1.74 (m)	3.93 (m) 3.63 (m)
Ile	172.5	60.0	36.5	25.7 16.4	11.7	8.87 (d) J = 9.5	4.62 (m)	2.48 (m)	1.71 (m) 1.35 (m) 1.08 (d) J = 6.0	0.861 (t) J = 7.0
Val_1	172.1	62.2	29.7	19.0 19.9		8.42 (d) J = 6.0	4.46 (m)	2.50 (m)	1.10 (d) J = 6.0 1.11 (d) J = 6.0	
Val_2	172.9	62.6	31.0	19.2 19.5		9.47 (br s)	4.72 (ca.)	2.38 (m)	1.12 (d) J = 6.5 1.14 (d) J = 6.5	
Leu_1	171.6	49.8	41.3	25.2	22.2[a] 23.3[b]	7.73 (d) J = 7.0	5.24 (ca.)	1.97 (m) 1.75 (m)	1.72 (m)	0.97 (d) J = 5.6 1.01[c] (d) J = 6.3
Leu_2	173.3	53.8	42.6	25.2	22.3[a] 23.4[b]	8.69 (d) J = 8.5	5.16 (ca.)	2.07 (m) 1.92 (m)	1.92 (m)	1.13 (d) J = 5.8 1.06[c] (d) J = 6.0

Assignments with the same superscripts (a, b and c) may be interchanged.

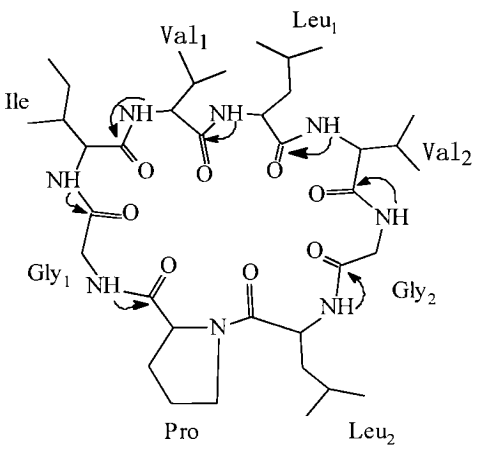

Fig. 1. Structure of polycarponin B (**1**); arrows show selected HMBC correlations.

and 1 650 cm^{-1}, respectively. The FABMS gave an $[M + H]^+$ ion at m/z 865 and the molecular formula $C_{45}H_{68}N_8O_9$ derived from the HR-FABMS ($[M + H]^+$ at m/z 865.525 0, calcd m/z 865.518 7), indicated the presence of 16 degrees of unsaturation. The ^{13}C and ^{1}H-NMR spectra (Table 2) showed the presence of eight amide carbonyl signals and five amide NH signals, respectively. From these facts, **2** was deduced to be a cyclopeptide.

Amino acid analysis[5] of the hydrolysate prepared from compound **2** with 6 mol/L HCl at 110 ℃ for 24 h in a sealed tube revealed the presence of three prolines, two leucines, one phenylalaine, one threonine and one valine, which was confirmed by analysis of the ^{1}H-^{1}H COSY spectrum. The NOESY and HMBC spectra provided the evidence for the linkage of amino acid residues (Fig. 2). The HMBC spectrum showed some correlations between amide CO and NH, indicating the presence of two peptide fragments (-Pro_3-Val-Leu_2-Phe-and-Pro_1-Thr-Leu_1-Pro_2-), and these two peptide fragments had to be linked in only one sequence. The NOESY spectrum also exhibited some important NOE correlations, indicating the presence of the peptide fragments (-Pro_3-Val-, Leu_2-Phe-Pro_1 and -Thr-Leu_1-). The combination of the results mentioned above gave rise to the structure of **2** as cyclo (-Pro_1-Thr-Leu_1-Pro_2-Pro_3-Val-Leu_2-Phe), and positive FABMS further confirmed the proposed structure.

Table 2 ^{1}H and ^{13}C-NMR spectral data of polycarponin C (**2**) in pyridine-d_5

	CO	Cα	Cβ	Cγ	Cδ	H_N	Hα	Hβ	Hγ	Hδ
Thr	171.2	62.4	67.0	22.3		7.40 (d)	4.55 (m)	4.64 (m)	1.36 (d)	
						J = 6.4			*J* = 6.0	
Phe	172.2	53.3	38.2	137.3	129.6	11.04 (d)	5.06 (m)	3.09 (m)		7.33 (m)
					129.2	*J* = 7.6		2.80 (m)		7.28 (m)
					127.5					7.15 (d)
										J = 7.2
Val	171.5	62.6	31.3	19.1		8.30 (d)	4.74 (m)	2.40 (m)	1.05 (d)	
				19.9		*J* = 9.2			*J* = 6.8	
									1.07 (d)	
									J = 7.2	
Leu_1	171.2	50.1	41.7	25.2	22.8	8.81 (d)	5.30 (m)	2.16 (ca.)	1.74 (ca.)	0.782 (d)
					22.9	*J* = 8.8				*J* = 5.6
										0.808 (d)
										J = 5.6
Leu_2	173.5	51.4	43.2	25.2	22.8	8.05 (d)	5.36 (m)	1.74 (ca.)	1.90 (ca.)	0.925 (d)
					23.2	*J* = 9.2				*J* = 6.8
										0.931 (d)
										J = 6.8
Pro_1	173.1	62.3	32.2	22.2	46.7		4.59 (d)	2.16 (ca.)	1.74 (ca.)	3.68 (ca.)
							J = 7.6	1.40 (m)		
Pro_2	171.1	61.6	31.3	22.2	46.7		4.49 (d)	1.90 (ca.)	1.64 (m)	3.68 (ca.)
							J = 7.6	2.72 (m)		3.55 (m)
Pro_3	170.8	59.9	28.8	25.5	47.9		4.35 (m)	1.90 (ca.)	2.16 (ca.)	4.08 (m)
								1.74 (ca.)	1.92 (ca.)	3.94 (m)

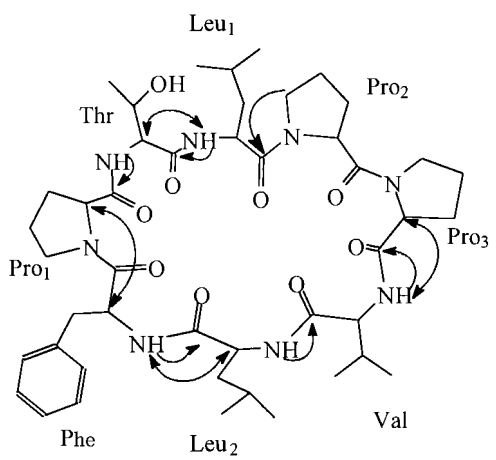

Fig. 2. Structure of polycarponin C (**2**)
Arrows (→) show selected HMBC correlations; arrows (↔) show selected NOESY correlations.

2 Experimental

2.1 Plant materials

The whole plants of *Polycarpon prostratum* (Forssk.) Aschers. *et* Schwein. *ex* Aschers were collected in Xishuangbanna, Yunnan Province, China, in July 1997, and identified by senior engineer WANG Hong. A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

2.2 Instruments and reagents

Melting points were determined on a Kofler block and uncorrected. Optical rotations were measured with a SEPA-300 polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer. NMR spectra were obtained on Bruker AM-400 and DRX-500 spectrometers. A VG Autospec-3000 spectrometer was used to record FABMS spectra. 200 - 300 mesh and 300 - 400 mesh silica gel and Diaion HP-20 were used for column chromatography.

2.3 Extraction and isolation

Plant material (17.8 kg) was extracted with hot ethanol four times to afford an EtOH extract that was suspended in water, extracted with EtOAc to afford an EtOAc residue (168 g), which was subjected to Diaion HP-20 column chromatography using a H_2O-MeOH gradient system (1∶0 - 0∶1). The fraction eluted with 70 % MeOH was further subjected to silica gel column chromatography ($CHCl_3$∶MeOH = 9∶1) to afford B (**1**, 53 mg) and C (**2**, 180 mg), respectively.

Polycarponin B (1) Colorless needles (MeOH). Mp 182 - 184 °C; $[\alpha]_D^{26}$ - 87.9° (*c* 0.44, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3 290 and 1 640. FABMS *m/z*: 749 $[M + H]^+$ (37), 636 (3), 537 (3), 424 (5), 325 (3), 268 (13), 155 (17), 86 (100). HR-FABMS $[M + H]^+$ at *m/z* 749.487 4 (calcd for $C_{37}H_{64}N_8O_8$, 749.492 5).

^{1}H, ^{13}C-NMR spectral data see Table 1.

Polycarponin C (2) Colorless needles (MeOH). Mp 183 - 186 ℃; $[\alpha]_D^{25}$ - 124.8° (*c* 0.57, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3 300 and 1 650. FABMS m/z: 865 [M + H]$^+$ (20), 554 (2), 407 (4), 294 (5), 195 (4), 120 (14), 70 (100); HR-FABMS [M + H]$^+$ at m/z 865.525 0 (calcd for $C_{45}H_{68}N_8O_9$, 865.518 7). ^{1}H, ^{13}C-NMR spectral data see Table 2.

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