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Leiocyclocin A and B, Two Cyclopeptides from *Goniothalamus leiocarpus*

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Abstract: Two new cyclopeptides, leiocyclocin A (**1**), B (**2**), were isolated from the seeds of *Goniothalamus leiocarpus*. Their structures were elucidated by means of spectral and chemical methods.

Keywords: Annonaceae, *Goniothalamus*, *G. Leiocarpus*, cyclopeptide, leiocyclocin A and B.

Ongoing phytochemical studies on *Goniothalamus* species have led to the isolation of a number of annonaceous acetogenins and unusual new styryllactones which were found to possess significant cytotoxic activities against several hunan tumour cell lines[1,2]. Firstly, cyclopeptides were reported to be isolated from genus *Goniothalamus* in this paper.

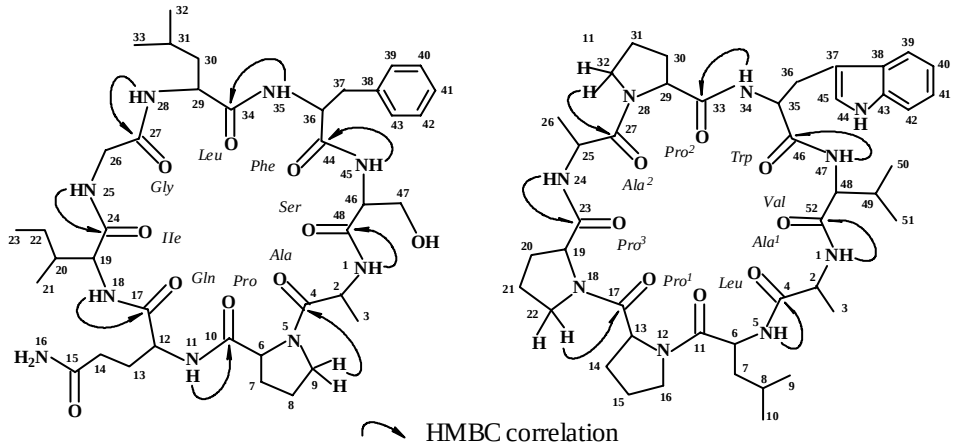
Goniothalamus leiocarpus is an evergreen tree which grows in the south of Yunnan, China. Four new anticancer styryllactones[3,4] from the stem bark and four known acetogenins[5] from the seeds of this spices were isolated. In our further investigation, we have isolated four new cyclopeptides from the seeds of *G. Leiocarpus* and identified the two of them in this paper. Their structures were elucidated by means of spectral and chemical methods.

410 g of the seeds of *Goniothalamus leiocarpus,* which were collected in the end of August in south of Yunnan province, were extracted with ethanol (500 mL×5) at room temperature. After removing the solvent at 50°C, 80 g of brown resin was obtained and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract (46 g) was subjected to silica gel chromatography eluting with gradient Petrol-EtOAc and EtOAc-MeOH. Further purification by silica gel chromatography gave two cyclopeptides, **1** (40 mg) and of **2** (36 mg), and their structures were identified as two octacyclopeptides.

Leiocyclocin A (**1**) was isolated as needles that gave a $[M+H]^+$ peak in the HRFABMS at *m/z* 814.4507 (calcd.814.4463) appropriate for a molecular formula of $C_{39}H_{59}N_9O_{10}$. Signals from 3.5 to 5.5 ppm and 7.5 to 10.5 ppm in the ^1H NMR spectrum (**Table 1**) showed the presence of protons belonging to methines (or methene) and NH groups, respectively, and the ^{13}C NMR spectra (**Table 1**) gave the presence of eight

carbonyls. Analysing of the ^{1}H, ^{13}C spectral data, HMQC-TOCSY and HMBC spectrum, **1** was identified to be composed by eight amino acid residues of alanine, proline, glutamine, isoleucine, glycine, phenylalanine and serine[6,7]. Meanwhile, amino acid analysis after hydrolyzing of **1** at 110°C with 6 mol/L HCl gave the result that the compound contained the amino acid residues of Ala (1eq), Pro (1eq), Gln (1eq), Ile (1eq), Gly (1eq), Phe (1eq), and Ser (1eq), which agreed with the analysis of the spectral data.

Figure 1 Structures of Leiocyclocin A(**1**) and B (**2**)



leiocyclocin A (**1**) leiocyclocin B (**2**)

Considering all 14 unsaturation degrees of the identified amino acid residues, the excess of one unsaturation degree for **1** demanded that there is another cycle in the molecule of **1**. In the chemical test, **1** showed negative reaction when tested with ninhydrin but positive after being hydrolyzed with concentrated HCl. Thus, **1** was a cyclopeptide.

Each α-methine (or methene) proton and NH proton of all amino acid residues in **1** was attributed by interpretation of the HMQC-TOCSY spectrum, and each carbonyl group of the identified amino acid residues was assigned by the correlation between each corresponding carbon of carbonyl and its α-CH proton (**Table 1**) in the HMBC spectrum (**Figure 1**). Finally, the amino acid sequence in **1** was determined by analysis of the correlation between the NH group protons and the carbon of carbonyl group of its neighbor amino acid residue in the HMBC spectrum, as shown in **Figure 1**.

The structure of **1** was supported by the cleavages in the FABMS spectrum as follows:

226 [Gln-Pro+H]$^{+}$, 339 [Ile-Gln-Pro+H]$^{+}$, 396 [Gly-Ile-Gln-Pro+H]$^{+}$, 509 [Leu-Gly-Ile-Gln-Pro+H]$^{+}$, 656 [Phe-Leu-Gly-Ile-Gln-Pro+H]$^{+}$, 743 [Ser-Phe-Leu-Gly-Ile-Gln-Pro+H]$^{+}$, 814 cyclo-[Ala-Ser-Phe-Leu-Gly-Ile-Gln-Pro+H]$^{+}$.

Leiocyclocin B (**2**) was isolated as needles that gave a [M+H]$^{+}$ peak in the HRFABMS at *m/z* 832.4724 (calcd.832.4721) appropriate for a molecular formula of $C_{43}H_{61}N_9O_8$ ([M]$^{+}$ 831). Eight signals from 4.0 to 5.4 ppm and several signals from 7.2 to 8.2 ppm in the ^{1}H NMR spectrum (**Table 1**) showed the presence of protons belonging

Table 1 ^{1}H (400MHz) and ^{13}C (100MHz) NMR Spectral Data of **1** and **2** (δ, ppm, J, Hz, in C_5D_5N)

Leiocyclocin A (**1**)					Leiocyclocin B (**2**)				
residue			H	C	Residue			H	C
Ala	NH	1	7.78 d, 6.5		Ala[1]	NH	1	8.19 d, 7.0	
	αCH	2	5.25 t, 6.5	49.11		αCH	2	3.98 q, 7.0,	51.35
	βCH_3	3	1.58 d, 6.5	17.17		βCH_3	3	1.68 d, 7.0	14.88
	CO	4		172.55		CO	4		171.12
Pro	N	5			Leu	NH	5	7.25 d, 10.2	
	αCH	6	4.94 t,	47.96		αCH	6	5.36 t, 10.2	48.94
	βCH_2	7	2.36 m; 1.98 m	25.57		βCH_2	7	1.50 t, 10.2;	43.30
	γCH_2	8	1.88 m; 1.22 m	25.39				1.25 t, 10.2	
	δCH_2	9	3.98 t	47.96		γCH	8	2.22 m	23.84
	CO	10		175.39		δCH_3	9	1.05 d, 6.4;	21.37
Gln	NH	11	8.22 br s			δCH_3	10	0.86 d, 5.6	23.84
	αCH	12	4.63 dd, 4.3, 10.5	56.88		CO	11		174.48
	βCH_2	13	2.35 m; 2.31 m	25.70	Pro[1]	N	12		
	γCH_2	14	2.82 ddd, 17.1, 8.0, 2.6;	31.86		αCH	13	4.41 dd, 10.4, 7.8	65.22
			2.75 ddd, 17.1, 10.1, 2.6			βCH_2	14	1.99 m; 1.91 m	30.08
	δCO	15		177.66		γCH_2	15	2.02 m	25.32
	εNH_2	16	10.53 d, 4.4; 9.01 br s			δCH_2	16	3.75 m; 3.39 m	46.98
	CO	17		172.85		CO	17		170.19
Ile	NH	18	8.21 br d, 10.2		Pro[3]	N	18		
	αCH	19	5.20 dd, 10.2, 2.6	58.18		αCH	19	4.93 dd, 4.5, 7.8	61.69
	βCH	20	2.69 m	36.45		βCH_2	20	2.02 m,	29.20
	γCH_2	21	1.94 m, 1.37 m	16.66		γCH_2	21	1.91 m	25.60
	γCH_3	22	1.00 d, 6.8	25.57		δCH_2	22	3.35 m	46.85
	δCH_3	23	0.92 d, 5.6	12.58		CO	23		171.04
	CO	24		172.35	Ala[2]	NH	24	7.31 d, .67	
Gly	NH	25	8.32 t, 5.9			αCH	25	4.08 q, 6.7	46.98
	αCH_2	26	4.47 dd, 16.9, 5.9;	44.48		βCH_3	26	0.94 d, 6.7	17.70
			3.81 dd, 16.9, 6.9			CO	27		172.31
	CO	27		169.68	Pro[2]	N	28		
Leu	NH	28	7.61 d, 9.6			αCH	29	4.58 dd, 6.6, 8.6	63.62
	αCH	29	5.48 td, 9.6, 3.3	50.33		βCH_2	30	2.40 m; 2.02 m	30.08
	βCH_2	30	1.95 m	43.74		γCH_2	31	1.68 m	25.32
	γCH	31	1.95 m	24.86		δCH_2	32	3.78 m, 3.38 m	47.90
	δCH_3	32	0.89 d, 6.0	20.97		CO	33		174.74
	δCH_3	33	0.90 d, 6.0	23.80	Trp	NH	34	7.52 t, 5.6	
	CO	34		177.28		αCH	35	5.06 dd, 10.9, 5.6	57.77
Phe	NH	35	10.48 d, 2.3			βCH_2	36	3.67 dd, 15.0, 5.6	25.95
	αCH	36	5.25 br t, 8.0	62.02		C	37		108.75
	βCH_2	37	3.41 dd, 14.0, 8.0;	37.08		C	38		128.44
			3.31 dd, 14.0, 8.0			CH	39	7.71 d, 8.0	113.02
	iC	38		137.21		CH	40	7.52 t, 8.0	122.75
	oCH	39, 43	7.20 m	128.90		CH	41	7.32 t, 8.0	120.20
	mCH	40 ,42	7.32 m	129.54		CH	42	7.80 d, 8.0	117.80
	pCH	41	7.20 m	127.29		C	43		137.55
	CO	44		172.55		NH	44	7.61 d, 5.6	
Ser	NH	45	8.21 br d, 7.0			CH	45	3.53 dd, 15.0, 5.6	125.64
	αCH	46	4.83 td, 7.0, 3.0	56.88		CO	46		172.75
	βCH_2	47	4.68 dd, 10.8, 3.0;	62.02	Val	NH	47	7.91 d, 10.0	
			4.17 dd, 10.8, 3.0			αCH	48	5.10 dd, 10.1, 3.0	58.46
	CO	48		170.90		βCH_2	49	2.92 dq, 6.9, 3.0	28.76
						γCH_3	50	1.01 d, 6.9	17.86
						γCH_3	51	1.12 d, 6.9	20.42
						CO	52		170.77

to methines (or methlene) and NH groups, respectively.

The ^{13}C NMR spectra (**Table 1**) gave the presence of eight carbonyls from *ca* 170 to 174 ppm. Analysing of the ^{1}H, ^{13}C spectral data, HMQC-TOCSY and HMBC spectrum, **2** was identified possessing eight amino acid residues of 2 alanine, 3 proline, 1 leucine, 1 tryptophan and 1 valine[8]. Among them, the presence of the residue of one tryptophan was indicated by the eight specified resonance signals, 108.75 (C), 113.02 (CH), 117.80 (CH), 120.20 (CH), 122.75 (CH), 125.37 (CH), 128.44 (C) and 137.55 (C) ppm in the ^{13}C NMR spectrum, and determined by the careful observation of the HMQC-TOCSY and HMBC spectrum of **2**. Meanwhile, amino acid analysis after hydrolyzing of **2** at 110°C with 6 mol/L HCl gave the result that the compound contained the amino acid residues of Ala (2eq), Pro (3eq), leu (1eq), val (1eq), which supported the analysis of the spectral data.

Each α-methine (or methlene in the Gly) proton and NH proton of all amino acid residues in **2** was attributed by the observation of the HMQC-TOCSY spectrum, and each carbonyl group of the identified amino acid residues was assigned by the analysis of the correlation between the corresponding carbonyl and its α-CH proton (**Table 1**) in the HMBC spectrum (**Figure 1**). Finally, the amino acid sequence was determined by analysis of the correlation between the NH protons and their neighbor carbon of the carbonyl groups of amino acid residues, in the HMBC spectrum, as shown in **Figure 1**.

The structure of leiocyclocin B was supported by the cleavages of **2** in the FABMS spectrum as follows:

284 $[Trp\text{-}Val\text{-}H]^+$, 381 $[Pro^2\text{-}Trp\text{-}Val\text{-}H]^+$, 452 $[Pro^2\text{-}Trp\text{-}Val\text{-}Ala^1\text{-}H]^+$, 565 $[Pro^2\text{-}Trp\text{-}Val\text{-}Ala^1\text{-}Leu\text{-}H]^+$, 665 $[Pro^2\text{-}Trp\text{-}Val\text{-}Ala^1\text{-}Leu\text{-}Pro^1+2H]^+$, 761 $[Pro^2\text{-}Trp\text{-}Val\text{-}Ala^1\text{-}Leu\text{-}Pro^1\text{-}Pro^3+H]$, 832 $[Pro^2\text{-}Trp\text{-}Val\text{-}Ala^1\text{-}Leu\text{-}Pro^1\text{-}Pro^3\text{-}Ala^2+H]^+$.

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