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Leiocyclocin C and D, two cyclopeptides from *Goniiothalamus leiocarpus*

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Two new cyclopeptides, leiocyclocin C (**1**) and D (**2**), were isolated from the seeds of *Goniiothalamus leiocarpus* (Annonaceae). Their structures were determined as cyclo-(Gly¹-Ser-Pro²-Tyr²-Gly²-Tyr¹-Pro¹-Pro³) and cyclo-(Gly¹-Leu-Pro¹-Gly²-Phe-Tyr-Pro²), respectively, by means of spectral and chemical methods.

1. Introduction

Goniiothalamus leiocarpus is an evergreen tree which grows in the south of Yunnan, China. Four styrylactones with anticancer activity have been reported [1] from its stem bark. In a further investigation, we obtained two cyclopeptides, leiocyclocin A and B [2], from the seeds of *G. leiocarpus*. In this paper, two cyclopeptides, leiocyclocin C (**1**) and D (**2**), are reported in minute quantity from the same plant. Their structures were elucidated by means of spectral and chemical methods.

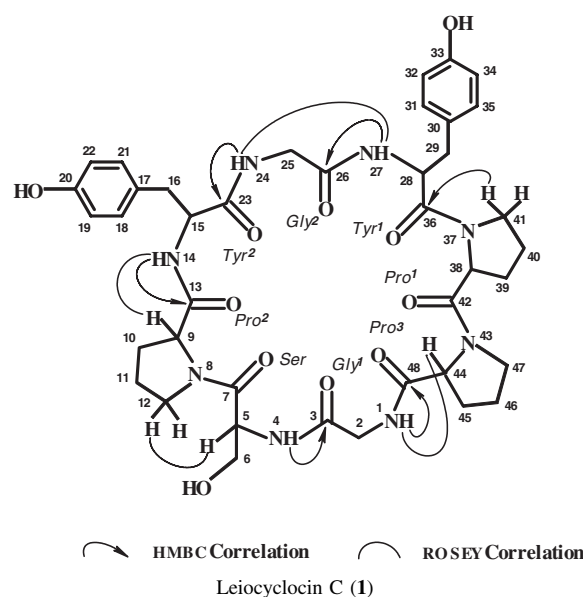
2. Investigations, results and discussion

Leiocyclocin C (**1**) was isolated as needles that gave a $[M+H]^+$ peak in the HRFABMS at m/z 819.3653 (calcd. 819.3677), appropriate for a molecular formula of $C_{40}H_{50}N_8O_{11}$ ($[M]^+$ 818). Signals from 4.5 to 5.5 ppm and 7.5 to 10.5 ppm in the ¹H NMR spectrum (Table) showed the presence of protons belonging to methines (or methylene) and NH groups, respectively, and the ¹³C NMR spectra (Table) gave the presence of eight carbonyls.

HMQC-TOCSY spectral data of **1** gave six amino acid residues involving 1 Ser, 2 Gly and 3 Pro. The presence of two other Tyr residues was indicated by the two resonance signals 158.11 (Ph-C-OH) and 157.62 (Ph-C-OH) ppm in the ¹³C NMR spectrum, and was confirmed by the correlation of 158.11/7.14 (d, 8.5 Hz), 7.31 (d, 8.5 Hz) ppm and 157.62/8.45 (d, 8.5 Hz), 7.29 (d, 8.5 Hz) in HMBC spectrum of **1**.

Considering all 19 unsaturation of identified amino acid residues, the excess of one unsaturation demanded that **1** had a different molecular structure. In chemical tests, **1** showed a negative reaction when tested with ninhydrin but not after being hydrolyzed with concentrated HCl. Thus, **1** was determined to be a monocyclic cyclopeptide consisting of the amino acid residues of 1 Ser, 2 Gly, 3 Pro, and 2 Tyr, and this molecular composition was consistent with the molecular ion peak at 819 m/z $[MH]^+$ in the FABMS spectrum of **1**.

Each α -methine (or methylene in the Gly) proton and NH proton of all the amino acid residues in **1** was attributed



by the examination of the HMQC-TOCSY spectrum, and each carbonyl group of the identified amino acid residues was assigned by the analysis of the correlations between the corresponding carbonyls and α -CH protons in the HMBC spectrum. Finally, the amino acid sequence in **1** was determined by analysis of the correlations between the NH protons and their neighbor carbonyl groups of the amino acid residue in HMBC, and correlations between NH protons and their neighbor α -H in the ROSEY spectra, as shown.

FABMS cleavages $[Pro^3-Gly^1]^+$ (m/z 155), $Pro^1-Pro^3-Gly^1$ (m/z 251), $[Pro^1-Pro^3-Gly^1-Ser+H]^+$ (m/z 339), and $[Pro^2-Tyr^2-Gly^2-Tyr^1-H]^+$ (m/z 479), $[Pro^2-Tyr^2-Gly^2-Tyr^1-Pro^1]^+$ (m/z 574), $[Ser-Pro^2-Tyr^2-Gly^2-Tyr^1-Pro^1]^+$ (m/z 664) supported the amino acid sequence derived from the NMR data of **1**.

Leiocyclocin D (**2**) was isolated as an amorphous solid that gave a $[M+H]^+$ peak in the HRFABMS at m/z 731.3600 (calcd. 731.3643) appropriate for a molecular

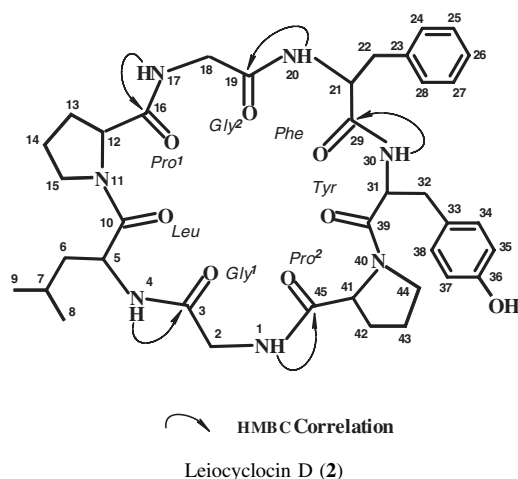
Table: ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1 and **2** (δ , ppm, Hz, in $\text{C}_5\text{D}_5\text{N}$)**

Leiocyclocin C (1)					Leiocyclocin D (2)				
Residue			H	C	Residue			H	C
Gly ¹	NH	1	10.25 dd, 9.0, 3.0		Gly ¹	NH	1	10.04 br d, 6.4	
	αCH ₂	2	4.97 dd, 16.1, 9.0; 3.78 dd, 16.1, 3.0	43.14		αCH ₂	2	5.00 dd, 17.2, 6.4 3.93 dd, 17.2, 3.2	43.77
	CO	3		168.98		CO	3		169.63
Ser	NH	4	8.65 d, 6.8		Leu	NH	4	9.06 d, 8.8	
	αCH	5	5.53 m	53.26		αCH	5	5.28 m	49.52
	βCH ₂	6	4.43 m; 4.25 m,	63.07		βCH ₂	6	1.88 m; 1.73 m	41.51
Pro ²	CO	7		174.05		γCH	7	1.73 m	25.21
	N	8				δCH ₃	8	0.95 d, 6.4	22.69
	αCH	9	4.57 dd, 8.7, 5.4	62.42		δCH ₃	9	0.95 d, 6.4	23.01
	βCH ₂	10	1.93 m, 1.70 m	29.65	Pro ¹	CO	10		170.80
	γCH ₃	11	1.60 m	24.94		N	11		
	δCH ₂	12	4.10 m, 3.39 m	48.37		αCH	12	4.42 m	62.43
Tyr ¹	CO	13		172.18		βCH ₂	13	2.13 m; 2.02 m	29.11
	NH	14	7.15 dd, 8.4			γCH ₂	14	1.80 m, 1.27 m	25.21
	αCH	15	5.20 m,	56.56		δCH ₂	15	3.67 m	47.91
	βCH	16	3.95 m; 3.45 m	37.13	Gly ²	CO	16		171.85
	iC	17		128.00		NH	17	9.21 d, 5.2	
	oCH	18, 22	7.14 d, 8.5	116.73		αCH ₂	18	4.69 dd, 16.8, 5.2 3.66 m	43.32
	mCH	19, 21	7.31 d, 8.5	130.11	Phe	CO	19		170.40
	pC	20		158.11		NH	20	9.21 d, 10.0	
	CO	23		173.82		αCH	21	5.34 m	58.40
Gly ²	NH	24	8.51 dd, 8.0, 4.8			βCH ₂	22	3.47 dd, 3.24 m	37.45
	αCH ₂	25	4.83 dd, 16.5, 8.0 3.68 dd, 16.5, 4.8	43.94		iC	23,		138.55
	CO	26		168.98		oCH	24, 28	7.20 m,	128.82
Tyr ²	NH	27	7.51 d, 7.7			mCH	25, 27	7.50 m,	130.16
	αCH	28	4.75 m	54.65		pCH	26	7.10 m	126.94
	βCH ₂	29	3.23 dd, 12.6, 4.4	40.75		CO	29		171.31
	iC	30		129.31	Tyr	NH	30	8.58 d, 5.7	
	oCH	31, 35	8.45 d, 8.5	116.59		αCH	31	5.23 m	54.31
	mCH	32, 34	7.29 d, 8.5	130.57		βCH ₂	32	3.47 dd; 3.24 m	37.45
Pro ¹	pC	33		157.62		iC	33		126.88
	CO	36		171.56		oCH	34, 38	7.59	132.28
	N	37				mCH	35, 37	7.20	116.42
	αCH	38	5.08 m	59.26	Pro ²	pC	36		158.06
	βCH ₂	39	1.93 m; 1.60 m	30.18		CO	39		170.88
	γCH ₂	40	1.43 m	23.14		N	40		
	δCH ₂	41	3.75 m; 3.58 m	47.65		αCH	41	4.44 m	63.13
	CO	42		170.15		βCH ₂	42	2.13 m, 2.02 m	29.11
	Pro ³	N	43				γCH ₂	43	1.79 m, 1.61 m
	αCH	44	4.61 t, 8.3	62.42		δCH ₂	44	3.67 m,	47.91
	βCH	45	2.10 m; 2.00 m	29.65		CO	45		172.31
	γCH	46	1.70 m; 1.55 m	25.85					
	δCH ₂	47	3.32 ddd, 16.0, 8.0, 4.5	46.85					
	CO	48		172.54					

formula of $\text{C}_{38}\text{H}_{49}\text{N}_7\text{O}_8$. Nine signals from 3.6 to 5.5 ppm and five signals from 8.5 to 10.5 ppm in the ^1H NMR spectrum (Table) showed the presence of protons belonging to methines (or methylene) and NH groups, respectively, and the ^{13}C NMR spectra (Table) gave the presence of seven carbonyls. HMQC-TOCSY spectral data of **2** gave seven amino acid residues including 2 Gly, 1 Phe, 1 Leu, 1 Tyr, and 2 Pro, which agreed with the $[\text{MH}]^+$ peak at m/z 731 in the FABMS of **2**.

Considering all 17 unsaturation of identified amino acid residues, the excess of one unsaturation demanded that **2** existed in a different molecular structure. In chemical tests, **2** showed a negative reaction when tested with ninhydrin but not after being hydrolyzed with concentrated HCl. Thus, **2** was determined to be a monocyclic cyclopeptide.

Each α -methine (or methylene in the Gly) proton and NH proton of all amino acid residues in **2** was attributed by examination of the HMQC-TOCSY spectrum. Each carbo-



nyl group of the identified amino acid residues was assigned by the analysis of the correlations between the corresponding carbonyls and α -CH protons (Table). Among them, the two groups of resonance signals that were attributed to the amino acids of Tyr and Phe, respectively, were unambiguously assigned via the specified signal 158.06 ppm of Tyr residue by means of the HMBC spectrum of **2**.

Finally, the amino acid sequence was determined by analysis of the correlations between the NH protons and the neighbor carbonyl groups in the HMBC spectrum, as shown.

In the FABMS spectrum of **2**, peaks at m/z 210 gave the linkage of Pro-Leu, and the cleavages of Pro-Lue-Gly¹ (m/z 267), Pro-Gly² (m/z 154), Pro-Gly²-Phe (m/z 302), Pro-Gly²-Phe-Tyr (m/z 465), Lue-Pro-Gly²-Phe-Tyr (m/z 578), and Lue-Pro-Gly²-Phe-Tyr-Pro (m/z 675) supported the amino acid sequence derived from the NMR data.

3. Experimental

3.1. Instruments and materials

MS was performed on a Autospec-3000 Spectrometer and EIMS under 70 eV. ¹H and ¹³C NMR spectra were recorded at 500 and 125 Hz, respectively, with a Bruker AM-400 Spectrometer. Silica gel-H (made by Qingdao Marine Chemical and Industrial Factory, China) was used for column chromatography and pre-coated Silica-G plates were employed for analytical TLC. The *Goniiothalamus leiocarpus* plants used in this investigation were collected in the south of Yunnan province, China. A voucher specimen of this plant was deposited at the Kunming Institute of Botany, Kunming, China.

3.2. Extraction, isolation and purification

Seeds of *Goniiothalamus leiocarpus*, (410 g) which were collected at the end of August in the south of Yunnan province, were extracted with alco-

hol (500 mL $\times$ 5) at room temperature. After removing the solvent at 50 °C, 80 g of brown resin was obtained and was partitioned between CHCl₃ and H₂O. The CHCl₃ extract (46 g) obtained was subjected to silica gel chromatography eluting with gradient Petrol-EtOAc and EtOAc-MeOH. The fraction in EtOAc-MeOH was subjected to RP-18 chromatography eluting with MeOH-H₂O and yielded **1** and **2**. Further purification by silica gel chromatography eluted with Ben-MeOH (85:15) gave white amorphous powder of **1** (<1 mg) and **2** (2 mg).

3.3. Characteristics of cyclopeptides

3.3.1. Leiocyclocin C (**1**)

FABMS m/z : 479 [Pro²-Tyr²-Gly²-Tyr¹-H]⁺, 576 [Pro²-Tyr²-Gly²-Tyr¹+Pro¹-H]⁺, 155 [Pro³-Gly¹+H]⁺, 251 [Pro¹-Pro³-Gly¹]⁺, 339 [Pro¹-Pro³-Gly¹-Ser+H]⁺, 436 [Pro¹-Pro³-Gly¹-Ser-Pro²+H]⁺, 600 [Tyr¹-Pro¹-Pro³-Gly¹-Ser-Pro²+2H]⁺, 656 [Gly²-Tyr¹-Pro¹-Pro³-Gly¹-Ser-Pro²+H]⁺, 819 cyclo-[Tyr²-Gly²-Tyr¹-Pro¹-Pro³-Gly¹-Ser-Pro²+H]⁺.

3.3.2. Leiocyclocin D (**2**)

FABMS m/z : 154 [Pro¹-Gly²]⁺, 154 [Pro²-Gly¹]⁺, 268 [Pro²-Gly¹-Leu+H]⁺, 365 [Pro²-Gly¹-Leu-Pro¹+H]⁺, 422 [Pro²-Gly¹-Leu-Pro¹-Gly²+H]⁺, 570 [Pro²-Gly¹-Leu-Pro¹-Gly²-Phe+2H]⁺, 732 [Pro²-Gly¹-Leu-Pro¹-Gly²-Phe+Tyr+H]⁺, 204 [Gly²-Phe]⁺, 302 [Pro²-Gly²-Phe]⁺, 465 [Pro²-Gly²-Phe-Tyr]⁺, 578 [Lue-Pro²-Gly²-Phe-Tyr]⁺, 635 [Gly¹-Lue-Pro²-Gly²-Phe-Tyr]⁺.

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