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CYCLOPEPTIDES FROM *STELLARIA YUNNANENSIS*

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Key Word Index—*Stellaria yunnanensis*; Caryophyllaceae; cyclopeptides; stellarin B and C.

Abstract—In a previous paper, we reported the structural elucidation of stellarin A, a new cyclic heptapeptide, from the fresh roots of *Stellarina yunnanensis* Franch. Further chemical study on this plant led to the isolation of another two new cyclopeptides named stellarin B and C. Their structures were established to be cyclo(Gly–Ser–HOIle–Phe–Phe–Ala) and cyclo(Gly–Ser–HOIle–Phe–Phe–Ser), respectively, by spectral methods.

INTRODUCTION

As part of a search for biologically active cyclopeptides from plant resources [1–4]. We have investigated the constituents of the fresh roots of *Stellaria yunnanensis* Franch (M), a plant which is only distributed in Yunnan, People's Republic of China where it is used as a lung tonic [5]. In a previous paper [6], we reported on the structure of stellarin A, a new cyclic heptapeptide from this plant. Further chemical studies on this plant have led to the isolation of two more new cyclopeptides named stellarin B (**1**) and C (**2**).

RESULTS AND DISCUSSION

Stellarin B (**1**), α_D^{19} + 15.0 (c 0.153, MeOH), was negative to ninhydrin reagent but a positive reaction was obtained after hydrolysis with 6 M HCl. Its molecular formula was assigned as $C_{32}H_{42}O_{86}$ (NMR and FABMS) ([M + 1]$^+$ at *m/z* 639]. The IR spectrum exhibited intense N–H and C=O absorptions at 3300 and 1600 cm^{-1}, respectively. The ^{13}C NMR spectrum showed the presence of six amide carbonyls (δ168.67, 169.57, 170.39, 172.24, 170.65, 170.98) and five methines (δ50.09, 53.73, 59.27, 55.27, 52.95) in the range δ45–65 [7]. The results mentioned above suggested that **1** was a cyclopeptide.

Amino acid analysis of the hydrolysate prepared from stellarin B (**1**) with 6 M HCl revealed the presence of Phe (2 eq.), Ser (1 eq.), Ala (1 eq.) and Gly (1 eq.). The ^{1}H NMR spectrum showed that one conformation dominated in DMSO. Extensive analyses of the NMR spectra indicated the presence of an atypical amino acid residue, in addition to the amino acid residues established by amino acid analysis. The atypical amino acid residue (δ–HO–Ile) was identified by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and DEPT techniques [^{1}H NMR (DMSO–d_6): δ7.468 (*d*, 7.8 Hz, H_N), 4.026 (*m*, H_α), 1.851 (*m*, $H_{\beta1}$), 0.451 (*d*, 6.6, $3H_{\beta2}$), 1.527 (*m*, $H_{\gamma1}$), 1.503 (*m*, $H_{\gamma2}$), 3.348 (*m*, $2H_\delta$)]. The HMBC and NOESY correlations summarized as Scheme 1 further confirmed its structure.

Evidence for the linkage of the amino acid residues was provided by the HMBC [8] and NOESY [9] correlations summarized in Scheme 2 and give rise to the structure of 1 as cyclo(Gly–Ser–HOIle–Phe$_1$–Phe$_2$–Ala). The proposed structure was further confirmed by FABMS.

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

Stellarin C (**2**), $[\alpha]_D^{19}$–12.2 (c 0.143, MeOH), showed a negative reaction to ninhydrin. Its molecular formula was determined as $C_{32}H_{42}O_9N_6$ (NMR and FABMS: ([M + 1]$^+$ at *m/z* 655). Its spectral data were very similar to those of **1**. Amino acid analysis of the hydrolysate prepared from stellarin C (**2**) by heating at 110° indicated the presence of Gly (1 eq.), Ser (2 eq.) and Phe (2 eq.). The above results indicated that the alanine residue was replaced by serine residue on going from stellarin B (**1**) to stellarin C (**2**).

The sequence of the amino acid residues in stellarin C (**2**) was determined by HMBC and NOESY correlations to be cyclo(Gly–Ser$_2$–OHIle–Phe$_1$–Phe$_2$–Ser$_1$). The proposed structure was further confirmed by FABMS.

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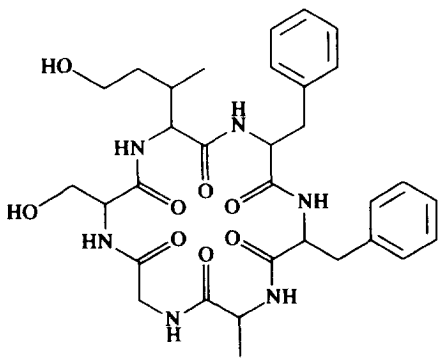
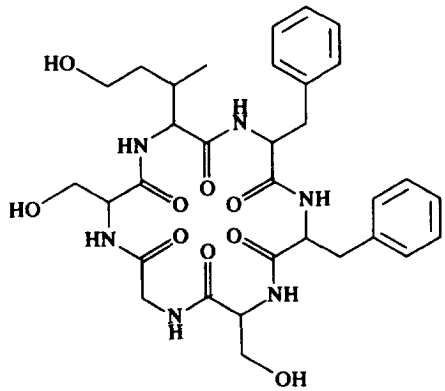
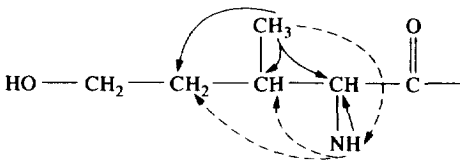


1 stellarin B



2 stellarin C



Scheme 1. Selected HMBC and NOESY for the amino acid residue (HOIle) in DMSO-d_6.

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

EXPERIMENTAL

General. ^1H, ^{13}C and 2D-NMR: TMS as int. standard; FABMS: ZAB–HS mass spectrometer.

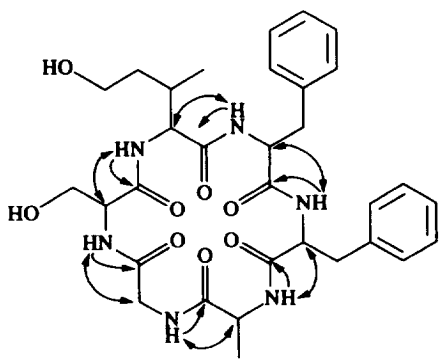
Table 1. ^1H NMR data of stellarin B and C (600 MHz, DMSO-d_6, TMS as int. standard)

C		H_N	α	β	γ	δ
B	Gly	8.623 (*d*, 12.0)	3.926 (*dd*, 12.0, 3.1)			
			3.423 (*dd*, 3.4, 12.0)			
	Ala	8.539 (*d*, 3.0)	3.943 (*dd*, 3.0, 6.6)	1.223 (*d*, 6.6)		
	Ser	8.090 (*d*, 8.4)	4.384 (*m*)	3.551 (*m*)		
					1.527 (*m*)	
	HOIle	7.823 (*d*, 7.8)	4.026(*m*)	1.850 (*m*)	1.503 (*m*)	3.348 (*m*)
					0.451 (*d*, 6.6)	
				2.913 (*dd*, 3.0, 15.0)		
	Phc_1	8.059 (*d*, 8.4)	4.255 (*m*)		ArH: 7.055–7.167	
				2.660 (*dd*, 3.6, 15.0)		
				3.125 (*dd*, 14.0, 4.2)		
	Phc_2	7.468 (*d*, 7.2)	4.427 (*m*)		ArH: 7.249–7.313	
				2.787 (*dd*, 14.0, 4.8)		
			3.928 (*m*)			
C	Gly	6.821 (*br s*)				
			3.456 (*m*)			
	Ser_1	8.452 (*d*, 3.5)	3.952 (*m*)	3.624 (*m*)		
				3.606 (*m*)		
	Ser_2	8.146 (*d*, 8.5)	4.319 (*m*)			
				3.548 (*m*)		
					1.526 (*m*)	
	HOIle	7.834 (*d*, 8.4)	3.963 (*m*)	1.840 (*m*)	1.036 (*m*)	3.360 (*m*)
					0.459 (*d*, 6.6)	
				2.888 (*dd*, 5.4, 10.3)	—	
	Phe_1	8.104 (*d*, 8.40)	4.253 (*m*)		ArH: 7.054–7.287	
				2.500 (*dd*, 3.6, 10.3)		
				3.144 (*dd*, 4.2, 13.8)		
	Phe_2	7.549 (*d*, 6.6)	4.496 (*m*)		ArH: 7.255–7.544	
				2.688 (*dd*, 3.0, 17.8)		

Coupling constants (Hz) are given in parentheses.

Table 2. ^{13}C NMR data of stellarin B and C (150 MHz, DMSO-d_6, TMS as int. standard)

		C=O	α	β	γ	δ
B	Gly	168.67	42.55			
	Ala	172.24	50.09	16.25		
	Ser	169.57	53.73	60.46		
					35.02	
	HOIle	170.65	59.27	31.47		59.28
					15.68	
						128.18
	Phe_1	170.39	55.27	37.46	137.55	128.77
						126.30
						126.46
	Phe_2	170.98	52.95	37.81	137.27	129.26
						126.06
C	Gly	168.63	42.56			
	Ser_1	170.20	57.11	60.45		
	Ser_2	169.50	53.95	60.41		
					34.97	
	HOIle	170.63	59.21	28.98		58.28
					15.69	
						126.03
	Phe_1	170.47	55.22	37.43	137.55	129.26
						126.37
						126.03
	Phe_2	171.26	53.24	37.76	137.40	129.26
						126.37



Scheme 2. Selected HMBC and NOESY for stellarin B in DMSO-d_6.

Plant material. Roots of *Stellaria yunnanensis* Franch (M) were collected in Kunming, Yunnan province of P.R. China in June 1992 and identified by Prof. Z. Y. Wu (Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China). A voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany (P.R. China).

Extraction and isolation of the cyclopeptides. The fresh roots of *S. yunnanensis* (19.6 kg) were extracted (× 3) with MeOH under reflux. Removal of the solvent by evaporation *in vacuo* yielded a syrup (480 g). The syrup was suspended in H_2O, extracted with petrol, EtOAc and *n*-BuOH, respectively. The EtOAc extracts were concentrated to afford a residue (37 g). The residue was subjected to CC on silica gel eluting with $CHCl_3$ containing increasing proportions of MeOH. Fractions were monitored by TLC. Further chromatographic purification gave stellarin A (180 mg, 0.028%), B (24 mg, 0.00037%), C (31 mg, 0.00047%), respectively.

Stellarin B (**1**). $C_{32}H_{42}O_8N_6$, amorphous powder, ninhydrin reaction (—). $[\alpha]_D^{19}$ + 15.0 (*c* 0.153, MeOH); UV λ_{max}^{EtOH} nm (log ε): 205 (4.46); IR ν_{max}^{KBr} cm^{-1}: 3300, 2900, 1650, 1515; FABMS *m/z* (rel. int.): 662 $[M + Na + 1]^+$, (base peak), 639 $[M + 1]^+$ (90), 492 (10), 421 (8), 364 (10), 267 (10), 217 (18); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Amino acid analysis of stellarin B (**1**). Amino acid analysis of the hydrolysate prepared by heating stellarin B with 6 M HCl at 110° for 24 hr in a sealed tube indicated the presence of Gly (1 eq.), Ser (1 eq.), Phe (2 eq.), Ala (1 eq.).

Stellarin C (**2**). $C_{32}H_{42}O_9N_6$, amorphous powder, ninhydrin reaction (—), $[\alpha]_D^{19}$ −12.29 (*c* 0.143, MeOH). UV λ_{max}^{EtOH} nm (log ε): 203 (4.48); IR ν_{max}^{KBr} cm^{-1}: 3300, 1650, 1525, 1470; FABMS *m/z* (rel. int.): 677 $[M + Na]^+$ (base peak), 655 $[M + 1]^+$ (70), 634 (20), 587 (10), 509 (15), 364 (20), 267 (50), 239 (52); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Amino acid analysis of stellarin C (**2**). Amino acid analysis of the hydrolysate prepared by heating stellarin C with 6 M HCl at 110° for 24 hr in a sealed tube indicated the presence of Gly (1 eq.), Phe (2 eq.), Ser (2 eq.).

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