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Two New Cyclic Peptides from *Psammosilene tunicoides*

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Abstract: From *Psammosilene tunicoides* W. C. Wu *et* C. Y. Wu, two cyclic octapeptides (named psammosilenins A and B) were isolated. Their structures were determined as cyclo(-Pro$_1$-Phe$_1$-Pro$_2$-Phe$_2$-Phe$_3$-Ala-Pro$_3$-Leu-) and cyclo(-Pro$_1$-Gly-Phe$_1$-Val-Pro$_2$-Phe$_2$-Thr-Ile-) by spectroscopic methods.

Keywords: *Psammosilene tunicoides*, Caryophyllaceae, cyclic peptide, psammosilenins A, psammosilenins B.

Psammosilene tunicoides W. C. Wu *et* C. Y. Wu, a monotype genus plant belonging to Caryophyllaceae, is a famous medicinal herb in Yunnan province. It is used as anodyne and haemastatic agent [1]. Studies on its saponins have been reported [2-4]. As a part of our investigation on the new bioactive cyclopeptides from higher plants [5-11], the chromatographic purification of the acetone-soluble fraction of the dried roots of the title plant (25Kg) led to the isolation of two new cyclic octapeptides, psammosilenins A (10mg) and B (6mg). This paper deals with the elucidation of their structures.

Psammosilenin A (**1**): white powder, $[\alpha]_D^{24}$ =-108.1 (c 0.39, MeOH), negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its molecular formula was assigned as $C_{51}H_{64}N_8O_8$ by HR-FABMS [$(M+1)^+$ at *m/z* 917.4859, calcd. *m/z* 917.4925], indicating 24 degrees of unsaturation. The IR absorptions at 3290 cm^{-1} and 1640 cm^{-1} were attributed to amino and amide carbonyl groups respectively. The ^{13}C NMR spectrum contained eight signals due to amide carbonyls at δ 174.1, 173.5, 173.1, 172.8, 171.7, 171.5, 171.5, 170.5. The ^1H NMR spectrum exhibited five amide protons at δ 9.22, 9.35, 9.43, 9.55, 9.70. These facts indicated that **1** was a cyclopeptide.

In order to identify spin systems of different amino acid residues, 2D-NMR techniques were used. By analysis of ^1H-^1H COSY, HMQC, HMBC spectra, these amino acid residues were revealed to be one alanine, one leucine, three phenylalanine, three proline units. The molecular weight of these amino acid residues was identical with that observed in FABMS. Unambiguous assignment of ^1H and ^{13}C NMR signals (**Table 1**) were carried out by means of 2D-NMR techniques including ^1H-^1H COSY,

HMQC and HMBC.

HMBC spectrum provided the evidences for the linkage of the amino acid residues. It showed the connectivity of NH_{Ala} to $C=O_{phe3}$, NH_{Phe3} to $C=O_{Phe2}$, NH_{Phe2} to $C=O_{Pro2}$, δ-H_{pro2} to $C=O_{Phe1}$, NH_{Phe1} to $C=O_{Pro1}$ and NH_{Leu} to $C=O_{Pro3}$ (**Figure 1**), which implied the presence of two peptide fragments of (-Pro_1-Phe_1-Pro_2-Phe_2-Phe_3-Ala-) and (-Pro_3-Leu-). These two peptide fragments had to be linked in only one sequence. Consequently, the structure of **1** was determined to be cyclo(-Pro_1-Phe_1-Pro_2-Phe_2-Phe_3-Ala-Pro_3-Leu-). The proposed structure was further confirmed by FABMS.

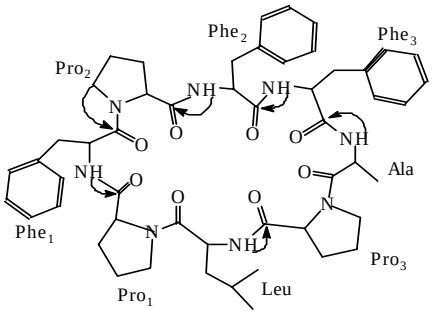
Table 1. ^1H and ^{13}C NMR Data of Psammosilenin A (**1**) in pyridine-d_5 (500MHz for ^1H NMR, 125MHz for ^{13}C NMR)

	CO	C_α	C_β	C_γ	C_δ	H_N	H_α	H_β	H_γ	H_δ
										0.91(d)
Leu	170.6	52.6	31.9	26.7	21.3 23.6	9.35(d) J=8.5	5.02(m)	2.79(m)	2.07(m)	J=6.5 0.1(d) J=6.5
Ala	172.8	49.5	15.6			9.70 (br.s)	5.31(m)	1.31(d) J=6.5		
Phe_1	171.7	55.9	37.9	140.3	126.5- 130.4	9.43(d) J=8.5	5.07(m)	3.79(m) 3.70(m)		6.95- 7.46
Phe_2	171.5	54.6	38.3	136.9	126.5- 130.4	9.55 (br.s)	5.24(m)	3.12(m) 3.26(m)		6.95- 7.46
Phe_3	174.1	55.4	39.1	138.4	126.5- 130.4	9.22(d) J=6.5	5.33(m)	3.60(m) 3.28(m)		6.95- 7.46
Pro_1	171.5	61.6	30.0	21.9	47.4		4.36(m)	1.23(m)	0.87(m)	3.54(m) 3.39(m)
Pro_2	173.5	59.7	29.6	25.7	47.0		4.89(m)	2.34(m) 2.07(m)	1.53(m) 1.95(m)	3.78(m)
Pro_3	173.1	61.0	30.7	22.8	47.5		4.74(m)	2.03(m)	1.73(m)	3.74(m)

Psammosilenin B (**2**): white power, $[\alpha]_D^{24}$ =-73.6 (c 023, MeOH), negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its FABMS gave a $[M+1]^+$ ion at *m/z* 859. The IR spectrum exhibited intense N-H and C=O absorptions at 3300 cm^{-1} and 1650 cm^{-1} respectively. The ^{13}C NMR spectrum showed the signals of eight amide carbonyls between δ 169.1 and 173.3. The ^1H NMR spectrum showed six amide protons between δ 8.58 and 9.56. From these facts, **2** was deduced to be a cyclopeptide.

Figure 1. Selected HMBC for **1**

Figure 2. Selected ROESY for **2**



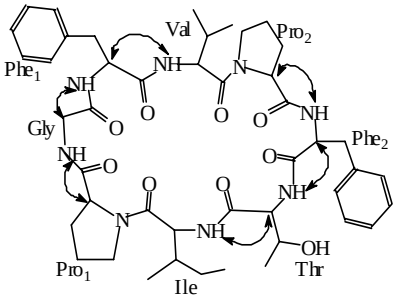


Table 2. ^{1}H and ^{13}C NMR Data of Psammosilenin B (**2**) in pyridine-d_5 (600MHz for ^{1}H NMR, 150MHz for ^{13}C NMR)

	CO	C_α	C_β	C_γ	C_δ	H_N	H_α	H_β	H_γ	H_δ
Gly	169.1	43.6				9.28 (br.s)	4.56(dd) J=16.8, 9 3.36(d) J=16.8			
Val	171.5[a]	55.2[b]	33.2	19.0 20.1		9.08 (br.s)	5.23(m)	2.56(m)	1.35(d) J=6.6 1.28(d) J=6.0	
Ile	171.5[a]	56.9[b]	40.1	25.2 15.3	10.9	8.70 (ca.)	5.23(m)	2.45(m)	1.80(m) 1.33(d) J=5.8	0.94(t) J=7.2
Thr	172.0[a]	63.0	68.4	20.9		8.58 (br.s)	5.38(m)	4.45(m)	1.57(d) J=6.0	
Pro_1	172.3[a]	62.5	29.6	25.2	48.6[c]		4.19(m)	2.06(m) 1.95(m)	1.88(m) 1.39(m)	3.83(m) 3.74(m)
Pro_2	172.3[a]	62.0	29.6	25.2	49.0[c]		3.89(m)	1.90(m) 1.69(m)	1.51(m) 1.46(m)	3.84(m) 3.73(m)
Phe_1	173.3[a]	58.0	41.8	138.0[d]	126.9-130.0	8.59 (br.s)	5.59(m)	3.44(d) J=13,4.2 3.15(m)		7.0-7.6
Phe_2	172.1[a]	60.5	35.9	139.5[d]	126.9-130.0	9.56 (br.s)	4.02(m)	4.00(m) 3.61(m)		7.0-7.6

[a,b,c,d] assignments with the same superscripts may be interchanged.

By analysis of the TOCSY, HMQC, DQF-COSY spectra, the eight amino acid residues were identified as one glycine, one isoleucine, one threonine, one valine, two phenylalanine and two proline units. The molecular weight of these amino acid residues was identical with that observed in FABMS. Assignment of the ^{1}H and ^{13}C NMR signals (**Table 2**) of **2** were accomplished using combination of 2D-NMR experiments such as DQF-COSY, HMQC and TOCSY.

The sequence of these amino acid residues was determined by NOESY spectrum. The NOESY correlations suggested the presence of two peptide fragments (-Pro_1-Gly-Phe_1-Val- and -Pro_2-Phe_2-Thr-Ile-), and these two peptide fragments had to be linked in only one sequence. These facts together with the information provided by FABMS led to the structure of **2** as cyclo(-Pro_1-Gly-Phe_1-Val-Pro_2-Phe_2-Thr-Ile-).

Acknowledgments

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