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瓦草中三个新环肽*

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A 摘要 从云南民间中草药瓦草(*Silene szechuensis*)的根中分离并鉴定了3个新的环肽, 分别命名为瓦草环肽 A, B, C(silenins A, B, C). 用光谱和化学方法确定它们均为环八肽, 其结构分别为: silenin A——cyclo-(Pro-Leu-Ser-Phe-Pro-Tyr-Leu-Val), silenin B——cyclo-(Phe-Leu-Ala-Pro-Leu-Pro-Phe-Pro), silenin C——cyclo-(Tyr-Ala-Phe-Pro-Gly-Phe-Tyr-Pro).

关键词 瓦草、石竹科、瓦草环肽 A, 瓦草环肽 B, 瓦草环肽 C 环肽,

THREE NEW CYCLOPEPTIDES FROM SILENE SZECHUENSIS

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Abstract From the roots of *Silene szechuensis*, a folk herb of Yunnan named "wa cao", three new cyclopeptides silenins A-C were isolated. The structures were elucidated by the spectral and chemical methods as silenin A ——cyclo-(Pro-Leu-Ser-Phe-Pro-Tyr-Leu-Val), silenin B ——cyclo-(Phe-Leu-Ala-Pro-Leu-Pro-Phe-Pro), silenin C —— cyclo-(Tyr-Ala-Phe-Pro-Gly-Phe-Tyr-Pro).

Key words *Silene szechuensis*, Caryophyllaceae, silenin A, silenin B, silenin C

Silene szechuensis Williams (Caryophyllaceae) is indigenous to Southwest China. It has been used as antipyretic, analgesic, diuretic, and so on in Yunnan for a long time (Jiangsu Institute of Botany *et al*, 1990). As parts of our investigation on cyclopeptides in Caryophyllaceae plants (Tan *et al*, 1993; Zhao *et al*, 1995; Zhang *et al*, 1995), in this paper we report three new cyclopeptides named silenins A(1), B(2), C(3) from the roots of the plant.

RESULTS AND DISCUSSION

The EtOH extract of the dried roots of *Silene szechuensis* was extracted with EtOAc in a Soxhlet apparatus. Removal of solvent furnished an EtOAc fraction (167g). The EtOAc fraction was repeatedly chromatographed on a silical gel, a MCI gel or a RP-18 column and afforded silenin A (126 mg), silenin B

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(710 mg) and silenin C (184 mg).

Silenin A (I), crystals, $[\alpha]_D^{20}$ −68.94° (c, 0.359, C_5H_5N). Its moleclular formula was deduced as $C_{48}H_{68}O_{10}N_8$ by means of DEPT spectral analysis and FAB−MS [(M+2)$^+$ at m / z 918]. The IR spectrum in KBr disc showed intense amide C=O at 1630 cm^{-1}and amide N−H at 3300 cm^{-1}. In the DEPT spectrum (C_5D_5N), a total of eight amide CO signals could be seen between 171.2 and 174.2 ppm. Meanwhile, the middle and high field signals of eleven methines, eleven methylenes, six methyls, and twelve low field signals between 116.0 and 157.3 ppm were identified. The low field signals showed the presence of one phenyl and one p−hydroxyphenyl. From these data, silenin A appeared to be a octapeptide.

By means of 2D NMR techniques, the amino acid composition could be identified to be two prolines, two leucines, one serine, one phenylalanine, one tyrosine and one valine, which were in correspondence with that of amino acid analysis after complete acidic hydrolysis (6N HCl, 110℃, 24 hrs). For the compound gave a negative response to ninhydrin test, it must be a cyclic octapeptide.

The amino acid sequence could be determined preliminarily by positive FAB−MS which showed the fragments of I to XII as following:

I m / z 211 [Pro−Leu+H]$^+$

II m / z 298 [Pro−Leu−Ser+H]$^+$

III m / z 445 [Pro−Leu−Ser−Phe+H]$^+$

IV m / z 542 [Pro−Leu−Ser−Phe−Pro+H]$^+$

V m / z 705 [Pro−Leu−Ser−Phe−Pro−Tyr+H]$^+$

VI m / z 818 [Pro−Leu−Ser−Phe−Pro−Tyr−Leu+H]$^+$

VII m / z 918 [Pro−Leu−Ser−Phe−Pro−Tyr−Leu−Val+2H]$^+$

VIII m / z 261 [Pro−Tyr+H]$^+$

IX m / z 374 [Pro−Tyr−Leu+H]$^+$

X m / z 473 [Pro−Tyr−Leu−Val+H]$^+$

XI m / z 570 [Pro−Tyr−Leu−Val−Pro+H]$^+$

XII m / z 683 [Pro−Tyr−Leu−Val−Pro−Leu+H]$^+$

XIII m / z 770 [Pro−Tyr−Leu−Val−Pro−Leu−Ser+H]$^+$

XIV m / z 918 [Pro−Tyr−Leu−Val−Pro−Leu−Ser−Phe+2H]$^+$

The fragments I−VII and VIII−XIV could give the same gross structure as cyclo−(Pro−Leu−Ser−Phe−Pro−Tyr−Leu−Val).

Further evidences were provided by ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, TOSCY and COLOC spectra. At first we assigned proton and carbon singals of every amino acid residues with those 2D NMR experiments (The data are shown in Table 1), and then determined the partial sequence of amino acid residues as following peptides (1) and (2) based on the correlations between amide CO and NH in COLOC spectra.

(1) −NH−Pro^1−Leu^1−Ser−CO−

(2) −NH−Pro^2−Tyr−Leu^2−CO−

Table 1 ^{1}H and ^{13}C NMR spectral data of silenins A (1), B (2), and C (3) [in C_5D_5N, 400HMz for δ, 100HMz for δ, TMS]

silenin A (1)			silenin B (2)			silenin C (3)		
Amino acid residues	H	C	Amino acid residues	H	C	Amino acid residues	H	C
Pro[1]			Pro[3]			Pro[1]		
α	4.72(1H,d,7.6)	62.4	α	4.54(1H,dd,2.4,5.2)	61 0	α	3 00(1H,m)	60 0
β	2.43(1H,br.),1.64(1H,m)	31.6	β	1.85(1H,m),1.36(1H,m)	29.7	β	1 70(1H,m),0 96(1H,m)	30 1
γ	1.64(2H,m)	22.8	γ	1 71(1H,m)	25 3	γ	1.46(1H,m),1.29(1H,m)	21 5
δ	3.60(1H,d,14.0),3.38(1H,m)	46.8	δ	3.55(1H,m),3 35(1H,m)	47.3	δ	3.31(1H,m),3.20(1H,m)	46 3
CO		171.2	CO		171.2	CO		171.5
Leu[1]			Phe[1]			Gly		
α	4.74(1H,m)	55.5	α	4.89(1H,m)	53.5	α	3.06(1H,m),2.95(1H,m)	42.3
β	1.90(1H,m),1.66(1H,m)	41 0	β	3.16(1H,dd,5.2,12.0)	40 2	NH	9.02(1H,t,6.1)	
				2.85(1H,dd,8.0,11.0)		CO		167 7
γ	I 68(1H,m)	26.0	ψ	7.10～7.29(5H,m)	126 2	Phe[2]		
					128 6	α	4.55(1H,t,11.0)	52 8
					129.2	β	2 67(2H,t,12 4)	35.3
					136 4	ψ	7 12～7.39(5H,m)	126 0
δ	0.69(3H,d,6.6),0.64(3H,d,6.6)	23 1,22 1	NH	8.34(1H,d,7.3)				128 0
								129.2
								136.1
NH	9 08(1H,d,8.0)		CO		172.0	NH	7.79(1H,d,8 8)	
CO		173.2	Leu[1]			CO		172.3
Ser			α	4.00(1H,m)	54.0	Tyr[2]		
α	5.18(1H,d1,5.5,8.0)	55.8	β	1.30(2H,m)	39.4	α	4.24(1H,m)	54 6
β	4.12(2H,m)	63.0	γ	1.77(1H,m)	25.5	β	3.03(1H,m),2 85(1H,m)	37.7
NH	8 52(1H,d,8.0)		δ	0.97(3H,d,6.6),0.92(3H,d,6.6)	23 3,20.8	ψ	7.18(2H,m),6.62(2H,d,8.4)	114 6
CO		172.0	NH	5.75(1H,d,5.3)				129.3
								130 0
								155.6
Phe			CO		171.8	NH	8 68(1H,s)	
α	4 98(1H,br.)	53.6	Ala			CO		169.9
β	2.94(1H,t,8.0),2.76(1H,dd,8.0,4.0)	39 4	α	4.69(1H,m)	49 1	Pro[2]		
ψ	6.90～7.04(5H,m)	127.5	β	1.36(3H,d,7.0)	15.9	α	3 07(1H,m)	60 0
		129.2	NH	6 88(1H,s)		β	1 79(1H,m),0.92(1H,m)	29.9
		129 9	CO		172 8	γ	1.54(1H,m),0.81(1H,m)	21.2
		136 9	Pro[1]			δ	3.21(1H,m),3 02(1H,m)	46.2
NH	10 56(1H,d,6.8)		α	4 26(1H,d,7.8)	61.1	CO		170 4
CO		171.8	β	2 07(1H,m),1 75(1H,m)	26.1	Tyr[1]		

Table 1 continued

Pro[2]			γ	1.84(2H,m)	22.0	α	4.03(1H,m)	55.8
α	3 77(1H,d,6.7)	62.4	δ	3.41(1H,m),2.43(1H,m)	47.7	β	2.94(2H,m)	38.0
β	1.64(1H,m),0 82(1H,m)	32.0	CO		173.5	ψ	6.94(2H,d,8.3),6.69(2H,d,8.3)	115.5
γ	0.87(2H,m)	22.0	Leu[2]					129.3
								130.0
								156 5
δ	3.38(1H,m),3.04(1H,m)	46.8	α	4.44(1H,m)	52 6	NH	8.86(1H,d,8.2)	
CO		171.6	β	1.21(2H,m)	37.6	CO		170 3
Tyr			γ	1.39(1H,m)	25.0	Ala		
α	4.78(1H,m)	57.3	δ	0 84(3H,m),0.73(3H,m)	22.2,21.1	α	4.18(1H,m)	47 9
β	3.44(1H,t,6.0),3.02(1H,d,9.0)	38.0	NH	8.92(1H,d,7.1)		β	1.28(3H,d,7.1)	18 0
ψ	7.05(2H,d,8.4),6 84(2H,d,8.4)	116.0	CO		174.3	NH	7.86(1H,d,6.9)	
		129.1	Pro[2]			CO		173.0
		130 7	α	4.08(1H,t,7.2)	63.5	Phe[1]		
		157.3	β	2.18(1H,m),1.70(1H,m)	31.4	α	4.45(1H,t,5.2)	53 1
NH	9 25(1H,br.)		γ	1.38(2H,m)	25.1	β	3.06(1H,m),2.90(1H,m)	36.6
CO		171.6	δ	3.52(1H,m),3.29(1H,m)	46.9	ψ	7.12～7.39(5H,m)	127.1
Leu[2]			CO		171 8			128.6
								138.3
α	4.83(1H,d,6 0)	52.0	Phe[2]			NH	8.54(1H,s)	
β	1 86(1H,m),1.54(1H,m)	41.2	α	5.23(1H,t,11.0)	51 7	CO		170.4
γ	1 63(1H,m)	25 8	β	3.79(1H,dd,3 3,12.7)	38.0			
				2.59(1H,dd,11.8,14.5)				
δ	0.64(3H,d,6.0),0.62(3H,d,6.0)	23.1,23.1	ψ	7.10～7.29(5H,m)	127.3			
					128.1			
					129.6			
					137.6			
NH	8.93(1H,br.)		NH	7 75(1H,d,9.8)				
CO		174.2	CO		171.5			
Val								
α	4.30(1H,dd,5.4,8 2)	59.5						
β	2 13(1H,m)	31.2						
γ	0.87(3H,d,6.6),0.79(3H,d,6.4)	19.8,19 8						
NH	7.75(1H)							
CO		172.3						

Therefore, the structure of the compound named silenin A, a octacyclopeptide, was elucidated as cyclo-(prolyl-leucyl-seryl-phenyl-prolyl-tyrosyl-leucyl-valyl).

Silenin B (2) amorphous powder, $[\alpha]_D^{20}$ -131.33 ° (c, 0.316, $CHCl_3$). Its moleclular formula was deduced as $C_{48}H_{66}O_8N_8$ by means of DEPT spectral analysis and FAB-MS [$(M+1)^+$ at m / z 883]. The IR spectrum in KBr disc showed intense amide C=O at 1629 cm^{-1} and amide N-H at 3292 cm^{-1}. In the DEPT spectrum ($CDCl_3$), a total of eight amide CO signals could be seen between 171.2 and 174.3 ppm. Meanwhile, the middle and high field signals of ten methines, thirteen methylenes, five methyls, and the low field signals of two phenyls were identified. Applications of 2D NMR techniques and amino acid analysis after hydrolysis, the amino acid composition could be identified to be three prolines, two leucines, two phenylalanines, one alanine. These data indicated that silenin B appeared to be a cyclic octapeptide, too.

The amino acid sequence could be determined preliminarily by positive FAB-MS which showed the fragments of I to VII as following:

I m / z 245 $[Pro-Phe+H]^+$

II m / z 358 $[Pro-Phe-Leu+H]^+$

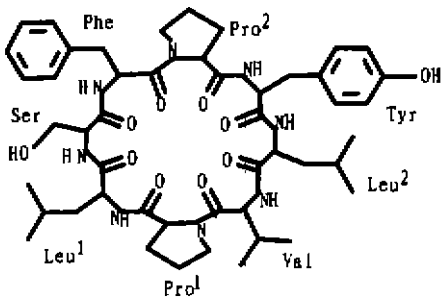
III m / z 429 $[Pro-Phe-Leu-Ala+H]^+$

IV m / z 526 $[Pro-Phe-Leu-Ala-Pro+H]^+$

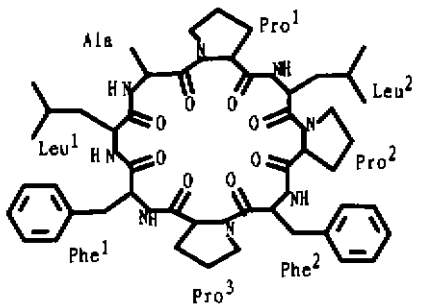
V m / z 602 $[Pro-Phe-Leu-Ala-Pro-Leu+H]^+$

VI m / z 211 $[Pro-Leu+H]^+$

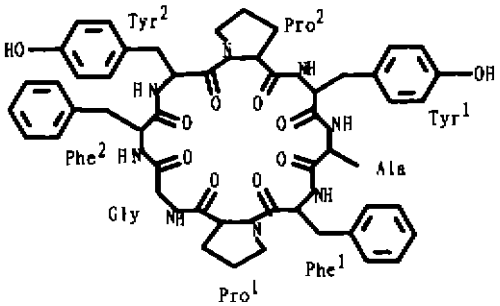
VII m / z 455 $[Pro-Leu-Pro-Phe+H]^+$



silenin A (1)



silenin B (2)



silenin C (3)

Therefore, the gross structure of the compound could be deducde as cyclo-(Pro-Phe-Leu-

Ala-Pro-Leu-Pro-Phe).

With the same methods mentioned above, we could determine the partial sequence of amino acid residues as following peptides (1), (2) and (3) based on the correlations between amide CO and NH in COLOC spectra.

(1) -N-Pro^2-Phe^2-CO-

(2) -N-Pro^3-Phe^1-CO-

(3) -NH-Leu^1-Ala-CO-

Then the structure of the compound named silenin B, a octacyclopeptide, was elucidated as cyclo-(prolyl-phenyl-leucyl-alanyl-prolyl-leucyl-prolyl-phenyl).

Silenin C (3), amorphous powder, $[\alpha]_D^{20}$-81.82° (c, 0.330, CH_3OH). Its moleclular formula was deduced as $C_{51}H_{58}O_{10}N_8$ by means of DEPT spectral analysis and FAB-MS [(M+1)+ at m/z 943]. The IR spectrum in KBr disc showed intense amide C=O at 1628 cm^{-1} and amide N-H at 3286 cm^{-1}. In the DEPT spectrum (CD_3OD), a total of eight amide CO signals could be seen between 167.7 and 173.0 ppm. Meanwhile, the middle and high field signals of seven methines, eleven methylenes, one methyl, two phenyls and two p-hydroxyphenyls were identified. Applications of 2D NMR techniques and amino acid analysis after hydrolysis, the amino acid composition could be identified to be two prolines, two phenylalanines, two tyrosines, one alanine, one glycine. These data indicated that silenin C appeared to be a cyclic octapeptide too.

The amino acid sequence could be determined preliminarily by positive FAB-MS which showed the fragments of I to VI as following:

I m/z 155 $[Pro-Gly+H]^+$

II m/z 302 $[Pro-Gly-Phe+H]^+$

III m/z 465 $[Pro-Gly-Phe-Tyr+H]^+$

IV m/z 261 $[Pro-Tyr+H]^+$

V m/z 332 $[Pro-Tyr-Ala+H]^+$

VI m/z 479 $[Pro-Tyr-Ala-Phe+H]^+$

So the gross structure of the compound could be deducded as cyclo-(Pro-Gly-Phe-Tyr-Pro-Tyr-Ala-Phe).

With the same methods mentioned above, we could determined the partial sequence of amino acid residues as following peptides (1) and (2) based on the correlations between amide CO and NH in COLOC spectra.

(1) -N-Pro^1-Gly-Phe^2-Tyr2-CO-

(2) -N-Pro^2-Tyr1-Ala-Phe^1-CO-

Therefore, the structure of the compound named silenin C, an octacyclopeptide, was elucidated as cyclo-(prolyl-glycyl-phenyl-tyrosyl-prolyl-tyrosyl-alanyl-phenyl).

EXPERIMENT

General Mps. uncorr. Optical rotations were recorded on SEPA-300 with 2 cm cell. IR were taken for KBr disc. NMR were measured with AMX-400 spectrometer using TMS as the internal standard. FAB-MS were determined with VG Autospec-3000 mass spectrometer.

Extraction and isolation The powdered roots of *Silene szechuensis* (20 kg) were extracted with 95%

EtOH three times at the refluex condition. The EtOH extracts (2.4 kg) was extracted with EtOAc in Soxhlet apparatus. Then the EtOAc solutions were evaporated and the residues (167 g) were subjected to a silica gel column eluting with $CHCl_3$–MeOH. Then by combination of a MCI gel and RP-18 column we obtained silenin A (126 mg), silenin B (710 mg), and silenin C (184 mg), respectively.

Silenin A (1) $C_{48}H_{68}O_{11}N_8$, white needles (MeOH), mp 264～266℃, $[\alpha]_D^{20}$–68.94° (c, 0.359, C_5H_5N). $\nu_{max}^{KBr}cm^{-1}$: 3300, 1630. Pos. FAB-MS m/z: 918[(M+2)$^+$, 74], 818(1.2), 770(0.6), 705(1.6), 683(0.4), 570(2.0), 542(4.8), 473(23), 445(24), 374(56), 298(81), 261(87), 211(81). ^1H and ^{13}C NMR: see Table 1. Amino acid analysis (standard method): Pro (2eq), Leu (2eq), Ser (1eq), Phe (1eq), Tyr (1eq), and Val (1eq).

Amino acid analysis of Silenin A (1): The hydrolysate of silenin A after hydrolysis with 6N HCl at 110℃ for 24 hrs was analysed for amino acids using the standard method.

Silenin B (2) $C_{48}H_{66}O_8N_8$, white amorphous powder, $[\alpha]_D^{20}$–131.33° (c, 0.316, CHCl3). $\nu_{max}^{KBr}cm^{-1}$: 3292, 1650, 1629. Pos. FAB-MS m/z: 883[(M+1)$^+$, 100], 602(1.0), 526(2.0), 455(5.0), 429(5.0), 358(6.0), 245(18), 211(19). ^1H and ^{13}C NMR: see Table 1. Amino acid analysis (standard method): Pro (3eq), Leu (2eq), Phe (2eq), and Ala (1eq).

Silenin C (3) $C_{51}H_{58}O_{10}N_8$, white amorphous powder, $[\alpha]_D^{20}$–81.82° (c, 0.330, CH_3OH). $\nu_{max}^{KBr}cm^{-1}$: 3286, 1628. Pos. FAB-MS m/z: 943[(M+1)$^+$, 13], 479(1.0), 465(1.2), 332(3.2), 302(5.0), 261(3.2), 155(9.4). ^1H and ^{13}C NMR: see Table 1. Amino acid analysis (standard method): Pro (2eq), Phe (2eq), Tyr (2eq), Ala (1eq), and Gly (1eq).

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