

## 王不留行环肽研究\*

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**摘要** 从中药王不留行(*Vaccaria segetalis*)种子中分离并鉴定了4个环肽化合物, 分别命名为王不留行环肽A, B, C, D(vaccarins A, B, C, D), 其中王不留行环肽A为新环肽化合物。其结构通过光谱和化学方法分别确定为: vaccarin A — cyclo-(Trp-Ala<sup>1</sup>-Gly-Val-Ala<sup>2</sup>), vaccarin B — cyclo-(Pro-Gly-Leu-Ser-Phe<sup>1</sup>-Ala-Phe<sup>2</sup>), vaccarin C — cyclo-(Pro<sup>1</sup>-Gly-Tyr-Val-Pro<sup>2</sup>-Leu-Trp), vaccarin D — cyclo-(Pro-Val<sup>1</sup>-Trp-Ala-Gly-Val<sup>2</sup>)。

**关键词** 王不留行, 石竹科, 王不留行环肽A, 王不留行环肽B, 王不留行环肽C, 王不留行环肽D  
**分类号** Q 946

## Cyclopeptides from *Vaccaria segetalis*

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**Abstract** From the seeds of *Vaccaria segetalis*, a well-known traditional Chinese medicine named “wang bu liu xing”, four cyclopeptides vaccarins A-D were isolated in which only vaccarin A is a new cyclopeptide. Their structures were elucidated by spectral and chemical methods as vaccarin A — cyclo-(Trp-Ala<sup>1</sup>-Gly-Val-Ala<sup>2</sup>), vaccarin B — cyclo-(Pro-Gly-Leu-Ser-Phe<sup>1</sup>-Ala-Phe<sup>2</sup>), vaccarin C — cyclo-(Pro<sup>1</sup>-Gly-Tyr-Val-Pro<sup>2</sup>-Leu-Trp), vaccarin D — cyclo-(Pro-Val<sup>1</sup>-Trp-Ala-Gly-Val<sup>2</sup>).

**Key words** *Vaccaria segetalis*, Caryophyllaceae, vaccarin A, vaccarin B, vaccarin C, vaccarin D

*Vaccaria segetalis* (Neck.) Garcke (Caryophyllaceae), distributed all over China except South China, is used as a well-known traditional Chinese medicine treated in amenorrhea, milk secretion blocking, dystocia, carbuncle, and bleeding (Jiangsu College of New Medicine, 1995). As parts of our investigations on cyclopeptides in Caryophyllaceae plants (Tan *et al.*, 1993; Zhao *et al.*, 1995; Zhang *et al.*, 1997; Wang *et al.*, 1998), in the communication (Zhang *et al.*, 1995) we reported one new cyclopeptide vaccarin A from *Vaccaria segetalis* which is the first cyclopeptide found in the genus of *Vaccaria*. In this paper we describe the isolation and structure determination of vaccarin A and other three cyclopeptides vaccarins B, C, D

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from the plant based on spectral and chemical methods.

## RESULTS AND DISCUSSION

The EtOH extract of the dried seeds of *Vaccaria segetalis* was suspended in H<sub>2</sub>O and extracted with petroleum, EtOAc and n-BuOH respectively. Removal of solvent furnished an EtOAc fraction (147 g). The EtOAc fraction was repeatedly chromatographed on a silical gel and a RP-18 column and afforded vaccarin A (538 mg), vaccarin B (205 mg), vaccarin C (317 mg) and vaccarin D (316 mg), respectively.

**Vaccarin A (1)** disc crystals,  $[\alpha]_D^{25.5} -104.23^\circ$  (c, 0.662, DMSO), gave a negative ninhydrin reaction, but positive when hydrolyzed with 6 mol/L HCl. Its molecular formula was deduced as C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>N<sub>6</sub> by means of DEPT spectral analysis and FAB-MS [(M+1)<sup>+</sup> at m/z 485]. The IR spectrum in KBr disc showed intense amide C=O at 1666, 1650 cm<sup>-1</sup> and amide N-H at 3336 cm<sup>-1</sup>. In the DEPT spectrum (DMSO), a total of five amide CO signals could be seen between 169.5 and 172.1 ppm. Meanwhile, the middle and high field signals of five methines, two methylenes, four methyls, and seven low field signals between 109.9 and 136.0 ppm were identified. The low field signals showed the presence of one indole group. From these data, vaccarin A appeared to be a pentapeptide.

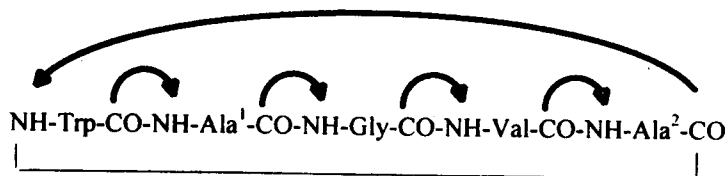
By means of 2D NMR techniques, the amino acid composition could be identified to be one tryptophan, two alanines, one glycine, one valine, which were in correspondence with that of amino acid analysis after complete acidic hydrolysis (6 mol/L HCl, 110 °C, 24 h). For the compound gave a negative response to ninhydrin test, it must be a cyclic pentapeptide.

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	228	[Ala-Gly-Val+H] <sup>+</sup>
II	m/z	299	[Ala-Gly-Val-Ala+H] <sup>+</sup>
III	m/z	258	[Ala-Trp+H] <sup>+</sup>
IV	m/z	329	[Ala-Trp-Ala+H] <sup>+</sup>
V	m/z	386	[Ala-Trp-Ala-Gly+H] <sup>+</sup>
VI	m/z	485	[Ala-Trp-Ala-Gly-Val+H] <sup>+</sup>

These fragments could give the gross structure as cyclo-(Trp-Ala-Gly-Val-Ala).

Further evidences were provided by <sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>13</sup>C COSY, TOSCY and COLOC spectra. At first we assigned proton and carbon signals of every amino acid residues with those 2D NMR experiments (The data are shown in Table 1), and then determined the sequence of amino acid residues based on the correlations between amide CO and NH in COLOC spectra as following (Tan *et al*, 1993):



Therefore, the structure of the compound named vaccarin A, a new pentacyclopeptide, was elucidated as cyclo-(tryptophyl-alanyl-glycyl-valyl-alanyl).

**Vaccarin B (2)** crystals,  $[\alpha]_D^{20} +5.58^\circ$  (c, 0.448, CHCl<sub>3</sub>). Its molecular formula was deduced as C<sub>37</sub>H<sub>49</sub>O<sub>8</sub>N<sub>7</sub> by means of DEPT spectral analysis and FAB-MS [(M+1)<sup>+</sup> at m/z 720]. The IR spectrum in

KBr disc showed intense amide C=O at 1650, 1616  $\text{cm}^{-1}$  and amide N-H at 3310  $\text{cm}^{-1}$ . In the DEPT spectrum (DMSO), a total of seven amide CO signals could be seen between 168.6 and 171.3 ppm. Meanwhile, the middle and high field signals of seven methines, eight methylenes, three 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 one proline, one glycine, one leucine, one serine, two phenylalanines, one alanine. These data indicated that vaccarin B appeared to be a cyclic heptapeptide.

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] <sup>+</sup>
II	m/z	268	[Pro-Gly-Leu+H] <sup>+</sup>
III	m/z	355	[Pro-Gly-Leu-Ser+H] <sup>+</sup>
IV	m/z	502	[Pro-Gly-Leu-Ser-Phe+H] <sup>+</sup>
V	m/z	573	[Pro-Gly-Leu-Ser-Phe-Ala+H] <sup>+</sup>
VI	m/z	720	[Pro-Gly-Leu-Ser-Phe-Ala-Phe+H] <sup>+</sup>

Therefore, the gross structure of the compound could be deduced as cyclo-(Pro-Gly-Leu-Ser-Phe-Ala-Phe).

With the same methods mentioned above, we could determine the sequence of amino acid residues as the following peptide based on the correlations between amide CO and NH in COLOC spectra:



Then the structure of the compound named vaccarin B, a heptacyclopeptide, was elucidated as cyclo-(prolyl-glycyl-leucyl-seryl-phenylalanyl-alanyl-phenylalanyl).

**Vaccarin C (3)** amorphous powder,  $[\alpha]_D^{19} -47.85^\circ$  (c, 0.768, CH<sub>3</sub>OH). Its molecular formula was deduced as C<sub>43</sub>H<sub>56</sub>O<sub>8</sub>N<sub>8</sub> by means of DEPT spectral analysis and FAB-MS [(M+1)<sup>+</sup> at m/z 813]. The IR spectrum in KBr disc showed intense amide C=O at 1660~1611  $\text{cm}^{-1}$  and amide N-H at 3346~3240  $\text{cm}^{-1}$ . In the DEPT spectrum (DMSO), a total of seven amide CO signals could be seen between 171.1 and 174.6 ppm. Meanwhile, the middle and high field signals of eight methines, ten methylenes, four methyls, one indole group and one *p*-hydroxyphenyl were identified. Applications of 2D NMR techniques and amino acid analysis after hydrolysis, the amino acid composition could be identified to be two prolines, one glycine, one tyrosine, one valanine, one leucine, one tryptophan. These data indicated that vaccarin C appeared to be a cyclic heptapeptide too.

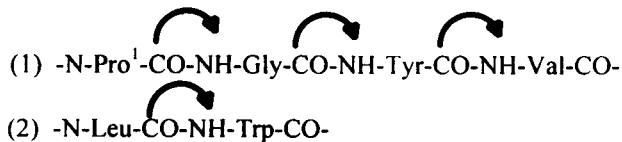
The amino acid sequence could be determined preliminarily by positive FAB-MS which showed the fragments of I to V as following:

I	m/z	318	[Pro-Gly-Tyr+H] <sup>+</sup>
II	m/z	417	[Pro-Gly-Tyr-Val+H] <sup>+</sup>
III	m/z	514	[Pro-Gly-Tyr-Val-Pro+H] <sup>+</sup>
IV	m/z	627	[Pro-Gly-Tyr-Val-Pro-Leu+H] <sup>+</sup>
V	m/z	813	[Pro-Gly-Tyr-Val-Pro-Leu-Trp+H] <sup>+</sup>

So the gross structure of the compound could be deduced as cyclo-(Pro-Gly-

Tyr-Val-Pro-Leu-Trp).

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



Therefore, the structure of the compound named vaccarin C, a heptacyclopeptide, was elucidated as cyclo-(prolyl-glycyl-tyrosyl-valyl-prolyl-leucyl-tryptophyl).

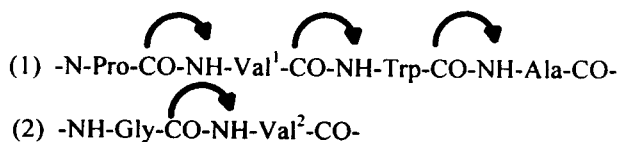
**Vaccarin D (4)** amorphous powder,  $[\alpha]_D^{24} -95.26^\circ$  (c, 0.454,  $\text{CHCl}_3$ ). Its molecular formula was deduced as  $\text{C}_{31}\text{H}_{43}\text{O}_6\text{N}_7$  by means of DEPT spectral analysis and FAB-MS  $[(M+1)^+ \text{ at } m/z 610]$ . The IR spectrum in KBr disc showed intense amide  $\text{C}=\text{O}$  at 1658, 1630  $\text{cm}^{-1}$  and amide  $\text{N}-\text{H}$  at 3330, 3234  $\text{cm}^{-1}$ . In the DEPT spectrum (DMSO), a total of six amide CO signals could be seen between 169.2 and 172.8 ppm. Meanwhile, the middle and high field signals of seven methines, five methylenes, five methyls and one indole group were identified. Applications of 2D NMR techniques and amino acid analysis after hydrolysis, the amino acid composition could be identified to be one proline, two valanines, one tryptophan, one alanine, one glycine. These data indicated that vaccarin D appeared to be a cyclic hexapeptide.

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

I	m/z 258	$[\text{Trp-Ala}+\text{H}]^+\text{ }^{\text{§}}$
II	m/z 315	$[\text{Trp-Ala-Gly}+\text{H}]^+$
III	m/z 414	$[\text{Trp-Ala-Gly-Val}+\text{H}]^+$
IV	m/z 511	$[\text{Trp-Ala-Gly-Val-Pro}+\text{H}]^+$
V	m/z 610	$[\text{Trp-Ala-Gly-Val-Pro-Val}+\text{H}]^+$

So the gross structure of the compound could be deduced as cyclo-(Trp-Ala-Gly-Val-Pro-Val).

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



Therefore, the structure of the compound named vaccarin D, a hexacyclopeptide, was elucidated as cyclo-(prolyl-valyl-tryptophyl-alanyl-glycyl-valyl).

## EXPERIMENTAL

**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.

Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of vaccarin A (1) and B (2) [in DMSO, 400MHz for  $\delta_{\text{H}}$ , 100MHz for  $\delta_{\text{C}}$ , TMS]

vaccarin A (1)			vaccarin B (2)		
Amino acid residues	H	C	Amino acid residues	H	C
Trp			Pro		
$\alpha$	4.21(1H,m)	56.1	$\alpha$	4.12(1H,m)	61.0
$\beta$	3.20(2H,dd,14.4,6.4)	26.3	$\beta$	2.04(1H,m),1.72(1H,m)	28.9
indole 1	10.86(1H,d,2.0)		$\gamma$	1.82(2H,m)	24.4
2	7.12(1H,d,2.0)	123.5	$\delta$	3.48(1H,m),2.92(1H,m)	47.4
3		109.9	CO		171.1
4		127.4	Gly		
5	7.54(1H,d,7.6)	118.2	$\alpha$	4.13(1H,m)	42.5
6	7.06(1H,dd,7.6,6.8)	120.9		3.33(1H,dd,16.8,4.3)	
7	6.97(1H,dd,8.0,7.2)	118.2	NH	8.84(1H,m)	
8	7.33(1H,d,8.4)	111.3	CO		168.6
9		136.0	Leu		
NH	7.93(1H,m)		$\alpha$	4.53(1H,d,10.0)	52.4
CO		171.0	$\beta$	1.55(2H,m)	42.9
Ala <sup>1</sup>			$\gamma$	1.56(1H,m)	24.0
$\alpha$	4.07(1H,m)	49.7	$\delta$	0.77(3H,d,5.7),0.72(3H,d,5.7)	22.6,21.6
$\beta$	1.22(3H,d,7.2)	17.0	NH	8.06(1H,d,10.3)	
NH	7.98(1H,d,8.8)		CO		171.1
CO		171.3	Ser		
Gly			$\alpha$	4.41(1H,d,6.1)	54.4
$\alpha$	4.10(1H,m),3.28(1H,m)	43.4	$\beta$	4.12(1H,m),3.75(1H,d,22.0)	62.2
NH	8.44(1H,t,5.6)		NH	8.43(1H,d,6.4)	
CO		169.5	CO		170.3
Val			Phe <sup>1</sup>		
$\alpha$	3.90(1H,t,7.6)	59.4	$\alpha$	4.19(1H,m)	56.6
$\beta$	1.96(1H,m)	29.6	$\beta$	3.02(2H,d,6.4)	35.7
$\gamma$	0.87(3H,d,5.6),0.85(3H,d,6.4)	19.0,18.0	$\varphi$	7.16~7.32(5H,m)	126.4
NH	7.75(1H,d,8.0)				128.2
CO		170.3			128.9
Ala <sup>2</sup>					137.3
$\alpha$	4.19(1H,m)	48.4	NH	8.51(1H,d,4.5)	
$\beta$	1.19(3H,d,6.8)	16.9	CO		170.3
NH	7.95(1H,m)		Ala		
CO		172.1	$\alpha$	4.11(1H,m)	49.0
			$\beta$	1.04(3H,d,7.3)	17.8
			NH	7.73(1H,d,8.6)	
			CO		171.3
			Phe <sup>2</sup>		
			$\alpha$	4.72(1H,dd,14.0,8.0)	51.8
			$\beta$	2.99(1H,m)	38.5
				2.65(1H,dd,13.2,5.8)	
			$\varphi$	7.16~7.32(5H,m)	126.4
					128.2
					129.3
					136.7
			NH	7.22(1H,m)	
			CO		169.0

Table 2  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of vaccarins C (3) and D (4) [in DMSO, 400MHz for  $\delta_{\text{H}}$ , 100HMz for  $\delta_{\text{C}}$ , TMS]

vaccarin C (3)			vaccarin D (4)		
Amino acid residues	H	C	Amino acid residues	H	C
Pro <sup>1</sup>			Pro		
$\alpha$	4.15(1H,m)	64.0	$\alpha$	4.33(1H,d,8.0)	60.3
$\beta$	2.23(1H,m),1.83(1H,m)	29.8	$\beta$	2.09(1H,m),1.90(1H,m)	31.3
$\gamma$	1.92(1H,m),1.52(1H,m)	22.6	$\gamma$	1.90(1H,m),1.70(1H,m)	21.4
$\delta$	3.51(1H,m),3.28(1H,m)	47.5	$\delta$	3.56(2H,dd,8.8,4.8)	46.7
CO		174.6	CO		171.5
Gly			Val <sup>1</sup>		
$\alpha$	3.69(2H,m)	43.6	$\alpha$	4.11(1H,d,10.0)	59.8
NH	7.97(1H,m)		$\beta$	2.02(1H,m)	30.0
CO		171.1	$\gamma$	0.75(3H,d,3.6),0.74(3H,d,3.6)	19.1,18.4
Tyr			NH	7.34(1H,d,8.4)	
$\alpha$	4.61(1H,m)	55.8	CO		172.5
$\beta$	2.70(1H,t,12.0)	36.8	Trp		
	2.60(1H,dd,18.0,4.0)		$\alpha$	4.25(1H,d,6.4)	55.6
$\psi$	6.63(2H,d,6.0),6.94(2H,d,8.4)	116.0	$\beta$	3.10(2H,m)	25.9
		130.2	indole 1	10.9(1H,s)	
		131.4	2	7.13(1H,d,1.6)	123.3
		156.4	3		109.2
NH	8.31(1H,t,6.5)		4		127.0
CO		173.1	5	7.54(1H,d,7.6)	118.3
Val			6	7.06(1H,t,7.2)	121.0
$\alpha$	3.92(1H,d,7.4)	60.1	7	6.96(1H,t,7.2)	118.3
$\beta$	1.95(1H,m)	30.9	8	7.33(1H,m)	111.3
$\gamma$	1.01(3H,d,6.7),0.93(3H,d,7.0)	19.5,19.1	9		136.1
NH	8.39(1H,d,4.0)		NH	8.51(1H,d,6.0)	
CO		173.2	CO		172.8
Pro <sup>2</sup>			Ala		
$\alpha$	4.59(1H,m)	62.5	$\alpha$	3.65(1H,m)	49.4
$\beta$	2.41(1H,dd,16.0,10.0)	32.0	$\beta$	1.12(3H,d,6.8)	15.4
	1.89(1H,m)		NH	8.98(1H,d,6.8)	
$\gamma$	2.06(1H,m),1.84(1H,m)	26.7	CO		171.2
$\delta$	3.68(1H,m),3.50(1H,m)	48.4	Gly		
CO		173.2	$\alpha$	3.65(1H,m),3.35(1H,m)	42.8
Leu			NH	7.44(1H,t,5.2)	
$\alpha$	4.17(1H,m)	56.5	CO		169.2
$\beta$	1.58(1H,m),1.26(1H,m)	41.3	Val <sup>2</sup>		
$\gamma$	1.43(1H,m)	26.3	$\alpha$	4.51(1H,m)	55.3
$\delta$	0.81(3H,d,6.5),0.77(3H,d,6.5)	23.3,21.3	$\beta$	2.17(1H,m)	29.7
NH	8.39(1H,d,4.0)		$\gamma$	0.92(3H,d,10.4)	19.4,17.4
CO		174.6		0.80(3H,d,6.8)	
Trp			NH	7.41(1H,d,9.2)	
$\alpha$	5.00(1H,m)	54.1	CO		170.7
$\beta$	3.53(1H,m)	26.7			
	3.23(1H,dd,13.4,5.4)				
indole 1					
2	7.26(1H,d,9.2)	125.7			
3		109.4			
4		129.4			
5	7.02(1H,m)	120.1			
6	7.02(1H,m)	122.5			
7	7.63(1H,d,7.2)	119.3			
8	7.26(1H,d,9.2)	112.6			
9		137.5			
NH	7.74(1H,d,7.2)				
CO		172.7			



$\nu_{\max}^{\text{KBr}}$ : 3330, 3234, 1658, 1630. Pos. FAB-MS  $m/z$ : 610[(M+1)<sup>+</sup>, 77], 511(4), 414(1.5), 315(4), 258(2.5). <sup>1</sup>H and <sup>13</sup>C NMR: see Table 2. Amino acid analysis (standard method): Trp (1eq), Ala (1eq), Pro (1eq), Gly (1eq), and Val (2eq).

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