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Structure of a new cyclic nonapeptide, segetalin F, and vasorelaxant activity of segetalins from *Vaccaria segetalis*

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Abstract—A new cyclic nonapeptide, segetalin F, has been isolated from the seeds of *Vaccaria segetalis* and the structure including absolute stereochemistry was elucidated by using 2D NMR and chemical means. A series of segetalins showed a vasorelaxant activity against norepinephrine (NE)-induced contractions of rat aorta.
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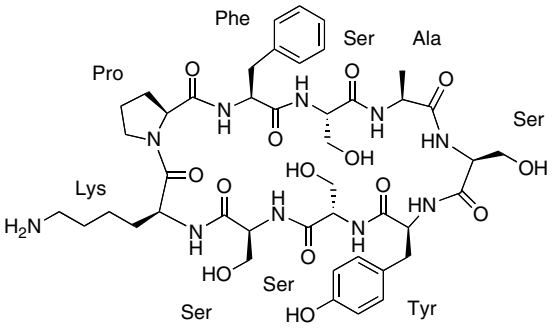
Cyclic peptides comprise a class of naturally occurring molecules, which exhibit a range of biological activities.[1] Their cyclic nature often provides more lipophilicity and membrane permeability, because of reduced zwitterionic character. Furthermore, restricted bond rotation results in rigid backbone conformation with more affinity and selectivity for binding proteins.[2] We previously isolated some new types of bioactive cyclic peptides[3–6] from higher plants such as *Rubia cordifolia*,[3] *Aster tataricus*,[4] *Vaccaria segetalis*,[5] *Celosia argentea*,[6] *Stellaria dichotoma* var. *lanceolata*,[7] and *Leonurus heterophyllus*.[8]

Our continuing search for structurally unique and biogenetically interesting peptides resulted in the isolation of a new cyclic nonapeptide, segetalin F, from the seeds of *Vaccaria segetalis* (Caryophyllaceae), which have been used as a Chinese drug for invigorating blood circulation, regulating menstrual disturbance, and to dispel edema.[9] In this paper, we describe the isolation, structure elucidation, and a vasorelaxant activity of a series of segetalins on rat aorta.

The seeds of *V. segetalis* were extracted with MeOH, and the MeOH extract was in turn partitioned with EtOAc and *n*-BuOH. Chromatographic purification of



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EtOAc materials gave segetalins A–E (**1**–**5**) as described in the previous papers.[5] *n*-BuOH-soluble materials were subjected to a Diaion HP-20 column ($MeOH/H_2O$, 0:1 → 1:0), in which a fraction eluted with 80% MeOH was purified by C_{18} HPLC (15% CH_3CN/0.1% CF_3CO_2H) followed by an amino silica gel column ($CHCl_3/MeOH/H_2O$, 7:3:0.5 → 6:4:1) to afford segetalin F (**6**, 0.002% yield) as colorless solids together with segetalins G (**7**) and H (**8**).[5]

ESIMS data of segetalin F (**6**) {$[\alpha]_D^{20}$ −73° (*c* 0.4, MeOH)} showed the pseudomolecular ion at *m*/*z* 955 $(M+H)^+$, and the molecular formula, $C_{44}H_{62}N_{10}O_{14}$, was established by ESITOFMS [*m*/*z* 955.4500, $(M+H)^+$, Δ −2.6 mmu]. IR absorptions implied the presence of hydroxyl (3310 cm^{-1}) and amide carbonyl groups (1685 cm^{-1}). Standard amino acid analysis of

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Table 1. ^{1}H and ^{13}C NMR data for segetalin F (**6**) in DMSO-d_6

Position	δ_H (int.; mult.; J (Hz))	δ_C
Pro[1]		
α	4.05 (1H, dd, 6.9, 6.9)	60.2
β	1.63 (1H, m)	28.8
	1.88 (1H, m)	
γ	1.84 (1H, m)	24.5
	1.93 (1H, m)	
δ	3.54 (1H, m)	47.2
	3.78 (1H, m)	
C=O		171.5
Phe[2]		
α	3.90 (1H, m)	55.9
β	3.15 (1H, dd, 13.2, 13.2)	33.9
	3.23 (1H, m)	
γ		139.0
δ	7.14 (2H, d, 7.2)	128.9
ε	7.27 (2H, t, 7.2)	128.1
ζ	7.20 (1H, t, 7.2)	126.1
NH	8.37 (1H, m)	
C=O		169.8
Ser[3]		
α	4.32 (1H, ddd, 6.6, 6.9, 6.0)	54.9
β	3.66 (1H, dd, 10.8, 6.0)	62.2
	3.96 (1H, m)	
NH	7.41 (1H, br d, 6.9)	
C=O		170.5
Ala[4]		
α	4.21 (1H, m)	48.8
β	1.27 (3H, d, 7.2)	17.0
NH	8.39 (1H, m)	
C=O		172.2
Ser[5]		
α	4.16 (1H, m)	56.4
β	3.31 (2H, m)	61.8
NH	7.85 (1H, br d, 6.0)	
C=O		169.1
Tyr[6]		
α	4.55 (1H, m)	53.8
β	2.68 (1H, dd, 14.1, 10.2)	35.8
	2.98 (1H, br d, 14.1)	
γ		127.7
δ	7.01 (2H, d, 8.7)	130.0
ε	6.63 (2H, d, 8.7)	114.7
ζ		155.6
NH	7.81 (1H, br d, 8.4)	
C=O		171.6
Ser[7]		
α	4.16 (1H, m)	56.2
β	3.59 (1H, m)	61.1
	3.72 (1H, dd, 11.1, 5.7)	
NH	8.26 (1H, m)	
C=O		169.6
Ser[8]		
α	4.10 (1H, m)	55.4
β	3.62 (1H, m)	60.3
	3.79 (1H, m)	
NH	7.71 (1H, m)	
C=O		169.3
Lys[9]		
α	4.61 (1H, ddd, 6.9, 6.6, 6.6)	49.6
β	1.52 (1H, m)	30.8
	1.67 (1H, m)	
γ	1.29 (2H, m)	21.8
δ	1.36 (1H, m)	30.9
	1.41 (1H, m)	
ε	2.51 (2H, m)	39.9
NH	7.41 (1H, br d, 6.9)	
NH_2	7.76 (2H, m)	
C=O		170.2

the hydrolysates of **6** showed the presence of each 1 mol of tyrosine (Tyr), phenylalanine (Phe), alanine (Ala), proline (Pro), and lysine (Lys), and 4 mol of serine (Ser). The absolute configurations of the amino acid residues in **6** were assigned as all L-configurations by chiral HPLC analysis of the hydrolysates of **6**.[10] The ^{1}H NMR (Table 1) spectrum of **6** in DMSO-d_6 showed nine proton resonances (δ 3.90–4.61), which were indicative of α-protons of amino acid residues. The presence of one methyl group, thirteen methylenes, nine sp^3 methines, nine sp^2 methines, and three sp^2 quaternary carbons was indicated by the ^{13}C NMR (Table 1) spectrum. Among them, four methylenes (δ_C 60.3, 61.1, 61.8, and 62.2) and one sp^2 quaternary carbon (δ_C 155.6) were ascribed to those bearing an oxygen atom. The Pro residue was revealed by analysis of the ^{1}H and ^{13}C NMR data (Table 1) using 2D experiments. The presence of the Lys was implied by ^{1}H–^{1}H COSY and HOHAHA cross-peaks for Hα to NH_2 (Fig. 1) and the carbon signal (δ_C 39.9) was assigned as Cε by HMQC spectrum. The UV absorption (ε 2030) at 276 nm of **6** supported the Tyr residue. These data combined with observation of nine carbonyl signals (δ 169.1–172.2) in the ^{13}C NMR spectrum suggested that **6** was an nonapeptide (Fig. 2).

The sequence of the nine amino acids was elucidated by detailed analysis of HMBC correlations for each Hα and the next NH to the amide carbonyl carbon as shown in Figure 1. The cyclic peptide nature analyzed by the NOESY correlation between Hα of Lys and Hδ of Pro revealed the whole sequence of segetalin F to be **6** (Fig. 1).

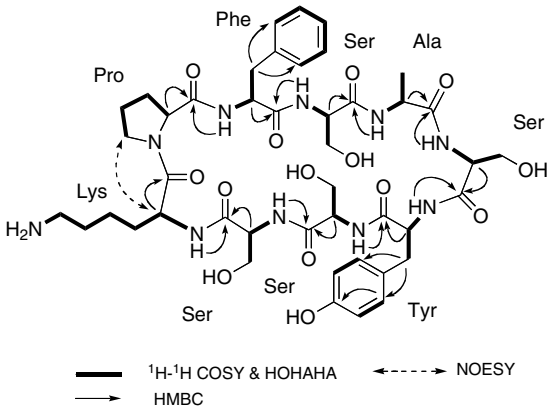

Figure 1. Selected 2D NMR correlations of segetalin F (**6**) in DMSO-d_6.

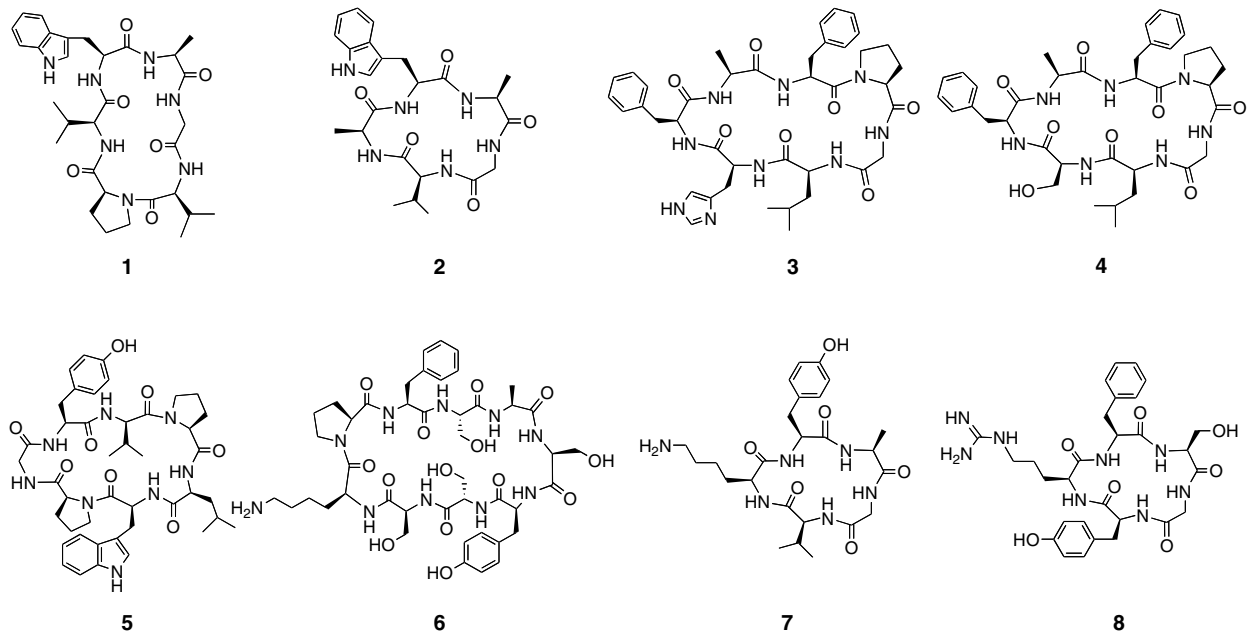

Figure 2. Structures of segetalins A–H (**1**–**8**).

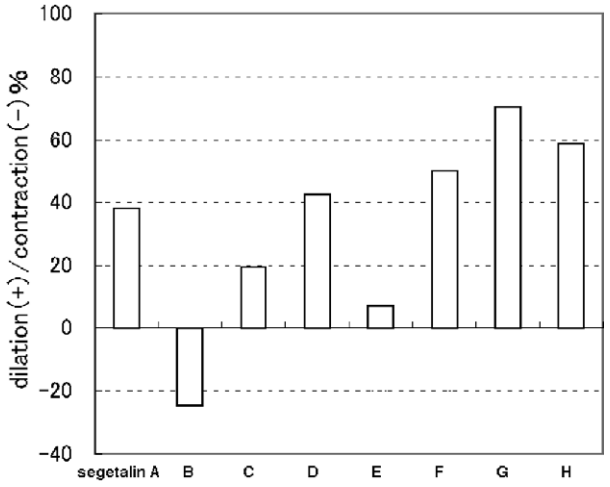

Figure 3. Relaxation Effects of 10^{-4} M segetalins A–H (**1**–**8**) on aortic rings precontracted with 3×10^{-7} M norepinephrine (NE). Positive values show vasodilator effects and negative ones show vasocontraction effects.

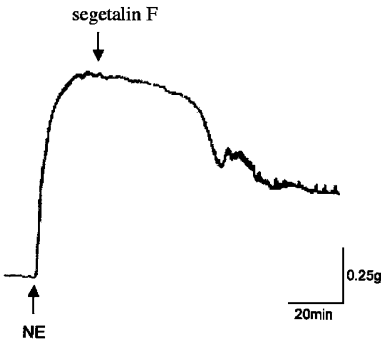

Figure 4. Typical recording of the slow relaxation effect of segetalin F (**6**, 10^{-4} M) on NE (3×10^{-7} M)—contracted aorta.

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. When NE 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, we added segetalins A–H (**1**–**8**) at 10^{-4} M (Fig. 3).[11] Segetalins A (**1**), D (**4**), F (**6**), G (**7**), and H (**8**) showed slow vasorelaxant actions (Fig. 4). Segetalins F (**6**), G (**7**), and H (**8**) with a basic amino acid such as Lys and Arg exhibited relatively potent relaxant activity. Interestingly, segetalin B (**2**) with a Trp residue and without a basic amino acid such as Lys and Arg residues showed contractile activity. In addition, vasodilation seems not to get influenced by the ring size. The same relaxant actions were seen in the sample of aortic rings without endothelium. The modes of action of segetalins on vasorelaxant and contractile activities are under investigation.

Acknowledgments

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