Pergamon

Tetrahedron Letters, Vol. 35, No 51, pp. 9593-9596, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02133-3

SEGETALIN A, A NEW CYCLIC HEXAPEPTIDE FROM *VACCARIA SEGETALIS* [1)]

Hiroshi Morita, Young Sook Yun, Koichi Takeya and Hideji Itokawa*

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432-1,
Hachioji, Tokyo 192-03, Japan

Abstract: A new cyclic hexapeptide, segetalin A, showing estrogen-like activity, has been isolated from the seeds of *Vaccaria segetalis* and the structure was elucidated by extensive 2D NMR, chemical and enzymatic degradations and ESI-MS/MS methods.

Recently a number of cyclic peptides with unique structures and biological activities have been isolated from natural origin. As part of our ongoing investigation of bioactive cyclic peptides from higher plants,[1-3)] we have isolated several cyclic peptides, named segetalins, showing potent estrogen-like activities, from the seeds of *Vaccaria segetalis* (Caryophyllaceae). The seeds of *V. segetalis* have been used to activate blood flow and promote milk secretion. In addition, it is commonly used to treat amenorrhea and breast infections.[4)] Extraction and purification examination of chemical constituents showing these follicle hormonic activity led us to isolation of a novel bioactive cyclic peptide. In this paper, we describe the isolation and structure elucidation of a major cyclic hexapeptide, named segetalin A (**1**) and its potent estrogen-like activity.

The methanolic extract of the seeds of *V. segetalis* was partitioned between EtOAc and H_2O. The EtOAc soluble material was subjected to silica gel column (CH_2Cl_2 - MeOH) and 80% CH_2Cl_2 eluted fraction was further chromatographed on a ODS HPLC column (35% CH_3CN/0.05% TFA) to yield several peptidic compounds as colorless needles, the major one of which, showing potent activity, is named as segetalin A (**1**: 0.02%).

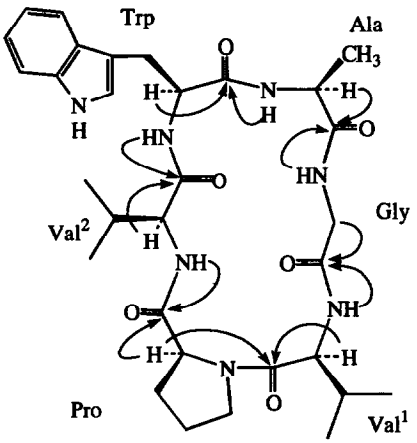

Fig. 1 Structure of Segetalin A (**1**), Arrows show some important HMBC correlations.

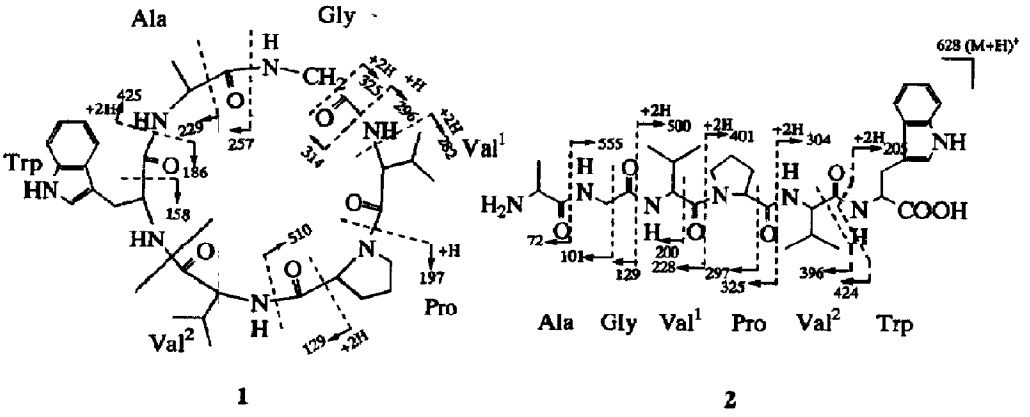

Fig. 2 ESI MS/MS Fragmentations of Segetalin A (**1**) and **2**

Table 1. ^{1}H and ^{13}C NMR Signal Assignments of Segetalin A (**1**) in pyridine-d_5.

assignment		δ_H (int. mult, J(Hz))	δ_C			δ_H	δ_C
Gly				Val^2			
	α	4.12 (1H, dd, 5.8, 16.5)	44.30		α	4.88 (1H, t, 10.2)	61.43
		4.42 (1H, dd, 5.8, 16.5)			β	2.38 (1H, m)	31.40
	NH	8.39 (1H, t, 5.8)			γ	0.93 (3H, d, 6.5)	19.05
	C=O		170.95			0.99 (3H, d, 6.6)	19.51
Val^1					NH	7.64 (1H, d, 10.2)	
	α	5.19 (1H, dd, 4.2, 10.0)	56.31		C=O		174.39
	β	2.72 (1H, m)	30.88	Trp			
	γ	1.28 (3H, d, 6.9)	17.92		α	4.92 (1H, ddd,5.7,7.5,8.6)	57.02
		1.58 (3H, d, 6.8)	20.09		β	3.27 (1H, dd, 7.5, 14.2)	26.42
	NH	8.09 (1H, d, 10.0)				3.43 (1H, dd, 8.6, 14.2)	
	C=O		172.40		NH	9.46 (1H, d, 5.7)	
Pro					1(NH)	11.91 (1H, s)	
	α	4.96 (1H, d, 8.3)	61.25		2	7.14 (1H, d, 2.2)	124.39
	β	1.96 (1H, m)	32.26		3		109.94
		2.13 (1H, dd, 6.2, 12.2)			4	7.56 (1H, d, 8.0)	118.86
	γ	1.62 (2H, m)	22.17		5	7.27 (1H, t, 8.0)	121.92
	δ	2.65 (1H, br t, 10.0)	47.57		6	7.08 (1H, t, 8.0)	119.33
		3.46 (1H, m)			7	7.59 (1H, d, 8.0)	112.01
	C=O		172.97		8		137.47
					9		127.88
					C=O		175.16
				Ala			
					α	3.99 (1H, m)	50.96
					β	1.72 (3H, d, 7.1)	16.05
					NH	10.55 (1H, d, 6.9)	
					C=O		171.86

Segetalin A (1),[5] colorless needles, mp.183.0 - 185.0 °C, $[\alpha]_D$ -73.4° (c 0.41, MeOH), showed a molecular formula, $C_{31}H_{43}N_7O_6$, which was permitted by HR FAB MS spectrum, indicating 14 degrees of unsaturation. The IR absorptions at 3315 and 1657 cm^{-1} were attributed to amino and amide carbonyl groups, respectively, and the UV absorptions at 272 (ε 6070), 280 (6470), 289 (5660) to an indole skeleton. Amino acid analysis of **1** showed the presence of Gly, Ala, Val × 2, Pro and Trp, which were confirmed to be all L-configuration by Marfey's derivatization,[6] followed by HPLC analysis.

In ^1H NMR spectrum (pyridine-d_5), existing in a single stable conformational state, the presence of five amide protons was clearly observed. Therefore, the other amino acid was assumed to belong to Pro and **1** was hexapeptide, which was also suggested by the presence of six carbon signals due to amide carbonyl. Since this composition accounted for 14 degrees of unsaturation, the other degree of unsaturation for **1** suggested cyclic nature of the peptide.

Extensive 2D NMR analyses, including COSY and HMQC[7] spectra, were used to determine the identity of the six amino acids and to assign the ^1H and ^{13}C signals. The sequence of the cyclic peptide was established based on data from HMBC experiment.[8] As can be seen from Fig. 1, which showed some important correlations, the whole structure was deduced to be Cyclo(Gly-Val-Pro-Val-Trp-Ala) and was confirmed by ESI-MS/MS experiment, as shown below.

ESI is a very soft ionization technique, which generates chiefly ions related to the molecular weight.[9] We have already reported that the possibility of using ESI MS/MS techniques as a tool for sequence determination of the peptides was tested.[3] The ESI MS spectra of **1** and **2** which is a corresponding acyclic peptide generated by digestion of **1** with α-chymotrypsin,[10] produced $(M+H)^+$ ion, which was then analyzed in a second mass spectrometer. As can be seen from fragmented ions of **1** and **2** (Fig. 2), the sequence of **1** was surely confirmed to be Gly-Val-Pro-Val-Trp-Ala.

From the examination of the effects of segetalin A on reproductive organs in female rats without ovary, **1** showed potent estrogen-like activity as follows. Following the administration of **1** (2.5 mg/kg, s.c., for consecutive 14 d from day 1 to 14), the weight of uterus was significantly increased (3.7 times against control uterus). The presence of many cyclic peptides, showing hormonic activities like oxytocin, vasopressin and so on, is well known. However, the presence of cyclic peptides as non-steroidal estrogens, showing estrogen-like activity, is not known yet. Furthermore, It is interesting that this estrogen-like activity well reflects the medicinal usage of the seeds of *V. segetalis*.

Studies on the structure analyses and biological evaluations of a series of segetalins are in progress and conformational informations will be described in a following paper as well as more detailed biological activity.

Acknowledgments: We are grateful to Dr. Mamoru Mieda of TEIKOKU HORMONE MFG. CO., LTD. for the biological evaluations.

References and Notes

1. Cyclic Peptides from Higher Plants. Part 13.; Part 12, H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, in press.
2. H. Itokawa, H. Morita, K. Takeya, N. Tomioka, A. Itai and Y. Iitaka, *Tetrahedron*, **1991**, *47*, 7007; H. Itokawa, H. Morita, K. Takeya, N. Tomioka and A. Itai, *Chem. Lett.*, **1991**, 2217; H. Itokawa, T. Yamamiya, H. Morita and K. Takeya, *J. Chem. Soc. Perkin Trans. 1*, **1992**, 455; H. Morita, T. Yamamiya, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, **1992**, *40*, 1352; H. Itokawa and K. Takeya, *Heterocycles*, **1993**, *35*, 1467; K. Takeya, T. Yamamiya, H. Morita and H. Itokawa, *Phytochemistry*, **1993**, *33*, 613; H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, **1993**, *41*, 992; H, Morita, S. Nagashima, O. Shirota, K. Takeya and H. Itokawa, *Chem. Lett.*, **1993**, 1877; H. Morita, H. Kobata, K. Takeya and H. Itokawa, *Tetrahedron Lett.*, **1994**, *35*, 3563; H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Heterocycles*, in press.; H. Morita, S. Nagashima, K. Takeya and H. Itokawa, *Tetrahedron*, in press; H. Morita, T. Kayashita, K. Takeya and H. Itokawa, *Tetrahedron*, in press; H, Morita, S. Nagashima, K. Takeya and H. Itokawa, *Chem. Lett.*, in press.
3. H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya and H. Itokawa, *Tetrahedron*, **1994**, *50*, 6797; H. Morita, T. Kayashita, H. Kobata, A. Gonda, K. Takeya and H. Itokawa, *ibid.*, **1994**, *50*, 9975.
4. The Pharmacology of Chinese Herbs, ed. by Kee Chang Huang, CRC press, Inc., USA, **1993**, p 254-255.
5. Segetalin A (1): HR FAB MS m/z: 610 (M^++1, Calcd for $C_{31}H_{44}N_7O_6$ 610.3353, Found 610.3343), ν_{max} (KBr)/cm^{-1} 3315 (NH), 3061, 2967, 2934, 1657 (amide C=O), 1526, 1458 and 1176; λ_{max} (MeOH) / nm 272 (ε 6070), 280 (6470), 289 (5660).
6. P. Marfey, Carlsberg Res. Commun., **1984**, *49*, 591.
7. A. Bax and S. Subramanian, J. Magn. Reson., **1986**, *67*, 565.
8. A. Bax and D. G. Davis, J. Magn. Reson., **1985**, *65*, 355.
9. A. P. Bruins, T. R. Corey and J. D. Henion, *Anal. Chem.*, **1987**, *59*, 2642; "Tandem Mass Spectrometry," ed. by F. W. McLafferty, Wiley, New York (1983).
10. α-Chymotrypsin (500 μg) was added to **1** (1 mg) in NH_4HCO_3 solution (1%, 0.9 ml) and the digestion was performed at 30 °C at pH 8.0. After 24 h, the reaction was stopped by 1N HCl and the digestion mixture was lyophilized to dryness. The hydrolysates were subjected to HPLC to give **2** (0.1 mg), amorphous powder.

(*Received in Japan* 1 *August* 1994; *accepted* 30 *September* 1994)