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PSEUDOSTELLARIN G, A NEW TYROSINASE INHIBITORY CYCLIC OCTAPEPTIDE FROM *PSEUDOSTELLARIA HETEROPHYLLA* [1)]

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Abstract: A new potent tyrosinase inhibitory cyclic octapeptide, pseudostellarin G, has been isolated from the roots of *Psedostellaria heterophylla* and the structure was elucidated by extensive 2D NMR methods and chemical degradation.

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,[1,2)] we have isolated a novel cyclic octapeptide, named pseudostellarin G, showing potent tyrosinase and melanin formation inhibitory activities, from the roots of *Psedostellaria heterophylla* (Caryophyllaceae). Tyrosinase inhibitors may control over production of the dermal melanin pigment since tyrosinase, which is a bifunctional copper protein widely distributed in animals and plants, plays an important role in the process of melanin biosynthesis.[3)] In this paper, we describe the isolation and structure elucidation of pseudostellarin G (**1**) and its potent activity of a novel tyrosinase inhibitor.

The methanolic extract of the roots of *P. heterophylla* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH soluble material was subjected to Diaion HP-20 column (H_2O - MeOH) and 80% MeOH eluted fraction was chromatographed on a silica gel column, followed by HPLC on ODS (70% MeOH) to yield several peptidic compounds as colorless needles, which one of them, showing most potent activity, is named as pseudostellarin G (**1**: 0.001%).

Pseudostellarin G (**1**), colorless needles, dec.265 °C, $[\alpha]_D$ -57.7° (c 0.78, MeOH), showed a molecular formula, $C_{42}H_{56}N_8O_9$, which was permitted by HR FAB MS spectrum,[4)] indicating 19 degrees of unsaturation. Amino acid analysis of **1** showed the presence of Pro × 2, Phe × 2, Ser, Ala, Gly and Leu, which were confirmed to be all L-configuration by Marfey's derivatization,[5)] followed by HPLC analysis. In ^1H NMR spectrum, **1** existed in two stable conformational states (e.g. 5:1 in pyridine-d_5; 1:1 in methanol-d_4) in polar solvents, which this phenomenon might have resulted from the isomerization about the proline amide bond with an isomerization rate slow enough to give separate signals in the NMR spectra. In major conformer (pyridine-d_5), the

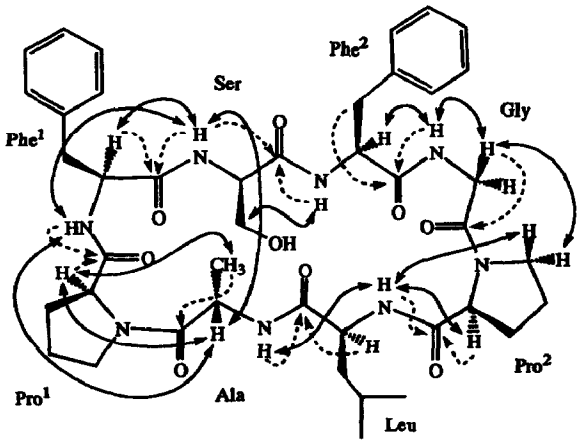

Fig. 1 Structure of Pseudostellarin G, Arrows show ROE relationship and dashed arrows show HMBC correlations.

presence of six amide protons was clearly observed. Therefore, the other amino acids were assumed to belong to Pro and 1 was octapeptide, which was also suggested by the presence of eight carbon signals due to amide carbonyl. Since this composition accounted for 18 degrees of unsaturation, the other degree of unsaturation for 1 suggested cyclic nature of the peptide.

Extensive 2D NMR analyses, including DQF-COSY, HOHAHA[6] and HMQC[7] spectra, were used to determine the identity of the eight amino acids and to assign the ^{1}H and ^{13}C signals.[8] The sequence of the cyclic peptide was established based on data from HMBC experiment.[9] As can be seen from Fig. 1, which showed some important correlations, two structural units, Pro-Phe-Ser-Phe-Gly and Pro-Leu-Ala, were established. Therefore, the whole sequence was determined to be Cyclo(Pro-Phe-Ser-Phe-Gly-Pro-Leu-Ala) and was confirmed by ROESY experiment.[10] From the results of ROE relationship (Fig. 1), it was considered to adopt two β-turn conformations between Pro and Gly, and Phe and Pro in solution.

Pseudostellarin G showed potent inhibitory activity against mushroom tyrosinase; the concentration causing 50% inhibition (IC_{50}) was 75 μM. This activity was stronger than that of arbutin (1.2 mM) and also cyclo(Pro-Tyr-Pro-Val) (1.5 mM), which was recently isolated from the lactic bacterium *Lactobacillus helveticus*.[11] Furthermore, pseudostellarin G showed potent inhibitory effect on the melanogenesis using cultured B16 melanoma cells (IC_{50} 102 μM).

Studies on the structure analyses and biological evaluations of a series of pseudostellarins are in progress.

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8. Pseudostellarin G (1): ^{1}H-NMR data (pyridine-d_5, 500MHz): Pro^{1}: 4.66 (1H, d, 8.1, Hα), 1.87 (1H, m, Hβ), 2.38 (1H, dd, 6.2, 12.2, Hβ), 0.94 (1H, m, Hγ), 1.39 (1H, m, Hγ), 3.42 (1H, m, Hδ), 3.51 (1H, m, Hδ); Phe^{1}: 4.94 (1H, m, Hα), 3.43 (1H, dd, 7.6, 12.7, Hβ), 3.52 (1H, m, Hβ), 7.17 - 7.39 (5H, m), 8.96 (1H, d, 7.7, NH); Ser: 4.57 (1H, m, Hα), 4.10 (1H, t, 12.0, Hβ), 4.55 (1H, m, Hβ), 7.69 (1H, d, 5.4, NH); Phe^{2}: 4.93 (1H, m, Hα), 3.13 (1H, dd, 9.6, 13.9, Hβ), 3.35 (1H, dd, 5.2, 13.9, Hβ), 7.17 - 7.39 (5H, m), 8.69 (1H, d, 5.8, NH); Gly: 3.70 (1H, dd, 6.6, 14.7, Hα), 4.26 (1H, dd, 3.1, 14.7, Hα), 10.14 (1H, br s, NH); Pro^{2}: 4.72 (1H, dd, 3.5, 8.7, Hα), 2.06 (1H, m, Hβ), 2.20 (1H, m, Hβ), 1.81 (1H, m, Hγ), 1.87 (1H, m, Hγ), 3.51 (1H, m, Hδ), 4.14 (1H, m, Hδ); Leu: 5.07 (1H, dt, 2.9, 9.4, Hα), 2.03 (1H, m, Hβ), 2.15 (1H, m, Hβ), 1.87 (1H, m, Hγ), 0.87 (3H, d, 6.7, Hδ), 0.96 (3H, d, 6.7, Hδ), 7.49 (1H, d, 9.4, NH); Ala: 5.03 (1H, dd, 2.7, 6.9, Hα), 1.80 (3H, d, 6.9, Hβ), 8.47 (1H, d, 2.7, NH). ^{13}C-NMR data (pyridine-d_5, 100MHz): Pro^{1}: 61.19 (α), 31.68 (β), 21.63 (γ), 46.86 (δ), 171.43 (C=O); Phe^{1}: 58.33 (α), 37.65 (β), 137.43 (γ), 128.73 (δ), 129.34 (ε), 126.86 (ζ), 172.22 (C=O); Ser: 53.89 (α), 61.19 (β), 171.25 (C=O); Phe^{2}: 55.33 (α), 39.07 (β), 138.56 (γ), 128.84 (δ), 129.37 (ε), 127.18 (ζ), 174.24 (C=O); Gly: 42.85 (α), 170.51 (C=O); Pro^{2}: 61.81 (α), 29.85 (β), 24.68 (γ), 48.25 (δ), 171.20 (C=O); Leu: 51.30 (α), 40.06 (β), 25.77 (γ), 20.84 (δ), 23.27 (δ), 174.24 (C=O); Ala: 50.35 (α), 15.80 (β), 171.98 (C=O).
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