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# CYCLIC PEPTIDES FROM HIGHER PLANTS, PART 15.[1] PSEUDOSTELLARIN H, A NEW CYCLIC OCTAPEPTIDE FROM *PSEUDOSTELLARIA HETEROPHYLLA*

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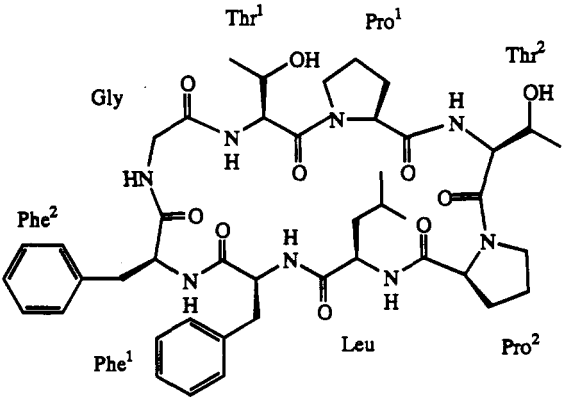
ABSTRACT.—A new cyclic octapeptide, pseudostellarin H [**1**], was isolated from the roots of *Pseudostellaria heterophylla*. Based on spectral evidence and chemical degradation, the primary structure of **1** was established as cyclo(Gly-Thr-Pro-Thr-Pro-Leu-Phe-Phe).

A series of cyclic peptides that are potent tyrosinase inhibitors, named pseudostellarins, have been isolated from the roots of *Pseudostellaria heterophylla* Miq. (Pax) (Caryophyllaceae). Their structures and enzyme inhibition profiles, along with conformational studies of pseudostellarin A, have been reported (2–5). It is interesting to note that the pseudostellarins differ in residue number and amino acid composition but all show similar tyrosinase inhibition. As part of our continuing studies in search of new biologically active cyclic peptides from higher plants (1–13), the further examination of the minor peptide compounds contained in the roots of *P. heterophylla* led us to the isolation of a new cyclic octapeptide, pseudostellarin H [**1**]. In this paper, we present a detailed account of the structure elucidation of **1**.

The roots of *P. heterophylla* were extracted with MeOH and then partitioned into *n*-BuOH/$H_2O$. The *n*-BuOH extract which showed tyrosinase inhibition was chromatographed on HP-20, and the fractions eluted with 80–100% MeOH/$H_2O$ were further separated by Si gel and ODS cc and detected by Dragendorff's reagent on tlc, to give pseudostellarin H [**1**].

Pseudostellarin H [**1**] was obtained as colorless needles, mp 171–172°, from MeOH, $[\alpha]_D$ −51.9° ($c$=0.11, MeOH). The relatively large molecular ion peak was observed by fabms and the molecular formula $C_{44}H_{61}N_8O_{10}$, showing 19 degrees of unsaturation, was established by hrfabms. The ir (1640 and 3392 $cm^{-1}$) spectrum indicated the presence of amide groups. Acid hydrolysis of **1** with subse-




**1**

[1]For Part 14, see Morita *et al.* (1).

quent hplc analysis of the resulting hydrolysate suggested the presence of Gly, Thr×2, Leu, Pro×2, and Phe×2 moieties. Derivatization of the acid hydrolysate with Marfey's reagent (14), followed by hplc analysis, indicated that all amino acid residues were L-isomers. In the nmr spectra of **1** in DMSO-$d_6$, irrespective of the presence of two proline residues, the presence of a single stable conformation was suggested by the occurrence of well-resolved sharp signals. The following spectroscopic data indicated the cyclic peptide nature of **1**. The 500 MHz $^1$H-nmr spectrum of **1** showed four doublet methyl signals (δ 0.71, 0.79, 0.98, and 1.14) assignable to Leu and Thr×2 residues, respectively. The $^{13}$C-nmr spectrum showed signals ascribable to eight amide carbons, corresponding to the amino acid composition. The $^1$H-$^1$H COSY and HOHAHA (15) nmr experiments allowed the identification of each amino acid resonance (Table 1). The complete $^1$H- and $^{13}$C-nmr assignments were made by a combination of 2D nmr techniques, such as analysis of their $^1$H-$^1$H COSY and HMQC (16) spectra (Table 1). The sequence of the above amino acids was established as follows. The presence of four peptide fragments A–D (A: Thr, B: Pro-Thr, C: Pro-Leu-Phe, D: Phe-Gly) was revealed by the HMBC (17) correlations as shown in Figure 1. For instance,

TABLE 1. $^1$H- and $^{13}$C-Nmr Assignments for **1** in DMSO-$d_6$.

| Position | $^1$H nmr $\delta_H$ [int., mult., $J$ (Hz)] | $^{13}$C nmr $\delta_C$ |
|---|---|---|
| Gly | | |
| α | 3.79 (1H, dd, 6.5, 16.9) | 42.47 |
| NH | 8.84 (1H, br s) | |
| C=O | | 168.17 |
| Thr[1] | | |
| α | 4.90 (1H, br d, 6.1) | 55.76 |
| β | 4.28 (1H, m) | 67.04 |
| γ | 0.98 (3H, d, 6.3) | 18.74 |
| OH | 5.40 (1H, d, 12.0) | |
| NH | 7.46 (1H, br s) | |
| C=O | | 168.94[d] |
| Pro[1] | | |
| α | 4.49 (1H, dd, 4.9, 8.4) | 59.42 |
| β | 2.07 (1H, m) | 27.93 |
| | 1.98 (1H, m) | |
| γ | 1.83 (2H, m) | 24.73[b] |
| δ | 3.67 (2H, m) | 46.96 |
| C=O | | 171.01 |
| Thr[2] | | |
| α | 4.59 (1H, dd, 7.4, 8.3) | 55.76 |
| β | 3.97 (1H, m) | 66.56 |
| γ | 1.14 (3H, d, 6.2) | 19.72 |
| OH | 4.94 (1H, d, 6.6) | |
| NH | 8.05 (1H, br s) | |
| C=O | | 169.19 |
| Pro[2] | | |
| α | 4.13 (1H, t, 7.5) | 60.58 |
| β | 2.09 (1H, m) | 29.12 |
| | 1.76 (1H, m) | |
| γ | 1.86 (2H, m) | 24.64[b] |
| δ | 3.83 (1H, m) | 47.55 |
| | 3.69 (1H, m) | |
| C=O | | 170.90 |

TABLE 1. Continued.

| Position | $^1$H nmr $\delta_H$ [int., mult., $J$ (Hz)] | $^{13}$C nmr $\delta_C$ |
|---|---|---|
| Leu | | |
| α | 3.58 (1H, ddd, 4.3, 6.9, 11.0) | 53.30 |
| β | 1.66 (1H, ddd, 4.3, 11.0, 13.7) | 38.31 |
| | 1.13 (1H,m) | |
| γ | 1.35 (1H, m) | 24.41 |
| δ | 0.79 (3H, d, 6.6) | 22.94 |
| | 0.71 (3H, d, 6.6) | 20.77 |
| NH | 7.65 (1H, d, 6.9) | |
| C=O | | 170.71 |
| Phe$^1$ | | |
| α | 4.82 (1H, m) | 52.65 |
| β | 2.81 (1H, dd, 10.0, 13.5) | 37.78 |
| | 2.75 (1H, dd, 3.4, 13.5) | |
| γ | | 137.57 |
| δ | 7.19 (2H, m) | 129.48 |
| ϵ | 7.29 (2H, m) | 127.65[c] |
| ζ | 7.16 (1H, m) | 126.04[c] |
| NH | 7.33 (1H, d, 9.4) | |
| C=O | | 172.08[d] |
| Phe$^2$ | | |
| α | 4.19 (1H, ddd, 4.1, 6.7, 7.6) | 56.03 |
| β | 2.96 (1H, dd, 8.3, 14.0) | 36.36 |
| | 2.88 (1H, dd, 6.7, 14.0) | |
| γ | | 136.98 |
| δ | 7.28 (2H, m) | 128.97 |
| ϵ | 7.18 (2H, m) | 128.15[c] |
| ζ | 7.22 (1H, m) | 126.52[c] |
| NH | 8.84 (1H, br s) | |
| C=O | | 171.81 |

[a]Peak not discernible.
[b–c]Assignments may be interchanged.

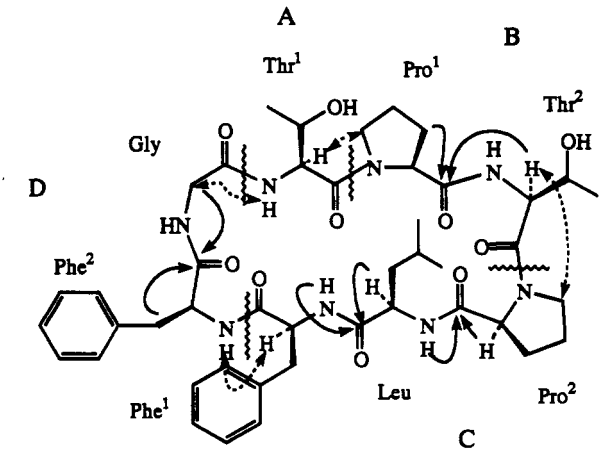



FIGURE 1. Structure of pseudostellarin H [**1**]; solid arrows show HMBC correlations and dashed arrows show nOe correlations in DMSO-$d_6$.

long-range $^2J_{H\text{-}C}$ correlations from Pro$^2$-Hα and Leu-NH to the amide carbonyl carbon at δ 170.90, and those from Leu-Hα and Phe$^1$-NH to the amide carbonyl carbon at δ 170.71 indicated the presence of fragment C. Furthermore, in the NOESYPH (18) spectrum (Figure 1), nOes were observed between the following proton pairs: Thr$^1$-Hα/Pro$^1$-Hδ, Thr$^2$-Hα/Pro$^2$-Hδ, Phe$^1$-Hα/Phe$^2$-NH, and Gly-Hα/Thr$^1$-NH. From the accumulated evidence described above, the structure of **1** was established as cyclo(Gly-Thr-Pro-Thr-Pro-Leu-Phe-Phe). The $^{13}$C-nmr chemical shifts (β: δ 27.93 and 29.12; γ: 24.73 and 24.64) of the β and γ positions in the two Pro residues suggested the proline amide bonds were trans (19).

Recently, a number of naturally occurring cyclic peptides with unique structures having biological activities have been isolated. Despite their importance, only very few studies of cyclic peptide-containing higher plants have been reported. Pseudostellarin H showed only very weak tyrosinase inhibition when tested by a dopachrome method (15% inhibition at 800 μM) (4). The other pharmacological activities of **1** are now under investigation.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp was obtained with a Yanagimoto MP-3 micro-melting point apparatus and was uncorrected. The optical rotation was measured on a Jasco DIP-4 polarimeter. The ir spectrum (KBr) was obtained on a Perkin-Elmer 1710 spectrophotometer. Mass spectra were recorded on a VG Autospec instrument. Hplc was performed on an Inertsil PREP-ODS packed with 10 μm ODS. Tlc was conducted on precoated Kieselgel 60 $F_{254}$ (Art. 5715; Merck) plates and the spots were detected by spraying with Dragendorff's reagent. $^1$H- and $^{13}$C-nmr spectra were run in DMSO-$d_6$ using a Bruker AM 500 instrument, shifts (δ) are reported in ppm. A 6-mg sample of **1** in a 5-mm tube (0.5 ml DMSO-$d_6$, degassed) was used for the homonuclear and heteronuclear measurements. The spectra were recorded at 303° K. NOESYPH experiments were acquired with mixing times of 0.6 sec. The value of the delay to optimize one-bond correlations in the HMQC spectrum and suppress them in the HMBC spectrum was 3.2 Hz and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 msec.

PLANT MATERIAL.—The roots of *P. heterophylla* were purchased in Shanghai, People's Republic of China, in May 1993. The botanical identification was made by Dr. Zhi-Sheng Qiao, Department of Pharmacognosy, College of Pharmacy, Second Military Medical University, Shanghai. A voucher specimen has been deposited in the herbarium of the Tokyo University of Pharmacy and Life Science.

EXTRACTION AND ISOLATION.—The roots of *Pseudostellaria heterophylla* (10.0 kg) were extracted with hot MeOH three times to give a MeOH extract that was partitioned with *n*-BuOH/$H_2O$. The *n*-BuOH-soluble fraction (167 g), showing tyrosinase inhibition, was subjected to Diaion HP-20 cc using a $H_2O$/MeOH gradient system (1:0–0:1). The fractions eluted with 80% and 100% MeOH, respectively, were further subjected to Si gel cc using a $CH_2Cl_2$-MeOH gradient system (1:0–0:1). The fraction eluted with 10% MeOH was subjected to ODS hplc with a 30% $CH_3CN$/0.05% TFA solvent system to give **1** (6.0 mg) as colorless needles: mp 171–172°; $[\alpha]_D$ −51.9° (*c*=0.11, MeOH); ir ν max (KBr) 1640 and 3392 cm$^{-1}$; $^1$H- and $^{13}$C-nmr data, see Table 1; fabms *m/z* $[M+H]^+$ 861 (base peak); hrfabms *m/z* found 861.4534, calcd for $C_{44}H_{61}N_8O_{10}$, 861.4511.

ACID HYDROLYSIS OF **1**.—A solution of **1** (1 mg) in 6 N HCl was heated at 110° for 24 h. After cooling, the solution was concentrated to dryness. The hydrolysates were dissolved in 0.02 N HCl and applied to an amino acid analyzer (Hitachi L-8500 Amino Acid Analyzer).

ABSOLUTE CONFIGURATION OF AMINO ACIDS.—A solution of **1** (1 mg) in 6 N HCl was heated at 110° for 12 h. The solution was concentrated to dryness. The residue was dissolved in $H_2O$ and treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) and 1 M $NaHCO_3$ at 35° for 1 h. After cooling, 2 M HCl was added and then concentrated to dryness. This residue was subjected to hplc [Lichrospher 100, RP-18 (10 μm), Merck], flow rate 1 ml/min, detection 340 nm, solvent: 10–50% $CH_3CN$/50 mM triethylamine phosphate (TEAP) buffer. The *R*, values (min) were L-Pro 28.04, L-Phe 40.79, L-Thr 21.75, and L-Leu 41.08, respectively.

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