Synthesis of a New Cyclic Peptide, Pseudostellarin G

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A new biologically active cyclic peptide, Pseudostellarin G was synthesized and the structure was established on the basis of analytical, IR, NMR and mass spectral data. The newly synthesized compound was screened for its antimicrobial and pharmacological activities.

Key words: Cyclic Peptides, Pseudostellarin G, Antimicrobial Activity, Pharmacological Activity

Introduction

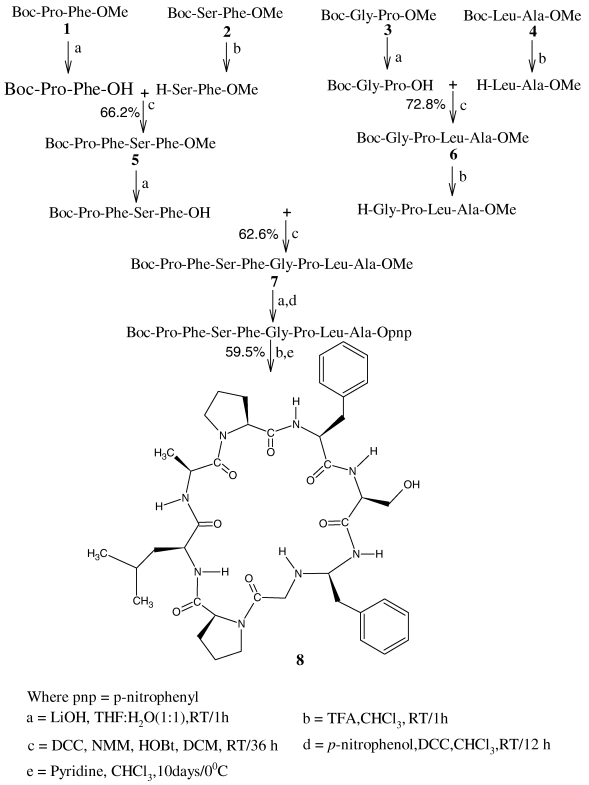
In the past two decades, a wide variety of naturally occurring bioactive cyclic peptides have been isolated from plants, marine sponges and tunicates [1]. Recently a large number of these cyclic peptides are emerging as an important class of organic compounds due to their unique structure and biological activities. The wide spread increase of bacterial resistance towards conventional antibiotics encourages the exploration of novel antimicrobial molecules with unexploited mechanisms. Initially discovered as a defensive system in invertebrates and vertebrates, antimicrobial peptides are attracting increase interest as potential therapeutics [2 – 4]. Unlike classical antibiotics, which must penetrate the target cell, the principle mode of action of peptides involves perturbation and permealization of the cell membrane. This mechanism confers activity towards a broad spectrum of microbial cells, but is also responsible for undesired lytic activity against mammalian cells such as erythrocytes [5 – 7].

Recently, Itakawa *et al.* [8] isolated a new biologically active cyclic peptide Pseudostellarin G from the rots of Pseudostellaria heterophylla and the structure was elucidated by extensive NMR, chemical and enzymatic degradations and mass spectrometric analysis.

In continuation of our research work of synthesizing natural cyclic peptides of biological interest [9], an attempt was made towards the synthesis of Pseudostellarin G to prove its identity with naturally isolated one. Keeping in view of significant biological activities exhibited by various cyclic peptides, the synthesized product was further subjected to antibacterial and pharmaceutical activity studies.

Results and Discussion

Pseudostellarin G is cyclo(-Pro-Phe-Ser-Phe-Gly-Pro-Leu-Ala-), a cyclic octa-peptide. In order to carry out the total synthesis of this cyclic peptide, four dipeptide units Boc-Phe-Pro-OMe **(1)**, Boc-Ser-Phe-OMe **(2)**, Boc-Gly-Pro-OMe **(3)** and Boc-Leu-Ala-OMe **(4)** were prepared by coupling Boc-amino acids with the respective amino acid ester hydrochlorides using DCC, HOBt and N-methyl morpholine according to Bodanszky procedure [10] with suitable modifications [11]. The ester group of dipeptide **(1)** was removed with LiOH and the Boc-group of dipeptide **(2)** was removed with trifluoroacetic acid. Both the deprotected units were coupled to get the tetrapeptide, Boc-Pro-Phe-Ser-Phe-OMe **(5)**. The remaining two dipeptides (**3** and **4**) were also coupled similarly to obtain the another tetrapeptide, Boc-Gly-Pro-Leu-Ala-OMe **(6)**. These tetrapeptides are the coupled after proper deprotection using DCC, HOBt and NMM to get the octapeptide, Boc-Pro-Phe-Ser-Phe-Gly-Pro-Leu-Ala-OMe **(7)**. Finally, the cyclization of the linear segment was carried out by the p-nitrophenyl ester method [12] as depicted in Scheme 1. The intermediates and final product were purified by column chromatography using dichloromethane-methanol system and recrystallized from EtOAc-*n*-hexane. The newly synthesized compounds was analyzed for C,H,N and the structure was confirmed by IR, ^{1}H NMR and mass spectral data. The characteristic IR and NMR spectra



Scheme 1.

of all the intermediate compounds were analyzed. The characteristic IR absorption bands of –CO-NH- moiety were present in the cyclized product. ^{1}H NMR and ^{13}C NMR spectra of all the cyclized product clearly indicates the presence of all respective amino acid moieties. Further more, the mass spectra of Pseudostellarin G showed the [M$^+$+H] peak at *m/z* 817 which is consistent with the molecular formula $C_{42}H_{56}N_8O_9$.

Biological Activity Studies

The synthesized cyclic peptide, Pseudostellarin G was also screened for its antibacterial, antifungal, anti-inflammatory and anthelmintic activity. The antibacterial and antifungal activity are carried out against four bacterial (*S. aureus, B. subtilis, P. aeruginosa and E. coli*) and two fungal strains (*C. albicans* and *A. niger*). These activity studies were carried out according to disc diffusion method [13]. Penicillin and Griseofulvin were used as standards against bacteria and fungal strains at 10 and 25 μg/disc respectively. The results summarized in Table 2 indicates that the compound is active against only the bacterial strains *E. coli* and

Table 1. Antimicrobial activity data of Pseudostellarin G.

Compound	Diameter of zone of inhibition					
	Antibacterial activity studies				Antifungal	
	P.aer	*E.coli*	*B.sub*	*S.aur*	*C.alb*	*A.niger*
Pseudostellarin G **8**	–	14	–	16	09	10
Penicillin	12	12	18	18	–	–
Greseofulvin	–	–	–	–	20	20

'–' indicates no activity.

Table 2. Antiinflammatory activity data of Pseudostellari G.

Compound	Increase in paw volume ±SE (ml) after 3 hr	Inhibition of oedema (%) after 3 hr
Pseudostellarin G **8**	0.68±0.04	24.45
Ibuprofen	0.55±0.03	38.89
Control	0.90±0.04	–

'–' indicates no activity.

Table 3. Anthelmintic activity data of Pseudostellari G.

Compound	Conc of the compd (mg)	Mean paralyzing time ±SE (min)	Mean death time ±SE (min)
Pseudostellarin G	100	42.15±1.49	74.30±1.10
	200	30.12±2.06	64.05±1.08
Mebendazole	100	18.01±2.01	55.20±2.00
	200	12.55±1.02	32.01±1.10
Control	–	–	–

S. aureus. The anti-inflammatory activity was carried out according to the method of Winter *et al.* [14] using Ibuprofen as the standard and the results are presented in Table 3. The antiinflammatory data reveals that the compound is moderately active. The anthelmintic activity was carried out against the earthworms (pontoscotex corethruses) according to Garg's method [15] (Table 4) using Mebendazole as standard drug. The compound is found to be less active as compared to the standard.

Experimental Section

Melting points were taken in open capillary and are uncorrected. IR spectra (in $CHCl_3$) were recorded on a Perkin-Elmer infrared spectrophotometer. NMR spectra were recorded in $CHCl_3$-d_6/DMSO-d_6 on a 300 MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a FAB mass spectrometer. The progresses of the reactions were checked by TLC on silica gel G plates and the products were purified by silica gel column chromatography.

The four dipeptides, Boc-Pro-Phe-OMe (**1**), Boc-Ser-Phe-OMe (**2**), Boc-Gly-Pro-OMe (**3**) and Boc-Leu-Ala-OMe (**4**) were prepared and coupled, after proper deprotection using LiOH and trifluoroacetic acid according to Bodanszky procedure with suitable modifications [11] to get two tetrapeptides,

Boc-Pro-Phe-Ser-Phe-OMe (**5**) and Boc-Gly-Pro-Leu-Ala-OMe (**6**). The resulting tetrapetides were then condensed to obtain the linear segment of Pseudostellarin G, Boc-Pro-Phe-Ser-Phe-Gly-Pro-Leu-Ala-OMe (**7**) according to the procedure used for tetrapeptides.

*Boc-Pro-Phe-Ser-Phe-OMe (**5**)*

IR($CHCl_3$): ν=3610 (br.s, O-H str.), 3420 (br.s, N-H str.), 3050 (m, =C-H str.), 2940 (s, C-H str.), 2770 (s), 1710 (s, C=O str. ester), 1685 (s, C=O str. amide), 1670 (s, C=O str. amide), 1650 (s, C=O str. amide), 1610 (s), 1600 (m), 1520 (s), 1500 (m), 1450 (s), 1370 (s), 1330 (s), 1230 (s), 1170 (s), 1020 (s), 1000 (s), 670 (s) cm^{-1}. – ^1H *NMR* (300 MHz, $CDCl_3$): δ = 8.6 (br.s, 1H, NH), 8.3 (br.s, 1H, NH), 7.9 (br.s, 1H, NH), 7.5 – 7.0 (m, 10H, Ar-H), 5.2 (s, 1H, α-OH), 4.8 – 4.6 (m, 2H, α-CH), 4.5 – 4.3 (m, 2H, α-CH), 4.1 – 4.0 (m, 2H, β-CH_2), 3.7 (s, 3H, O-CH_3), 3.6 – 3.4 (m, 2H, -N-CH_2), 3.2 – 3.0 (m, 4H, β-CH_2), 2.2 – 1.6 (m, 4H, -CH_2-CH_2-), 1.4 (s, 9H, C $(CH_3)_3$. – $C_{32}H_{42}N_4O_8$ (610.7): calcd. C 62.94, H 6.93, N 9.17; found C 62.70, H 6.85, N 9.13.

*Boc-Gly-Pro-Leu-Ala-OMe (**6**)*

IR($CHCl_3$): ν = 3200 (br.s, N-H str.), 2970 (s, C-H str.), 2800 (s), 1735 (s, C=O str. ester), 1685 (s, C=O str. amide), 1660 (s, C=O str. amide), 1655 (s, C=O str. amide), 1600 (s), 1535 (s), 1440 (s), 1370 (s), 1310 (s), 1265 (s), 1200 (s), 1170 (s), 1030 (s), 870 (s) cm^{-1}. – ^1H *NMR* (300 MHz, $CDCl_3$): δ = 8.3 (br.s, 2H, NH), 7.8 (br.s, 1H, NH), 4.7 – 4.5 (m, 2H, α-CH), 4.4 – 4.2 (m, 1H, α-CH), 4.1 – 3.9 (m, 2H, β-CH_2), 3.7 (s, 3H, O-CH_3), 3.6 – 3.4 (m, 2H, -N-CH_2), 2.2 – 1.8 (m, 4H, -CH_2-CH_2-), 1.7 – 1.6 (m, 2H, CH_2), 1.4 (s, 9H, C $(CH_3)_3$, 1.3 (d, J = 7.0 Hz, 3H, -CH_3), 1.2 – 1.1 (1H, m, γ-CH), 0.9 (d, J = 7.0 Hz, 6H, C $(CH_3)_2$). – $C_{22}H_{38}N_4O_7$ (470.6): calcd. C 56.15, H 8.14, N 11.90; found C 56.02, H 8.08, N 11.67.

*Boc-Pro-Phe-Ser-Phe-Gly-Pro-Leu-Ala-OMe (**7**)*

IR($CHCl_3$): ν = 3580 (br.s, O-H str.), 3200 (br.s, N-H str.), 3060 (m,=C-H str.), 2980 (s, C-H str.), 2800 (s, C-H str.), 1720 (s, C=O str. ester), 1670 (s, C=O str. amide), 1665 (s, C=O str. amide), 1650 (s, C=O str. amide), 1645 (s, C=O str. amide), 1605 (s), 1600 (s), 1520 (s), 1500 (s), 1445 (s), 1370 (s), 1280 (s), 1170 (s), 1020 (s), 980 (s), 720 (s) cm^{-1}. – ^1H *NMR* (300 MHz, *($CDCl_3$)*): δ = 8.4 (br.s, 2H, NH), 8.0 (br.s, 2H, NH), 7.5 (br.s, 2H, NH), 7.3 – 6.9 (m, 10H, Ar-H), 5.15 (s, 1H, -OH), 4.8 – 4.6 (m, 4H, α-CH), 4.45 – 4.3 (m, 3H, α-CH), 4.2 – 4.0 (m, 3H, α-CH_2 and α-CH), 3.95 – 3.8 (m, 2H, β-CH_2), 3.75 (s, 3H, OCH_3), 3.6 – 3.4 (m, 4H, N-CH_2), 3.2 – 3.0 (m, 4H, β-CH_2), 2.2 – 1.8 (m, 8H, -CH_2-CH_2-), 1.7 – 1.6 (m, 2H, -CH_2), 1.5 (d, 3H, J = 7.5 Hz, CH_3), 1.45 (s, 9H, C $(CH_3)_3$), 1.3 – 1.1 (m, 1H, γ-CH), 0.95 (d, 6H, J = 6.5 Hz, -C$(CH_3)_2$). – $C_{48}H_{68}N_8O_{12}$ (949.11): calcd. C 60.74, H 7.22, N 11.81; found C 60.64, H 7.02, N 11.74.

*Pseudostellarin G (**8**)*

To the solution of Boc-octapeptide *p*-nitrophenyl ester (1.2 mmol) in chloroform (15 ml), trifluoroacetic acid (0.274 g, 2.4 mmol) was added, stirred for 1 h at room temperature and washed with 10% sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulpate. To the resulting Boc-deprotected peptide-pnp ester in THF (15 ml), pyridine (1.4 ml, 2 mmol) was added and kept at 4 °C for seven days. The reaction mixture was washed with 10% sodium bicarbonate solution until the byproduct *p*-nitrophenol was removed completely and finally washed with 5% HCl (5 ml). The organic layer was dried over anhydrous sodium sulphate. THF and pyridine were distilled under reduced pressure to get Pseudostellarin G. The crude product was purified by silica gel column chromatography using the dichloromethane-methanol system and finally recrystallized from EtOAc-*n*-hexane.

M. p. 267 °C dec. (Lit. [8] 268 °C dec). - *IR*($CHCl_3$): ν = 3600 (br.s, O-H str.), 3420 (br.s, N-H str.), 3050 (m, =C-H str.), 2950 (s, C-H str.), 2820 (s, C-H str.),1690 (s, C=O str. amide), 1680 (s, C=O str. amide), 1650 (s, C=O str. amide), 1610 (s, C=C str.), 1605 (s, N-H def.), 1445 (s, C-H def.), 1050 (s, C-H def.), 915 (s, C-H def.) cm^{-1}. – ^1H *NMR* (300 MHz, DMSO-d_6): δ = 8.6 – 8.4 (br.s, 4H, NH), 7.8 – 7.6 (br.s, 2H, NH), 7.3 – 6.9 (m, 10H, Ar-H), 5.1 (s, 1H, -OH), 4.9 – 4.7 (m, 4H, α-CH), 4.6 – 4.4 (m, 3H, α-CH), 4.2 – 3.9 (m, 4H, α-CH_2 and β-CH_2), 3.6 – 3.4 (m, 4H, N-CH_2), 3.3 – 3.1 (m, 4H, β-CH_2), 2.2 – 1.8 (m, 8H, -CH_2-CH_2-), 1.8 – 1.7 (m, 1H, γ-CH), 1.5 (d, 3H, J = 6.6 Hz, CH_3), 1.3 – 1.1 (m, 2H, β-CH_2), 0.9 (d, 6H, J = 6.5 Hz, -C$(CH_3)_2$). – ^{13}C *NMR (DMSO-d_6):* δ = 173.0 (s, C=O), 172.2 (s, C=O), 172.0 (s, C=O), 171.4 (s, C=O), 171.1 (s, C=O), 170.5 (s, C=O), 138.6 (s, Ar-γ-C), 137.4 (s, Ar^1-γ-C), 129.3 (d, Ar and Ar^1-ε-C), 128.8 (d, Ar and Ar^1-δ-C), 127.2 (d, Ar-ζ-C), 126.6 (d, Ar^1-ζ-C), 61.8 (d, α-CH), 61.2 (d, α-CH), 61.0 (t, β-CH_2), 58.4 (d, α-CH), 55.4 (d, α-CH), 53.9 (d, α-CH), 51.3 (d, α-CH), 50.4 (d, α-CH), 48.3 (t, N-CH_2), 46.8 (t, N-CH_2), 42.9 (t, α-CH_2), 40.0 (t, β-CH_2), 39.0 (t, β-CH_2), 37.6 (t, β-CH_2), 31.6 (t, β-CH_2), 29.8 (t, β-CH_2), 25.7 (t, γ-CH), 25.7 (t, γ-CH_2), 24.7 (t, γ-CH_2), 23.3 (q, -CH_3), 21.6 (t, γ-CH_2), 20.8 (q, -CH_3), 15.8 (q, -CH_3). – *FAB mass*: m/z = 817 [M^++H]. – $C_{42}H_{56}N_8O_9$ (817): calcd. C 61.75, H 6.91, N 13.72; found C 61.70, H 6.89, N 13.68.

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