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Short Communication

Synthesis and biological evaluation of pseudostellarin B

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Abstract

A new potent bioactive cyclic peptide pseudostellarin B has been synthesised. The structure was elucidated by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and FAB mass spectral data. The synthesised compound was also screened for its antibacterial, antifungal, antiinflammatory and anthelmintic activities.

Keywords: Pseudostellarin B; Spectral studies; Bioactivities

1. Introduction

Recently a number of cyclic peptides with unique structures and biological activities have been isolated from natural sources [1]. Peptides appear to be important potential drugs: cyclotheonamides serve as a model compound for antithrombin drugs, discodermins are potential antitumor promoting drugs and calyculins are useful biochemical reagents [1]. A new potent tyrosinase inhibitory cyclic peptide, pseudostellarin B, has been isolated from the roots of *Pseudostellaria heterophylla* and the structure was elucidated by extensive 2D NMR methods, chemical and enzymatic degradations and tandem MS spectroscopic analyses by Hiroshi Morita et al. [2].

As part of our ongoing study on the synthetic aspects of bioactive cyclic peptides [3], we planned to synthesise pseudostellarin B and study its biological activities. In this paper, we describe the synthesis, characterisation and biological studies of pseudostellarin B.

2. Chemistry

For the present work, the cyclic octapeptide was disconnected into four dipeptides **1**–**4**. These dipeptides were coupled after proper deprotection using dicyclohexyl carbodiimide (DCC) as a coupling agent to get tetrapeptides **5** and **6**. These two tetrapeptides were coupled to get octapeptide **7**. Finally cyclisation of **7** employing the *p*-nitrophenyl ester method resulted in expected product **8** (Scheme 1).

For the protection of the amino group of the L-amino acids, we used di-*tert*-butyl pyrocarbonate to get N-^{t}Boc amino acids [4]. The carboxyl group of L-amino acids was protected by esterification in the presence of thionyl chloride. Dipeptides **1**–**4**, tetrapetides **5** and **6** and octapeptide **7** were prepared by the Bodanszky method [5]. All these intermediates were purified by recrystallisation from $CHCl_3$–*n*-hexane. CF_3COOH–$CHCl_3$ was used for the removal of the ^{t}Boc group and LiOH–H_2O for the removal of the ester group. Cyclisation of linear segment **7** was carried out by using *p*-nitrophenyl ester method [6] to yield pseudostellarin B **8**.

The newly synthesised compound was characterised by elemental analyses, IR, ^{1}H NMR, and mass spectral data (Table 1). The infrared spectrum of **8** shows absorption bands that are characteristic of the secondary amide groups, no absorption bands appear for free amino groups or carboxyl groups. Extensive application of NMR techniques was used to determine the

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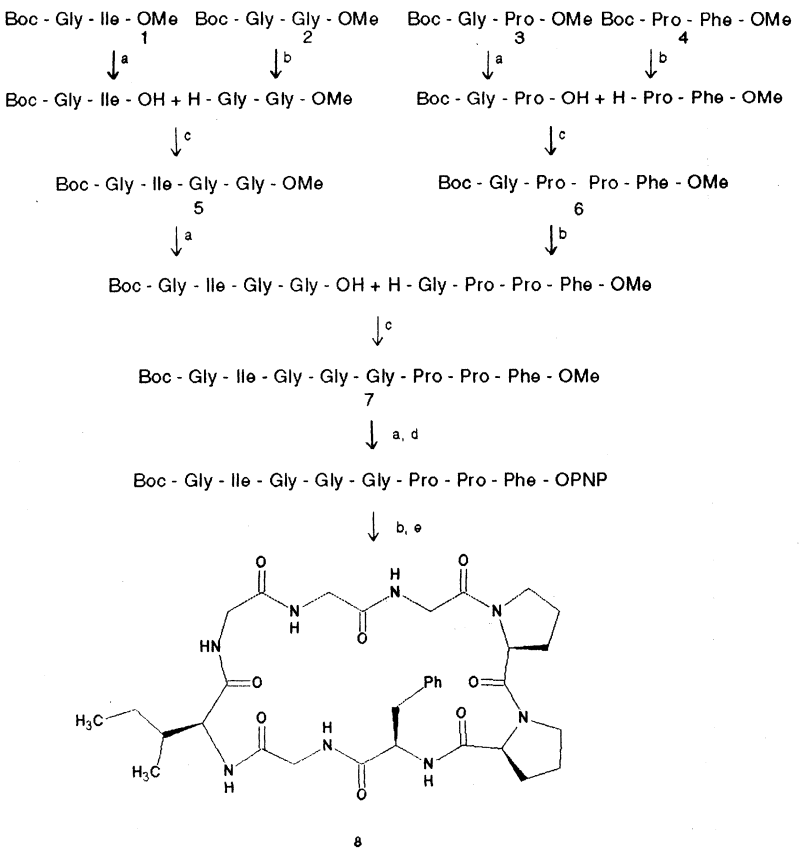
identity of these amino acid units. ^{1}H NMR clearly showed the presence of all the protons of eight amino acids. Moreover the mass spectrum of **8** showed the $M^+ + H$ peak at m/z 683, which corresponds to the molecular formula $C_{33}H_{48}N_8O_8$. The relative intensity of the molecular ion peak suggested the cyclic nature of the octapeptide.

3. Experimental

Melting points were taken in open capillary tubes and are uncorrected. IR spectra (in $CHCl_3$) were recorded on a Perkin–Elmer infrared spectrophotometer. NMR spectra were recorded in $CHCl_3$-d/DMSO-d_6 on a 300 MHz spectrophotometer using tetramethyl silane (TMS) as an internal standard. The mass spectra were recorded on a FAB mass spectrometer. The purity of all compounds was checked by thin layer chromatography (TLC) on silica gel G plates.

3.1. Pseudostellarin B **8**

To the solution of Boc-octapeptide-pnp 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 sulphate. To the 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 to get pseudostellarin B **8**. The crude product was purified by silica gel column chromatography using the dichloromethane–methanol system and finally recrystallised from EtOAc–*n*-hexane.



Scheme 1.

Table 1
Characterisation of **1–8**

Compound	Physical state	M.p. (°C)	Yield (%)	Molecular formula	Anal. (%N), found (calc.)
1 [a]	Semisolid mass	–	82.8	$C_{14}H_{26}N_2O_5$	9.34 (9.27)
2	Semisolid mass	–	71.8	$C_{10}H_{18}N_2O_5$	11.43 (11.38)
3	Viscous liquid	–	76.9	$C_{13}H_{22}N_2O_5$	9.84 (9.79)
4 [b]	Pale white solid	55–56	79.8	$C_{20}H_{28}N_2O_5$	7.40 (7.44)
5 [c]	Semisolid mass	–	75.6	$C_{18}H_{32}N_4O_7$	13.44 (13.46)
6 [d]	Semisolid mass	–	68.2	$C_{27}H_{38}N_4O_7$	10.60 (10.56)
7 [e]	Semisolid mass	–	70.0	$C_{39}H_{58}N_8O_{11}$	13.80 (13.76)
8 [f]	White solid	167–169	61.0	$C_{33}H_{46}N_8O_8$	16.48 (16.42)

[a] IR (cm^{-1}): 3320 (γ_{N-H}), 2930 (γ_{C-H}), 1710 ($\gamma_{C=O}$ ester), 1670 ($\gamma_{C=O}$ amide), 1051 (γ_{C-O}), 860 (γ_{C-H}); ^{1}H NMR ($CHCl_3$-*d*): δ 8.2 (br s, 1H, NH), 6.9 (br s, 1H, NH), 4.4–4.3 (m, 1H, α-CH), 4.1–3.9 (m, 2H, α-CH_2), 3.7 (s, 3H, –OCH_3), 1.7–1.6 (m, 1H, γ-CH), 1.4 (s, 9H, ^{t}Boc), 1.3–1.1 (m, 2H, β-CH_2), 0.95 (doublet overlapped with triplet, 6H, $2CH_3$).

[b] IR (cm^{-1}): 3540 (γ_{N-H}), 3310 (γ_{N-H}), 3010 ($\gamma_{=C-H}$), 2975 (γ_{C-H}), 1735 ($\gamma_{C=O}$ ester), 1675 ($\gamma_{C=O}$ amide), 1660 ($\gamma_{C=O}$ amide), 1595 ($\gamma_{C=C}$), 1020 (γ_{C-O}), 740 (γ_{C-H}); ^{1}H NMR ($CHCl_3$-*d*): δ 7.25 (m, 5H, ArH), 5.1–4.7 (br s, 1H, NH), 4.5–4.1 (m, 2H, 2α-CH), 3.7 (s, 3H, –OCH_3), 3.4–3.0 (m, 4H, N–CH_2 and β-CH_2), 2.2–1.6 (m, 4H, –CH_2–CH_2–), 1.4 (s, 9H, ^{t}Boc).

[c] IR (cm^{-1}): 3315 (γ_{N-H}), 2910 (γ_{C-H}), 1740 ($\gamma_{C=O}$ ester), 1690 ($\gamma_{C=O}$ amide), 1670 ($\gamma_{C=O}$ amide), 1420 (γ_{C-H}), 1100 (γ_{C-O}), 970 (γ_{C-H}); ^{1}H NMR ($CHCl_3$-*d*): δ 8.2–7.8 (br s, 3H, 3NH), 6.9 (br s, 1H, NH), 4.4–4.3 (m, 3H, α-CH and α-CH_2), 4.2–3.9 (m, 4H, 2α-CH_2), 3.7 (s, 3H, –OCH_3), 1.8–1.6 (m, 1H, –CH), 1.4 (s, 9H, ^{t}Boc),1.3–1.1 (m, 2H, γ-CH_2), 0.98 (doublet overlapped with triplet, 6H, $2CH_3$).

[d] IR (cm^{-1}): 3300 (γ_{N-H}), 3020 ($\gamma_{=C-H}$), 2950 (γ_{C-H}), 1720 ($\gamma_{C=O}$ ester), 1675 ($\gamma_{C=O}$ amide), 1620 ($\gamma_{C=C}$), 1320 (γ_{C-N}), 1140 (γ_{C-O}), 970 (γ_{C-H}); ^{1}H NMR ($CHCl_3$-*d*): δ 8.3 (br s, 1H, NH), 7.8 (br s, 1H, NH), 7.4–7.1 (m, 5H, ArH), 4.7–4.5 (m, 3H, 3α-CH), 4.2–4.0 (m, 2H, α-CH_2), 3.7 (s, 3H, –OCH_3), 3.6–3.4 (m, 4H, N–CH_2), 3.3–3.0 (m, 2H, β-CH_2), 2.2–1.6 (m, 8H, 2-CH_2–CH_2–), 1.4 (s, 9H, ^{t}BOc).

[e] IR (cm^{-1}): 3310 (γ_{N-H}), 3150 (γ_{N-H}), 3010 ($\gamma_{=C-H}$), 2950 (γ_{C-H}), 1720 ($\gamma_{C=O}$ ester), 1690 ($\gamma_{C=O}$ amide), 1685 ($\gamma_{C=O}$ amide), 1675 ($\gamma_{C=O}$ amide), 1620 ($\gamma_{C=C}$), 1470 (γ_{C-H}), 1280 (γ_{C-O}), 970 (γ_{C-H}); ^{1}H NMR ($CHCl_3$-*d*): δ 8.4–8.2 (br s, 4H, 4NH), 8.0–7.7 (br s, 2H, 2NH), 7.4–7.1 (m, 5H, ArH), 4.8–4.5 (m, 4H, α-CH), 4.3–4.0 (m, 6H, α-CH_2), 3.7 (s, 3H, –OCH_3), 3.5–3.1 (m, 6H, 2N –CH_2 and β-CH_2), 2.2–1.7 (m, 8H, –CH_2–CH_2–), 1.6–1.5 (m, 1H, β-CH), 1.4 (s, 9H, ^{t}BOC), 1.3–1.1 (m, 2H, γ-CH_2), 0.95 (doublet overlapped with triplet, 6H, $2CH_3$).

[f] IR (cm^{-1}): 3350 (γ_{N-H}), 3015 ($\gamma_{=C-H}$), 2985 (γ_{C-H}), 1690 ($\gamma_{C=O}$ amide), 1670 ($\gamma_{C=O}$ amide), 1615 ($\gamma_{C=C}$), 1510 (γ_{C-N}), 1445 (γ_{C-H}), 975 (γ_{C-H}); ^{1}H NMR (DMSO): δ 8.3–8.1 (br s, 4H, 4NH), 8.0–7.7 (br s, 2H, 2NH), 7.3–7.0 (m, 5H, ArH), 4.8–4.6 (m, 4H, 4α-CH), 4.4–4.1 (m, 6H, 3α-CH_2), 3.6–3.4 (m, 4H, 2-N–CH_2), 3.3–3.1 (m, 2H, β-CH_2) 2.2–1.8 (m, 8H, 2-CH_2–CH_2–), 1.7–1.6 (m, 1H, β-CH), 1.4–1.2 (m, 2H, γ-CH_2); FABMS (*m*/*z*): ($M^+ + H$) 683.

Table 2
Antibacterial and antifungal activity data

Comp. no.	Zone of inhibition (in mm) at 10 μg ml^{-1} concentration					
	P. aeruginosa	*E. coli*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. niger*
8	–	14	16	–	11	–
Penicillin	12	12	18	18	–	–
Streptomycin	16	16	12	12	–	–
Griseofulvin	–	–	–	–	20	18

4. Biological activity studies

The newly synthesised compound **8** was screened for its in vitro antibacterial, antifungal, anti-inflammatory and anthelmintic activity. Antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *C. albicans* and *A. niger* were carried out by employing the disc diffusion method [7]. The results are summarised in Table 2. The anti-inflammatory activity study was carried out according to the method of Winter et al. [8] (Table 3) and the anthelmintic activity study according to the method of Garg and Atal [9] (Table 4).

Table 3
Anti-inflammatory activity data

Comp. no.	Increase in Paw volume (ml) ± S.E.	Percentage inhibition of oedema
8	0.85 ± 0.04	5.56
Control	0.90 ± 0.02	–
Ibuprofen	0.55 ± 0.04	40.50

5. Results and discussion

Pseudostellarin B showed moderate antibacterial activity against *E. coli*, *B. subtilis*, and antifungal activity against *C. albicans*, whereas it showed very less anti-

Table 4
Anthelmintic activity data

Comp. no.	% Conc of comp. (mg)	Mean paralysing time (min) ± S.E	Mean death time (min) ± S.E
8	100	65.30 + 2.09	95.17 + 2.03
	200	56.58 + 2.05	85.24 + 2.16
Control	–	–	–
	–	–	–
Mebendazole	100	18.01 + 2.01	35.20 + 2.00
	200	12.55 + 1.02	32.01 + 1.09

inflammatory and anthelmintic activity as compared with the standard drug.

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