VOL. 51, NO. 11, NOV. 1998 THE JOURNAL OF ANTIBIOTICS pp. 1055 — 1058

COMMUNICATIONS TO THE EDITOR

Isolation and Structure of Peptaibolin, a New Peptaibol from *Sepedonium* Strains

Sir:

In the course of a screening aimed at new metabolites, we used electrospray mass spectrometry (ESI-MS, CID-MS/MS)[1,2] for the detection of linear peptides and peptaibols in microbial culture extracts[3~5]. Here we report the isolation and structure elucidation of peptaibolin (**1**, Fig. 1) as an unusual representative of the peptaibol family of lipophilic antibiotics.

1 was disclosed in two fungal strains, *Sepedonium* sp. HKI-0117 and *Sepedonium ampullosporum* HKI-0053 coproducing the neuroleptic peptaibol-type antibiotic ampullosporin[5]. In contrast to the known peptaibols[6], the compound **1** contains only five peptide bonds. However, it displays all of the structural features of the peptaibols such as the presence of α-aminobutyric acid moieties, of an acylated amino terminus, and an alcoholic group at the opposite end of the peptide chain.

The producing strains were obtained from the strain collection of the Hans-Knöll-Institute of Natural Products Research, Jena (Germany). *Sepedonium* sp. HKI-0117 was identified as an anamorphic representative of the genus *Hypomyces* (*Apiocrea*)[7]. The fungus forms aerial mycelium and conidia on malt agar. The hyaline hyphae are richly branched and septate with a diameter from 3 to 5 μm. The strain is characterized by two different types of conidia-aleuriospores (round, warted, orange- coloured, diameter from 16 to 21 μm) and blastospores (cylindric to conic 8 ~ 28 μm/3.5 ~ 5 μm).

For the production of peptaibolin the producer strains were cultivated as surface cultures at 25°C in 500 ml Erlenmeyer bottles containing 100 ml medium composed as follows: glycerol 3%, glucose 1%, peptone 0.5%, NaCl 0.5%, zeolite 0.01%, agar 0.1%, distilled water (sterilization 120°C, 20 minutes). Each bottle was inoculated with a $2\,cm^2$ area of a 21 days agar culture. After 21 days of cultivation as solid culture at 28°C, the mycelium cake was harvested from 10 liters of culture and extracted twice with 2 liters of EtOAc. The culture liquid was extracted twice with 8 liters of EtOAc. The combined extracts were dried and evaporated. The residue (approx. 2 g) was subjected to silica gel chromatography (silica gel 60 Merck, 0.063 ~ 0.1 mm, column 600 × 30 mm), using stepwise $CHCl_3$, and $CHCl_3$-MeOH (9 : 1, 8 : 2, v/v) as eluents. Fractions containing **1** as detected by *m/z* 590 during ESI-MS were pooled and evaporated. The residue (0.8 g) was dissolved in 10 ml methanol and subjected to chromatography on Sephadex LH-20 in methanol (column 600 × 25 mm). Fractions containing peptaibolin (**1**) were purified by preparative isocratic HPLC (Spherisorb ODS-2, RP_{18}; 25 × 250 mm, CH_3CN-H_2O 83 : 17, v/v, 10 ml/minute, 210 nm; retention time 8.7 minutes). Yield 12 mg (colourless microcrystalline material).

Acidic hydrolysis of **1**, derivatization of the amino acids by dimethylaminoazobenzenesulfonyl chloride (DABS) or Marfey's reagent[8,9], and HPLC analysis of the obtained derivatives[3~8] showed the presence of L-leucine, α-aminoisobutyric acid and L-phenylalaninol.

Thus 1 mg of **1** was hydrolysed by 1 ml of 6 N HCl for 24 hours at 120°C. The crude amino acids were derivatized by DABS and separation of derivatives was achieved using a gradient of sodium acetate buffer (pH 6.6) containing 4% DMF (Solvent A) and acetonitrile/2-propanol 2 : 3; v/v; Solvent B).

The gradient was started with 5% B for 4 minutes. Thereafter the gradient of 5% B to 100% B was run from 4 to 28 minutes followed by isocratic run using up to 100% B (31 minutes, 1.5 ml/minute; 45°C, 435 nm).

Marfey's reagent (N^2-(5-fluoro-2,4-dinitrophenyl)-L-alanine-amide)[8] was used to obtain the diasteromeric derivatives of chiral amino acids[9]. A linear gradient was established from 100% triethylammoniumphosphate (TEAP; mM)/acetonitrile (9 : 1; v/v) to 100% TEAP/acetonitrile (1 : 1; v/v) (45 minutes; 1.5 ml/minute; 340 nm)[3,5].

The molecular weight of **1** was determined by high-resolution fast atom bombardment (HR-FAB) mass spectrometry (double-focusing mass spectrometer AMD 402; Intectra Harpstedt, Germany) due to *m/z* 590.3895

Fig. 1. Structure of peptaibolin (**1**).

Ac-L-Leu-Aib-L-Leu-Aib-L-Pheol

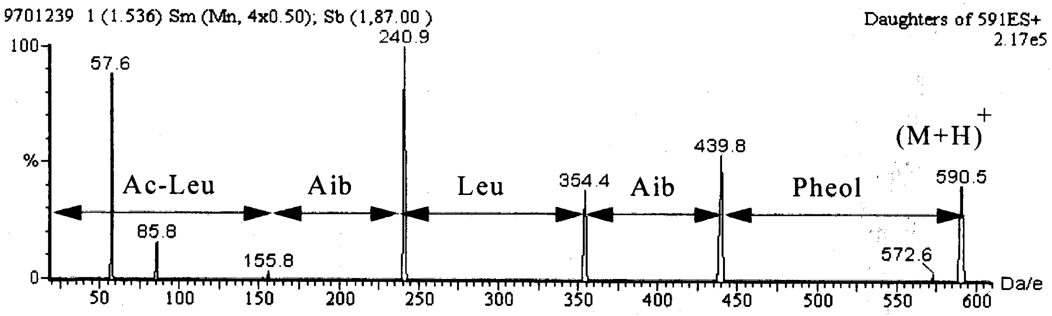
Ac-L-Leu: *N*-acetyl-L-leucine
Aib: α-aminoisobutyric acid
L-Pheol: L-phenylalaninol
L-Leu: L-leucine

Table 1. Physico-chemical properties of peptaibolin (**1**).

Appearance	Colorless microcrystals
Chemical formula	$C_{31}H_{51}N_5O_6$
HRFAB-MS *m/z*	590.3895 $[M+H]^+$; calcd. 590.3918
$[\alpha]_D^{25}$ (in MeOH)[a]	$-19.0°$
UV (λ_{max}; nm) in parantheses ε; (cm^2/Mol, in MeOH)[b]	232 (10.000)
IR (ν_{max}; cm^{-1}), in KBr[c]	1181, 1219, 1291, 1379, 1462, 1536, 1653, 2950, 3310
Rf on TLC ($CHCl_3$/MeOH 5 : 1)	0.6

[a] Propol polarimeter (Dr. KERNCHEN, SEELZE, Germany).
[b] Beckmann DU 60.
[c] Shimadzu IR-470.

Fig. 2. ESI-CID-MS/MS fragmentation pattern of **1** ($[M+H]^+$: *m/z* 590).



$[M+H]^+$ (calculated 590.3918 for $C_{31}H_{52}N_5O_6$).

During electrospray ionization mass spectrometry (triple quadrupole mass spectrometer Quattro 400; VG Biotech; Altrincham, U.K.) and collision-induced dissociation (CID-MS/MS, argon as target gas) of *m/z* 590 ($[M+H]^+$) a series of diagnostic daughter ions was generated suggesting the sequence of the amino acids as shown in Fig. 2.

Finally, the structure of peptaibolin was confirmed unambiguously by one- and two-dimensional NMR experiments (COSY; ^{13}C, DEPT, HMBC, HSQC, NOESY; Bruker Avance DRX 500, Rheinstetten, Germany; Table 1.). The ^{13}C NMR spectrum displays five carboxyl signals and six additional carbons attributable to an aromatic ring which is monosubstituted according to the ^1H, ^1H coupling data. The aliphatic $^3J_{H,H}$ couplings suggested the presence of several $-CH-(CH_3)_2$, $-C(CH_3)_2$ and $-CH-NH$ groups. Conclusive evidence for the chemical constitution of **1** was obtained from the HMBC spectrum of **1** (Fig. 3).

Compound **1** shows moderate antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC: 100 μg/ml) and yeasts such as *Candida albicans* (MIC: 100 μg/ml)$^{10)}$.

Acknowledgments

This work was supported by Thüringer Ministerium für Forschung, Wissenschaft und Kultur (Erfurt, Germany).

HEIKE HÜLSMANN
STEPHAN HEINZE
MICHAEL RITZAU
BRIGITTE SCHLEGEL
UDO GRÄFE

Hans-Knöll-Institute of Natural Products Research,
D-07745 Jena, Beutenbergstrasse 11, Germany

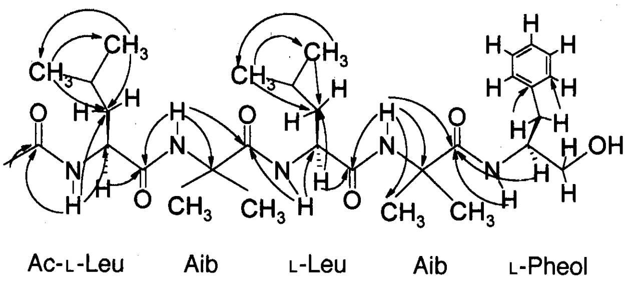
(Received June 22, 1998)

Table 2. Assigment of ^{1}H and ^{13}C chemical shift and coupling data of peptaibolin (in DMSO; concentration: 54 mM; chemical shifts in ppm).

		Position*	δ_C	δ_H
1	Ac-L-Leu	C-1′	170.8	
		C-2′	22.4	1.88 (s)
		N-H		8.15 (d)
		C-1	172.9	
		C-2	53.0	4.03 (m)
		C-3	39.6	1.46 (m)
		C-4	24.1	1.63 (m)
		C-5	22.7	0.90 (d) $^{3}J_{H,H}$ 6.6
		C-6	21.8	0.85 (d) $^{3}J_{H,H}$ 6.4
2	Aib	N-H		8.28 (s)
		C-1	174.9	
		C-2	55.9	
		C-3	25.2	1.24 (s)
		C-4	25.2	1.24 (s)
3	L-Leu	N-H		7.41 (d)
		C-1	171.7	
		C-2	52.8	3.90 (m)
		C-3	39.2	1.48 (m)
		C-4	24.2	1.55 (m)
		C-5	21.3	0.80 (d) $^{3}J_{H,H}$ 6.3
		C-6	22.9	0.86 (d) $^{3}J_{H,H}$ 5.1
4	Aib	N-H		7.55 (s)
		C-1	173.5	
		C-2	56.0	
		C-3	25.2	1.36 (s)
		C-4	24.6	1.36 (s)
5	L-Pheol	N-H		6.95 (d)
		O-H		4.43 (t)
		C-1	62.8	3.34 (m), 3.26 (m)
		C-2	52.7	3.82 (m)
		C-3	36.4	2.83 (dd), 2.57 (dd)
		C-4	139.5	
		C-5	129.1	
		C-6	127.9	7.19~7.21 (m)
		C-7	125.7	
		C-8	127.9	
		C-9	129.1	

* The DMSO ^{1}H and ^{13}C peaks at 2.49 ppm and 39.5 ppm, respectively, were used as reference signals. Multiplicity in parentheses (s: singlet; d: doublet; m: multiplet). For abbreviations of the amino acids see Fig. 1. Coupling constants in Hz.)

Fig. 3. Diagnostic C, H long-range couplings as detected in the HMBC spectrum of **1**.



References

1) SMITH, R. D.; J. A. LOO, C. G. EDMONDS, C. J. BARINAGA & H. R. UDSETH: New developments in biochemical mass spectrometry: electrospray ionization. Anal. Chem. 62: 882~899, 1990

2) SCHMIDLER, P. A.; A. VAN DORSELLAER & A. M. FALICK: Analysis of hydrophobic proteins and peptides by electrospray ionization mass spectrometry. Anal. Biochem. 213: 256~263, 1993

3) HEINZE, S.; M. RITZAU, W. IHN, B. SCHLEGEL, K. DORNBERGER, W. F. FLECK, M. ZERLIN, C. CHRISTNER, U. GRÄFE, G. KÜLLERTZ & G. FISCHER: Lipohexin, a new inhibitor of prolyl endopeptidase from *Moeszia lindtneri*

(HKI-0054) and *Paecilomyces* sp. (HKI-0055). I. Screening, isolation and structure elucidation. J. Antibiotics 50: 379~383, 1997

4) Gräfe, U.; W. Ihn, M. Ritzau, W. Schade, C. Stengel, B. Schlegel, W. F. Fleck, W. Künkel, A. Härtl & W. Gutsche: Helioferins, novel antifungal lipopeptides from *Mycogone rosea.* Screening, isolation, structures and biological properties. J. Antibiotics 48: 126~133, 1995

5) Ritzau, M.; S. Heinze, K. Dornberger, A. Berg, W. Fleck, B. Schlegel, A. Härtl & U. Gräfe: Ampullosporin, a new peptaibol-type Antibiotic from *Sepedonium ampullosporum* HKI-0053 with neuroleptic activity in mice. J. Antibiotics 50: 722~728, 1997

6) Laatsch, H.: Antibase, database of microbial products. VCH Weinheim, Edition 1998

7) ARX, M. A.: The genera of fungus, sporulating in pure culture. *Ed.* J. Cramer, p. 337, Vaduz, 1981

8) Szikat, G.; G. Mezö & M. Hudecz: Application of Marfey's reagent in racemization studies of amino acids and peptides. J. Chromatogr. 444: 115~122, 1988

9) Brückner, H. & C. Keller-Hoehl: HPLC separation of DL-amino-acids derivatized with N^2-(5-fluoro-2,4-dinitrophenyl)-L-amino acid-amides. Chromatographia 30: 621~629, 1990

10) Anonymous: Deutsches Arzneimittelbuch 9th Edition. pp. 47 and 424. Deutscher Apothekerverlag Stuttgart, 1986