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A NEW CYCLIC PEPTIDE FROM THE MARINE FUNGAL STRAIN *Aspergillus* sp. AF119

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*A new cyclic heptapeptide containing γ-aminobutyric acid in the ring, namely unguisin E (**1**), was isolated from the fermentation culture of* Aspergillus *sp. AF119. The structure was elucidated by spectroscopic analyses, including 1D and 2D NMR experiments, and HR ESI-Q-TOF mass spectrometry, and by comparison with those reported. Compound **1** was evaluated for its antimicrobial activities by the paper diffusion method.*

Keywords: heptapeptide, unguisin E, *Aspergillus* sp. AF119.

The fungal strain AF119 was isolated from the soil of Xiamen beach, and was identified as *Aspergillus* sp. based on its complete ITS1-5.8S-ITS2 gene sequences. Further chemical constituent studies led to a new cyclic heptapeptide, namely unguisin E (**1**). Here we report the isolation and structure elucidation of compound **1**.

Compound **1**, $[\alpha]_D^{20}$ + 48° (*c* 0.5, MeOH), was obtained as a white amorphous powder. UV (MeOH, λ_{max}, nm): 227.0 (2.42), 281.0 (0.70). IR (KBr, ν_{max}, cm^{-1}): 3306, 2964, 2933, 1643, 1452, 1530. The molecular formula was determined as $C_{41}H_{56}N_8O_7$ according to the HR ESI-Q-TOF MS ($[M + H]^+$ at *m/z* 773.4342) and NMR data (Table 1). The ^1H and ^{13}C NMR data of **1** suggested a peptide structure and confirmed the elemental composition. The ^1H NMR spectrum revealed the presence of seven amide NH signals between δ 8.33 and 7.64 and an additional NH signal at δ 10.83, which suggested the presence of an indole ring. The occurrence of seven amino acids was supported by amide carbonyl signals at 170.5, 171.5, 172.1, 172.3, 172.9, 173.0 and 173.2. Seven amino acid residues were assigned on the basis of ^1H–^1H COSY and HMBC experiments as Ala (2 eq.), Val (2 eq.), *β*-methyl-Phe (1 eq.), *γ*-aminobutyric acid (GABA) (1 eq.), and Trp (1 eq.). The peptide sequence for **1** was determined to be Val-1, Ala-1, GABA, Trp, Ala-2, Val-2, and *β*-methyl-Phe by key NOESY correlations (Fig. 1). The ^1H and ^{13}C NMR spectral data of **1** were very similar to those recorded for unguisin A [1], which suggested a close structural relationship between the two compounds. The only difference was that the amino acid Phe in unguisin A [1] was replaced by *β*-methyl Phe in **1**. The relative stereochemistry of compound **1** was determined by NOESY correlations. From these data, the structure of compound **1** was established as cyclo (alanyl–tryptophyl–4-aminobutanoyl–alanyl–valyl–*β*-methyl-phenylalanyl–valyl), and we named it unguisin E for consistency with the literature [1, 2].

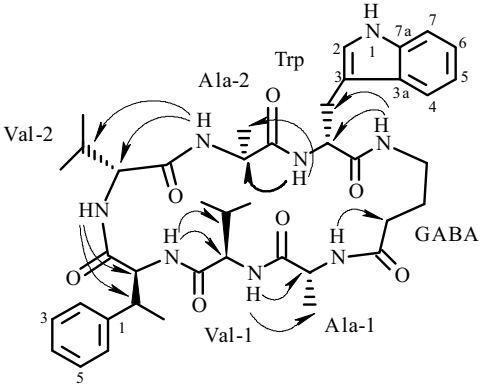

Fig. 1. Key NOESY correlations to determine the structure of compound **1**.

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TABLE 1. NMR Data for **1*** (600 MHz, DMSO-d_6)

Position	δ_H (mult., J/Hz)	δ_C	key-NOESY
Trp			
NH	7.97 (d, J = 6.5)		H-α, H-β (Ala-2)
α	4.05 (m)	55.3 (d)	
β	3.19 (2H, m)	25.8 (t)	
NH-1	10.83 (br.s)		
2	7.10 (s)	124.0 (d)	
3		111.1 (s)	
4	7.52 (d, J = 8.0)	118.7 (d)	
5	6.98 (t, J = 8.0)	118.7 (d)	
6	7.07 (t, J = 8.0)	121.4 (d)	
7	7.33 (d, J = 8.0)	111.8 (d)	
3a		127.6 (s)	
7a		136.3 (s)	
GABA			
NH	7.64 (t)		H-α, H-β (Trp)
α	2.97 (m), 3.09 (m)	38.8 (t)	
β	1.59 (m), 1.67 (m)	26.1 (t)	
γ	1.96 (m), 2.12 (m)	33.3 (t)	
Ala-1			
NH	7.85 (d, J = 6.3)		H-α (GABA)
α	4.20 (m)	48.3 (d)	
β	1.15 (3H, d, J = 6.7)	18.3 (q)	
Val-1			
NH	7.91		H-α, H-β (Ala-1)
α	3.89 (m)	60.1 (d)	
β	1.62 (m)	29.5 (d)	
γ	0.38 (3H, d, J = 6.7)	19.0 (q)	
γ	0.77 (3H, d, J = 6.7)	19.8 (q)	
β-Methyl-Phe			
NH	8.27 (d, J = 8.5)		H-α, H-β (Val-1)
α	4.41 (m)	59.3 (d)	
β	3.51 (m)	39.6 (d)	
γ	1.18 (3H, d, J = 6.7)	14.7 (q)	
1		143.3 (s)	
2	7.26 (d, J = 7.6)	128.2 (d)	
3	7.21 (t, J = 7.6)	128.4 (d)	
4	7.13 (t, J = 7.6)	126.6 (d)	
5	7.21 (t, J = 7.6)	128.4 (d)	
6	7.26 (d, J = 7.6)	128.2 (d)	
Val-2			
NH	7.87		H-α, H-β (β-methyl-Phe)
α	4.07 (m)	58.7 (d)	
β	2.01 (m)	30.2 (d)	
γ	0.61 (3H, d, J = 6.5)	19.2 (q)	
γ	0.62 (3H, d, J = 6.5)	18.9 (q)	
Ala-2			
NH	8.33		H-α (Val-2)
α	3.94 (m)	50.3 (d)	
β	1.19 (3H, d, J = 6.7)	17.7 (q)	

*The seven carbonyl carbon signals appear at δ 170.5, 171.5, 172.1, 172.3, 172.9, 173.0, and 173.2.

Besides the known unguisins A–D, unguisin E is another new cyclic heptapeptide containing a GABA-derived moiety in the ring, and this was isolated from the fungus *Aspergillus* sp. AF119.

EXPERIMENTAL

General Experimental Procedures. Precoated silica-gel GF_{254} plates for TLC and column chromatography (CC) silica gel (Qingdao Marine Chemical Factory, Qingdao, P. R. China); RP-18 gel (40–63 μm, Merck) or Sephadex LH-20 gel (Amersham Biosciences). UV spectrum: Genesys^{TM} 2Thermospectronic, λ_{max} (ε); in nm. IR spectrum: Thermo Nicolet 380 FT-IR spectrophotometer, with KBr cells; in cm^{-1}. NMR spectra: Bruker ARX 600 spectrometer operating, at 600/150 MHz, δ in ppm rel. to Me_4Si; J in Hz. HR-Q-TOF-MS: Bio TOF^{TM} Q mass spectrometer (Bruker); in *m/z*. Optical rotation: AUTOPOL$^{@}$IV automatic polarimeter.

Microbial Material. The fungus strain AF119 was isolated from the soil of Xiamen beach. It was identified as *Aspergillus* sp. by traditional morphology and ITS sequencing. The ITS sequence of strain AF119 was cloned and sequenced (GenBank accession number: AY373842.1). The blast search result showed that the sequence of strain AF119 was 99% homologous to the species of *Aspergillus candidus* ATCC 1002.

Extraction and Isolation. A stock of *Aspergillus* sp. AF119 was cultivated on ca.10 cm petri dishes with half seawater PDA medium (total 1.0 L) for 15 days at 28°C. The mycelium together with culture medium was extracted three times with EtOAc. The organic solvent was evaporated under reduced pressure to afford the crude extract (0.94 g). The extract was first subjected to MPLC over RP-18 SiO_2 (80 g) CC using a stepwise gradient of 30, 50, 70, and 100% (v/v) acetone in H_2O to afford Fr. 1 (184 mg) obtained from 50% acetone. Fraction 1 was subjected to Sephadex LH-20 eluted with MeOH to afford Fr. 1.1 (23 mg) and Fr. 1.2 (32 mg). Fraction 1.1 was purifed by CC ($CHCl_3$–MeOH) to yield **1** (20 mg).

Bioassay Procedures. The antifungal activity of **1** was tested at a concentration of 30 μg/disk against *Candida albicans* using the agar diffusion method. The results showed almost no inhibitory activity.

ACKNOWLEDGMENT

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