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A new cyclohexapeptide, penitropeptide and a new polyketide, penitropone from the endophytic fungus *Penicillium tropicum*

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ABSTRACT

The first chemical investigation of the endophytic fungus *Penicillium tropicum* isolated from leaves of *Sapium ellipticum* afforded a new cyclohexapeptide, penitropeptide (**1**), and a new polyketide, penitropone (**2**), in addition to two known compounds, 6-hydroxy-8-methoxy-3*S*,5-dimethyl-3,4-isocoumarin (**3**) and adametizine B (**4**). Their structures were unambiguously elucidated on the basis of 1D and 2D NMR spectroscopy as well as mass spectrometry, and by comparison with the literature. The absolute configurations of these two new compounds **1** and **2** were determined by Marfey's method and X-ray single crystal diffraction, respectively. All isolated compounds were tested for their cytotoxic and antibacterial activities. However, none of them showed significant activity.

Introduction

Secondary metabolites of endophytes have a long and rich history as an important source of biologically active molecules with unusual structures.[1–3] The genus *Penicillium* which comprises more than 300 species is one of the most ubiquitous fungal genera and has been intensely studied since the discovery of the penicillins.[4,5] Consequently, many structurally unique and bioactive secondary metabolites have been characterized from this genus, as exemplified by phenolic bisabolene sesquiterpenes peniciaculins A and B from *Penicillium aculeatum*,[6] azaphilone derivatives pinophilins A, B, and D–F from *Penicillium pinophilum*,[7,8] or novel alkaloids citrinadin A–B and perinadine A from *Penicillium citrinum*.[9–11] During our ongoing search for new secondary metabolites from endophytic fungi,[12–14] the ethyl acetate extract of *Penicillium tropicum* isolated from the leaves of *Sapium ellipticum* (Euphorbiaceae) attracted our attention due to the presence of structurally diverse compounds that did not match our *in house* LC–UV data base. Chemical examination of this fungus led to the isolation of a new cyclohexapeptide, penitropeptide (**1**), a new polyketide, penitropone (**2**), and two known compounds 6-hydroxy-8-methoxy-3*S*,5-dimethyl-3,4-isocoumarin (**3**) and adametizine B (**4**) (Fig. 1). Herein, we describe the structural elucidation of the new compounds **1** and **2**, including their absolute configurations, established by application of Marfey's method and by X-ray diffraction, respectively.

Results and discussion

Compound **1** was isolated as white, amorphous solid. Its HR-ESIMS spectrum gave a peak $[M+H]^+$ at *m*/*z* 698.3661 and the molecular formula $C_{38}H_{47}N_7O_6$, indicating 19 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) exhibited five amide protons at δ_H 10.83 (1H, d, *J* = 2.0 Hz, Trp^5-1′NH), 8.81 (1H, dd, *J* = 7.5, 4.8 Hz, Gly^1-NH), 8.60 (1H, d, *J* = 4.4 Hz, Leu^6-NH), 8.57 (1H, d, *J* = 9.0 Hz, Trp^5-NH), and 7.36 (1H, d, *J* = 6.8 Hz, Phe^2-NH), as well as α-amino protons between δ_H 3.40 and 4.50, suggesting a peptide structure for **1**. Detailed interpretation of the COSY and HMBC spectra (Table 1 and Fig. 2) revealed the presence of six amino acid residues in **1**, including a Gly (glycine), a Phe (phenylalanine), two Pro (proline), a Trp (tryptophan), and a Leu (leucine). The HMBC correlations from the amide protons Trp^5-NH, Leu^6-NH, Gly^1-NH, and Phe^2-NH to their adjacent carbonyls Pro^4-CO, Trp^5-CO,

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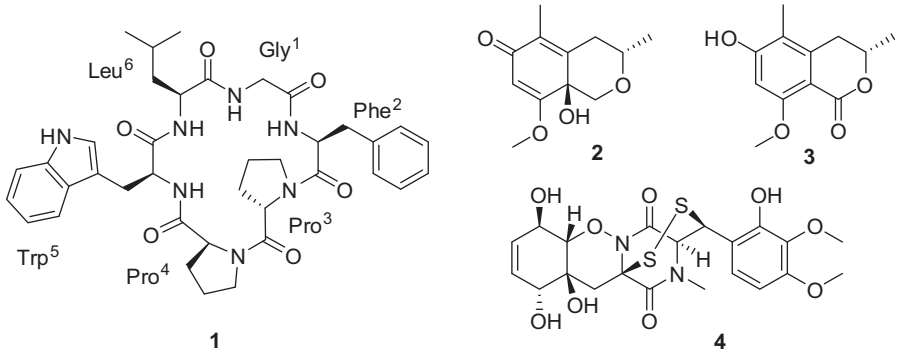

Figure 1. Structures of compounds **1**–**4**.

Table 1
^{1}H, ^{13}C, HMBC and ROESY NMR data of penitropeptide (**1**)[a]

Residue	Position	δ_C	δ_H (J in Hz)	HMBC	ROESY
Gly^1	NH		8.81, dd (7.5, 4.8)	Leu^6-CO, Gly^1-α	Leu^6-β, Leu^6-δ, Phe^2-NH
	CO	168.8, C			
	α	43.4, CH_2	4.01, dd (16.5, 7.5); 3.40, dd (16.5, 4.8)	Leu^6-CO, Gly^1-CO	Phe^2-NH
Phe^2	NH		7.36, d (6.8)	Gly^1-CO, Phe^2-CO, Phe^2-α	Gly^1-NH, Gly^1-α, Pro^3-δ
	CO	167.5, C			
	α	53.0, CH	4.50, m		Pro^3-δ
	β	36.4, CH_2	3.08, dd (13.1, 9.3); 2.86, dd (13.1, 3.8)	Phe^2-CO, Phe^2-α, Phe^2-1′, Phe^2-2′/6′	
	1′	137.7, C			
	2′	129.1, CH	7.28, m	Phe^2-β, Phe^2-4′, Phe^2-6′	
	3′	128.3, CH	7.28, m	Phe^2-1′, Phe^2-5′	
	4′	126.4, CH	7.20, m	Phe^2-2′/6′	
	5′	128.3, CH	7.28, m	Phe^2-1′, Phe^2-3′	
	6′	129.1, CH	7.28, m	Phe^2-β, Phe^2-2′, Phe^2-4′	
Pro^3	CO	169.5, C			
	α	59.1, CH	4.37, dd, (8.9, 7.3)	Pro^3-CO, Pro^3-β, Pro^4-CO	Pro^4-α
	β	28.0, CH_2	2.19, m; 1.62, m	Pro^3-CO	Pro^4-α
	γ	24.3, CH_2	1.94, m; 1.78, m		
	δ	46.1, CH_2	3.48, m; 3.29, m	Phe^2-CO	Phe^2-NH, Phe^2-α
Pro^4	CO	170.8, C			
	α	60.2, CH	4.22, dd (7.1, 2.3)	Pro^4-CO, Pro^4-γ	Pro^3-α, Pro^3-β, Trp^5-NH
	β	30.6, CH_2	1.84, m	Pro^4-CO, Pro^4-α, Pro^4-δ	
	γ	21.1, CH_2	1.39, m; 0.50, m		
	δ	46.1, CH_2	3.13, m		
Trp^5	NH		8.57, d (9.0)	Pro^4-CO	Pro^4-α, Trp^5-2′, Trp^5-4′
	CO	173.2, C			
	α	54.1, CH	4.48, m		Leu^6-NH
	β	26.8, CH_2	3.35, m; 3.13, m	Trp^5-α, Trp^5-2′, Trp^5-3′, Trp^5-3′a	Leu^6-NH
	1′ NH		10.83, d (2.0)	Trp^5-2′, Trp^5-3′, Trp^5-7′, Trp^5-3′a, Trp^5-7′a	
	2′	123.0, CH	7.16, d (2.0)	Trp^5-3′, Trp^5-3′a, Trp^5-7′a	Trp^5-NH
	3′	111.0, C			
	4′	118.5, CH	7.92, d (7.9)	Trp^5-3′, Trp^5-6′, Trp^5-7′a	Trp^5-NH
	5′	117.9, CH	6.98, t (7.4)	Trp^5-7′, Trp^5-3′a	
	6′	120.5, CH	7.06, t (7.4)	Trp^5-4′, Trp^5-7′a	
	7′	110.6, CH	7.33, d (8.0)	Trp^5-5′, Trp^5-3′a	
	3′a	127.5, C			
	7′a	135.7, C			
Leu^6	NH		8.60, d (4.4)	Trp^5-CO, Leu^6-α, Leu^6-β	Trp^5-α, Trp^5-β
	CO	173.1, C			
	α	53.6, CH	4.01, m	Leu^6-CO	
	β	39.0, CH_2	1.55, m	Leu^6-CO, Leu^6-α, Leu^6-γ, Leu^6-δ/δ'	Gly^1-NH
	γ	24.0, CH	1.62, m	Leu^6-β, Leu^6-δ/δ'	
	δ	22.3, CH_3	0.96, d (6.5)	Leu^6-β, Leu^6-γ, Leu^6-δ'	Gly^1-NH
	δ'	22.3, CH_3	0.90, d (6.5)	Leu^6-β, Leu^6-γ, Leu^6-δ	

[a] Recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C in DMSO-d_6.

Leu^6-CO, and Gly^1-CO, respectively, as well as key ROESY correlations (Table 1 and Fig. 2) between Pro^4-Hα/Trp^5-NH, Trp^5-Hα/Leu^6-NH, Gly^1-NH/Phe^2-NH, and Gly^1-Hα/Phe^2-NH, revealed Pro^4-Trp^5-Leu^6-Gly^1-Phe^2 as amino acid sequence. In addition, the HMBC correlations from Pro^3-Hδ to Phe^2-CO and the NOE correlations between Phe^2-Hα/Pro^3-Hδ, and Pro^3-Hα/Pro^4-Hα indicated the presence of a *trans* peptide bond between Phe^2-Pro^3 and a *cis* peptide bond between Pro^3-Pro^4, which was also supported by the carbon resonances of β and γ in Pro^3 ($\delta_{C\text{-}\beta}$ 28.0, $\delta_{C\text{-}\gamma}$ 24.3) and Pro^4 ($\delta_{C\text{-}\beta}$ 30.6, $\delta_{C\text{-}\gamma}$ 21.1).[15] Thus, **1** was determined to be the new *cyclo*(-Gly-Phe-Pro-Pro-Trp-Leu-), for which the name penitropeptide is proposed.

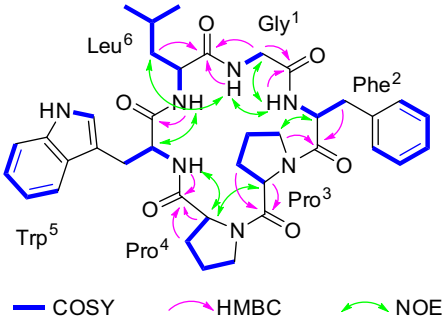

Figure 2. Key COSY, HMBC, and NOE correlations of **1**.

The absolute configurations of the amino acid residues were determined following acidic hydrolysis of **1** and subsequent derivatization according to Marfey's method.[16] Comparison of the resulting derivatives with those of standard amino acids using LC–MS led to the assignment of the L-configuration for all amino acid residues.

The molecular formula of compound **2** was established as $C_{12}H_{16}O_4$ on the basis of HR-ESIMS data (*m/z* 225.1122 $[M+H]^+$), implying 5 degrees of unsaturation. The ^1H NMR spectrum of **2** (Table 2) showed the presence of an olefinic methine proton at δ_H 5.60 (1H, s, H-7), an oxygenated methine proton at δ_H 4.45 (1H, ddq, J = 6.1, 1.8, 6.7 Hz, H-3), an oxygenated methylene group at δ_H 3.98 (1H, d, J = 11.9 Hz, H-1a) and 3.54 (1H, d, J = 11.9 Hz, H-1b), a methylene group at δ_H 3.07 (1H, ddq, J = 13.4, 6.1, 1.0 Hz, H-4a) and 2.57 (1H, dd, J = 13.4, 1.8 Hz, H-4b), three methyl groups, including a methoxy signal at δ_H 3.78 (3H, s, H_3-13), a vinyl methyl signal at δ_H 1.86 (3H, d, J = 1.0 Hz, H_3-12), and an aliphatic methyl signal at δ_H 1.12 (3H, d, J = 6.7 Hz, H_3-11). The ^{13}C NMR spectrum of **2** exhibited a total of 12 carbons (Table 2), including a carbonyl signal at δ_C 188.7 (C-6), three olefinic quaternary carbons at δ_C 176.5 (C-8), 149.2 (C-10), and 131.0 (C-5), an olefinic methine at δ_C 101.9 (C-7), an oxygenated methine at δ_C 72.9 (C-3), an oxygenated quaternary carbon at δ_C 70.2 (C-9), an oxygenated methylene at δ_C 70.1 (C-1), a methoxy group at δ_C 56.7 (C-13), a methylene at δ_C 33.4 (C-4), and two methyls at δ_C 16.4 (C-11), and 10.7 (C-12). The HMBC correlations from H_2-1 to C-8, C-9, and C-10, from H_2-4 to C-5, C-9, and C-10, from H_3-12 to C-5, C-6, and C-10, from H-7 to C-5, C-6, C-8, and C-9, and from H_3-13 to C-8 indicated the presence of the cyclohexadienone ring A and the attachment of methyl, methoxy, and two methylene groups at C-5, C-8, C-9, and C-10, respectively (Fig. 3). Moreover, the COSY correlations between H-3/H_2-4 and H-3/H_3-11, as well as the HMBC correlations from H_2-1 to C-3 and from H-3 to C-1 revealed the presence of the tetrahydropyran ring B, and consequently the attachment of a hydroxy group at C-9 to satisfy its molecular formula. Thus, the planar structure of **2** was elucidated as shown in Figure 1 and named penitropone.

Table 2
^1H, ^{13}C, and HMBC NMR data of penitropone (**2**)

Position	δ_C[a]	δ_H (J in Hz)[a]	HMBC[a]	δ_H (J in Hz)[b]
1	70.1, CH_2	3.98, d (11.9) 3.54, d (11.9)	C-3, C-8, C-9, C-10	3.93, d (11.8) 3.53, d (11.8)
3	72.9, CH	4.45, ddq (6.1, 1.8, 6.7)	C-1, C-10	4.40, ddq (6.1, 2.0, 6.7)
4	33.4, CH_2	3.07, ddq (13.4, 6.1, 1.0) 2.57, dd (13.4, 1.8)	C-3, C-5, C-9, C-10, C-11	3.05, ddq (13.4, 6.1, 1.0) 2.50, dd (13.4, 2.0)
5	131.0, C			
6	188.7, C			
7	101.9, CH	5.60, s	C-5, C-6, C-8, C-9	5.49, s
8	176.5, C			
9	70.2, C			
10	149.2, C			
11	16.4, CH_3	1.12, d (6.7)	C-3, C-4	1.10, d (6.7)
12	10.7, CH_3	1.86, d (1.0)	C-5, C-6, C-10	1.80, d (1.0)
13	56.7, CH_3	3.78, s	C-8	3.74, s
9-OH				4.81, s

[a] Recorded at 300 MHz for ^1H and 75 MHz for ^{13}C in CD_3OD;
[b] Recorded at 600 MHz in CD_3COCD_3.

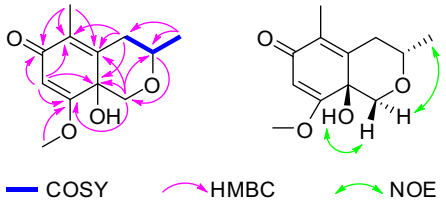

Figure 3. Key COSY, HMBC, and NOE correlations of **2**.

The relative configuration of **2** was deduced from the ROESY spectrum in CD_3COCD_3. The NOE correlations between H-1a (δ_H 3.93) and 9-OH (δ_H 4.81), and between H-1b (δ_H 3.53) and H_3-11 (δ_H 1.10) suggested a *trans* orientation of 9-OH and CH_3-11 (Fig. 3). Finally, the absolute configuration of **2** was determined as 3*S*, 9*S* according to X-ray diffraction of a single crystal using the Flack parameter of −0.08(7) with Cu-Kα radiation (Fig. 4).[17]

The two known compounds were identified as 6-hydroxy-8-methoxy-3*S*,5-dimethyl-3,4-isocoumarin (**3**),[18] and adametizine B (**4**).[19,20]

Compounds **1**–**4** were tested for their cytotoxicity against the human ovarian cell line A2780. However, none of them exhibited significant inhibitory activity at the concentration of 10.0 μM. Also, the compounds showed no inhibitory activity against *Staphylococcus aureus* ATCC 25923 and *Acinetobacter baumannii* ATCC BAA1605 even at the highest test concentration of 64 μg/mL.

In summary, four compounds were isolated during the first chemical examination of endophytic fungus *P. tropicum*. Penitropeptide (**1**) is a new cyclic hexapeptide and the configurations

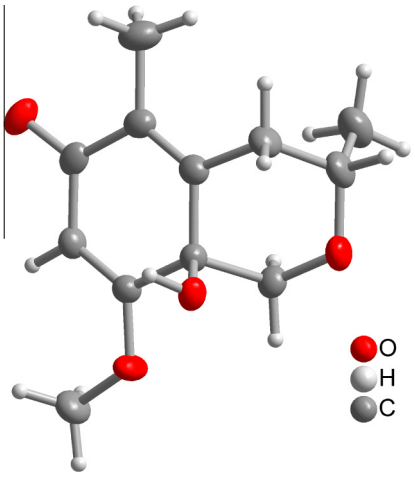

Figure 4. Molecular structure of **2** from single-crystal X-ray diffractometry (70% thermal ellipsoids, H atom of arbitrary radii).

of the amino acid residues were established using Marfey's method. Penitropone (**2**) and 6-hydroxy-8-methoxy-3*S*,5-dimethyl-3,4-isocoumarin (**3**) are polyketides while the absolute configuration of the new compound penitropone (**2**) was determined by X-ray single crystal diffraction. Adametizine B (**4**) is a novel epidithiodiketopiperazine derivative, which was first isolated from the hyper saline lake derived fungus *Penicillium* sp.[19] and later obtained from the sponge-derived fungus *Penicillium adametzioides*.[20]

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Supplementary data

Supplementary data (UV, MS and NMR spectra of **1** and **2** as well as X-ray data of **2**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.05.095.

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