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Pleosporin A, an antimalarial cyclodepsipeptide from an elephant dung fungus (BCC 7069)

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ABSTRACT

Cyclodepsipeptides SCH 217048 (**1**), SCH 218157 (**2**), and a new analog, pleosporin A (**3**), were isolated from cultures of an unidentified elephant dung fungus of the family Pleosporaceae. The structure of **3** was elucidated on the basis of detailed spectroscopic interpretation. The absolute configurations of **1**–**3** were determined by chiral column HPLC analysis and Marfey's method. Cyclodepsipeptides **1**–**3** exhibited antimalarial activity against *Plasmodium falciparum* K1 with respective IC_{50} values of 1.6, 6.4, and 1.6 μg/mL, while they did not show cytotoxicity against KB, MCF-7 and NCI-H187 cell-lines or non-cancerous Vero cells at 50 μg/mL.

The isolation of SCH 217048, a potent cyclodepsipeptide tachykinin (NK_2) receptor inhibitor, from an unidentified fungus was reported in 1998 by Puar and co-workers.[1] SCH 217048 exhibited a highly selective NK_2 antagonist-type activity (K_i 27.43 nM), while it showed no activity at 10 μM in NK_1 and NK_3 assays. This report was soon followed by the isolation of the closely related analogs, SCH 378161, SCH 378167, and SCH 378199 produced by the same fungal strain,[2] and SCH 218157 co-produced with SCH 217048 by another unidentified fungus.[3] The planar structures including the sequences of the amino acids and 2-hydroxy-3-methylpentanoic acid (Hmp) were elucidated by NMR and MS analyses, while their absolute configurations have not been determined. The assignment of the absolute configurations and the accessibility to these molecules would have been key requirements for further biochemical studies as leads for selective tachykinin receptor inhibitors.

As part of our research program on bioactive secondary metabolites from fungal sources in Thailand, we have been investigating the constituents of several coprophilous fungi isolated from elephant dung samples, primarily due to our interest in this unique ecological niche.[4] One of the unidentified coprophilous strains, BCC 7069 of the family Pleosporaceae,[5] was chemically explored in detail as a culture extract exhibited antimalarial activity against *Plasmodium falciparum* K1 with an IC_{50} value of 29 μg/mL, and it displayed unique ^1H NMR spectroscopic data suggesting the presence of a mixture of peptides. The study led to the isolation of SCH 217048 (**1**), SCH 218157 (**2**), and a new analog named pleosporin A (**3**). We report herein the structure elucidation of **3**, and the determination of the absolute configurations of **1**–**3** (Fig. 1), and the evaluation of their biological activities.

The fungus BCC 7069 was fermented in peptone yeast glucose medium (PYGM; 28 × 250 mL) at 25 °C for 17 days under static conditions. The cultures were filtered to separate mycelia (residue) and broth (filtrate). The EtOAc extract from the broth (1.20 g) was subjected to fractionation using Sephadex LH-20 (MeOH) and silica gel (0–30% MeOH in CH_2Cl_2) column chromatography, and the eluted fractions containing cyclodepsipeptides were further separated by preparative HPLC (ODS column, MeCN/H_2O = 50:50) to furnish **1** (56 mg), **2** (28 mg), and **3** (16 mg). The MeOH extract from mycelia (245 mg) was also fractionated using similar procedures to give **1** (12 mg), **2** (10 mg), and **3** (5 mg). The structures of **1** and **2** were elucidated by the analysis of their NMR spectroscopic data obtained in $CDCl_3$.[6,7] A comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** and **2** in DMSO-d_6 with those reported in the literature confirmed that these were SCH 217048 and SCH 218157, respectively.

Pleosporin A (**3**) was obtained as a colorless solid.[8] The molecular formula of **3** was established by HRMS (ESI-TOF) as $C_{56}H_{87}N_{11}O_{13}$, which suggested the presence of two *N*-methylglutamine units (*N*-Me-Gln) as in **2**. The ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$ were similar to those of **1** and **2**, and were indicative of a closely related structure. Specifically, the presence of four broad singlets due to carboxamide protons (δ_H 5.62, 5.59, 5.56, and 5.49) in the ^1H NMR spectrum was consistent with the presence of two *N*-Me-Gln residues. Nine amino acids were assigned by the analysis of COSY, HMQC, and HMBC data as two *N*-methylglutamine (*N*-Me-Gln-1, *N*-Me-Gln-2), two proline (Pro-1, Pro-2), phenylalanine (Phe), *N*-methylvaline (*N*-Me-Val), valine (Val), and isoleucine (Ile) (Table 1). The Hmp residue was also assigned on

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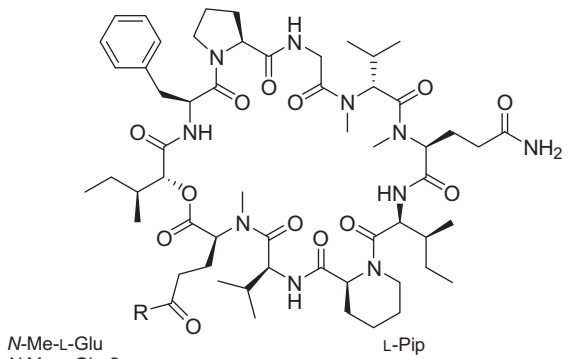
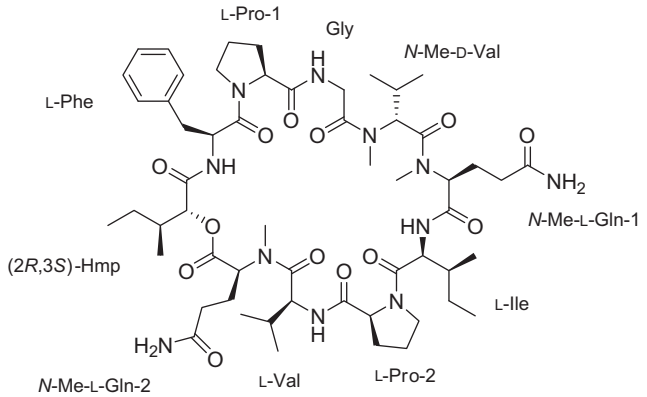


1 R = OH : SCH 217048
2 R = NH_2: SCH 218157



3

Figure 1. Structures of the cyclodepsipeptides isolated from BCC 7069.

the basis of the 2D NMR data. The ^{1}H and ^{13}C NMR spectroscopic data for Hmp were similar to those of **1** and **2**, which suggested that these compounds share the same absolute configuration. The sequence of nine amino acids and Hmp was assigned to be the same as in **1** and **2** by analysis of HMBC and NOESY correlations (Fig. 2). Thus, a HMBC correlation from the NH of Phe to the ester carbonyl (C-1) of Hmp indicated the Hmp–Phe amide linkage. An intense NOESY correlation between H-5 of Pro-1 (δ_H 3.78, m) and H-2 of Phe revealed the amide bonding of Phe–Pro-1. The linkage of Pro-1–Gly–*N*-Me-Val–*N*-Me-Gln-1–Ile was established by following HMBC correlations: from NH of Gly to C-1 of Pro-1, from N-CH_3 of *N*-Me-Val to C-1 of Gly and C-2 of *N*-Me-Val, from N-CH_3 of *N*-Me-Gln-1 to C-1 of *N*-Me-Val and C-2 of *N*-Me-Gln-1, and from NH of Ile to C-1 of *N*-Me-Gln-1. NOESY correlation be-

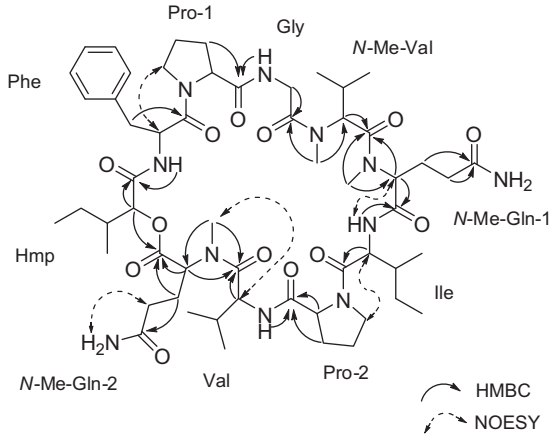


Figure 2. Selected HMBC and NOESY correlations for **3**, indicating the sequence of nine amino acids and Hmp.

Table 1
NMR spectroscopic data for **3** in $CDCl_3$ (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position	δ_C, Mult.	δ_H, Mult. (*J* in Hz)	Position	δ_C, Mult.	δ_H, Mult. (*J* in Hz)	Position	δ_C, Mult.	δ_H, Mult. (*J* in Hz)
Hmp			Gly			Pro-2		
1	169.2, qC		1	170.4,[a] qC	2.30, d (16.0)	1	172.9,[e] qC	
2	76.0, CH	5.20, d (2.1)	2	41.1, CH_2	4.54, dd (17.0, 8.4)	2	59.5, CH	4.67, m
3	36.3, CH	2.15, m			3.99, dd (17.0, 2.5)	3	29.8, CH_2	2.01, m; 1.77, m
4	26.4, CH_2	1.19, m; 1.18, m	N*H*		7.12, dd (8.4, 2.5)	4	25.1,[d] CH_2	2.14, m; 1.88, m
5	11.6, CH_3	0.84, t (7.5)	*N*-Me-Val			5	48.0, CH_2	3.70, m; 3.68, m
3-*CH_3*	14.8, CH_3	0.76, d (6.8)	1	170.4,[a] qC		Val		
Phe			2	57.9, CH	5.18, d (10.7)	1	172.8,[e] qC	
1	171.5, qC		3	27.6, CH	2.40, m	2	54.9,[d] CH	4.66, m
2	52.6, CH	4.88, m	4	19.7, CH_3	0.92, m	3	31.3, CH	2.16, m
3	37.6, CH_2	3.12, dd (14.0, 9.9)	4′	18.5, CH_3	0.89, m	4	18.2, CH_3	0.94, m
		3.05, dd (14.0, 4.6)	N-*CH_3*	29.1, CH_3	3.03, s	4′	19.4, CH_3	0.89, m
4	137.0, qC		*N*-Me-Gln-1			N*H*		9.02, d (10.1)
5, 9	129.3, CH	7.29, m	1	168.9, qC		*N*-Me-Gln-2		
6, 8	128.4, CH	7.28, m	2	59.0, CH	5.31, m	1	169.39,[c] qC	
7	126.8, CH	7.22, m	3	25.0,[b] CH_2	2.35, m; 1.86, m	2	62.4, CH	3.91, dd (7.4, 6.4)
N*H*		7.87, d (8.8)	4	31.7, CH_2	2.27, m; 2.17, m	3	24.5, CH_2	2.53, m; 2.28, m
Pro-1			5 (*CO*NH_2)	174.2, qC	5.56, br s; 5.49, br s	4	31.1, CH_2	2.51, m; 2.33, m
1	170.9, qC		N-*CH_3*	29.4, CH_3	2.79, s	5 (*CO*NH_2)	174.4, qC	5.62, br s; 5.59, br s
2	60.4, CH	4.38, dd (8.0, 3.6)	Ile			N-*CH_3*	38.7, CH_3	3.29, s
3	28.6, CH_2	2.12, m; 2.03, m	1	169.36,[c] qC				
4	25.3, CH_2	2.28, m; 1.99, m	2	55.0,[d] CH	4.72, dd (7.9, 3.5)			
5	47.3, CH_2	3.78, m; 3.50, m	3	38.9, CH	1.79, m			
			4	23.9, CH_2	1.42, m; 0.99, m			
			5	11.7, CH_3	0.86, m			
			3-*CH_3*	15.8, CH_3	0.89, m			
			N*H*		6.42, d (7.9)			

[a] The carbon resonances were superimposed.
[b–e] Carbon chemical shifts may be interchanged.

tween H-5 of Pro-2 and H-2 of Ile suggested the Ile–Pro-2 connection. HMBC correlations from NH of Val to C-1 of Pro-2, and from N-CH_3 of *N*-Me-Gln-2 to C-1 of Val and C-2 of *N*-Me-Gln indicated the connections of Pro-2–Val–*N*-Me-Gln. Finally, the ester linkage of *N*-Me-Gln–Hmp to form a cyclodecadepsipetide was revealed by the HMBC correlation from H-2 of Hmp to C-1 of *N*-Me-Gln-2. The structure of **3** is most closely related to SCH 378161, which possesses Pro instead of pipecolic acid (Pip) in **1**.

The absolute configurations of Hmp and the amino acid residues of **1**–**3** were determined by HPLC analysis of the acid hydrolyzate using a ligand-exchange-type chiral column. Thus, SCH 217048 (**1**) was hydrolyzed in 6 M HCl at 110 °C for 15 h. After cooling, the aqueous solution was extracted with Et_2O. The Et_2O solution and the aqueous layer were concentrated in vacuo and separately subjected to HPLC analysis. For the determination of the absolute configuration of the Hmp residue, standard samples of four Hmp isomers were prepared from L- and D-Ile, and L- and D-*allo*-Ile.[9] The ^{1}H NMR ($CDCl_3$, 400 MHz) spectroscopic data of the Et_2O extract were identical with those of (2*S*,3*R*)-Hmp/(2*R*,3*S*)-Hmp, and they were different from those of (2*S*,3*S*)-Hmp/(2*R*,3*R*)-Hmp. HPLC analysis using the chiral column established that the hydrolyzate contained (2*R*,3*S*)-Hmp.[10] The analysis of the aqueous layer of the hydrolyzate, employing standard L- and D-amino acids, revealed the presence of Gly, L-Pip, L-Pro, *N*-Me-D-Val, L-Val, (2*S*,3*S*)-L-Ile, and L-Phe.[11] By acid hydrolysis, the *N*-Me-Gln residue should be converted into *N*-Me-Glu. Due to the significant retention time flipping of standard *N*-Me-L-Glu and *N*-Me-D-Glu, they could not be assigned during the analysis. The absolute configurations of *N*-Me-Glu and *N*-Me-Gln in **1** were determined by application of Marfey's method.[12] The acid hydrolyzate of **1** was derivatized with N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA). HPLC analysis using a reverse-phase column revealed the presence of *N*-Me-L-Glu and the absence of *N*-Me-D-Glu.[13]

The HPLC chromatogram (chiral column) of the acid hydrolyzate (aqueous layer) of SCH 218157 (**2**) was indistinguishable from that of **1**, which indicated that both hydrolyzates possessed the same amino acids. Chiral column HPLC analysis of the hydrolyzate of **3** indicated the absence of L-Pip, and relatively higher peak intensity for L-Pro than other amino acids when compared with the chromatograms for **1** and **2**.

Compounds **1**–**3** exhibited antimalarial activity against *Plasmodium falciparum* K1 with IC_{50} values of 1.6, 6.4, and 1.6 μg/mL, respectively.[14] These compounds were also subjected to other biological assays: antitubercular activity (*Mycobacterium tuberculosis* H37Ra),[15] antifungal activity (*Candida albicans*),[15] antibacterial activity (*Bacillus cereus*),[15] and cytotoxicity to cancer cell-lines (KB, MCF-7, and NCI-H187)[16] and nonmalignant Vero cells.[15] They were inactive in these assays at 50 μg/mL.

In conclusion, a new antimalarial cyclodepsipeptide, pleosporin A (**3**), was isolated from an elephant dung fungus and its structure was elucidated. The absolute configurations of **3** and the known analogs **1** and **2** were determined by chiral column HPLC analysis and Marfey's method. Although the antimalarial activities of **1**–**3** were much weaker than the standard drug, dihydroartemisinin (IC_{50} 0.0044 μg/mL in our assay system), their good selectivity index suggested that they could serve as new antimalarial leads.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.11.063.

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5. The fungus used in this study was isolated from an elephant dung sample collected from the Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Thailand, by Dr. Nigel L. Hywel-Jones. The living culture was deposited in the BIOTEC Culture Collection as BCC 7069 on September 21, 1999. Results from the LSU and ITS gene sequences indicated that this fungus belongs to the family Pleosporaceae of the order Pleosporales, but it is not assignable to any genera. These sequencing data were deposited in GenBank (accession No. KF486914 for LSU gene, and KF482067 for ITS gene).
6. *SCH 217048 (**1**)*: Colorless solid; mp 199–201 °C; $[\alpha]_D^{26}$ −112 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.20) nm; IR (ATR) ν_{max} 3407, 3328, 1750, 1676, 1631, 1447 cm^{-1}; HRESI MS *m*/*z* 1159.6386 [M+Na]$^+$ (calcd for $C_{57}H_{88}N_{10}O_{14}Na$, 1159.6374); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopic data were consistent with those reported in the literature.
7. *SCH 218157 (**2**)*: Colorless solid; mp 187–189 °C; $[\alpha]_D^{26}$ −116 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.20) nm; IR (ATR) ν_{max} 3421, 1749, 1670 sh, 1631, 1449 cm^{-1}; HRESI MS *m*/*z* 1136.6719 [M+H]$^+$ (calcd for $C_{57}H_{90}N_{11}O_{13}$, 1136.6714); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopic data were consistent with those reported in the literature.
8. *Pleosporin A (**3**)*: Colorless solid; mp 124–126 °C; $[\alpha]_D^{26}$ −125 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.23) nm; IR (ATR) ν_{max} 3423, 1750, 1671, 1631, 1447 cm^{-1}; HRESI MS *m*/*z* 1122.6555 [M+H]$^+$ (calcd for $C_{56}H_{88}N_{11}O_{13}$, 1122.6558); see Table 1 for ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data.
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10. Chiral column: Phenomenex Chirex 3126 (D)-penicillamine, 4.6 × 250 mm; flow rate 0.5 mL/min, detection UV 235 nm. The Et_2O extract from the acid hydrolysis solution was analyzed using 15% 2-propanol in 2 mM aqueous $CuSO_4$ as the mobile phase. The retention times of (2*S*,3*R*)-Hmp and (2*R*,3*S*)-Hmp were 107 and 176 min, respectively. The Et_2O extract contained (2*R*,3*S*)-Hmp.
11. Three mobile phase conditions were employed due to the large retention time differences of the standard amino acids: (1) 5% MeCN in 2 mM aqueous $CuSO_4$, Gly (t_R 12.6 min), L-Pip (t_R 16.7 min), D-Pip (t_R 25.4 min), L-Pro (t_R 18.9 min), D-Pro (t_R 45 min), *N*-Me-L-Val (t_R 19.6 min), *N*-Me-D-Val (t_R 34 min), and L-Val (t_R 83 min); (2) 5% 2-propanol in 2 mM aqueous $CuSO_4$, L-Val (t_R 25 min), D-Val (t_R 40 min), L-*allo*-Ile (t_R 44 min), L-Ile (t_R 52 min), D-*allo*-Ile (t_R 71 min), and D-Ile (t_R 88 min); (3) 15% 2-propanol in 2 mM aqueous $CuSO_4$, L-Ile (t_R 29 min), L-Phe (t_R 61 min), D-Phe (t_R 94 min).
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13. Column: NovaPak C_{18} (3.9 × 150 mm, 4 μm), mobile phase MeCN/(0.05% TFA in H_2O) = 45:55, flow rate 0.5 mL/min, UV detection at 340 nm. Retention times of the FDAA-derivatized standard amino acids, *N*-Me-L-Glu and *N*-Me-D-Glu, were 8.1 and 7.4 min, respectively. The derivatized hydrolyzate contained *N*-Me-L-Glu.
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