Paecilodepsipeptide A, an Antimalarial and Antitumor Cyclohexadepsipeptide from the Insect Pathogenic Fungus *Paecilomyces cinnamomeus* BCC 9616

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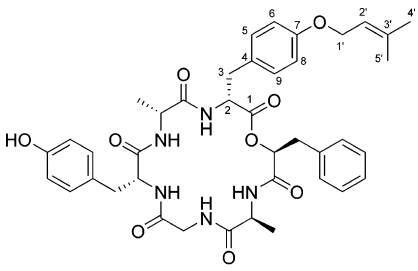
Paecilodepsipeptide A (**1**), a new cyclohexadepsipeptide possessing three D-amino acid residues, together with its linear analogues paecilodepsipeptides B (**2**) and C (**3**), was isolated from the insect pathogenic fungus *Paecilomyces cinnamomeus* BCC 9616. Structures of these compounds were elucidated primarily by NMR and mass spectroscopic analyses. The absolute configurations of the amino acid and hydroxy acid residues of **1** were addressed by HPLC analysis of its acid hydrolyzate using a chiral column and Marfey's method. Paecilodepsipeptide A (**1**) showed activity against the malarial parasite *Plasmodium falciparum* K1 with an IC_{50} value of 4.9 μM. This compound also showed cytotoxicity to two cancer cell lines, KB (IC_{50} 5.9 μM) and BC (IC_{50} 6.6 μM); however, it was inactive against noncancerous Vero cells up to 67 μM (50 μg/mL).

Fungi belonging to the genus *Paecilomyces* have been the source of a wide range of bioactive compounds.[1] Representative secondary metabolites are paecilotoxins (highly toxic linear peptides; also designated as leucinostatins) from *P. lilacinus*,[2] paeciloquinones (anthraquinones, protein tyrosine kinase inhibitors) from *P. carneus* P-177,[3] paecilosetin (a tetramic acid derivative, antibiotic) from *P. farinosus*,[4] and a series of trichothecanes from *P. tenuipes*.[5] As a part of our research program on bioactive metabolites of insect pathogenic fungi,[6] we have investigated the constituents of a scale insect pathogen, *Paecilomyces cinnamomeus* BCC 9616, as the extract of this strain had shown moderate cytotoxic activity. Chemical studies led to the identification of the new cyclohexadepsipeptide paecilodepsipeptide A (**1**), along with its linear derivatives paecilodepsipeptides B (**2**) and C (**3**). Details of the isolation, structure elucidation, and biological activities of these compounds are presented here.



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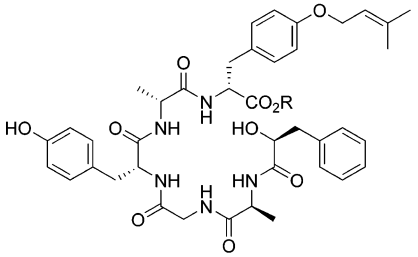

2 : R = H
3 : R = CH_3

Paecilodepsipeptide A (**1**) was isolated as a colorless solid from the MeOH extract of mycelia, whereas a trace of **1** was detected in the EtOAc extract of broth (culture filtrate). The molecular formula of **1** was established as $C_{40}H_{47}N_5O_9$ using HRESIMS in combination with the ^{13}C NMR data. The IR spectrum showed intense broad absorption bands of amides at ν_{max} 3250−3400 (NH) and 1630−1670 cm^{-1} (carbonyl) and a band of an ester carbonyl at 1739 cm^{-1}. The ^1H and ^{13}C NMR data (DMSO-d_6) suggested that **1** was a hexadepsipeptide composed of one 2-hydroxycarboxylic acid and five amino acid residues, showing six carbonyl carbon signals at δ_C 172.5, 172.3, 171.7, 170.6, 169.6, and 168.1 and five amide NH protons at δ_H 8.57 (d, J = 3.8 Hz), 8.23 (d, J = 8.3 Hz), 8.09 (dd, J = 5.7, 5.5 Hz), 7.66 (d, J = 6.3 Hz), and 7.64 (d, J = 7.5 Hz). These amide NH groups were attached respectively to C-2 carbons at δ_C 56.5 (δ_H 4.25, m), 56.1 (δ_H 4.18, m), 43.5 (δ_H 3.57, dd, J = 15.8, 6.4 Hz, and 3.34, m; glycine), 49.1 (δ_H 4.26, m), and 48.1 (δ_H 4.23, m). The 2-hydroxycarboxylic acid residue was assigned to be 3-phenyllactic acid (3-Ph-Lac) on the basis of the 2D NMR data (COSY, HMQC, and HMBC). An oxymethine at δ_C 73.6 (C-2; δ_H 5.13, t, J = 5.1 Hz) was attached with δ_C 37.4 methylene (C-3; δ_H 2.99, dd, J = 14.0, 5.8 Hz, and 2.59, dd, J = 14.0, 4.3 Hz), which was flanked by an unsubstituted phenyl group. Five amino acid residues were elucidated to be a glycine (Gly), two alanine (Ala), a tyrosine (Tyr), and an *O*-prenyltyrosine (*O*-prenyl-Tyr). Thus, one of the two tyrosine residues showed a phenolic proton signal at δ_H 9.20 (s, 7-O*H*), while the other formed a prenyl ether. HMBC correlation from the methylene protons of the prenyl group (δ_H 4.45, 2H, d, J = 6.6 Hz, H-1′) to δ_C 158.0 quaternary carbon (C-7) of Tyr and the NOESY cross-peak between H-1′ and H-6/H-8 (δ_H 6.88, d, J = 8.5 Hz) confirmed the local structure. The assignment of six carbonyl carbons (C-1) was established on the basis of HMBC correlations from H-3 and/or H-2 to C-1 for each residue (Figure 1). The sequence of the six residues was also established by analysis of HMBC data. Thus, amide protons of Ala(1), Gly, Tyr, Ala(2), and *O*-prenyl-Tyr were correlated respectively to carbonyl carbons of 3-Ph-Lac, Ala(1), Gly, Tyr, and Ala(2). In addition, HMBC correlations were observed from the diastereotopic α-protons of Gly (δ_H 3.57 and 3.34) to the Ala(1) carbonyl (C-1) and from H-2 of Ala(2) to the Tyr carbonyl (C-1). This sequence was further supported by NOESY correlations: H-2 of 3-Ph-Lac to NH of Ala(1); H-3 of Ala(1) to NH of Gly; H-2 of Gly to NH of Tyr; H-3 of Tyr to NH of Ala(2); and H-3 of Ala(2) to NH of *O*-prenyl-Tyr. The molecular formula of **1** (HRMS) required the ester linkage of *O*-prenyl-Tyr−3-Ph-Lac, which was in agreement with the downfield shift of H-2 (δ_H 5.13) of 3-Ph-Lac. This linkage was also supported by the appearance

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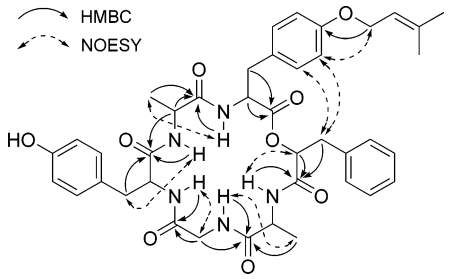

Figure 1. Selected HMBC and NOESY correlations for **1**, indicating the sequence of six residues.

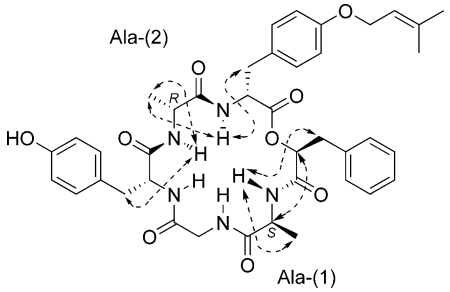

Figure 2. Selected NOESY correlations for **1**, suggesting the location of L- and D-Ala.

of weak NOESY cross-peaks from H-3 of 3-Ph-Lac (δ_H 2.59) to the *O*-prenyl-Tyr aromatic protons H-5/H-9 and H-6/H-8.

The absolute configurations of the amino acid residues and 3-Ph-Lac in **1** were determined by HPLC analysis of its acid hydrolyzate using a ligand-exchange-type chiral column.[7] Co-injection of the hydrolyzate with standard L- and D-amino acids and L- and D-3-Ph-Lac revealed that four residues in cyclohexadepsipeptide **1** are *O*-prenyl-D-Tyr, D-Tyr, Gly, and (2*S*)-L-3-Ph-Lac. However, L- and D-Ala peaks in the chromatogram could not be clearly distinguished due to their overlapping with solvent-derived noise signals. Consequently, Marfey's method[8,9] was employed to determine the absolute configuration of the two Ala residues. The hydrolyzate of **1** was derivatized with N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) and analyzed by HPLC using an ODS column. The HPLC chromatogram (UV 340 nm) of the FDAA-derivatized hydrolyzate exhibited both peaks of L-Ala and D-Ala with a close peak area, which indicated that paecilodepsipeptide A (**1**) possesses one L-Ala and one D-Ala residue. Although not certain, the location of L- and D-Ala in **1** was proposed on the basis of NOESY data, as shown in Figure 2. Amide N*H* of Ala(2) showed cross-peaks with methyl protons (H-3) of this residue and methylene protons (H-3) of D-Tyr. Amide N*H* of *O*-prenyl-D-Tyr showed NOESY correlations with methylene protons (H-2) of this residue and methyl protons (H-3) of Ala(2). These data suggested that amide protons of Ala(2) and *O*-prenyl-D-Tyr and the methyl group of Ala(2) are coplanar (α-face of the macrocyclic ring). On the other hand, N*H* of Ala(1) exhibited cross-peaks with methyl protons (H-3) of this residue and methylene protons (H-3) of L-3-Ph-Lac, consistent with β-face orientation of these protons. A weak NOESY cross-peak was observed between the H-2 of Ala(1) and L-3-Ph-Lac, which indicated a coplanar relationship (α-face). Therefore, the assignment of Ala(1) and Ala(2) respectively as L-Ala and D-Ala more reasonably accounts for the observed NOE data than its translocation.

The molecular formula of paecilodepsipeptide B (**2**), $C_{40}H_{49}N_5O_{10}$, was determined by HRESIMS. The ^{1}H and ^{13}C NMR data of **2** were similar to those of **1**. The significant difference was the upfield shift of H-2 of 3-Ph-Lac (δ_H 4.11, dd, J = 8.0, 3.1 Hz) when compared with **1** (H-2, δ_H 5.13). In addition, the carbonyl carbon (C-1) of *O*-prenyl-Tyr appeared as a broad signal at δ_C 174.3. Analysis of 2D-NMR data revealed that this compound possesses the same six residues as those of **1**. Therefore, paecilodepsipeptide B (**2**) was identified as a linear analogue of **1**, wherein the ester moiety is hydrolyzed. To correlate the structures between paecilodepsipeptides A (**1**) and B (**2**), alkaline hydrolysis of **1** was examined. Cyclodepsipeptide **1** was treated with 2 M NaOH in dioxane at rt for 2 h. The ^{1}H NMR spectrum of the reaction product was identical to that of **2** (isolate).

The ^{1}H and ^{13}C NMR spectra of paecilodepsipeptide C (**3**) were largely identical to those of **2**, with the only difference being the presence of methoxy group resonances at δ_H 3.56 (3H, s)/δ_C 52.3 in **3**. HMBC correlation from the methoxy protons to C-1 (δ_C 172.3) of *O*-prenyl-Tyr indicated that compound **3** forms a methyl ester at the *C*-terminal position. In addition, a hydroxyl proton appeared as a broad signal at δ_H 5.80, and it showed COSY correlation with H-2 (δ_H 4.10, dd, J = 7.9, 3.4 Hz) of 3-Ph-Lac. The structure designated as **3** was consistent with its molecular formula of $C_{40}H_{49}N_5O_{10}$ (HRESIMS).

A unique structural feature of paecilodepsipeptide A (**1**) is that it possesses three D-amino acid residues, including an unusual *O*-prenyl-D-Tyr, whereas it contains only one L-amino acid (L-Ala). Linear analogues **2** and **3** are considered respectively to be hydrolysis and methanolysis products of **1**. Probably these minor constituents are artifacts, formed during methanolic mycelia extraction processes and/or extensive column chromatography. To our knowledge, there has been no report on novel metabolites from the rare species *P. cinnamomeus* or its teleomorph *Torrubiella luteorostrata*.

Paecilodepsipeptide A (**1**) exhibited activity against the malarial parasite *Plasmodium falciparum* K1 with an IC_{50} value of 4.9 μM. This compound also showed cytotoxicity to two cancer cell lines, KB (IC_{50} 5.9 μM) and BC (IC_{50} 6.6 μM); however, it was inactive against Vero cells (African green monkey kidney fibroblasts) up to 67 μM (50 μg/mL). In contrast, linear analogues **2** and **3** were inactive in these assays, suggesting that cyclic depsipeptide structure is important for the biological activities. Cyclic despsipeptides are a large group of natural products produced by fungi, actinomycetes, cyanobacteria, higher plants, and marine organisms, and they exhibit a wide range of biological activities.[10] Although not closely related to **1**, enniatins and beauvericins are among the common cyclohexadepsipeptides produced by fungi. Antimalarial and cytotoxic activities of paecilodepsipeptide A (**1**) are similar to our previous data for these compounds.[11,12]

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a Varian CARY 1E UV−visible spectrophotometer. FT-IR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on a Bruker AV500D spectrometer. ESITOF mass spectra were measured with a Micromass LCT mass spectrometer.

Fungal Material. *Paecilomyces cinnamomeus* was isolated on a Homoptera scale insect collected in Khao Yai National Park, Nakorn Nayok Province, Thailand, and it was identified by Dr. Nigel L. Hywel-Jones, BIOTEC. This fungus was deposited in the BIOTEC Culture Collection (BCC) as BCC 9616 on August 3, 2001.

Fermentation and Isolation. *P. cinnamomeus* BCC 9616 was maintained on potato dextrose agar at 25 °C, and the agar was cut into plugs (1 × 1 cm) and inoculated into 2 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 8 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 8 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary cultures (in 2 flasks) was transferred into 20 × 1 L Erlenmeyer flasks each containing 250 mL

of a liquid medium (composition: sucrose 30.0 g, malt extract 20.0 g, bacto-peptone 2.0 g, yeast extract 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.5 g, per liter), and final fermentation was carried out at 25 °C for 24 days under static conditions. The cultures were harvested by filtration, and the residue (mycelium) was macerated in MeOH (1 L, rt, 2 days). The filtrate was defatted with hexane (700 mL), and the MeOH phase was evaporated to dryness. The residue was diluted with EtOAc (700 mL), washed with H_2O (200 mL), and concentrated under reduced pressure to leave a pale brown gum (8.20 g). This crude extract was triturated in MeOH (50 mL) for 15 h and filtered. The residual solid was mainly composed of the known triterpene zeorin. The filtrate was passed through a Sephadex LH-20 column (4.0 × 30 cm) with MeOH as eluent. The third fraction (2.49 g) contained the depsipeptides and zeorin, which was triturated in MeOH (10 mL, 16 h), then filtered. The residual solid (1.0 g) was mainly composed of zeorin. The filtrate (1.51 g) was subjected to column chromatography on silica gel (5 × 25 cm, step gradient elution, $MeOH/CH_2Cl_2$). The depsipeptide-containing fractions were repeatedly subjected to preparative HPLC using a reversed-phase column (NovaPak HR C_{18}, 2.5 × 10.0 cm, mobile phase $MeOH/H_2O$ and $MeCN/H_2O$, flow rate 8 mL/min) to afford **1** (174 mg; t_R 18 min, $MeCN/H_2O$, 45:55), **2** (20.1 mg; t_R 12 min, $MeOH/H_2O$, 40:60), and **3** (30.2 mg; t_R 14 min, $MeCN/H_2O$, 45:55).

Paecilodepsipeptide A (1): colorless solid; mp 140−141 °C; $[\alpha]^{25}_D$ +22 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.79), 226 (4.40), 277 (3.57), 284 (sh) (3.49) nm; IR (KBr) ν_{max} 3386, 3305, 1739, 1650 br, 1513, 1239, 1003, 701 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr 8.57 (1H, d, J = 3.8 Hz, N*H*), 7.02 (2H, d, J = 8.5 Hz, H-5 and H-9), 6.88 (2H, d, J = 8.5 Hz, H-6 and H-8), 5.34 (1H, br t, J = 6.6 Hz, H-2′), 4.45 (2H, d, J = 6.6 Hz, H-1′), 4.25 (1H, m, H-2), 2.91 (1H, dd, J = 13.2, 5.4 Hz, H-3a), 2.80 (1H, dd, J = 13.2, 9.2 Hz, H-3b), 1.65 (3H, br s, H-4′), 1.62 (3H, br s, H-5′); Ala(2) 7.64 (1H, d, J = 7.5 Hz, N*H*), 4.23 (1H, m, H-2), 1.27 (3H, d, J = 7.0 Hz, H-2); D-Tyr 9.20 (1H, s, 7-O*H*), 8.23 (1H, d, J = 8.3 Hz, N*H*), 6.96 (2H, d, J = 8.4 Hz, H-5 and H-9), 6.62 (2H, d, J = 8.4 Hz, H-6 and H-8), 4.18 (1H, m, H-2), 2.95 (1H, dd, J = 14.2, 4.3 Hz, H-3a), 2.72 (1H, dd, J = 13.9, 10.8 Hz, H-3b); Gly 8.09 (1H, dd, J = 5.7, 5.5 Hz, N*H*), 3.57 (1H, dd, J = 15.8, 6.4 Hz, H-2a), 3.34 (1H, m, H-2b); Ala-(1) 7.66 (1H, d, J = 6.3 Hz, N*H*), 4.26 (1H, m, H-2), 1.05 (3H, d, J = 6.8 Hz, H-2); L-3-Ph-Lac 7.18 (2H, m, H-6 and H-8), 7.17 (1H, m, H-7), 6.78 (2H, m, H-5 and H-9), 5.13 (1H, t, J = 5.1 Hz, H-2), 2.99 (1H, dd, J = 14.0, 5.8 Hz, H-3a), 2.59 (1H, dd, J = 14.0, 4.3 Hz, H-3b); ^{13}C NMR (125 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr 171.7 (s, C-1), 158.0 (s, C-7), 137.4 (s, C-3′), 130.8 (d, C-5 and C-9), 128.3 (s, C-4), 120.4 (d, C-2′), 115.0 (d, C-6 and C-8), 64.6 (t, C-1′), 56.5 (d, C-2), 35.4 (t, C-3), 25.8 (q, C-4′), 18.4 (q, C-5′); Ala(2) 172.5 (s, C-1), 48.1 (d, C-2), 19.1 (q, C-3); D-Tyr 170.6 (s, C-1), 156.2 (s, C-7), 130.2 (d, C-5 and C-9), 128.6 (s, C-4), 115.4 (d, C-6 and C-8), 56.1 (d, C-2), 36.0 (t, C-3); Gly 169.6 (s, C-1), 43.5 (t, C-2); Ala(1) 172.3 (s, C-1), 49.1 (d, C-2), 18.7 (q, C-3); L-3-Ph-Lac 168.1 (s, C-1), 136.0 (s, C-4), 130.2 (d, C-5 and C-9), 128.3 (d, C-6 and C-8), 127.1 (d, C-7), 73.6 (d, C-2), 37.4 (t, C-3); HRMS (ESITOF) *m/z* 764.3262 $[M + Na]^+$ (calcd for $C_{40}H_{47}N_5O_9Na$, 764.3271).

Paecilodepsipeptide B (2): colorless solid; mp 115−116 °C; $[\alpha]^{26}_D$ −28 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.71), 226 (4.33), 278 (3.54), 284 (sh) (3.48) nm; IR (KBr) ν_{max} 3387, 1642 br, 1514, 1384, 1239, 701 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr 7.22 (1H, m, N*H*), 7.00 (2H, d, J = 8.4 Hz, H-5 and H-9), 6.69 (2H, d, J = 8.5 Hz, H-6 and H-8), 5.38 (1H, m, H-2′), 4.39 (2H, d, J = 6.6 Hz, H-1′), 3.99 (1H, m, H-2), 3.02 (1H, dd, J = 13.5, 5.2 Hz, H-3a), 2.87 (1H, m, H-3b), 1.72 (3H, br s, H-4′), 1.66 (3H, br s, H-5′); Ala(2) 8.23 (1H, d, J = 7.6 Hz, N*H*), 4.16 (1H, m, H-2), 1.19 (3H, d, J = 7.1 Hz, H-2); D-Tyr 9.23 (1H, s, 7-O*H*), 7.99 (1H, d, J = 8.1 Hz, N*H*), 7.00 (2H, d, J = 8.4 Hz, H-5 and H-9), 6.62 (2H, d, J = 8.4 Hz, H-6 and H-8), 4.34 (1H, m, H-2), 2.89 (1H, m, H-3a), 2.61 (1H, dd, J = 13.8, 10.3 Hz, H-3b); Gly 8.32 (1H, m, N*H*), 3.67 (1H, dd, J = 16.6, 6.2 Hz, H-2a), 3.59 (1H, dd, J = 16.6, 4.8 Hz, H-2b); Ala(1) 7.80 (1H, d, J = 7.6 Hz, N*H*), 4.22 (1H, m, H-2), 1.12 (3H, d, J = 7.1 Hz, H-2); L-3-Ph-Lac 7.24 (2H, m, H-6 and H-8), 7.21 (2H, m, H-5 and H-9), 7.17 (1H, m, H-7), 4.11 (1H, dd, J = 8.0, 3.1 Hz, H-2), 2.98 (1H, dd, J = 13.8, 3.3 Hz, H-3a), 2.71 (1H, dd, J = 13.8, 8.3 Hz, H-3b); ^{13}C NMR (125 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr 174.3 (s, C-1), 157.1 (s, C-7), 137.1 (s, C-3′), 131.0 (s, C-4), 131.0 (d, C-5 and C-9), 120.7 (d, C-2′), 114.2 (d, C-6 and C-8), 64.5 (t, C-1′), 55.8 (d, C-2), 36.6 (t, C-3), 25.9 (q, C-4′), 18.4 (q, C-5′); Ala(2) 171.6 (s, C-1), 49.3 (d, C-2), 18.5 (q, C-3); D-Tyr 171.4 (s, C-1), 156.2 (s, C-7), 130.5 (d, C-5 and C-9), 128.4 (s, C-4), 115.3 (d, C-6 and C-8), 55.3 (d, C-2), 37.0 (t, C-3); Gly 169.0 (s, C-1), 42.0 (t, C-2); Ala(1) 172.9 (s, C-1), 48.4 (d, C-2), 18.9 (q, C-3); L-3-Ph-Lac 173.7 (s, C-1), 139.1 (s, C-4), 130.0 (d, C-5 and C-9), 128.3 (d, C-6 and C-8), 126.4 (d, C-7), 72.2 (d, C-2), 40.2 (t, C-3); HRMS (ESITOF) *m/z* 782.3387 $[M + Na]^+$ (calcd for $C_{40}H_{49}N_5O_{10}Na$, 782.3377).

Paecilodepsipeptide C (3): colorless solid; mp 168−169 °C; $[\alpha]^{25}_D$ −20 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.58), 227 (4.17), 278 (3.44), 284 (sh) (3.38) nm; IR (KBr) ν_{max} 3289, 1739, 1636, 1544, 1514, 1446, 1238, 829, 699 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr-OMe 8.20 (1H, d, J = 7.4 Hz, N*H*), 7.10 (2H, d, J = 8.6 Hz, H-5 and H-9), 6.81 (2H, d, J = 8.6 Hz, H-6 and H-8), 5.38 (1H, br t, J = 6.6 Hz, H-2′), 4.43 (2H, d, J = 6.7 Hz, H-1′), 4.38 (1H, m, H-2), 3.56 (3H, s, CO_2CH_3), 2.92 (1H, m, H-3a), 2.87 (1H, m, H-3b), 1.72 (3H, br s, H-4′), 1.66 (3H, br s, H-5′); Ala(2) 8.20 (1H, d, J = 7.4 Hz, N*H*), 4.29 (1H, m, H-2), 1.18 (3H, d, J = 7.1 Hz, H-2); D-Tyr 9.25 (1H, br s, 7-O*H*), 8.16 (1H, d, J = 7.6 Hz, N*H*), 7.00 (2H, d, J = 8.4 Hz, H-5 and H-9), 6.61 (2H, d, J = 8.4 Hz, H-6 and H-8), 4.41 (1H, m, H-2), 2.86 (1H, m, H-3a), 2.60 (1H, dd, J = 13.9, 10.2 Hz, H-3b); Gly 8.25 (1H, t, J = 5.8 Hz, N*H*), 3.71 (1H, dd, J = 16.7, 5.9 Hz, H-2a), 3.55 (1H, m, H-2b); Ala(1) 7.72 (1H, d, J = 7.5 Hz, N*H*), 4.27 (1H, m, H-2), 1.10 (3H, d, J = 7.0 Hz, H-2); L-3-Ph-Lac 7.24 (2H, m, H-6 and H-8), 7.20 (2H, m, H-5 and H-9), 7.18 (1H, m, H-7), 5.80 (1H, br s, 2-O*H*), 4.10 (1H, dd, J = 7.9, 3.4 Hz, H-2), 2.97 (1H, dd, J = 13.8, 3.5 Hz, H-3a), 2.72 (1H, dd, J = 13.8, 8.1 Hz, H-3b); ^{13}C NMR (125 MHz, DMSO-d_6) δ *O*-prenyl-D-Tyr-OMe 172.3 (s, C-1), 157.7 (s, C-7), 137.3 (s, C-3′), 130.5 (d, C-5 and C-9), 129.1 (s, C-4), 120.5 (d, C-2′), 114.8 (d, C-6 and C-8), 64.6 (t, C-1′), 54.3 (d, C-2), 36.3 (t, C-3), 25.9 (q, C-4′), 18.5 (q, C-5′); Ala(2) 172.6 (s, C-1), 48.4 (d, C-2), 18.5 (q, C-3); D-Tyr 171.3 (s, C-1), 156.2 (s, C-7), 130.5 (d, C-5 and C-9), 128.3 (s, C-4), 115.3 (d, C-6 and C-8), 54.6 (d, C-2), 37.2 (t, C-3); Gly 168.9 (s, C-1), 42.2 (t, C-2); Ala(1) 172.8 (s, C-1), 48.1 (d, C-2), 19.1 (q, C-3); L-3-Ph-Lac 173.2 (s, C-1), 138.9 (s, C-4), 130.0 (d, C-5 and C-9), 128.3 (d, C-6 and C-8), 126.5 (d, C-7), 72.4 (d, C-2), 40.6 (t, C-3); HRMS (ESITOF) *m/z* 774.3714 $[M + H]^+$ (calcd for $C_{41}H_{52}N_5O_{10}$, 774.3712).

Acid Hydrolysis of 1. Compound **1** (2.0 mg) was hydrolyzed by heating in 6 M HCl (1 mL) at 110 °C for 15 h. After cooling, the solution was evaporated to dryness and dissolved in MeOH (150 μL), which was subjected to HPLC analysis.

Preparation of Standard Amino Acids. *N*-Boc-*O*-prenyl-L-Tyr was synthesized in two steps from *N*-Boc-L-Tyr (Fluka) and prenyl bromide. A portion (1 mg) was dissolved in trifluoroacetic acid (TFA, 0.4 mL), and the solution was stirred at rt for 0.5 h, then evaporated to dryness. The residue was dissolved in MeOH (150 μL) and used for HPLC analysis as authentic standard *O*-prenyl-L-Tyr. *N*-Boc-*O*-prenyl-D-Tyr was synthesized in two steps from *N*-Boc-L-Tyr methyl ester (Fluka) and prenyl bromide. A portion (1 mg) was treated with TFA (0.4 mL) at rt for 1 h, then concentrated in vacuo. The residue was dissolved in MeOH (150 μL) and used for HPLC analysis as authentic standard *O*-prenyl-D-Tyr.

HPLC Analysis of the Hydrolyzate Using a Chiral Column. HPLC analysis of the depsipeptide hydrolyzate was performed using a ligand-exchange-type chiral column: Sumichiral OA-5000, 4.6 × 150 mm, 5 μm (Sumika Chemical Analysis Service, Ltd); flow rate 1 mL/min, UV detection at 235 nm.[7] Standard L- and D-amino acids and L- and D-3-Ph-Lac were used for co-injection experiments. Three mobile phase conditions were employed for polarity reasons: (1) 15% 2-propanol in 2 mM aqueous $CuSO_4$, L-3-Ph-Lac (t_R 58 min), D-3-Ph-Lac (t_R 61 min), *O*-prenyl-L-Tyr (t_R 64 min), *O*-prenyl-D-Tyr (t_R 67 min); (2) 5% MeOH in 2 mM aqueous $CuSO_4$, L-Tyr (t_R 24 min), D-Tyr (t_R 29 min); (3) 2 mM aqueous $CuSO_4$, Gly (t_R 3.8 min). The hydrolyzate of **1** contained (2*S*)-L-3-Ph-Lac, *O*-prenyl-D-Tyr, D-Tyr, and Gly; however, L- and D-Ala peaks could not be clearly distinguished due to their overlapping with solvent-derived noise signals at any elution conditions.

Preparation and Analysis of Marfey Derivatives. Paecilodepsipeptide A (**1**, 1.0 mg) was hydrolyzed as described above, dried, and redissolved in H_2O (150 μL). To this solution were added 1% (w/v) FDAA (Marfey's reagent, N_α-(2,4-dinitro-5-fluorophenyl)-L-alanina-

mide) in acetone (300 μL) and 1 M $NaHCO_3$ solution (70 μL), and the mixture was incubated at 40 °C for 1 h. The reaction was quenched by addition of HCl (70 μL, 1 M), and the resulting homogeneous solution was diluted with MeOH (1.0 mL). Standard L- and D-amino acids were also derivatized with FDAA in the same manner as that of the hydrolyzate of **1**. HPLC analysis was performed with the following conditions: NovaPak C_{18} (3.9 × 150 mm, 4 μm), mobile phase MeCN/(0.05% TFA in H_2O), 25:75, flow rate 0.5 mL/min, UV detection at 340 nm. Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: Gly (9.9), L-Ala (12.8), D-Ala (18.0), L-Tyr (19.4), and D-Tyr (26.1). The HPLC chromatogram of the derivatized hydrolyzate of **1** contained peaks of Gly, L-Ala, D-Ala, and D-Tyr.

Alkaline Hydrolysis of 1. To a solution of **1** (2.0 mg) in dioxane (1 mL) was added 2 M NaOH (0.2 mL), and the mixture was stirred at rt for 2 h. The mixture was evaporated, and the residue was dissolved in H_2O (2 mL), acidified with 1 M HCl (0.45 mL), and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to leave a colorless solid (1.7 mg). The ^{1}H NMR (DMSO-d_6) spectrum of the crude product was identical to that of **2** (isolate). The hydrolysis product and the natural products (isolates) **1** and **2** were subjected to HPLC/UV analysis using a reversed-phase column: NovaPak HR C_{18} (3.9 × 150 mm, 4 μm), eluent MeCN/H_2O, 35:65, flow rate 0.5 mL/min. The identification of the hydrolysis product as paecilodepsipeptide B (**2**) was confirmed by co-injection: **1**, t_R 10.3 min; **2**, t_R 3.4 min.

Biological Assays. The assay for activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique described by Desjardins.[13] The IC_{50} value of the standard antimalarial compound dihydroartemisinin was 0.0042 μM. Cytotoxicity against KB cells (oral human epidermoid carcinoma), BC cells (human breast cancer), and Vero cells (African green monkey kidney fibroblasts) was evaluated using the colorimetric method.[14] The IC_{50} values of the standard compound ellipticine were 0.97 μM for KB cells, 0.13 μM for BC cells, and 1.2 μM for Vero cells.

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