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Bioactive Compounds from the Scale Insect Pathogenic Fungus *Conoideocrella tenuis* BCC 18627

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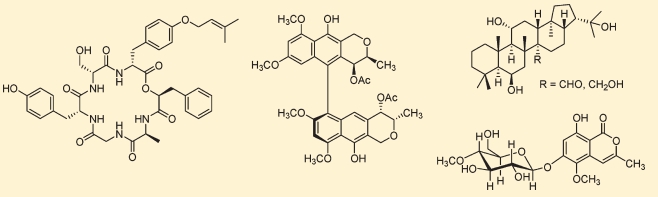
Supporting Information

ABSTRACT: A new cyclohexadepsipeptide, conoideocrellide A (**1**), its linear derivatives, conoideocrellides B−D (**2**−**4**), three new hopane triterpenoids (**5**−**7**), two new bioxanthracenes (**9** and **10**), and a new isocoumarin glycoside (**13**) were isolated from the scale insect pathogenic fungus *Conoideocrella tenuis* BCC 18627. Biological activities of the new compounds were evaluated.



Conoideocrella (family Clavicipitaceae, Hypocreales, Ascomycota) is a recently proposed genus of insect pathogenic fungi that specifically attacks scale insects.[1] There are only two described species, *C. luteorostrata* and *C. tenuis*, which were originally placed in *Torrubiella* as *T. luteorostrata* and *T. tenuis*, respectively. There have been a few reports of compounds from this genus: paecilodepsipeptide A and a naphthopyrone glycoside from *T. luteorostrata* BCC 9617,[2] torrubiellutins A−C from *T. luteorostrata* BCC 12904,[3] and isocoumarin glycosides from *T. tenuis* BCC 12732.[4] In our research on the metabolites of insect pathogenic fungi, we found an extract from *C. tenuis* strain BCC 18627 that exhibited a complex and unique ^{1}H NMR spectroscopic profile suggesting the co-occurrence of several types of secondary metabolites. Scale-up fermentation and chemical studies led to the isolation of four new depsipeptides, conoideocrellides A−D (**1**−**4**), three new hopane-type triterpenes (**5**−**7**) and the known zeorin (**8**), two new bioxanthracenes (**9** and **10**) and the known ES-242-1 (**11**)[5−7] and ES-242-2 (**12**),[6−8] and a new (**13**) and two known (**14** and **15**)[4] isocoumarin glycosides. We report here details of the isolation, structure elucidation, and biological activities of the new compounds.

RESULTS AND DISCUSSION

Conoideocrellide A (**1**) was isolated as a colorless solid, and the molecular formula was established as $C_{40}H_{47}N_5O_{10}$ by HRESIMS. The IR spectrum exhibited intense broad absorption bands at ν_{max} 3500−3360 (amide NH and OH) and 1670−1630 cm^{-1} (amide carbonyl) and a band of an ester carbonyl at 1738 cm^{-1}. The ^{1}H and ^{13}C NMR data (DMSO-d_6) suggested that **1** was a hexadepsipeptide composed of a hydroxycarboxylic acid and five amino acid residues, showing six carbonyl carbon signals at δ_C 172.5−168.2 and five amide NH protons at δ_H 8.27−7.68. Five amino acid residues were assigned on the basis of 2D NMR data (COSY, HMQC, and HMBC) as a glycine (Gly), an alanine (Ala), a serine (Ser), a tyrosine (Tyr), and an *O*-prenyltyrosine (*O*-prenyl-Tyr) (Table 1). Thus, one of the two tyrosine residues exhibited a phenolic proton signal at δ_H 9.21 (7-O*H*), while the other formed a prenyl ether. HMBC correlations from the methylene protons of the prenyl group at δ_H 4.45 to δ_C 158.0 of a quaternary carbon (C-7) and the NOESY cross-peak between H_2-1′ and H-6/H-8 confirmed the 7-*O*-prenyl group. The 3-phenyllactic acid (3-Ph-Lac) residue was also elucidated on the basis of 2D NMR data. An oxymethine at δ_C 74.4 (C-2) was attached to δ_C 37.4 of a methylene (C-3), which was flanked by a phenyl group (HMBC). The sequence of the six residues was also established by analysis of HMBC data (Figure 1). Amide protons of Ala, Gly, Tyr, Ser, and *O*-prenyl-Tyr were correlated respectively to carbonyl carbons of 3-Ph-Lac, Ala, Gly, Tyr, and Ser. HMBC correlation from H-2 of 3-Ph-Lac to C-1 of *O*-prenyl-Tyr indicated an ester linkage of *O*-prenyl-Tyr-3-Ph-Lac to form a cyclohexadepsipeptide. This sequence was further supported by NOESY correlations of H-2 of 3-Ph-Lac to NH of Ala; H_3-3 of Ala to NH of Gly; H_2-2 of Gly to NH of Tyr; H-3 of Tyr to NH of Ser; H_2-3 of Ser to NH of *O*-prenyl-Tyr; and H_2-3 of *O*-prenyl-Tyr to H-2 of 3-Ph-Lac.

The absolute configurations of the amino acid residues in **1** were determined by Marfey's method.[9,10] The acid hydrolysate 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 hydrolysate exhibited peaks of *O*-prenyl-D-Tyr, D-Ser, D-Tyr, L-Ala, and Gly. The absolute configuration of the Ph-Lac residue was addressed by isolation of this fragment from an acid hydrolysate. The specific rotation of 3-phenyllactic acid ($[\alpha]^{26}_D$ −22, *c* 0.18, EtOH), isolated from the hydrolysate, was consistent with that of the commercial product (Sigma-Aldrich) (2*S*)-L-3-phenyllactic acid. Conoideocrellide A (**1**) is structurally very similar to paecilodepsipeptide A (**16**), which was previously isolated in our laboratory from *T. luteorostrata* BCC 9617 and its anamorph *Paecilomyces*

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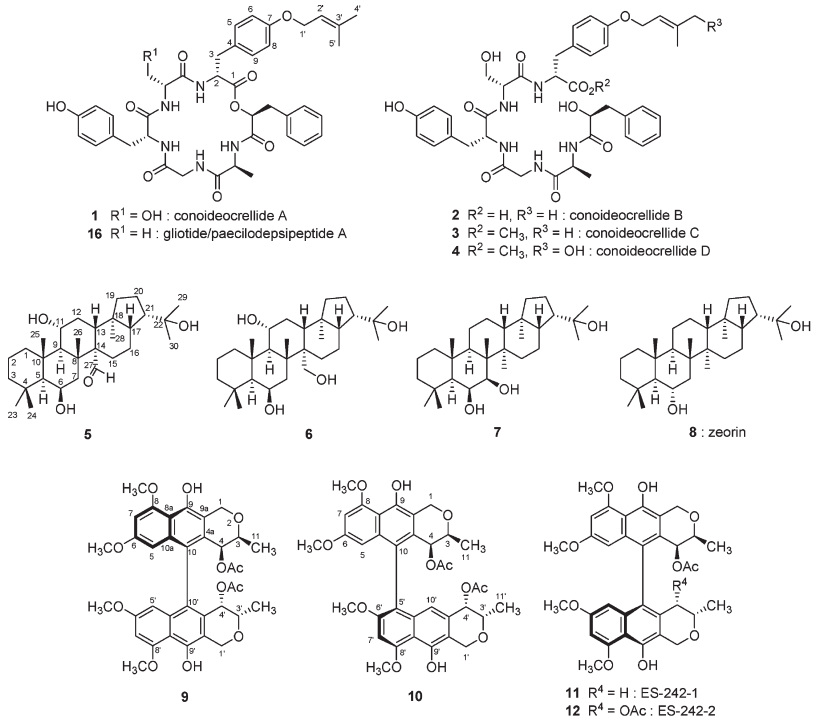

Table 1. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Conoideocrellide A (1)

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)
O-prenyl-D-Tyr			D-Ser			Gly		
1	171.6, qC		1	170.5, qC		1	169.5, qC	
2	55.8, CH	4.38, m	2	55.1, CH	4.26, m	2	43.4, CH_2	3.63, m; 3.40, m
3	35.5, CH_2	2.84−2.83, m	3	61.9, CH_2	3.62−3.58, m	N*H*		7.94, dd (5.8, 5.5)
4	128.3, qC		3-O*H*		5.08, t (5.5)	L-Ala		
5,9	130.8, CH	6.94, d (8.6)	N*H*		7.68, d (8.0)	1	172.5, qC	
6,8	115.0, CH	6.84, d (8.6)	D-Tyr			2	48.9, CH	4.28, m
7	158.0, qC		1	171.2, qC		3	18.6, CH_3	1.09, d (7.0)
1′	64.7, CH_2	4.45, d (6.6)	2	56.2, CH	4.19, m	N*H*		7.70, d (6.9)
2′	120.5, CH	5.35, m	3	36.1, CH_2	2.96, dd (14.0, 4.4)		L-3-Ph-Lac	
3′	137.4, qC				2.72, m	1	168.2, qC	
4′	25.8, CH_3	1.67, s	4	128.5, qC		2	74.4, CH	5.05, dd (6.3, 4.5)
5′	18.4, CH_3	1.64, s	5,9	130.2, CH	6.98, d (8.5)	3	37.4, CH_2	3.01, dd (14.0, 6.3)
N*H*		8.27, d (4.9)	6,8	115.5, CH	6.63, d (8.5)			2.74, dd (14.0, 4.5)
			7	156.2, qC		4	136.2, qC	
			7-O*H*		9.21, s	5,9	130.3, CH	6.89, d (7.9)
			N*H*		8.23, d (8.1)	6,8	128.4, CH	7.21, m
						7	127.1, CH	7.19, m

cinnamomeus BCC 9616.[2,11] Paecilodepsipeptide A is identical to gliotide, which was isolated from *Gliocladium* sp.[12,13] The absolute configuration of **1** proved to be identical to gliotide/paecilodepsipeptide A (**16**). The only structural difference is that **1** possesses a D-Ser residue instead of D-Ala in **16**.

The molecular formula of conoideocrellide B (**2**), $C_{40}H_{49}N_5O_{11}$, was determined by HRESIMS. The ^1H 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 = 8.5, 3.4 Hz) when compared with **1** (H-2, δ_H 5.05). In addition, C-1 of *O*-prenyl-Tyr appeared as a broad signal at δ_C 173.4. Therefore, conoideocrellide B (**2**) was identified as a linear analogue of **1**, wherein the ester moiety is hydrolyzed. This conclusion was confirmed by alkaline hydrolysis of **1**.

Conoideocrellide C (**3**) was assigned as the methyl ester variant of **2**. HMBC correlation from the methoxy protons (δ_H 3.56) to C-1 (δ_C 172.1) of *O*-prenyl-Tyr indicated that compound **3** forms a methyl ester at the *C*-terminal position. The structure designated as **3** was consistent with its molecular formula of $C_{41}H_{51}N_5O_{11}$ (HRESIMS).

Conoideocrellide D (**4**) was structurally related to **3**, with the only difference being that the prenyl group was oxygenated in **4**.

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Thus, CH_3-4′ of *O*-prenyl-Tyr was replaced by a hydroxymethyl group resonating at δ_C 66.0/δ_H 3.82 and 4.87. The *E*-geometry of the trisubstituted olefin was assigned on the basis of the NOESY correlations for H_2-1′/H_3-5′ and H-2′/H_2-4′.

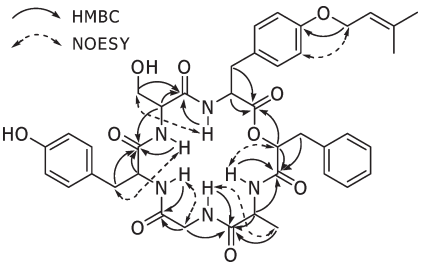

Figure 1. Selected HMBC and NOESY correlations for **1**, indicating the sequence of five amino acids and 3-Ph-Lac.

Similar to **16**, a unique structural feature of conoideocrellide 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 a methanolic mycelia extraction process and/or extensive column chromatography. We did not isolate the cyclic analogue of **4**, the plausible precursor.

Compound **5** was isolated as a colorless solid, and the molecular formula was established as $C_{30}H_{50}O_4$ by HRESIMS. The IR spectrum exhibited broad absorption bands of hydroxy groups at ν_{max} 3531 and 3484 cm^{-1} and a carbonyl absorption at 1697 cm^{-1}. The ^1H and ^{13}C NMR data in DMSO-d_6 suggested that **5** is a

Table 2. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Triterpenes 5−7

	5		6		7	
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)
1	44.5, CH_2	α 0.81, dt (3.2, 13.5); β 2.73, m	45.0, CH_2	α 0.82, m; β 2.68, br d (13.3)	42.8, CH_2	α 0.70, m; β 1.57, m
2	19.1, CH_2	α 1.24, m; β 1.59, m	19.2, CH_2	α 1.23, m; β 1.61, m	18.9, CH_2	α 1.31, m; β 1.59, m
3	44.23, CH_2	α 1.06, m; β 1.19, m	44.5, CH_2	α 1.11, dt (4.0, 13.4); β 1.21, m	44.0, CH_2	α 1.10, m; β 1.26, m
4	34.7, qC		34.8, qC		34.2, qC	
5	56.6, CH	0.55, br s	56.8, CH	0.70, br s	55.4, CH	0.68, br s
6	65.9, CH	4.18, m	66.4, CH	4.27, br s	72.11,[a] CH	4.04, m
7	42.6, CH_2	α 1.08, m; β 1.47, m	42.1, CH_2	α 1.76, dd (12.2, 4.5); β 1.37, m	72.13,[a] CH	3.44, m
8	43.9, qC		43.6, qC		46.8, qC	
9	57.7, CH	1.42, m	56.6, CH	1.21, m	50.9,[b] CH	1.13, m
10	39.9, qC		39.2, qC		37.2, qC	
11	69.4, CH	4.13, m	69.2, CH	3.83, m	20.9, CH_2	α 1.54, m; β 1.36, m
12	35.6, CH_2	α 2.12, m; β 1.80, m	36.7, CH_2	α 1.36, m; β 1.51, m	24.6, CH_2	α 1.38, m; β 1.30, m
13	49.2, CH	1.78, m	48.4, CH	1.44, m	49.5, CH	1.33, m
14	55.9, qC		45.1, qC		43.5, qC	
15	27.4, CH_2	α 1.98, m; β 0.90, m	28.7, CH_2	α 1.70, m; β 0.95, m	38.4, CH_2	α 1.65, m; β 1.47, m
16	22.1, CH_2	α 1.25, m; β 1.81, m	23.5, CH_2	α 1.21, m; β 1.79, m	22.3, CH_2	α 1.51, m; β 1.84, m
17	54.0, CH	1.38, m	54.6, CH	1.32, m	54.1, CH	1.30, m
18	44.21, qC		44.2, qC		44.3, qC	
19	39.7, CH_2	α 0.99, m; β 1.44, m	41.3, CH_2	α 0.90, m; β 1.45, m	41.9, CH_2	α 0.88, m; β 1.45, m
20	26.6, CH_2	α 1.45, m; β 1.62, m	26.6, CH_2	α 1.46, m; β 1.64, m	26.4, CH_2	α 1.45, m; β 1.60, m
21	50.5, CH	2.06, m	51.1, CH	2.11, m	51.0,[b] CH	2.09, m
22	71.7, qC		72.1, qC		72.0, qC	
23	34.0, CH_3	0.85, s	34.2, CH_3	0.89, s	33.3, CH_3	0.89, s
24	24.1, CH_3	1.14, s	24.3, CH_3	1.16, s	24.3, CH_3	1.14, s
25	18.3, CH_3	1.30, s	18.2, CH_3	1.28, s	17.4, CH_3	1.09, s
26	19.3, CH_3	1.29, s	19.4, CH_3	1.22, s	11.6, CH_3	1.12, s
27	210.7, CH	10.32, s	60.4, CH_2	3.70, dd (12.2, 5.3)	17.7, CH_3	0.93, s
				3.62, dd (12.2, 4.5)		
28	14.8, CH_3	0.58, s	15.3, CH_3	0.81, s	16.7, CH_3	0.71, s
29	31.3, CH_3	0.98, s	31.1, CH_3	1.04, s	31.2, CH_3	1.06, s
30	29.7, CH_3	1.04, s	29.6, CH_3	1.03, s	29.6, CH_3	1.02, s
6-O*H*		4.14, m		3.99, d (3.4)		4.03, m
7-O*H*						3.86, br d (7.5)
11-O*H*		4.17, d (6.0)		3.83, m		
22-O*H*		3.88, s		3.80, s		3.81, s
27-O*H*				4.19, dd (5.3, 4.5)		

[a,b] Carbon chemical shifts may be interchanged.

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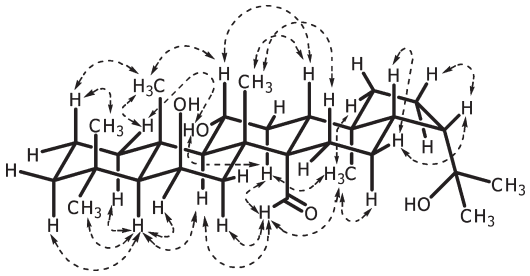

Figure 2. Key NOESY correlations for **5**.

Table 3. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for 9

position	δ_C, mult.	δ_H, mult. (J in Hz)
1, 1′	65.5, CH_2	5.19, d (15.7); 4.84, d (15.7)
3, 3′	73.4, CH	3.65, dq (1.8, 6.4)
4, 4′	65.6, CH	5.30, d (1.8)
4a, 4a′	131.8, qC	
5, 5′	96.8, CH	6.09, d (2.2)
6, 6′	157.8, qC	
7, 7′	98.2, CH	6.51, d (2.2)
8, 8′	157.3, qC	
8a, 8a′	110.9, qC	
9, 9′	149.8, qC	
9a, 9a′	115.6, qC	
10, 10′	125.1, qC	
10a, 10a′	134.9, qC	
11, 11′	16.6, CH_3	1.05, d (6.4)
4-OCOCH$_3$, 4′-OCOCH$_3$	169.3, qC	
4-OCO*CH*$_3$, 4′-OCO*CH*$_3$	20.4, CH_3	1.88, s
6-O*CH*$_3$, 6′-O*CH*$_3$	55.3, CH_3	3.51, s
8-O*CH*$_3$, 8′-O*CH*$_3$	56.2, CH_3	4.08, s
9-O*H*, 9′-O*H*		9.55, s

hopane-type triterpene related to the known co-metabolite, zeorin (**8**). The ^{1}H and ^{13}C NMR, DEPT135, and HMQC data for **5** supported the presence of an aldehyde (δ_C 210.7/δ_H 10.32), an oxygenated quaternary carbon (δ_C 71.7), five quaternary carbons, two oxymethines (δ_C 65.9/δ_H 4.18 and δ_C 69.4/δ_H 4.13), five methines, nine methylenes, and seven methyl groups (Table 2). The planar structure of **5** was deduced by analyses of COSY and HMBC data. The location of the aldehyde functionality was assigned by the HMBC correlations from the formyl proton (δ_H 10.32) to C-14 and C-15. The key HMBC correlations were those from seven methyl groups (H$_3$-23, H$_3$-24, H$_3$-25, H$_3$-26, H$_3$-28, H$_3$-29, and H$_3$-30) attached to quaternary sp^3 carbons C-4, C-4, C-10, C-8, C-18, C-22, and C-22, respectively. The relative configuration of **5** was addressed on the basis of J-values and NOESY correlations (Figure 2). Key NOESY correlations were those for protons and methyl protons at axial positions. NOESY cross-peaks at the β-face were observed from H$_3$-25 to H$_\beta$-2 and H-11 and from H$_3$-26 to H-11, H-13, and H$_\beta$-15. Important NOESY correlations on the α-face were those from H-5 to H$_\alpha$-1, H$_\alpha$-3, H-6, and H-9 and from H-27 to H$_\alpha$-7, H-9, H$_\alpha$-12, and H$_3$-28. H-5 resonated as a broad singlet with a narrow signal width and exhibited a weak COSY cross-peak and an intense NOESY cross-peak with H-6. These data indicated an equatorial orientation of H-6. An axial orientation of H-11 was evident from the NOESY correlations from this proton

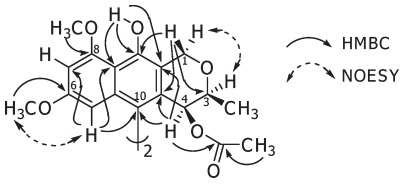

Figure 3. Selected HMBC and NOESY correlations for **9**.

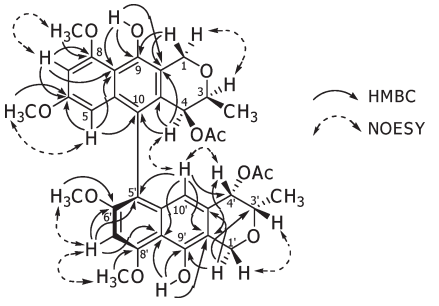

Figure 4. Selected HMBC and NOESY correlations for **10**.

(H-11) to H$_3$-25, H$_3$-26, and H-13 and from 11-OH to H$_\beta$-1 and H$_\alpha$-12. The unusual downfield shift of H$_\beta$-1 (δ_H 2.73) can be explained by the deshielding of this proton by the 11α-OH group. On the basis of these data, **5** was assigned as a new hopane-type triterpene, hopan-27-al-6β,11α,22-triol.

Compound **6** possessed the molecular formula $C_{30}H_{52}O_4$ (HRESIMS), containing two more hydrogen atoms than **5**. The ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **5**. Significant differences were the absence of the ^{1}H and ^{13}C resonances of a formyl group (CH-27 of **5**) instead of the presence of a hydroxymethyl group (δ_C 60.4, δ_H 3.70 and 3.62; 27-O*H* δ_H 4.19, dd, J = 5.3, 4.5 Hz). The location of the hydroxymethyl group was confirmed by the HMBC correlations from H$_2$-27 to C-8 and C-13 and from 27-OH to C-14.

The molecular formula of compound **7** was determined by HRESIMS as $C_{30}H_{52}O_3$. The ^{1}H and ^{13}C NMR spectroscopic data suggested that **7** is structurally related to **5** and **6**; however, the 11-OH functionality was absent and CH$_3$-27 was not oxygenated. Instead, **7** possessed a 7-OH group, as confirmed by a weak COSY correlation between H-7 (δ_H 3.44) and H-6 (δ_H 4.04) and an HMBC correlation from 7-OH to C-8. Similar to **5** and **6**, H-5 resonated as a broad singlet with a narrow peak width and exhibited an intense NOESY cross-peak to H-6, which confirmed an axial orientation of 6-OH. NOESY correlations from H-7 to H-5, H-9, and H$_3$-27 indicated that H-7 occupied an axial position. Therefore, compound **7** was assigned as hopane-6β,7β,22-triol.

The molecular formula of **9** ($C_{36}H_{38}O_{12}$), determined by HRESIMS, was the same as the known co-metabolite ES-242-2 (**12**).[6−8] The ^{1}H and ^{13}C NMR spectroscopic data of compound **9** were similar to those of **12** and also revealed that **9** is a symmetrical dimer (Table 3). The small coupling constant (J = 1.8 Hz) of H-3/H-4 indicated the *cis*-relation of CH$_3$-11 and the acetoxy group. Analysis of HMBC correlations (Figure 3) revealed that **9** is a C-10−C-10′ dimer. The intense NOESY correlation of H-4 (H-4′) and H-5′ (H-5) indicated that the helicity of the chiral axis is opposite that of **12**. Therefore, compound **9** was assigned as the atropisomer of **12**.

Compound **10** possessed the same molecular formula (HRESIMS) as **9** and **12**. Analysis of the 2D NMR data, in particular HMBC correlations, demonstrated that it is a C-10−C-

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Table 4. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for 10

position	δ_C, mult.	δ_H, mult. (J in Hz)	position	δ_C, mult.	δ_H, mult. (J in Hz)
1	65.0, CH_2	5.28, d (15.6)	1′	64.7, CH_2	5.21, d (15.6)
		4.90, d (15.6)			4.76, d (15.6)
3	72.8, CH	3.79, br q (6.4)	3′	72.4, CH	3.91, dq (1.6, 6.4)
4	67.3, CH	5.26, br s	4′	68.8, CH	5.63, d (1.6)
4a	132.0, qC		4a′	133.0, qC	
5	98.3, CH	6.00, d (2.1)	5′	113.8, qC	
6	157.1, qC		6′	155.5, qC	
7	97.9, CH	6.45, d (2.1)	7′	94.5, CH	6.67, s
8	157.3, qC		8′	157.7, qC	
8a	110.89,[a] qC		8a′	110.86,[a] qC	
9	149.64,[b] qC		9′	149.59,[b] qC	
9a	115.1, qC		9a′	114.6, qC	
10	123.8, qC		10′	116.3, CH	6.39, s
10a	135.6,[c] qC		10a′	135.5,[c] qC	
11	17.0, CH_3	1.08, d (6.4)	11′	16.8, CH_3	1.23, d (6.4)
4-OCOCH$_3$	169.5, qC		4′-OCOCH$_3$	170.4, qC	
4-OCOC*H*$_3$	20.8,[d] CH_3	1.98, s	4′-OCOC*H*$_3$	20.7,[d] CH_3	1.77, s
6-OC*H*$_3$	55.1, CH_3	3.45, s	6′-OC*H*$_3$	56.8, CH_3	3.69, s
8-OC*H*$_3$	56.4,[e] CH_3	4.07, s	8′-OC*H*$_3$	56.3,[e] CH_3	4.18, s
9-O*H*		9.53, s	9′-O*H*		9.51, s

[a−e] The assignments of carbons can be interchanged.

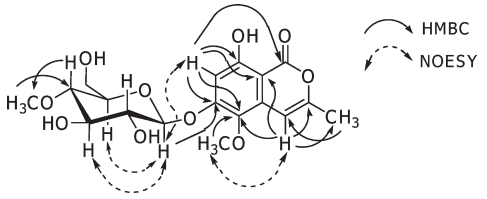

Figure 5. Selected HMBC and NOESY correlations for **13**.

5′ dimer (Figure 4, Table 4). H-4 (δ_H 5.26) resonated as a broad singlet, whereas H-4′ (δ_H 5.63) was a doublet with a small coupling constant (J = 1.6 Hz). These data indicated that the relative configurations of C-3/C-4 and C-3′/C-4′ are the same as other ES-242 derivatives. The intense NOESY correlation observed between H-4 and H-10′ (δ_H 6.39, s) revealed the helicity of the chiral axis as shown in the structural formula. The deacetyl analogue of **10** and its atropisomer were previously reported.[6,7]

The molecular formula of **13** was established as $C_{18}H_{22}O_{10}$ by HRESIMS. The ^1H and ^{13}C NMR spectroscopic data showed a resemblance to the known co-metabolite **14**.[4] Notable differences were the presence of a methoxy group (δ_C 60.9; δ_H 3.81, 3H, s) and the absence of one of the *meta*-coupled aromatic protons (H-5). Analysis of the 2D NMR data (COSY, HMQC, and HMBC) revealed that **13** was the 5-methoxy derivative of **14** (Figure 5, Table 5). The sugar moiety was assigned as 4′-*O*-methyl-β-glucopyranose, which was identical to **14** and **15**. Thus, the vicinal coupling constants of $J_{1',2'}$ = 7.7 Hz, $J_{2',3'}$ = 9.0 Hz, and $J_{3',4'} = J_{4',5'}$ = 9.3 Hz indicated that methine protons H-1′ to H-5′ all occupy axial positions. The intense HMBC correlation from methoxy protons (δ_H 3.56, 3H, s) to C-4′ (δ_C 79.1) and the correlation from H-4′ (δ_H 3.24) to the methoxyl carbon (δ_C 59.6) demonstrated that the 4′-OH group was methylated. The linkage of the sugar to C-6 (δ_C 157.8) of the aglycone was evident from the HMBC correlation from the anomeric proton (H-1′, δ_H 5.17) to C-6. The NOESY correlation found between H-7 and H-1′ further supported the sugar junction. The negative specific rotation of **13**, $[\alpha]^{23}_D$ −55 (c 0.17, MeOH), was similar to those of the known co-metabolites **14** and **15**.[4] Due to the sample shortage, further analysis to confirm the absolute configuration of the sugar moiety of **13** was not performed; however, the co-occurrence suggested that **13** also possesses a 4-*O*-methyl-D-glucose.

Table 5. NMR Spectroscopic Data (500 MHz, acetone-d_6) for 13

position	δ_C, mult.	δ_H, mult. (J in Hz)
1	166.0, qC	
3	154.3, qC	
4	98.7, CH	6.65, br s
4a	131.1, qC	
5	135.3, qC	
6	157.8, qC	
7	101.8, CH	6.74, s
8	159.2, qC	
8a	99.2, qC	
9	18.6, CH_3	2.28, d (0.6)
5-OC*H*$_3$	60.9, CH_3	3.81, s
8-O*H*	10.92, br s	
1′	100.2, CH	5.17, d (7.7)
2′	74.0, CH	3.57, t (8.4)
3′	77.1, CH	3.67, t (9.0)
4′	79.1, CH	3.24, t (9.3)
5′	76.3, CH	3.61, m
6′	61.1, CH_2	3.85, br d (11.9); 3.71, m
4′-OC*H*$_3$	59.6, CH_3	3.56, s

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Table 6. Biological Activities of Triterpenes 5−7 and Bioxanthracenes 9 and 10

	P. falciparum[a]	*M. tuberculosis*[b]	HSV-1[c]	cytotoxicity (IC_{50}, μM)[d]			
compound	(IC_{50}, μM)	(MIC, μM)	(IC_{50}, μM)	KB	MCF-7	NCI-H187	Vero
5	>21	>105	21	10	28	68	69
6	9.8	52	14	5.6	15	47	47
7	>22	>109	>109	>109	>109	>109	>109
9	10	>75	>75	4.4	22	71	>75
10	15	>75	>75	15	37	12	>75

[a] Antimalarial activity against *Plasmodium falciparum* K1. Standard antimalarial drug dihydroartemisinin showed an IC_{50} value of 0.0044 μM. [b] Antitubercular activity against *Mycobacterium tuberculosis* H37Ra. MIC values of standard anti-TB drug isoniazid were 0.17−0.34 μM. [c] Anti-HSV-1 activity. Standard antiviral compound Acyclovir showed an IC_{50} value of 17 μM. [d] IC_{50} values of a standard compound, doxorubicin hydrochloride, against the cancer cell-lines KB, MCF-7, and NCI-H187 were 0.27, 4.9, and 0.23 μM, respectively. The IC_{50} value of a standard compound, ellipticine, against Vero cells was 12 μM.

New compounds **1**−**7**, **9**, and **10** were subjected to our biological activity protocols inclusive of antiplasmodial (*Plasmodium falciparum* K1), antimycobacterial (*Mycobacterium tuberculosis* H37Ra), and antiviral (herpes simplex virus type 1, HSV-1) activities and cytotoxicity against the human cancer cell lines KB (oral epidermoid carcinoma), MCF-7 (breast cancer), and NCI-H187 (small cell lung cancer) and nonmalignant Vero cells (African green monkey kidney fibroblasts). Conoideocrellide A (**1**) and linear analogues **2**−**4** were inactive in these assays. In contrast, paecilodepsipeptide A (**16**) was reported to show antimalarial (IC_{50} 4.9 μM) and cytotoxic activities in the same assays.[11] Triterpene **6** and bioxanthracenes **9** and **10** exhibited weak antimalarial activity (Table 6). Triterpenes **6** and **7** also showed anti-HSV-1 and cytotoxic activities. ES-242 derivatives are known to exhibit antimalarial activity.[6a,7]

The present study demonstrates that the genus *Conoideocrella* is a potent source of novel bioactive compounds. Structural similarity and the occurrence in closely related species of **1** and **16** suggest that the nonribosomal peptide synthetases for these depsipeptides are almost identical, with the only difference being the recognition site for D-Ser (for **1**) or D-Ala (for **16**). Since **16** was also isolated from *Gliocladium* sp., these depsipeptides are not specific to *Conoideocrella*. Other scale insect pathogens, *Hypocrella* and *Moelleriella* and their anamorph *Ascherisonia*, are also reported as producers of hopane-type triterpenes.[14,15] While these genera commonly produce a mixture of three hopanoids, zeorin (**8**), dustanin (hopane-15α,22-diol), and 3β-acetoxyhopane-15a,22-diol, with a variety of relative compositions,[14] the genus *Conoideocrella* has been known to produce only zeorin.[2] A unique structural feature of the new hopanoids **5**−**7** is the axial 6-OH, while 6α-OH (equatorial) hopanoids such as zeorin (**8**) are well known. This is also the first report of the isolation of ES-242 derivatives from *Torrubiella* or *Conoideocrella*. Several compounds possessing a 4-*O*-methyl-β-glucopyranoside moiety have been previously isolated from insect pathogenic fungi.[2,4,16−18] However, isocoumarin aglycones are known only from *Conoideocrella*.[4]

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBC Cintra 404 spectrophotometer. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESITOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. *Conoideocrella tenuis* was isolated from a scale insect (Hemiptera) in Khao Yai National Park, Nakhon Nayok Province, and identified on the basis of the morphology of the fungus on the scale insect. The fungus produced white to cream mycelia surrounding the host and had conical perithecia. The ITS rDNA of this strain was sequenced and compared to the in-house database for invertebrate pathogens and was further subjected to a BLAST search in GenBank. The molecular data revealed it to be *Conoideocrella tenuis* and confirmed the morphological identification.[1] This fungus was deposited in the BIOTEC Culture Collection (BCC) as BCC 18627 on August 30, 2005.

Fermentation, Extraction, and Isolation. The fungus BCC 18627 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 6 × 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 4 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 4 days on a rotary shaker (200 rpm). These secondary cultures were pooled, and each 25 mL portion was transferred into 60 × 1 L Erlenmeyer flasks containing 250 mL of M102 medium (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; pH was not adjusted), and final fermentation was carried out at 25 °C for 20 days under static conditions (final pH 5.93). The cultures were filtered to separate mycelia and filtrate (broth). Culture broth was extracted with EtOAc (2 × 15 L), and the combined organic phase was concentrated to obtain a brown gum (extract A, 3.10 g). Wet mycelia were macerated in MeOH (1.5 L, rt, 2 days), then filtered. The filtrate was defatted by partitioning with hexanes (1 L). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (1 L), washed with H_2O (200 mL), and concentrated under reduced pressure to leave a brown solid (extract B, 5.10 g). Extract A was subjected to column chromatography (CC) on silica gel (5.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to obtain three pooled fractions: fractions A-1 (51 mg), A-2 (732 mg), and A-3 (1.70 g). Fraction A-1 was subjected to preparative HPLC using a reversed-phase column (LiChroCART, 10 × 250 mm, 10 μm; mobile phase MeCN/H_2O, 50:50, flow rate 4 mL/min) to furnish **11** (0.8 mg, t_R 10 min) and **12** (10.0 mg, t_R 24 min). Fraction A-2 was repeatedly fractionated by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution) to afford **4** (11.2 mg). Fraction A-3 was fractionated by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution) and Sephadex LH-20 (2.8 × 60 cm, MeOH) to give **14** (5.6 mg). Mycelial extract (extract B) was triturated in MeOH (3 mL, rt, 2 h) and filtered. Residual solid (B1, 2.5 g) and filtrate (B2) were separately subjected to chromatographic fractionations. The insoluble solid (B1) was fractionated by CC on silica gel (5.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to

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50:50) to obtain five pooled fractions: fractions B1-1 (700 mg), B1-2 (250 mg), B1-3 (250 mg), B1-4 (210 mg), and B1-5 (350 mg). Fraction B1-1 contained mainly fatty acids and a small amount of **8**. Fraction B1-2 was further fractionated by CC on silica gel (2.8 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to give four fractions, B1-2-1−B1-2-4. Fraction B1-2-2 (50 mg) was purified by CC on silica gel (1.8 × 15 cm, acetone/CH_2Cl_2, step gradient elution from 0:100 to 10:90) to furnish **7** (7.6 mg). Fraction B1-2-3 (150 mg) was fractionated by CC on Sephadex LH-20 (2.8 × 60 cm, MeOH), followed by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution), to obtain **8** (8.0 mg), **5** (15.4 mg), and **7** (13.8 mg). Fraction B1-3 was purified by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution) to obtain **6** (126 mg). The filtrate from trituration (B2) was subjected to preparative HPLC using a reversed-phase column (Phenomenex Luna 10u C18(2) 100A, 21.2 × 250 mm, 10 μm; mobile phase MeCN/H_2O, 50:50, flow rate 15 mL/min) to collect five fractions, B2-1−B2-5. Fraction B2-1 (739 mg) was further separated by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to furnish **3** (12.2 mg) and **2** (4.2 mg). Fraction B2-2 (697 mg) was further purified by preparative HPLC (Phenomenex Luna) to obtain **1** (520 mg). Fraction B2-3 (225 mg) was further purified by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) and preparative HPLC (Phenomenex Luna) to furnish **9** (4.9 mg, t_R 19 min) and **12** (5.0 mg, t_R 24 min). Fraction B2-4 (75 mg) was also purified by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) and preparative HPLC (Phenomenex Luna) to furnish **12** (27.1 mg, t_R 24 min), while fraction B2-5 (213 mg) gave **10** (10.8 mg, t_R 31 min).

Another fermentation batch (M102 medium, 40 × 250 mL), which was examined prior to the isolation described above, gave **1** (20 mg), **8** (not purified), **9** (8.3 mg), **10** (4.0 mg), **12** (8.1 mg), and **15** (70.5 mg). Mass fermentation (40 × 250 mL) in yeast extract sucrose medium (sucrose 150 g, yeast extract 20 g, per liter) was also performed. The extracts provided **1** (293 mg), **3** (9.0 mg), and **13** (2.1 mg), whereas several other compounds were not purified.

Conoideocrellide A (1): colorless solid; mp 141−142 °C; $[\alpha]^{23}_D$ +29 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.31), 277 (3.24), 284 sh (3.18) nm; IR (KBr) ν_{max} 3493, 3405, 3368, 1738, 1697, 1673, 1650, 1513, 1237 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1; HRMS (ESI-TOF) *m/z* 758.3397 $[M + H]^+$ (calcd for $C_{40}H_{48}N_5O_{10}$, 758.3396), 780.3216 $[M + Na]^+$ (calcd for $C_{40}H_{47}N_5O_{10}Na$, 780.3215).

Conoideocrellide B (2): colorless, amorphous solid; $[\alpha]^{25}_D$ −20 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.05), 277 (3.22), 284 sh (3.14) nm; IR (KBr) ν_{max} 3418, 1638, 1544, 1515, 1241 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Supporting Information; HRMS (ESI-TOF) *m/z* 774.3356 $[M - H]^-$ (calcd for $C_{40}H_{48}N_5O_{11}$, 774.3358).

Conoideocrellide C (3): colorless solid; mp 163−164 °C; $[\alpha]^{25}_D$ −20 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.27), 278 (3.43), 284 sh (3.37) nm; IR (KBr) ν_{max} 3423, 3283, 1741, 1633, 1544, 1514, 1237 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Supporting Information; HRMS (ESI-TOF) *m/z* 812.3467 $[M + Na]^+$ (calcd for $C_{41}H_{51}N_5O_{11}Na$, 812.3477).

Conoideocrellide D (4): colorless solid; mp 181−182 °C; $[\alpha]^{25}_D$ −20 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.36), 277 (3.55), 284 sh (3.47) nm; IR (KBr) ν_{max} 3371, 3281, 1739, 1634, 1514, 1235 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Supporting Information; HRMS (ESI-TOF) *m/z* 828.3414 $[M + Na]^+$ (calcd for $C_{41}H_{51}N_5O_{12}Na$, 828.3426).

Hopan-27-al-6β,11α,22-triol (5): colorless solid; mp 234−235 °C; $[\alpha]^{25}_D$ −10 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3531, 3484, 2945, 1697 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m/z* 497.3605 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3601).

Hopane-6β,11α,22,27-tetraol (6): colorless solid; mp 220−221 °C; $[\alpha]^{24}_D$ +7 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3456, 2941 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m/z* 499.3759 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_4Na$, 499.3763).

Hopane-6β,7β,22-triol (7): colorless solid; mp 245−246 °C; $[\alpha]^{24}_D$ +12 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3423, 2946 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m/z* 483.3821 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_3Na$, 483.3809).

Compound 9 (atropisomer of ES-242-2): yellow powder; $[\alpha]^{25}_D$ −26 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.74), 312 (3.95), 341 (3.78), 356 (3.82) nm; IR (KBr) ν_{max} 3406, 1735, 1625, 1365, 1233, 1096 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 3; HRMS (ESI-TOF) *m/z* 685.2264 $[M + Na]^+$ (calcd for $C_{36}H_{38}O_{12}Na$, 685.2261).

Compound 10: yellow powder; $[\alpha]^{25}_D$ +66 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.75), 311 (3.90), 354 (3.82) nm; IR (KBr) ν_{max} 3384, 1730 sh, 1726, 1626, 1361, 1236, 1095 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 4; HRMS (ESI-TOF) *m/z* 685.2256 $[M + Na]^+$ (calcd for $C_{36}H_{38}O_{12}Na$, 685.2261).

6,8-Dihydroxy-5-methoxy-3-methylisocoumarin 6-*O*-(4-*O*-methyl-β-D-glucopyranoside) (13): colorless, amorphous solid; $[\alpha]^{23}_D$ −55 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.44), 242 (4.44), 263 (3.94), 279 sh (3.70), 338 (3.69) nm; IR (KBr) ν_{max} 3442, 1694, 1649, 1619, 1488, 1107, 980 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 5; HRMS (ESI-TOF) *m/z* 421.1108 $[M + Na]^+$ (calcd for $C_{18}H_{22}O_{10}Na$, 421.1105).

Acid Hydrolysis of 1. Conoideocrellide A (**1**, 20 mg) was hydrolyzed by heating in 6 M HCl (6 mL)/MeOH (2 mL) at 110 °C for 15 h. After cooling, the aqueous solution was extracted with Et_2O (3 × 5 mL), and the combined organic phase was dried over $MgSO_4$, evaporated, and dried in vacuo. The residual solid was purified by CC on Sephadex LH-20 (1.5 × 30 cm, MeOH) to furnish (2*S*)-L-3-phenyllactic acid (3.7 mg): colorless solid; $[\alpha]^{26}_D$ −22 (*c* 0.18, EtOH); ^1H NMR (400 MHz, acetone-d_6) and MS data were identical to those of the commercial sample (Sigma-Aldrich).

Preparation and Analysis of Marfey Derivatives. Conoideocrellide A (**1**, 3.0 mg) was hydrolyzed by heating in 6 M HCl (1 mL) at 110 °C for 14 h. After cooling, the solution was evaporated and dried in vacuo. The hydrolysate was 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-alaninamide) 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 1 M HCl (70 μL), 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 for the hydrolysate of **1**. *O*-Prenyl-L-Tyr and *O*-prenyl-D-Tyr were previously synthesized for structure elucidation of paecilodepsipeptide A.[9] HPLC analysis was performed with the following conditions: NovaPak C_{18} (3.9 × 150 mm, 4 μm), mobile phase MeCN/(0.05% TFA or 20 mM ammonium phosphate in H_2O), flow rate 0.5 mL/min, UV detection at 340 nm. Three mobile phase conditions were employed for polarity and separation reasons: (1) MeCN/(20 mM ammonium phosphate in H_2O) = 20:80, L-Ser (t_R 4.6 min), D-Ser (t_R 5.3 min); (2) MeCN/(0.05% TFA in H_2O) = 25:75, Gly (t_R 9.8 min), L-Ala (t_R 12.2 min), D-Ala (t_R 18.3 min), L-Tyr (t_R 19.8 min), D-Tyr (t_R 27.6 min); (3) MeCN/(0.05% TFA in H_2O) = 45:55, L-Tyr (t_R 6.6 min), D-Tyr (t_R 8.7 min), *O*-prenyl-L-Tyr (t_R 9.1 min), *O*-prenyl-D-Tyr (t_R 12.2 min). The derivatized hydrolysate of **1** contained D-Ser, Gly, L-Ala, D-Tyr, and *O*-prenyl-D-Tyr.

Alkaline Hydrolysis of 1. To a solution of **1** (3.0 mg) in dioxane (1 mL) was added 2 M NaOH (0.2 mL), and the mixture was stirred at

room temperature for 2 h. The mixture was evaporated, and the residue was diluted with H_2O, 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, amorphous solid (3.2 mg), whose MS and ^{1}H NMR (DMSO-d_6) data were identical to those of **2**.

Biological Assays. The assay for activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique.[19] Growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra, antiviral activity against herpes simplex virus type-1, and cytotoxicity to Vero cells were assessed using the green fluorescent protein microplate assay.[20] Cytotoxic activities against KB, MCF-7, and NCI-H187 cells were evaluated using the resazurin microplate assay.[21]

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of **1**−**7**, **9**, **10**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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