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Two New Lipodepsipeptides from the Endophytic Fungus *Colletotrichum aotearoa* Y41

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Abstract: Two closely related lipodepsipeptides, with the same 12-membered oxodepsipeptide ring system, aotearolides A (**1**) and B (**2**), together with 1*H*-indole-3-carbaldehyde (**3**) and 2-(1*H*-indol-3-yl)acetic acid (**4**) were isolated from the endophytic fungal isolate *Colletotrichum aotearoa* Y41 of *Huperzia serrata*. The structures were elucidated on the basis of their high-resolution ESI/MS, 1D and 2D NMR spectroscopic data.

Keywords: Lipodepsipeptide; aotearolides A and B; *Colletotrichum aotearoa*; *Huperzia serrata*.

1. Introduction

Colletotrichum is a genus of fungi symbionts to plants as endophytes or phytopathogens. Many species of this genus are plant pathogenes, but some may have a mutualistic relationship with hosts [1]. The chemical constituents of *Colletotrichum* genus have been investigated and types of structures including alkaloids, benzenoids, flavones, isocoumarins, phenalenones, steroids, tetralones and triterpenes have been reported, and their bioactivities including the free radical scavenging, antioxidant activities [2,3] and the inhibition of nitric oxide (NO) production in lipopolysaccharide activated murine macrophage RAW264.7 cells also have been evaluated [4].

During our screening on the chemistry of endophytic fungi of *Huperzia serra*ta, the strain *Colletotrichum aotearoa* Y41 was selected for further investigation based on the results of HPLC-DAD analysis. Subsequent fractionation studies identified the known compounds 1*H*-indole-3-carbaldehyde (**3**) [5, 6] and 2-(1*H*-indol-3-yl)acetic acid (**4**) [7, 8]. Also detected and isolated was a family of novel lipodepsipeptides, aotearolides A and B (**1** and **2**). Herein, the isolation and structure elucidation of the new compounds (**1**, **2**/**2a**) from *Colletotrichum aotearoa* Y41 were reported (Figure 1), which were determined by analysis of the high-resolution ESI/MS (HR-ESI/MS), 1D and 2D NMR spectroscopic data.

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2. Materials and Methods

2.1. Material

The Y41 strain was isolated from the leaves of *H. serrata* collected from Meihua mountain (Longyan, Fujian, China) and was identified as *Colletotrichum aotearoa* by analyzing of the internal transcribed spacer (ITS) regions (GenBank accession number KC297063) and morphology.

Optical rotations were carried out using an Anton Paar MCP200 automatic polarimeter; The nuclear magnetic resonance (NMR) spectra were measured using a DRX-400 spectrometer (Bruker Daltonics Inc., Billerica, Massachusetts), with ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz. HR-ESI/MS was recorded on a LTQ Velos Pro HRMS instrument.

2.2. Fermentation and Isolation

The Y41 strain was cultured for 10 d on potato dextrose agar (PDA) media (potato 200 g, agar 15 g, dH_2O 1000 mL, pH natural) at 28 °C. The fermented agar cakes were diced and extracted three times overnight with EtOAc : MeOH (80 : 20, *v*/*v*) at room temperature to afford crude extract. The extract was dissolved in 80 mL 95% MeOH, and extracted with an equal volume of petroleum ether (PE) to afford MeOH soluble extract (1.28 g) and PE soluble extract (0.3 g), respectively.

The MeOH soluble extract was subjected to column chromatography (CC) over Sephadex LH-20 (140 g, MeOH) to obtain Fr. 1–8. Fr. 4 (120 mg) was subjected to medium pressure liquid chromatography (MPLC) over RP-18 SiO_2 (30 g), eluted with H_2O, then a stepwise gradient of 50, 70, 85, 90 and 100% (*v*/*v*) MeOH in H_2O to afford Fr. 4a–e. Fr. 4b (33 mg) was subjected to CC (SiO_2, 1.5 g) eluted with CH_2Cl_2-MeOH (100 : 1, 60 : 1, 50: 1, 40: 1, 30: 1) to yield **2** (9 mg).

Fr. 4c (32 mg) was subjected to CC (SiO_2, 1.0 g) eluted with PE-acetone (15 : 1, 10 : 1, 8 : 1, 6: 1, 5 : 1, 3 : 1, 2 : 1) to obtain **1 (**10 mg). Fr. 7 (93 mg) was purified by MPLC over RP-18 SiO_2 (30 g), eluted with 30% (1-11#, 200 mL), 40% (12-22#, 200 mL), 50% (23-36#, 250 mL) and 60% (37-45#, 150 mL) MeOH in H_2O to afford Fr. 7a–~~7~~d. Fr. 7b (25 mg) was finally purified by semi-preparative reversed-phase high pressure liquid chromatography (HPLC) (Agilent 1260 instrument; ZORBAX Eclipse XDB-C_{18} 5 μm, column ID: 9.4 × 250 mm, flow rate: 4 mL/min, elution: CH_3CN/H_2O (40 : 60, *v*/*v*), UV detections at 254, 320 nm) to afford **3** (t_R 8.6 min, 2.5 mg) and **4** (t_R 9.7 min, 5 mg), respectively.

2.3. Spectroscopic Data

*Aotearolide A (**1**):* White powder; $[\alpha]_D^{20}$ - 63.3 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data (Table 1); HR-ESI/MS: *m/z* 703.3779 $[M + Na]^+$, (calculated as 703.3776 for $C_{35}H_{56}N_2O_{11}$ Na^+).

*Aotearolide B (**2**):* White powder; $[a]_D^{20}$ -49 (*c* 0.09, MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3) and ^{13}C NMR (100 MHz, CD_3COCD_3) spectral data (Table 1); HR-ESI/MS: *m/z* 717.3935 $[M + Na]^+$, (calculated as 717.3933 for $C_{36}H_{58}N_2O_{11}Na^+$)

3. Results and Discussion

3.1. Structure elucidation

Aotearolide A (**1**) was isolated as white powder with the molecular formula of $C_{35}H_{56}N_2O_{11}$ determined by the HR-ESI/MS $[M + Na]^+$ at *m/z* 703.3779, which was consistent with the ^{1}H and ^{13}C NMR data (Table 1) and corresponded to 9 degrees of unsaturation. The ^{1}H NMR spectrum of **1** was well dispersed in $CDCl_3$ and displayed a pattern of chemical shifts typical for peptides (an NH doublet

at δ_H 7.50 and two α-amino protons at δ_H 4.93 and 5.28, respectively), a mono-substituted phenyl unit (δ_H 7.29 (2H), 7.28 (2H) and 7.23 (1H), and an α-glucose moiety (δ 4.69 (s), 3.82, 3.28, 3.72, 3.52 and 3.79 and 3.89). The ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra (Figure S1-S7) revealed the presence of a phenylalanine and an isoleucine residues, an α-glucose, a 5-oxygenated-2,6-dimethyl-3,11-dihydroxy-dodecanoic acyl moiety and three methyl groups (δ 1.00 d, 0.79 d and 1.13 d, Me-2a, Me-6a and Me-12).

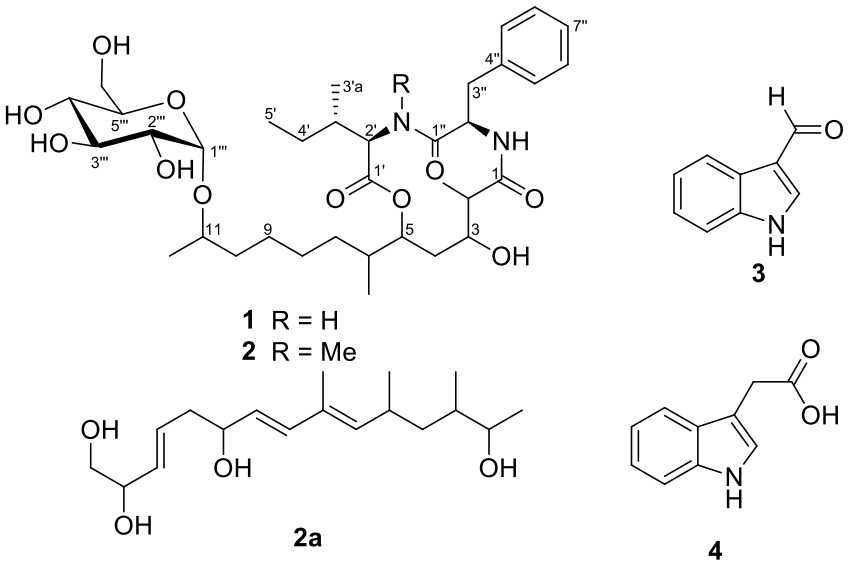

Figure 1. Chemical structures of compounds **1**–**4** and **2a**

The ^{13}C NMR spectrum of **1** displayed all 35 carbon resonances, and three of the nine sp^2 equivalents were attributable to the ester/amide carbonyl resonances at δ_C 169.5, 174.3 and 174.7, respectively. The phenylalanine (Phe) residue was confirmed by HMQC, HMBC and COSY correlations and thus accounted for an additional four degrees of unsaturation. A set of COSY correlations from H-2 (δ_H 2.75) to H-6 (δ 2.01) and from H-12 (δ_H 1.13) to H-10 (δ_H 1.38 and 1.61) together with the ^{13}C NMR (Table 1) identified a significant structure fragment and positioned the Me-2, 3-oxy, 5-oxy, Me-6 and 11-oxy, respectively (Figure 1). HMBC correlations of H-2 to C-1 and C-3, H-2a to C-2, C-1 and C-3, OH-3 to C-4 and C-3, H-5 to C-5 and C-1', H-6a to C-7, C-6 and C-5, H-11 to C-9 and C-10, and H-12 to C-11 and C-10 extended C-1 to C-12 and the structure fragment to include the isoleucine (Ile) and Phe residues. The downfield shift of H-5 (δ_H 4.75) required a lactone linkage to the isoleucine residue, which was confirmed by the HMBC correlation between H-5 and C-1'. The downfield shift of C-11 (δ_H 3.95, δ_C 73.5) confirmed that this carbon was oxygenated, while the HMBC correlation between H-1''' and C-11 further corroborated the connection of α-glucose with C-11. Overall, the α-glucose was connected to the dodecanoic unit at C-11, and the Ile carboxyl group connected to C(5)-OH to form an ester, and the Phe amino group connected to the C-1 carboxyl functionality of the dodecanoic acid to form an amide. The above connections created a 12-membered lipodepsipeptide, satisfying the nine degrees of unsaturation and all NMR assignments, thus completing the gross structure of aotearolide A (**1**).

Compound **2** was isolated as white powder. The 1D, 2D NMR spectra of **2** was found to be similar with that of **1** except for an additional methyl group (δ_H 2.32, δ_C 30.2). The HMBC correlations of the methyl protons to C-2' and C-1" confirmed its position at 2'-NCH_3 (Figure S12), Therefore, **2** was identified as a N-CH_3 analog of **1**, a closely related compound of **1** and named aotearolide B (**2**), which was confirmed by its HR-ESI/MS data at *m/z* 717.3935 $[M + Na]^+$ with the molecular formula $C_{36}H_{58}N_2O_{11}$ (Figure S13), which was supported by the NMR data (Table 1). Meanwhile, careful

inspection of the HMBC and ^{1}H-^{1}H COSY correlations (Figure S12 and S11), another structure was deduced to be **2a** as a mixture of **2** (Figure 1). The ratio of **2**/**2a** was about 1 : 1 based on the integrals of protons in the ^{1}H NMR spectra.

Table 1. ^{1}H and ^{13}C NMR data of **1** (in $CDCl_3$) and **2** (in CD_3COCD_3, δ in ppm, *J* in Hz).

position	**1**		**2**	
	δ_H (Mult., *J* Hz)	**δ_C**	**δ_H (Mult., *J* Hz)**	**δ_C**
NH	7.50(d, 9.8)			
1		174.7 (s)		174.7 (s)
2	2.75 (m)	48.2 (d)	2.75 (m)	48.2 (d)
2a	1.00 (d, 6.7)	7.9 (q)	0.99 (d, 6.7)	7.8 (q)
3	4.15 (m)	68.6 (d)	4.15 (m)	68.4 (d)
3-OH	4.05 (d, 5.4)			
4	1.74 (m), 1.59 (m)	32.2 (t)	0.93 (m)	32.5 (t)
5	4.73 (d, 9.3)	77.3 (d)	4.73 (br d, 11.4)	77.3 (d)
6	2.01 (m)	34.5 (d)	2.01 (m)	34.5 (d)
6a	0.79 (d, 7.6)	16.2 (q)	0.79 (d, 6.5)	16.2 (q)
7	1.74 (m), 1.96 (m)	32.5 (t)	1.58 (m)	32.2 (t)
8	1.33 (m)	26.8 (t)	1.37 (m)	26.7 (t)
9	1.38 (m)	25.8 (t)	1.427 (m)	25.8 (t)
10	1.38 (m), 1.61(m)	37.7 (t)	1.39 (m), 1.67 (m)	37.6 (t)
11	3.95 (m)	73.5 (d)	3.91 (m)	73.4 (d)
12	1.13 (d, 7.1)	19.8 (q)	1.12 (d, 6.0)	19.8 (q)
1'		169.5 (s)		169.6 (s)
2'	4.93 (d, 11.3)	61.1 (d)	4.92 (d, 11.7)	61.1 (d)
2'-NCH_3			2.72 (s)	30.2 (q)
3'	1.90 (m)	31.2 (d)	1.89 (m)	31.2 (d)
3'a	0.86 (d, 6.4)	15.7 (q)	0.86 (m)	15.7 (q)
4'	1.00 (m), 0.76 (m)	24.5 (t)	0.76 (m)	24.5 (t)
5'	0.75 (d, 4.0)	10.2 (q)	0.75 (d, 3.6)	10.1 (q)
1"		174.3 (s)		174.3 (s)
2"	5.28 (m)	51.1 (d)	5.25 (m)	51.1 (d)
3"	2.88 (m), 3.20 (dd, 10.9,13.0)	37.4 (t)	2.90 (dd, 12.7, 5.5), 3.22 (m)	37.4 (t)
4"		138.5 (s)		138.6 (s)
5"	7.29 (m)	130.1 (d)	7.28 (m)	130.1 (d)
6"	7.28 (m)	129.1 (d)	7.27 (m)	129.1 (d)
7"	7.23 (m)	127.3 (d)	7.23 (m)	127.3 (d)
8"	7.28 (m)	129.1 (d)	7.27 (m)	129.1 (d)
9"	7.29 (m)	130.1 (d)	7.28 (m)	130.1 (d)
1'"	4.69 (s)	98.5 (d)	4.68 (s)	98.5 (d)
2'"	3.82 (m)	72.8 (d)	3.79 (m)	72.7 (d)
3'"	3.28 (br s)	77.4 (d)	3.47 (m)	75.4 (d)
4'"	3.72 (t, 9.2)	69.0 (d)	3.68 (t, 9.3)	69.0 (d)
4'"-OH	4.23 (d, 1.6)			
5'"	3.52 (m)	75.5 (d)	3.25 (m)	77.4 (d)
6'"	3.79 (m), 3.89 (m)	62.8 (t)	3.78 (m), 3.88 (dd, 11.6, 2.2)	62.7 (t)

In order to purify compounds **2** and **2a** from the mixture of **2**/**2a**, the sample was acetylated and purified by CC (SiO_2) to obtain acetylated compound **2'**, and the ^{1}H NMR spectrum of **2'** was obtained (Figure S14), but it was not pure. It was not possible to further purify the acetylated product because of the amount limitation.

To solve the configurations of **1** and **2**, many solvents were tested to grow crystals, but all resulted with failure. The acetylated derivative of **1** was obtained by treatment of **1** (10 mg) with

Ac_2O/Py (1 : 1) at room temperature, but only flaky solid was obtained, which was not suitable for single crystal X-ray diffraction. However, the D-Ile and D-Phe moieties of **1** and **2** could be determined by comparing the NMR data with that of the corresponding literature [9-11].

Table 2. The NMR data of **2a** (in CD_3COCD_3, δ in ppm, *J* in Hz).

position	δ_H (Mult., *J* Hz)	δ_C	COSY	HMBC
1	1.06 (d, 6.3)	20.4 (q)	3.58 (m)	C-2, C-3
2	3.58 (m)	71.2 (d)	1.06 (d), 1.42 (m)	
3	1.42 (m)	38.6 (d)		
3a	0.85 (m)	14.9 (q)	1.42 (m)	C-3, C-4, C-2
4	1.46 (m)	41.4 (t)	1.46 (m)	
5	2.64 (m)	30.9 (d)	0.96 (d), 5.19 (d)	
5a	0.96 (d, 6.7)	22.2 (q)	2.64 (m)	C-5, C-4, C-6,
6	5.19 (d, 9.7)	139.6 (d)	1.75 (s), 2.64 (m)	C-7a, C-8
7		132.7 (s)		
7a	1.75 (s)	12.9 (q)		C-7, C-8, C-6
8	6.22 (d, 15.6)	135.2 (d)	5.60 (m)	C-7a, C-11, C-7, C-6
9	5.60 (m)	130.8 (d)	4.12 (m), 6.22 (d)	C-7
10	4.12 (m)	72.4 (d)	2.26 (m), 5.60 (m)	C-12, C-8
11	2.26 (m)	41.9 (t)		C-10, C-12, C-9, C-14
12	5.80 (m)	128.7 (d)	2.26 (m), 5.54 (m)	C-11
13	5.54 (m)	133.7 (d)	5.80 (m)	C-11
14	4.08 (m)	73.5 (d)	3.45 (m)	C-15
15	3.45 (m), 3.39 (m)	67.3 (t)	4.08 (m)	

As a conclusion, the 12-membered cyclic lipodepsipeptides are rare in nature. Hapalosin [12], the first reported one, was isolated from the cyanobacterium *Hapalosiphon welwitschii* [13], acremolides A-D [14] from an Australian marine-derived fungus strain *Acremonium* sp, and stereocalpins from the dry lichen, *Stereocaulon alpinum* [15], as well as the recent reported taumycins A and B from the Madagascar sponge *Fascaplysinopsis* sp. [9]. Here, aotearolides A and B from the endophytic fungal strain *C. aotearoa* Y41 of *H. serra*ta, not only increase new additions to this small group of lipodepsipeptides, but also widen the source of this type of natural products.

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Supporting Information

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