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# Agonodepsides A and B: Two New Depsides from a Filamentous Fungus F7524

Shugeng Cao,\* Alex S. Y. Lee, Yicun Huang, Horst Flotow, Siewbee Ng, Mark S. Butler, and Antony D. Buss

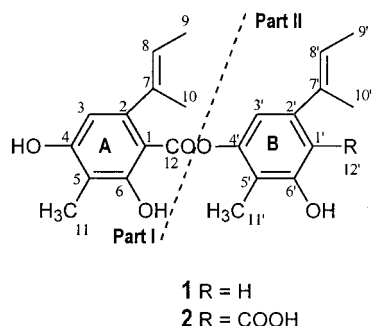
Centre for Natural Product Research, Institute of Molecular and Cell Biology, 59A Science Park Drive, The Fleming, Singapore Science Park 118240, Singapore

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Two new compounds, agonodepsides A (**1**) and B (**2**), were isolated from a nonsporulating filamentous fungus, F7524. The compounds were purified via reversed-phase chromatography and their structures determined by spectroscopic methods. Agonodepside A (**1**) was found to inhibit the mycobacterial InhA enzyme with an IC<sub>50</sub> value of 75  $\mu$ M, while **2** was inactive at 100  $\mu$ M.

InhA is an enoyl reductase essential for mycolic acid synthesis and growth in *Mycobacterium tuberculosis* and is the molecular target of the antitubercular drug isoniazid.<sup>1–3</sup> Isoniazid must be activated in vivo to form an InhA-inhibitory complex with NADH. Resistance to InhA has emerged due to mutations affecting isoniazid activation. Potent inhibitors of InhA are sought that do not require in vivo activation and that bind directly to InhA. Such inhibitors should be active against both isoniazid-susceptible and -resistant strains.

In our continuing search for biologically active products from natural sources, two new depsides, **1** and **2**, were obtained from a filamentous fungus through InhA assay-guided isolation. Many related depsides and depsidones have been isolated from fungi, but only a few contain two 1-methylprop-1-enyl groups in one molecule.<sup>4–8</sup>



Compound **1** was obtained as a yellow oil with a molecular formula of C<sub>23</sub>H<sub>26</sub>O<sub>5</sub> based on the negative ion ESI mass spectrometry (*m/z* 381) and <sup>13</sup>C NMR. In the <sup>1</sup>H NMR spectrum of **1** (DMSO-*d*<sub>6</sub>), three hydroxyl singlets ( $\delta_{\text{H}}$  10.72, s; 10.30, s; 9.59, s), one aromatic singlet ( $\delta_{\text{H}}$  6.26, s), two aromatic doublets ( $\delta_{\text{H}}$  6.77, d, *J* = 1.7 Hz; 6.51, d, *J* = 1.7 Hz), and two aromatic methyl singlets ( $\delta_{\text{H}}$  1.98, s; 1.87, s) were observed. In addition, two olefinic multiplets ( $\delta_{\text{H}}$  5.80, m; 5.30, m), two broad methyl singlets ( $\delta_{\text{H}}$  1.90, br s; 1.88, br s), and two broad methyl doublets ( $\delta_{\text{H}}$  1.74, br d, *J* = 6.7 Hz; 1.63, br d, *J* = 6.7 Hz) were also observed (Table 1), indicating the presence of two 1-methylprop-1-enyl groups. In the HMBC spectrum of **1** (Table 1) both H-9 (3H,  $\delta_{\text{H}}$  1.63, br d, *J* = 6.7 Hz) and H-10 (3H,  $\delta_{\text{H}}$  1.88, br s) correlated with C-7 ( $\delta_{\text{C}}$  137.6) and C-8 ( $\delta_{\text{C}}$  120.7). H-10 (3H,  $\delta_{\text{H}}$  1.88, br s) also showed a correlation with C-2 ( $\delta_{\text{C}}$  146.2), which was the carbon connected to one of the 1-methylprop-

**Table 1.** NMR Spectral Data<sup>a</sup> of Agonodepside A (**1**) and Agonodepside B (**2**)

position	<b>1</b>		<b>2</b>	
	<sup>13</sup> C <sup>b</sup>	<sup>1</sup> H <sup>c</sup> (mult. <i>J</i> = Hz)	<sup>13</sup> C <sup>b</sup>	<sup>1</sup> H <sup>c</sup> (mult. <i>J</i> = Hz)
1	104.5		104.6	
2	146.2		146.0	
3	108.4	6.26 (s)	108.4	6.22 (s)
4	159.8		159.8	
5	109.1		109.4	
6	159.8		159.8	
7	137.6		137.5	
8	120.7	5.30 (m)	120.9	5.27 (m)
9	13.6	1.63 (3H, br d, 6.7)	13.5	1.58 (3H, br d, 6.8)
10	18.4	1.88 (3H, br s)	18.3	1.83 (3H, br s)
11	8.1	1.98 (3H, s)	8.1	1.95 (3H, s)
12	168.6		168.1	
1'	109.2	6.77 (d, 1.7)	112.4	
2'	141.5		145.6	
3'	109.1	6.51 (d, 1.7)	113.2	6.34 (s)
4'	149.7		151.2	
5'	115.1		116.4	
6'	156.1		159.5	
7'	134.1		137.4	
8'	121.6	5.80 (m)	121.0	5.27 (m)
9'	14.1	1.74 (3H, br d, 6.7)	13.7	1.61 (3H, br d, 6.8)
10'	15.0	1.90 (3H, br s)	18.2	1.81 (3H, br s)
11'	8.9	1.87 (3H, s)	9.0	1.91 (3H, s)
12'			171.7	
6-OH		10.72 (s)		10.43 (s)
6'-OH		9.59 (s)		10.14 (s)
4-OH		10.30 (s)		

<sup>a</sup> Assignments based on COSY, ROESY, HSQCED, and HMBC.

<sup>b</sup> Recorded at 125 MHz with DMSO-*d*<sub>6</sub> as internal standard at  $\delta$  39.5. Chemical shifts ( $\delta$ ) in ppm. <sup>c</sup> Recorded at 500 MHz with DMSO-*d*<sub>6</sub> as internal standard at  $\delta$  2.39. Chemical shifts ( $\delta$ ) in ppm. One proton unless otherwise stated.

1-enyl groups. The singlet at  $\delta_{\text{H}}$  6.26 was assigned to be H-3 on the basis of HMBC correlations with C-7 ( $\delta_{\text{C}}$  137.6), C-1 ( $\delta_{\text{C}}$  104.5), C-4 ( $\delta_{\text{C}}$  159.8), C-5 ( $\delta_{\text{C}}$  109.1), and C-12 ( $\delta_{\text{C}}$  168.6). It was clear that the aromatic methyl at  $\delta_{\text{H}}$  1.98 (H-11) and hydroxyl group ( $\delta_{\text{H}}$  10.72) at C-6 were *meta* and *para*, respectively, to H-3, as the former correlated with C-4 ( $\delta_{\text{C}}$  159.8), C-5 ( $\delta_{\text{C}}$  109.1), and C-6 ( $\delta_{\text{C}}$  159.8), while 6-OH correlated with C-1 ( $\delta_{\text{C}}$  104.5), C-5 ( $\delta_{\text{C}}$  109.1), and C-6 ( $\delta_{\text{C}}$  159.8). The chemical shift value of  $\delta_{\text{C}}$  159.8 for C-4 indicated that it must be oxygenated. Thus, part I of the molecule was determined to be 4,6-dihydroxy-5-methyl-2-(1-methylprop-1-enyl)benzoate. Similarly, the substituents at C-2', C-5', and C-6' in ring B were determined to be 1-methylprop-1-enyl, methyl, and hydroxyl groups, respectively. Two *meta*-coupled aromatic doublets ( $\delta_{\text{H}}$  6.77, d, *J* = 1.7 Hz; 6.51, d, *J* = 1.7 Hz) in ring B showed correlations with C-7' in the HMBC spectrum, which indicated that

\* To whom correspondence should be addressed. MerLion Pharmaceuticals. Tel: (65) 6871-9721. Fax: (65) 6773-7072. E-mail: shugeng@merlionpharma.com.

1-methylprop-1-enyl at C-2 was flanked by these two protons (H-1' and H-3'). The appearance of a carbonyl group (C-12) at low field ( $\delta_C$  168.6) rather than high field ( $\delta_C$  164.1)<sup>5</sup> indicated that the molecule was a depside.<sup>4</sup> The negative ESI mass spectrum of **1** exhibited a significant peak at  $m/z$  203 that corresponded to part I of the molecule. The configurations of both 1-methylprop-1-enyl groups were assigned as *E* on the basis of the chemical shift values of  $\delta_C$  13.6 (C-9),  $\delta_C$  18.4 (C-10),  $\delta_C$  14.1 (C-9'), and  $\delta_C$  15.0 (C-10').<sup>4-8</sup> Selective refocusing of 10-Me and 10'-Me in 1D NOESY experiments gave enhancements to 9-Me and 9'-Me, respectively, which confirmed the assignments of *E* geometries for the C-2 and C-2' side chains. Thus, **1** is an ester of monomers I and II, for which we propose the trivial name agonodepside A (**1**).

A molecular formula of  $C_{24}H_{26}O_7$  was established by HREIMS and  $^{13}C$  NMR for **2**. The mass 426 of **2** was 44 more than that of **1**, which suggested that **2** was a carboxylic acid derivative of **1**. The  $^1H$  NMR spectrum of **2** (Table 1) differed from that of **1** only in positions 1' and 3'. These two aromatic doublets ( $\delta_H$  6.77, d,  $J = 1.7$ , H-1';  $\delta_H$  6.51, d,  $J = 1.7$ , H-3') in **1** were replaced by an aromatic singlet ( $\delta_H$  6.34, s, H-3') in **2**. In the HMBC spectrum, H-3' ( $\delta_H$  6.34, s) showed a correlation with C-12' ( $\delta_C$  171.7), which suggested that the carboxylic acid group was located at C-1'. Like **1**, part I of **2** was connected to part II via an ester bridge ( $\delta_C$  168.1, C-12) and the stereochemistry of 1-methylprop-1-enyl substituents at C-2 and C-2' was *E*. The above analysis allowed the NMR assignment (Table 1) of structure **2** to agonodepside B.

When tested in the InhA assay, agonodepside A (**1**) proved to be marginally active, with an  $IC_{50}$  value of 75  $\mu M$ , while **2** was not active at 100  $\mu M$  (highest concentration tested).

## Experimental Section

**General Experimental Procedures.** General experimental procedures have been reported elsewhere.<sup>9</sup>

**Microorganism and Fermentation.** The fungal strain F7524 is an endophyte isolated from dry leaves of *Derris thyrsoiflora* and deposited in the Centre for Natural Product Research culture collection. The strain was subcultured on malt extract agar (CM057B, Oxoid) for 7 days at 24 °C. It was used to inoculate 250 mL Erlenmeyer flasks each containing 50 mL of seed medium composed of 0.4% glucose, 1% malt extract, and 0.4% yeast extract. The pH of the medium was adjusted to 5.5 before sterilization. The seed flasks were incubated for 5 days at 24 °C on a rotary shaker at 200 rpm. A volume of 20 mL of seed culture was used to inoculate 400 mL of liquid medium in a 2 L Erlenmeyer flask. The medium was composed of 3% maltose, 1% glucose, 0.008% yeast extract, and 0.2% peptone. It also contained trace amounts of  $KH_2PO_4$ ,  $MgSO_4 \cdot 7H_2O$ ,  $FeCl_3$ ,  $ZnSO_4$ , and  $CaCl_2$ . The pH was adjusted to 6.0. The fermentation was carried out at 200 rpm for 9 days at 24 °C under static conditions.

**Biological Assays.** This was a fluorometric assay, which measured the amount of NADH left in the reaction. Test samples in 12.5% aqueous DMSO were incubated for 20 min in 50  $\mu L$  of reaction mixture containing 12 ng of InhA enzyme, 0.25 mM DDC (dodecenoyl-CoA), 100  $\mu M$  NADH (nicotinamide adenine dinucleotide), and 30 mM PIPES (piperazine-*N,N'*-bis(2-ethanesulfonic acid)), at pH 6.8. Fluorescence was measured at 1 min intervals over a period of 8 min (excitation = 360 nm and emission = 465 nm)<sup>10</sup> and the rate of NADH depletion calculated. Triclosan was used as the positive control ( $IC_{50} = 3 \mu M$ ).

**Extraction and Isolation.** The freeze-dried fermentation material was extracted three times (10 mL of solvent for each 1 g of dry material) with  $CH_2Cl_2$ -MeOH (1:1) and evaporated to dryness under vacuum. The dry extract (3.3 g) was partitioned three times between hexane and 90% MeOH in  $H_2O$  (1:1). The 90% MeOH portion was adjusted to 70% with  $H_2O$  and partitioned three times with  $CH_2Cl_2$ . A 200 mg aliquot of the active  $CH_2Cl_2$  fraction (1.2 g) was subjected to reversed-phase preparative HPLC using gradient elution (flow rate, 20 mL/min; 100% (0.1%  $HCOOH$  in  $H_2O$ ) to 100% (0.1%  $HCOOH$  in  $CH_3CN$ ) in 30 min) to give compounds **1** (0.5 mg, 1.5 mg/L) and **2** (20 mg, 60 mg/L).

**Agonodepside A (1):** white powder; UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 214 (4.57), 275 (4.16), 315 (2.82) nm; IR  $\nu_{max}$  (KBr) 3400, 2960, 1725, 1635  $cm^{-1}$ ;  $^1H$  NMR (DMSO- $d_6$ , 500 MHz, Table 1);  $^{13}C$  NMR (DMSO- $d_6$ , 125 MHz, Table 1); ESIMS (negative)  $m/z$  381  $[M - H]^-$ , 203; HRMS (negative)  $m/z$  381.1689 (calcd for  $C_{23}H_{25}O_5$ , 381.1697,  $[M - H]^-$ ).

**Agonodepside B (2):** white powder; UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 216 (4.55), 274 (4.18), 312 (3.75) nm; IR  $\nu_{max}$  (KBr) 3400, 2960, 1720, 1640  $cm^{-1}$ ;  $^1H$  NMR (DMSO- $d_6$ , 500 MHz, Table 1);  $^{13}C$  NMR (DMSO- $d_6$ , 125 MHz, Table 1); ESIMS (negative)  $m/z$  425  $[M - H]^-$ , 221, 203; HRMS (negative)  $m/z$  425.1576 (calcd for  $C_{24}H_{25}O_7$ , 425.1595,  $[M - H]^-$ ).

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