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Drychampones A–C: Three Meroterpenoids from *Dryopteris championii*

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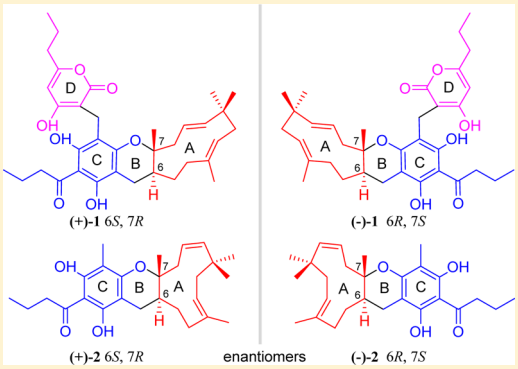
Supporting Information

ABSTRACT: Three novel sesquiterpenoid-based meroterpenoids, drychampones A–C (**1**–**3**, respectively), were isolated from *Dryopteris championii*. Compounds **1** and **3** possessed a novel carbon skeleton which was constructed by an 11/6/6 ring system coupled with a pyronone moiety, and **1**–**3** were three racemates. Their structures and absolute configurations were elucidated by NMR, MS, and computational methods. The hypothetical biosynthetic pathways of these meroterpenoids and their antibacterial activities were also discussed.



The *Dryopteris* genus is one of the largest fern of the Dryopteridaceae, which consists of 230 species and widely distributes throughout the world.[1,2] *Dryopteris championii* is mainly distributed throughout China, and some places of Japan and Korea.[3] As a traditional Chinese medicine, *D. championii* is used for the treatment of cold, asthma, hemafecia, dysmenorrhea, ancylostomiasis, etc.[4] Previous phytochemical investigations on the plants of *Dryopteris* genus had led to the isolation of phloroglucinols, terpenoids, and flavonoids.[5−7] Additionally, some alkanes and saturated fatty acids were identified from the volatile constituents in the roots and leaves of *D. championii* by GC-MS,[8] and seven phloroglucinols and three other compounds were isolated from the extract of this plant.[4] The phloroglucinols were considered to be the main components of the *Dryopteris* genus plants and they were proved to possess the antibacterial, antitumor, and antiviral activities.[9−11]

To discover structurally novel and biologically interesting compounds, the present study was undertaken to investigate the chemical constituents of *D. championii*. As a result, three novel sesquiterpenoid-based meroterpenoids, drychampones A–C (**1**–**3**, respectively), along with three known compounds (Figure 1), aspidin BB (**4**),[4] desaspidin BB (**5**),[12] and desaspidin PB (**6**),[13] were isolated from the ethanol extract of the aerial part of *D. championii*. Compounds **4**–**6** were methylene-bridged phloroglucinol derivatives constructed by a filicinic acid ring and an aromatic phloroglucinol ring,[12] and they have been previously reported from *Dryopteris*,[4,13,14] including **4** from *D. championii*. Compounds **1** and **3** featured a new carbon skeleton with the incorporation of a sesquiterpenoid moiety to an unusual phloroglucinol derivative via a hetero-Diels–Alder cycloaddition to form the unexpected 11/6/6 ring system, and compounds **1**–**3** were three racemates. In this article, we would like to report the isolation, structural elucidation, antibacterial activity, and plausible biogenetic pathway of these isolates.

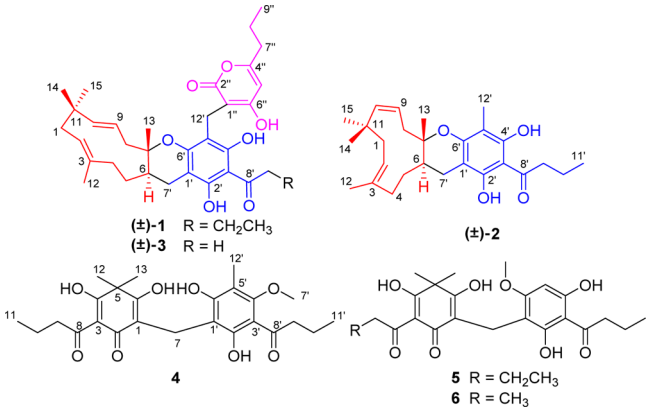


Figure 1. Chemical structures of **1**–**6**.

Compound **1** was obtained as yellow powder. The HR-ESI-MS of **1** showed the quasi-molecular ion peak at *m*/*z* 579.3319 $[M+H]^+$ (calcd for $C_{35}H_{47}O_7$ *m*/*z* 579.3316), consistent with the molecular formula $C_{35}H_{46}O_7$ with 13 degrees of unsaturation. The ^1H NMR spectrum of **1** revealed the presence of the signals due to three hydroxyls [δ_H 16.26, 10.25, and 9.95 (each 1H, s)]; four olefinic protons [δ_H 5.92 (1H, s),

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Table 1. NMR Data of 1−3[a]

	1		2		3	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	1.75	41.6	1.74 (dd, 12.5, 4.2)	41.6	1.74 (dd, 12.5, 4.2)	41.5
1b	2.16 (t, 12.3)		2.17 (t, 12.5)		2.17 (t, 12.5)	
2	4.98 (d, 8.6)	123.1	5.00 (dd, 8.7, 4.2)	123.3	5.00 (dd, 8.7, 4.2)	123.3
3		136.9		136.8		136.8
4a	1.84 (t, 12.1)	37.9	1.84	38.0	1.87	37.8
4b	2.08 (t, 12.6)		2.10 (m)		2.10 (m)	
5a	1.16 (m)	30.5	1.16 (m)	30.6	1.22 (m)	30.6
5b	1.28 (m)		1.28 (m)		1.29 (m)	
6	1.77	34.8	1.83	34.8	1.87	35.5
7		83.0		82.3		86.3
8a	2.34 (m)	42.9	2.35 (m)	43.0	2.47 (d, 10.0)	43.0
8b	2.55 (d, 14.5)		2.56 (d, 14.5)		2.61 (d, 14.5)	
9	5.09 (m)	120.1	5.15 (m)	120.3	5.10 (m)	119.2
10	5.13 (d, 16.2)	143.2	5.14 (d, 16.2)	143.1	5.17 (d, 16.2)	143.9
11		38.4		38.4		38.5
12	1.60 (s)	17.3	1.61 (s)	17.4	1.61 (s)	17.4
13	1.11 (s)	20.3	1.12 (s)	20.1	1.18 (s)	20.2
14	0.96	24.4	1.00 (s)	24.4	1.00 (s)	24.4
15	1.03 (s)	30.4	1.04 (s)	30.4	1.05 (s)	30.3
1′		103.2		99.5		102.3
2′		161.0		154.8		162.1
3′		105.0		105.9		106.5
4′		160.9		162.9		158.1
5′		104.9		101.5		103.7
6′		156.1		157.6		155.8
7′α	2.04	24.0	2.05	23.9	3.05 (dd, 16.8, 5.2)	23.1
7′β	3.04		2.90 (dd, 16.8, 5.2)	30.6	2.04 (m)	
8′		206.8		206.5		205.2
9′	2.98 (m)	46.1	2.99 (td, 7.5, 3.0)	46.8	2.72 (s)	33.6
10′	1.68 (m)	19.0	1.67 (m)	19.0		
11′	0.96	14.2	0.95 (t, 7.5)	14.3		
12′	3.56 (d, 3.5)	17.3	2.04 (s)	7.3	3.58 (d, 3.5)	17.5
1″		102.6				102.3
2″		170.1				169.9
4″		164.6				165.1
5″	5.92 (s)	101.8			5.93 (s)	101.1
6″		167.8				167.2
7″	2.41 (t, 7.5)	35.5			2.44	35.5
8″	1.64 (m)	20.4			1.66 (m)	20.3
9″	0.93 (t, 7.5)	13.6			0.94 (t, 7.5)	13.6

[a]Measured at 500 (^{1}H) and 125 (^{13}C) MHz in $CDCl_3$. δ in parts per million, J in hertz. Overlapped signals are reported without designating multiplicity.

5.13 (1H, d, J = 16.2 Hz), 5.09 (1H, m), and 4.98 (1H, d, J = 8.6 Hz)]; six methyls [δ_H 1.60, 1.11, 1.03 (each 3H, s), 0.96 (6H, overlapped), and 0.93 (3H, t, J = 7.5 Hz)]. The ^{13}C and DEPT NMR spectra of **1** displayed thirty-five signals, including six methyls, ten methylenes, five methines, and 14 quaternary carbons. Detailed analysis of the ^{1}H and ^{13}C NMR data of **1** (Table 1) showed that **1** possessed the same pyronone ring as that of phloropyron BB.[12] The signals assignable to one hexasubstituted benzene ring (δ_C 161.0, 160.9, 156.8, 105.0, 104.9, and 103.2), two phenolic hydroxyls (δ_H 16.26, 10.25), together with one carbonyl (δ_C 206.8) and one propyl (δ_C 46.1, 19.0, and 14.2) revealed that an aromatic phloroglucinol ring replaced the 3-butyrylfilicinic acid moiety in phloropyron BB to construct a new dimerous acylphloroglucinol (**1a**) in **1**. The remaining resonances assignable to four methyls, four methylenes, four methines (including three olefinic carbons), and three quaternary carbons (including an olefinic and an oxygenated carbons) were in good agreement with the humulene moiety of guajadial B,[15] indicating that the presence of the same partial structure (**1b**) in **1**. In the HMBC spectrum (Figure 2), the observed correlations between H-7′ and C-5, C-

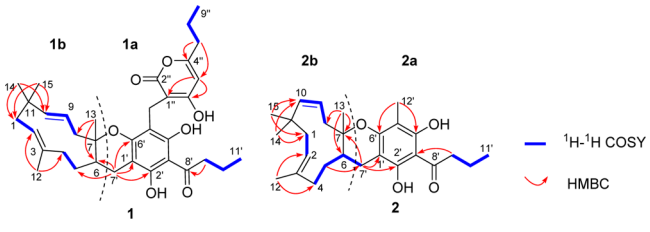

Figure 2. Key ^{1}H−^{1}H COSY and HMBC correlations of **1** and **2**.

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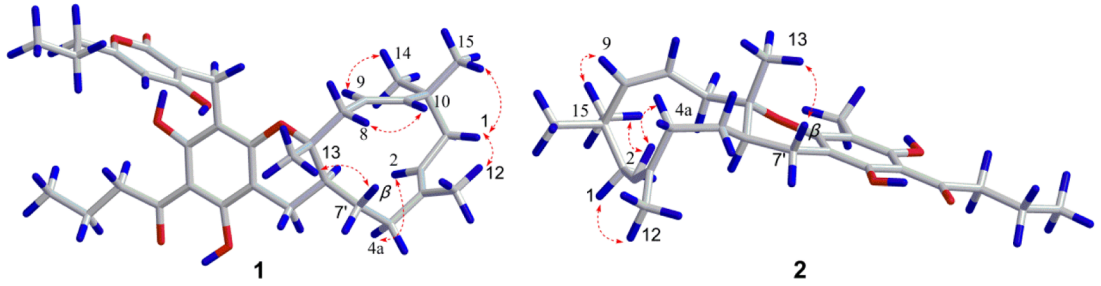

Figure 3. Key NOESY correlations of **1** and **2**.

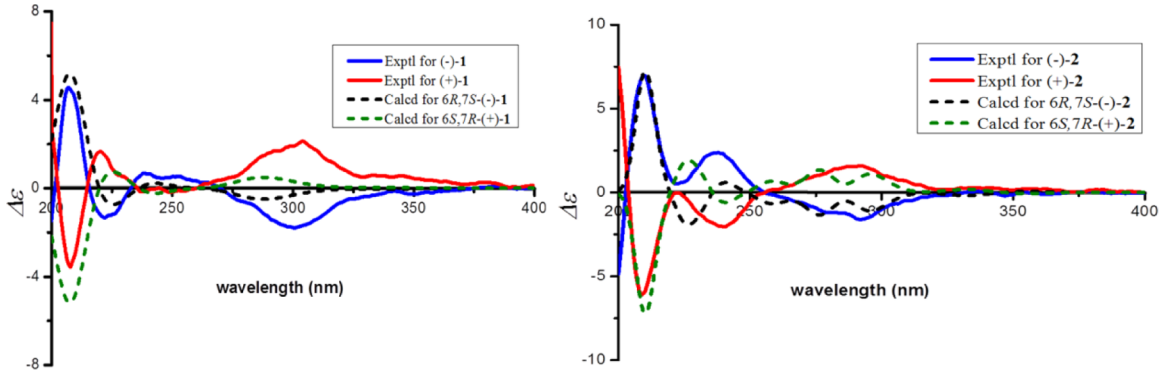

Figure 4. Calculated and experimental CD spectra of (±)-**1** and (±)-**2**.

6, C-1′, C-2′, and C-6′ suggested that **1a** and **1b** were linked through the C-7′−C-6 bond. Moreover, the oxygenated quaternary carbon C-7 (δ_C 83.0) and the obvious downfield chemical shift of C-6′ (δ_C 156.1) revealed that **1a** and **1b** were connected via a pyran ring. So the planar structure of **1** was finally established as depicted and named drychampone A (Figure 1).

The relative stereochemistry of **1** was established by analysis of its NOESY data (Figure 3) and coupling constants of protons. In the NOESY spectrum of **1**, the cross-peaks between H-7′β and H_3-13 as well as no correlation between H-6 and H_3-13 implied that H-6 and H_3-13 had different orientations. Similarly, the cross-peaks between H_3-12 and H-1b supported the *E*-geometry of the C-2/C-3 olefin. And the *E*-geometry of C-9/C-10 olefin was consistent with the coupling constant observed (J_{9-10} = 16.2 Hz).

Although there were two chiral centers (C-6 and C-7) in **1**, the optical activity and Cotton effect of **1** were too weak to be detected, indicating that **1** might be a racemate. And it was further confirmed by chiral HPLC analysis, in which two distinct chromatographic peaks appeared with a ratio of 1:1 (see the Supporting Information). Subsequently, a pair of enantiomers [(+)-**1** and (−)-**1**] were successfully separated by a chiral HPLC column, and the measured specific rotation values were +25.3 and −25.6, respectively. To determine the absolute configurations of (+)-**1** and (−)-**1**, a comparison between the experimental and calculated CD spectra using the time-dependent DFT method of each enantiomer was performed (Figure 4). The measured CD spectrum of (−)-**1** showed negative Cotton effect at 295 nm ($\Delta\varepsilon$ −1.6), positive one at 207.2 nm ($\Delta\varepsilon$ + 4.5), which were consistent with the calculated CD spectrum for 6*R*, 7*S* isomer. Whereas, the CD spectrum of (+)-**1** displayed reverse Cotton effects at the same wavelengths, which corresponded to 6*S*, 7*R* isomer. Based on the above evidence, the absolute configurations of (−)-**1** and (+)-**1** were established, respectively.

Compound **2** was obtained as yellow powder and its molecular formula was determined to be $C_{27}H_{38}O_4$ with 9 degrees of unsaturation by the HR-ESI-MS at *m*/*z* 427.2845 $[M+H]^+$ (calcd for $C_{27}H_{39}O_4$ *m*/*z* 427.2843). A careful and detailed comparison of the ^1H and ^{13}C spectra data of **2** (Table 1) with those of **1**, suggesting that **2** was also a humulene-based meroterpenoid except for the absence of the pyronone unit and the methylene at C-12′ in **1**, and the presence of an extra methyl [δ_H 2.04 (3H, s); Me-12′] in **2**. The HMBC correlations between H_3-12′ and C-4′, C-5′, and C-6′ verified that the extra methyl was connected to C-5′ (Figure 2).

In the NOESY spectrum (Figure 3), the cross-peaks between H-7′β and H_3-13, between H-2 and H-4a, as well as between H_3-12 and H-1b, together with the protons coupling constant observed (J_{9-10} < 10 Hz) established the relative configuration of **2**. Additionally, the lack of optical activity and Cotton effect revealed that **2** was also a racemate, which was confirmed by chiral HPLC analysis (see the Supporting Information). Furthermore, a pair of enantiomers, (+)-**2** and (−)-**2**, were obtained, and their CD curves were completely reversed (Figure 4). Finally, the absolute configurations were established to be 6*R*, 7*S* for (−)-**2** and 6*S*, 7*R* for (+)-**2**, respectively, by the CD calculation experiment as that of **1**.

The molecular formula of **3** was deduced as $C_{33}H_{42}O_7$ by the quasi molecular ion at *m*/*z* 551.3003 $[M+H]^+$ (calcd for $C_{33}H_{43}O_7$ *m*/*z* 551.3003) in its HR-ESI-MS. Comparison of the 1D NMR data of **3** with those of **1** showed that their chemical shifts were similar except for the signals of the side chain connected with the carbonyl (δ_C 205.2, C-8′). It was plausible to deduce that the propyl group attached to C-8′ in **1** was replaced by a methyl [δ_H 2.72 (3H, s); C-9′], which was subsequently confirmed by the HMBC correlations from H_3-9′ to C-3′ and C-8′. Accordingly, compound **3** was elucidated and

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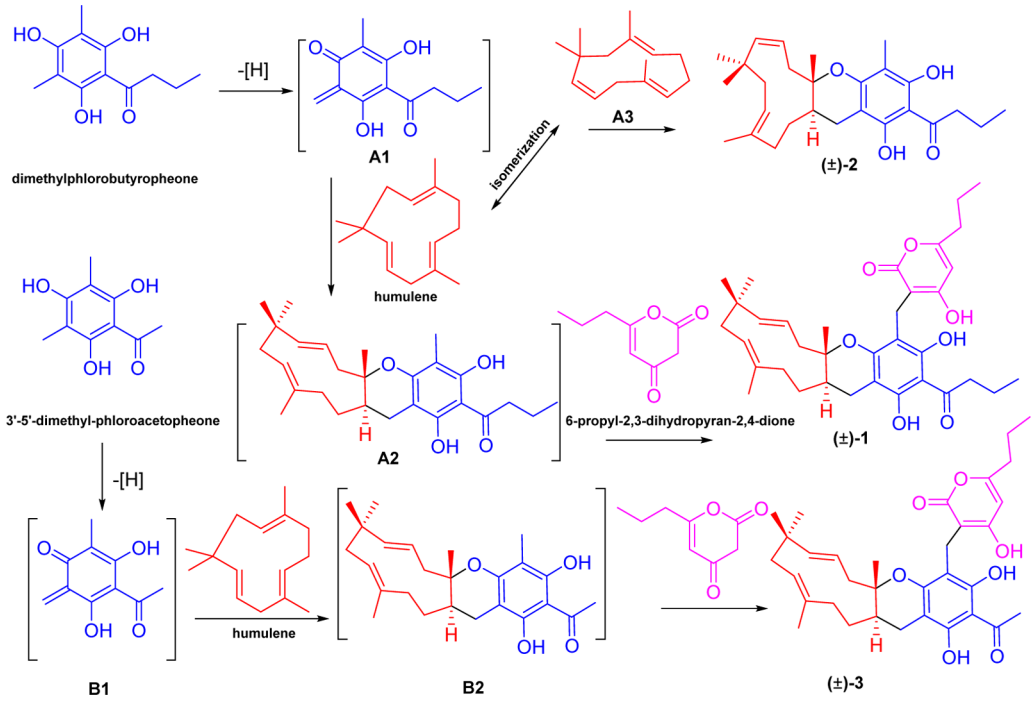
Scheme 1. Hypothetical Biogenetic Pathways of 1−3



named drychampone C. Similarly, the weak optical activity and Cotton effect (see the Supporting Information) indicated that **3** was a racemic mixture as well. However, **3** could not be separated by the present chiral conditions.

In our work, three novel humulene-based meroterpenoids, drychampones A−C (**1−3,** respectively), were obtained from *D. championii* and possessed the hybrid structures bearing unusual 11/6/6 ring system which consisted of diverse phloroglucinol derivatives and the sesquiterpenoid moiety. The previous literatures[16,17] had reported some acyl phloroglucinols from *Dryopteris* genus. Moreover, the key precursors were detected by the analysis of LC-MS and GC-MS (see the Supporting Information). Thus, the plausible biosynthetic pathways for compounds **1−3** were proposed as shown in Scheme 1. Compound **1** was considered to be derived from dimethylphlorobutyrophenone.[16] First, dimethylphlorobutyrophenone was dehydrogenated to generate intermediate **A1**.[18] Then, **A1** could couple with humulene[19] and a isomer of humulene (**A3**) to yield **A2** and **2**, respectively, by the hetero-Diels−Alder-machanism.[18] Finally, **A2** was coupled with 6-propyl-2,3-dihydropyran-2,4-dione[17] to yield **1**. Compound **3** was considered to be originated from 3′,5′-dimethyl-phloroacetophenone,[20] which was reduced to yield **B1**. The intermediate **B1** was coupled with humulene and then 6-propyl-2,3-dihydropyran-2,4-dione to afford **3**.[18]

Phloroglucinol derivatives from Dryopteridaceae plants exhibited diverse and potent bioactivities,[9−11,21] especially for the noteworthy antibacterial activity.[9,22] In our experiments, compounds (±)-**1**, (±)-**2**, and **3**, along with three known phloroglucinols (**4−6**), antibacterial activities were tested against the *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Dickeya zeae*. Compounds **4−6** showed obvious antibacterial activities with the minimal inhibition concentration (MIC) values ranging from 4 to 16 μg/mL (Table 2), and compound **4** displayed the activities with the MIC values ranging from 8 to 16 μg/mL consistent with the data reported.[22] The antibacterial activities of **5** and **6** were reported for the first time. However, the meroterpenoids, (±)-**1**, (±)-**2**, and **3**, were virtually inactive with the (MIC) values more than 128 μg/mL. The same trend was observed for the inhibition of the growth of the above four kinds of bacteria, which might be attributed to the sesquiterpenoid structure of humulene.

Table 2. Minimum Inhibitory Concentration (MIC) of Compounds against Selected Microorganism

	name of the microorganism			
	MIC ± SD [μg/mL][a]			
compounds	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Dickeya zeae*
(−)-**1**	> 128	> 128	> 128	> 128
(+)-**1**	> 128	> 128	> 128	> 128
(−)-**2**	> 128	> 128	> 128	> 128
(+)-**2**	> 128	> 128	> 128	> 128
3	> 128	> 128	> 128	> 128
4	8 ± 1.4	16 ± 1.7	8 ± 1.3	8 ± 0.7
5	8 ± 0.5	4 ± 0.9	16 ± 1.1	16 ± 0.3
6	4 ± 0.7	8 ± 0.5	16 ± 1.3	8 ± 0.7
CPFX[b]	1 ± 0.1	1 ± 0.3	2 ± 0.2	1 ± 0.4

[a]Values present mean ± SD of triplicate experiments. [b]Positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Column chromatography (CC) was performed using silica gel (80−100/200−300/300−400 mesh), Sephadex LH-20 and ODS (50 μm). Thin-layer chromatography (TLC) was performed using precoated silica gel plates (GF254). Analytical HPLC, preparative HPLC, and chiral HPLC isolation were performed using a solvent delivery system with a DAD detector, and an analytical C_{18} analytical column (5 μm, 4.6 × 250 mm), a preparative C_{18} column (5 μm, 20 × 250 mm), and a chiral column (5 μm, 10 × 250 mm), respectively. UV spectra were determined by a UV/vis spectrophotometer using MeOH as the solvent. IR spectra

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were measured using the ATR (attenuated total reflection) method on a FT-IR spectrometer with KBr disks. Optical rotations were recorded on a digital polarimeter. ECD spectra were taken on a spectropolarimeter. NMR spectra were obtained on 500 MHz spectrometer with TMS as an internal standard. The chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz. NMR peak assignments are based on ^{1}H−^{1}H COSY, HSQC, and HMBC spectroscopic data. HR-ESI-MS data were performed on an ESI/TOF mass spectrometer. LC-MS analysis was performed on an analytical HPLC system coupled with a LC/MSD TOF mass spectrometer equipped with an ESI source in positive mode. GC-MS analysis was performed on a GC system coupled with a quadrupole mass spectrometer equipped with an EI source.

Plant Material. The aerial part of plant of *Dryopteris championii* was collected in Haikou City, Hainan Province, China, in February of 2014. The plant was authenticated by Prof. G.-X. Zhou (College of Pharmacy, Jinan University). A voucher specimen (no. 2014021705) was deposited in the Institute of Traditional Chinese Medicine and Natural Products of Jinan University.

Extraction and Isolation. The air-dried and powdered plant (10.0 kg) was extracted with 95% ethanol for 4 times at room temperature (4 × 40 L). The combined extracts were concentrated to afford the crude extract (400.0 g) which was then suspended in water and successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol. The petroleum ether part (250.0 g) was fractionated by silica gel column chromatography (CC) eluted with petroleum ether containing increasing amount of ethyl acetate [100:0 to 0:100 (v/v)] and then MeOH to give eight fractions (A−H). Fraction B was separated by Sephadex LH-20 [2:1 $CHCl_3/CH_3OH$, (v/v)] to give compound **4** (151.2 mg). Fraction C (36.0 g) was rechromatographed on silica gel CC to afford four subfractions (C1−C4). Subfraction C2 (2.1 g) were purified by Sephadex LH-20 [1:1 $CHCl_3/CH_3OH$, (v/v)] to yield compounds **5** (5.6 mg) and **6** (3.7 mg). Fraction D (10.6 g) was chromatographed over an ODS CC using CH_3OH/H_2O in a gradient [60:40 to 100:0, (v/v)] and preparative HPLC [99:1 CH_3OH/H_2O, (v/v)] to yield compounds **1** (4.3 mg) and **3** (8.6 mg). Fraction E was successively separated by ODS [60:40 to 100:0 CH_3OH/H_2O, (v/v)] and preparative HPLC [90:1 CH_3OH/H_2O, (v/v)] to afford **2** (7.5 mg). Finally, two pairs of enantiomers [(+)-**1** (1.2 mg), (−)-**1** (1.1 mg), (+)-**2** (3.1 mg), and (−)-**2** (3.1 mg)] were successfully separated by chiral HPLC column using CH_3CN/CH_3COOH [100:0.1, (v/v)] and $CH_3OH/H_2O/CH_3COOH$ [92:8:0.1, (v/v)] as the mobile phase, respectively.

Drychadial A (**1**). Yellow powder; mp 102−103 °C; [α]27 D + 25.3 [(+)-**1**] (c 0.25, CH_3Cl); [α]27 D −25.6 [(−)-**1**] (c 0.37, CH_3Cl); UV (MeOH) λ_{max} 206, 295 nm; IR (KBr) ν_{max} 3426, 2962, 2937, 1637, 1603, 1409, 1369, 1189, 1110 cm^{-1}; HR-ESI-MS m/z 579.3319 [M+H]$^+$ (calcd for $C_{35}H_{47}O_7$ m/z 579.3316); ^{1}H and ^{13}C NMR data in Table 1.

Drychadial B (**2**). Yellow powder; mp 110−112 °C; [α]27 D + 32.1 [(+)-**2**] (c 0.30, CH_3Cl), [α]27 D −31.8 [(−)-**2**] (c 0.34, CH_3Cl); UV (MeOH) λ_{max} 204, 295 nm; IR (KBr) ν_{max} 3426, 2962, 2934, 1606, 1412, 1369, 1191, 1108 cm^{-1}; HR-ESI-MS m/z 427.2845 [M+H]$^+$ (calcd for $C_{27}H_{39}O_4$ m/z 427.2843); ^{1}H and ^{13}C NMR data in Table 1.

Drychadial C (**3**). Yellow powder; mp 106−107 °C; [α]27 D + 1.2 (c 0.47, CH_3Cl); UV (MeOH) λ_{max} 205, 296 nm; IR (KBr) ν_{max} 3427, 2959, 2934, 1634, 1603, 1409, 1372, 1189, 1108 cm^{-1}; HR-ESI-MS m/z 551.2997 [M+H]$^+$ (calcd for $C_{33}H_{43}O_7$ m/z 551.3003); ^{1}H and ^{13}C NMR data in Table 1.

Aspidin BB (**4**).[4] Needle crystal ($CHCl_3$); mp 123−124 °C; HR-ESI-MS m/z 461.2178 [M+H]$^+$ (calcd for $C_{25}H_{33}O_8$ m/z 461.2170); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.50 (1H, s, 4-OH), 10.00 (1H, s, 6′−OH), 3.73 (3H, s, H-7′), 3.56 (2H, br s, H-7), 3.17 (2H, t, J = 7.3 Hz, H-9′), 3.08 (2H, t, J = 7.3 Hz, H-9), 2.14 (3H, s, H-12′), 1.75 (4H, m, H-10, 10′), 1.51 (3H, s, H-13), 1.45 (3H, s, H-12), 0.99 (6H, m, H-11, 11′); ^{13}C NMR (125 MHz, $CDCl_3$), 111.1 (C-1), 187.7 (C-2), 108.3 (C-3), 198.8 (C-4), 44.4 (C-5), 172.0 (C-6), 17.3 (C-7), 206.8 (C-8), 43.2 (C-9), 18.4 (C-10), 14.2 (C-11), 24.0 (C-12), 25.4 (C-13), δ 112.6 (C-1′), 163.0 (C-2′), 107.7 (C-3′), 159.8 (C-4′), 109.6 (C-5′), 160.4 (C-6′), 61.7 (C-7′), 207.0 (C-8′), 44.4 (C-9″), 18.2 (C-10′), 14.1 (C-11′), 9.4 (C-12′).

Desaspidin BB (**5**).[12] Yellow powder; mp 151−153 °C; HR-ESI-MS m/z 447.2014 [M+H]$^+$ (calcd for $C_{24}H_{31}O_8$ m/z 447.2013); ^{1}H NMR (500 MHz, $CDCl_3$) δ 18.55 (1H, s, 4-OH), 13.89 (1H, s, 4′−OH), 11.50 (1H, s, 2′−OH), 8.93 (1H, s, 6-OH), 6.07 (1H, s, H-5′), 3.97 (3H, s, H-7′), 3.52 (2H, br s, H-7), 3.17 (4H, m, H-9, 9′), 1.69 (4H, m, H-10, 10′), 1.48 (6H, s, H-12, 13), 0.99 (6H, m, H-11, 11′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.4 (C-1), 187.7 (C-2), 108.2 (C-3), 198.7 (C-4), 44.3 (C-5), 171.3 (C-6), 17.0 (C-7), 207.0 (C-8), 43.2 (C-9), 18.3 (C-10), 14.2 (C-11), 24.8 (C-12, 13), 105.0 (C-1′), 160.3 (C-2′), 107.3 (C-3′), 165.6 (C-4′), 92.0 (C-5′), 160.9 (C-6′), 56.6 (C-7′), 206.8 (C-8′), 46.6 (C-9′), 18.4 (C-10′), 14.1 (C-11′).

Desaspidin PB (**6**).[13] Yellow powder; mp 150−152 °C; HR-ESI-MS m/z 433.1862 [M+H]$^+$ (calcd for $C_{23}H_{28}O_8$ m/z 433.1857); ^{1}H NMR (500 MHz, $CDCl_3$) δ 18.55 (1H, s, 4-OH), 13.88 (1H, s, 4′−OH), 11.45 (1H, s, 2′−OH), 8.94 (1H, s, 6-OH), 6.07 (1H, s, H-5′), 3.97 (3H, s, H-7′), 3.53 (2H, br s, H-7), 3.22 (2H, q, J = 7.3, H-9), 3.14 (2H, t, J = 7.3 Hz, H-9′), 1.70 (2H, m, H-10′), 1.48 (6H, s, H-12, 13), 1.17 (3H, t, J = 7.3 Hz, H-10), 0.99 (3H, t, J = 7.3 Hz, H-11′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.4 (C-1), 187.7 (C-2), 108.2 (C-3), 198.1 (C-4), 44.1 (C-5), 171.3 (C-6), 17.0 (C-7), 207.7 (C-8), 35.1 (C-9), 8.6 (C-10), 24.8 (C-12, 13), 105.0 (C-1′), 160.3 (C-2′), 107.3 (C-3′), 165.6 (C-4′), 92.0 (C-5′), 160.9 (C-6′), 56.6 (C-7′), 207.7 (C-8′), 46.6 (C-9′), 18.2 (C-10′), 14.2 (C-11′).

Antibacterial Assay. The minimum inhibitory concentration (MIC) was determined by 96-well microtiter plate assay method. Four kinds of bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Dickeya zeae* (provided by Plant Protection Research Institute Guangdong Academy of Agricultural Sciences) were tested. All the glassware and media used were sterilized in an autoclave at 121 °C, 15 psi pressure for 20 min, and experiment was performed under strict aseptic conditions. The active bacterial strain was obtained after inoculated in 100 mL of LB broth in a conical flask and incubated for 24 h at 37 °C and 200 rpm. The 96-well microtiter plate assay method was undertaken to determine the MIC of each compound with double broth dilution method, using standard antibiotic [Ciprofloxacin hydrochloride (CPFX)] as the positive drug. A volume of 5 μL of test compound solution (dissolved in dimethyl sulfoxide) with 195 μL LB broth was mixed uniformly and filled in relevant wells. Then, serial dilutions were attained with the double broth dilution method. Finally, 10 μL of the bacterial suspension (1 × 10^8 CFU/mL) was added to each well except for the negative control. As a result, four kinds of bacterial were treated with the concentration of 128, 64, 32, 16, 8, 4, and 2 μg/mL of tested compound. The plates were prepared and incubated at 37 °C for 24 h. Each experiment was performed in triplicate, and the MIC is the lowest concentration without visible growth.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01720.

Detailed NMR, HR-ESI-MS, UV, and IR spectra of **1**−**3**; chemical calculation details for (±)-**1** and (±)-**2** (PDF)

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Notes

The authors declare no competing financial interest.

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