JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Phthalide and Isocoumarin Derivatives Produced by an *Acremonium* sp. Isolated from a Mangrove *Rhizophora apiculata*

Vatcharin Rukachaisirikul,*[,†] Aekkachai Rodglin,[†] Yaowapa Sukpondma,[†] Souwalak Phongpaichit,[‡] Jirayu Buatong,[‡] and Jariya Sakayaroj[§]

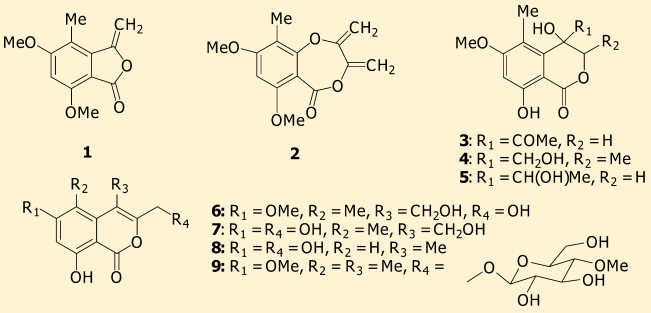
[†]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[‡]Natural Products Research Center and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[§]National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand

Supporting Information

ABSTRACT: Nine new fungal metabolites, one phthalide derivative, acremonide (**1**), and eight isocoumarin derivatives, acremonones A–H (**2**–**9**), were isolated from the mangrove-derived fungus *Acremonium* sp. PSU-MA70 together with 10 known compounds. Their structures were determined by NMR analysis. The known 8-deoxytrichothecin and trichodermol exhibited moderate antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, respectively.



The genus *Acremonium* produces various bioactive compounds, such as antibacterial acremoxanthone A,[1] antifungal dihydroresorcylide,[2] antioxidant acremonin A,[3] cytotoxic awajanoran,[4] and anti-inflammatory ascofuranone.[5] During our ongoing search for bioactive metabolites from mangrove-derived fungi, we discovered that the crude extract from a culture broth of the endophytic fungus *Acremonium* sp. PSU-MA70 isolated from a branch of *Rhizophora apiculata*, a mangrove plant, exhibited antifungal activity against standard *Candida albicans* NCPF3153 and *Cryptococcus neoformans* ATCC90113. We describe herein the isolation and structural elucidation of nine new compounds including one phthalide derivative, acremonide (**1**), and eight isocoumarin derivatives, acremonones A–H (**2**–**9**), together with 10 known compounds, (+)-brefeldin A,[6] guangomide A,[7] guangomide B,[7] 8-deoxytrichothecin,[8] trichodermol,[9] 4-methyl-1-phenyl-2,3-hexanediol,[10] (2*R*,3*R*)-4-methyl-1-phenyl-2,3-pentanediol,[11] 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide,[12] Sch 54794,[13] and Sch 54796.[13] Their antifungal activity against *C. albicans* and *C. neoformans* was evaluated.

All compounds were isolated using chromatographic techniques, and their structures were elucidated on the basis of UV, IR, NMR, and MS analysis. For the known compounds, their ^{1}H and ^{13}C NMR data were compared with previously reported data. The absolute configuration of the known compounds was determined by comparison of their specific rotations with those previously reported.

RESULTS AND DISCUSSION

Acremonide (**1**) was obtained as a white solid and had the molecular formula $C_{12}H_{12}O_4$. The IR spectrum exhibited an absorption band at 1760 cm^{-1} for a phthalide carbonyl group.[14] Comparison of the ^{1}H and ^{13}C NMR (Table 1) and HMBC (see Supporting Information) data of **1** with those of 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide revealed the replacement of a methyl signal (H_3-10, δ_H 1.82; δ_C 25.7)[12] and a dioxyquaternary sp^3 carbon (C-3, δ_C 104.5) in 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide with signals of a *gem*-olefinic alkene (H_2-10, δ_H 5.30 and 5.19, each 1H, d, J = 2.7 Hz; δ_C 95.2) and an oxyquaternary sp^2 carbon (C-3, δ_C 152.4) in **1**, respectively. The HMBC correlations from H_2-10 to C-3 and C-4 (δ_C 139.1) confirmed the location of the terminal double bond. Consequently, acremonide (**1**) was a dehydrate derivative of 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide.

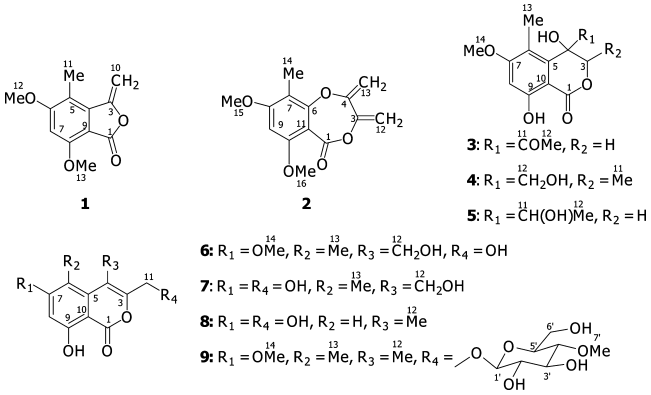
Acremonone A (**2**), a white solid, had the molecular formula $C_{14}H_{14}O_5$, representing eight degrees of unsaturation. The IR spectrum exhibited an absorption band at 1745 cm^{-1} for a lactone carbonyl group. The ^{1}H NMR (Table 1) and HMBC (see Supporting Information) data indicated that it possessed a pentasubstituted benzene, similar to that of **1**. In addition, the ^{1}H NMR spectrum displayed signals for two sets of terminal olefinic protons, δ_H 5.16 and 5.11 (each 1H, d, J = 1.8 Hz) and 4.95 and 4.86 (each 1H, d, J = 1.5 Hz). Apart from the carbon

Received: July 30, 2011
Published: April 23, 2012

Table 1. ^{1}H and ^{13}C NMR Data for Acremonide (1) and Acremonones A (2), B (3), C (4), and D (5) in $CDCl_3$

	1[a]		2[b]		3[c]		4[c]		5[c]	
position	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)
1	157.8, C		162.4, C		169.4, C		169.9, C		169.9, C	
3	152.4, C		156.2, C		71.4, CH_2	4.39, d (12.0) 4.19, d (12.0)	79.5, CH	4.44, q (6.5)	70.4, CH_2	4.46, d (10.5) 4.14, d (10.5)
4	139.1, C		148.9, C		76.1, C		73.4, C		74.3, C	
4-OH						4.50, s		2.97, s		2.75, s
5	114.1, C				136.9, C		141.0, C		139.0, C	
6	164.3, C		153.3, C		118.3, C		117.3, C		117.9, C	
7	96.0, CH	6.50, s	114.2, C		165.3, C		165.2, C		165.1, C	
8	157.8, C		162.3, C		99.9, CH	6.52, s	99.0, CH	6.39, s	98.7, CH	6.46, s
9	105.5, C		92.5, CH	6.31, s	164.4, C		163.4, C		163.5, C	
9-OH						11.59, s		11.52, s		11.64, s
10	95.2, CH_2	5.30, d (2.7) 5.19, d (2.7)	158.9, C		100.2, C		100.3, C		99.6, C	
11	11.0, CH_3	2.30, s	105.0, C		206.4, C		14.7, CH_3	1.51, d (6.5)	69.0, CH	4.17, q (6.5)
12	56.4, CH_3	3.96, s	106.6, CH_2	5.16, d (1.8) 5.11, d (1.8)	25.4, CH_3	2.26, s	62.6, CH_2	4.02, d (11.0) 3.72, d (11.0)	17.4, CH_3	1.20, d (6.5)
13	56.1, CH_3	4.01, s	97.3, CH_2	4.95, d (1.5) 4.86, d (1.5)	11.4, CH_3	1.96, s	12.2, CH_3	2.27, s	12.3, CH_3	2.42, s
14			7.9, CH_3	2.10, s	56.0, CH_3	3.87, s	55.9, CH_3	3.79, s	55.9, CH_3	3.88, s
15			55.8, CH_3	3.88, s						
16			56.4, CH_3	3.89, s						

[a]Data were measured at 300 MHz (^{1}H) and 125 MHz (^{13}C). [b]Data were measured at 300 MHz (^{1}H) and 75 MHz (^{13}C). [c]Data were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).



resonances of the pentasubstituted benzene, the ^{13}C NMR spectrum (Table 1) consisted of one carbonyl carbon (δ_C 162.4) and four carbons of two *gem*-disubstituted alkenes (δ_C 156.2/106.6 and 148.9/97.3). All terminal olefinic protons, H_2-12 (δ_H 5.16 and 5.11) and H_2-13 (δ_H 4.95 and 4.86), exhibited HMBC correlations with C-3 (δ_C 156.2) and C-4 (δ_C 148.9), constructing a 1,3-butadienyl unit with two oxy substituents at C-3 and C-4. This unit was linked to C-1 and C-6 through ester and ether linkages, respectively, on the basis of the HMBC correlations of H_2-12/C-1 (δ_C 162.4), the chemical shift of C-6 (δ_C 153.3), and the degrees of unsaturation. Hydrogenation of **2** with H_2 and Pd/C provided a tetrahydro derivative of which the ^{1}H and ^{13}C data for the right ring (see Supporting Information) are comparable with a synthetic compound.[15] Thus, the structure **2** was assigned for acremonone A.

Acremonone B (**3**), with the molecular formula $C_{13}H_{14}O_6$, was obtained as a pale yellow gum. The IR spectrum exhibited absorption bands at 3432, 1716, and 1673 cm^{-1} for hydroxyl, ketone carbonyl, and conjugated lactone carbonyl groups, respectively. Detailed analysis of the ^{1}H and ^{13}C NMR (Table 1) and HMBC (Figure 1) data indicated that **3** has a pentasubstituted benzene similar to that in **1** but carrying a

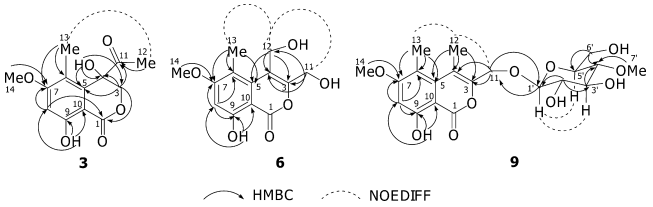


Figure 1. Selected HMBC and NOEDIFF data for compounds **3**, **6**, and **9**.

chelated hydroxyl group (9-OH, δ_H 11.59, s) at a *peri* position to the lactone carbonyl group instead of a methoxyl group. In addition, the ^{1}H NMR spectrum (Table 1) displayed signals for one hydroxyl proton (δ_H 4.50, s), one set of nonequivalent oxymethylene protons (δ_H 4.39 and 4.19, each 1H, d, J = 12.0 Hz), and one methyl group (δ_H 2.26, s). The ^{13}C NMR spectrum (Table 1) showed resonances for one ketone carbonyl (δ_C 206.4), one lactone carbonyl (δ_C 169.4), one oxygenated quaternary (δ_C 76.1), one oxymethylene (δ_C 71.4), and one methyl (δ_C 25.4). The HMBC correlations of the nonequivalent oxymethylene protons (H_2-3, δ_H 4.39 and 4.19), the methyl protons (H_3-12, δ_H 2.26), and 4-OH (δ_H 4.50) (Figure 1) established a hydroisocoumarin derivative with a hydroxyl group and an acetyl moiety at C-4. Irradiation of H_3-12 in the NOEDIFF experiment enhanced the signal intensity of H_3-13 (δ_H 1.96), supporting the above assignment. Thus, acremonone B had the structure **3**.

Acremonone C (**4**), a pale yellow gum, had the molecular formula $C_{13}H_{16}O_6$. The IR spectrum was similar to that of **3** with the absence of an absorption band for a ketone functional group. The ^{1}H NMR data (Table 1) were almost identical to

dx.doi.org/10.1021/np200885e | J. Nat. Prod. 2012, 75, 853–858

Table 2. ^{1}H and ^{13}C NMR Data for Acremonones E (6), F (7), G (8), and H (9)[a]

	6[b]		7[b]		8[b]		9[c]	
position	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)	δ_C	δ_H mult (J, Hz)
1	167.3, C		167.0, C		167.0, C		167.0, C	
3	156.8, C		156.8, C		153.0, C		147.0, C	
4	116.8, C		117.0, C		111.8, C		115.3, C	
5	137.7, C		138.8, C		142.0, C		137.5, C	
6	114.8, C		113.9, C		102.2, CH	6.56, d, 2.0	114.5 C	
7	166.7, C		165.4, C		166.6, C		165.5, C	
8	99.1, CH	6.67, s	102.6, CH	6.58, s	102.7, CH	6.46, d, 2.0	98.8, CH	6.58, s
9	163.9, C		163.5, C		165.0, C		162.9, C	
9-OH		11.93, s		11.84, s		11.49, s		11.85, s
10	101.2, C		101.0, C		100.5, C		100.5, C	
11	60.3, CH_2	4.60, d, 5.5	60.4, CH_2	4.61, d, 5.5	59.6, CH_2	4.51, d, 5.5	66.5, CH_2	4.68, d (12.5)
								4.62, d (12.5)
11-OH		4.94, br s		4.84, t, 5.5		4.65, br s		
12	57.6, CH_2	4.76, d, 4.5	57.6, CH_2	4.78, d, 4.5	12.0, CH_3	2.19, s	17.2, CH_3	2.41, s
12-OH		4.49, br s		4.38, t, 4.5				
13	12.1, CH_3	2.56, s	12.1, CH_3	2.58, s			13.7, CH_3	2.38, s
14	56.7, CH_3	3.98, s					56.0, CH_3	3.92, s
1′							102.1, CH	4.43, d (8.0)
2′							74.0, CH	3.41, dd (9.0, 8.0)
3′							76.4, CH	3.66, t (9.0)
4′							79.1, CH	3.24, t (9.0)
5′							75.7, CH	3.36, ddd (9.0, 4.5, 3.0)
6′							62.0, CH_2	3.92, m
								3.76, br d, (12.0)
7′							60.7, CH_3	3.60, s

[a]Data were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Data were measured in acetone-d_6. [c]Data were measured in $CDCl_3$.

those of **3** except for the replacement of signals for H_2-3 and H_3-12 in **3** with those for a 1-substituted ethoxyl group (H-3, δ_H 4.44, q, J = 6.5 Hz, and H_3-11, δ_H 1.51, d, J = 6.5 Hz) and nonequivalent hydroxymethyl protons (H_2-12, δ_H 4.02 and 3.72, each 1H, d, J = 11.0 Hz). The HMBC correlations from H_2-12 to C-3 (δ_C 79.5), C-4 (δ_C 73.4), and C-5 (δ_C 141.0) and those from H-3 to C-1 (δ_C 169.9), C-5, C-11 (δ_C 14.7), and C-12 (δ_C 62.6) established an isochromanone derivative with the methyl group at C-3 and both hydroxyl and hydroxymethyl groups at C-4. Irradiation of H_2-12 affected the signal intensity of H_3-11 in the NOEDIFF experiment, indicating a *cis* relationship between the hydroxymethyl and methyl groups. Therefore, acremonone C possessed the structure **4**.

Acremonone D (**5**) was obtained as a pale yellow gum and displayed the molecular formula $C_{13}H_{16}O_6$, indicating two mass units higher than that of **3**. A ketone carbonyl absorption band was absent in the IR spectrum of **5**. Analysis of the ^{1}H NMR data (Table 1) indicated the replacement of the acetyl signal (H_3-12, δ_H 2.26) in **3** with signals for a 1-substituted 1-hydroxyethyl unit (H-11, δ_H 4.17, q, J = 6.5 Hz and H_3-12, δ_H 1.20, d, J = 6.5 Hz). These results revealed that the acetyl group in **3** was reduced to a corresponding alcohol in **5**. The HMBC correlations of H-11 with C-3 (δ_C 70.4) and C-5 (δ_C 139.0) confirmed the above conclusion. Irradiation of H_3-12 enhanced the signal intensity of H_3-13 (δ_C 2.42) in the NOEDIFF experiment, supporting above assignment. Thus, acremonone D (**5**) was an 11-hydroxy derivative of **3**.

Acremonone E (**6**) was obtained as a pale yellow gum and had the molecular formula $C_{13}H_{14}O_6$ (seven degrees of unsaturation). The IR spectrum exhibited absorption bands similar to those of **4** and **5**. The ^{1}H and ^{13}C NMR (Table 2) and HMBC (Figure 1) data indicated that **6** possessed a pentasubstituted benzoyl unit, identical to that of compounds **3**, **4**, and **5**. In addition, the ^{1}H NMR spectrum consisted of signals for two sets of hydroxymethyl protons (δ_H 4.76, d, J = 4.5 Hz, 2H and 4.49, br s, 1H, and δ_H 4.60, d, J = 5.5 Hz, 2H and 4.94, br s, 1H). The ^{13}C NMR spectrum contained, apart from the ^{13}C signals of the pentasubstituted benzoyl moiety, two quarternary sp^2 (δ_C 156.8 and 116.8) and two oxymethylene (δ_C 60.3 and 57.6) carbons. The hydroxymethyl protons (H_2-11, δ_H 4.60) exhibited HMBC correlations with C-3 (δ_C 156.8), C-4 (δ_C 116.8), and C-12 (δ_C 57.6), while the other hydroxymethyl protons (H_2-12, δ_H 4.76) displayed the same correlations with C-3, C-4, and C-5 (δ_C 137.7). Accordingly, a vinyl (C3/C4) fragment with the hydroxymethyl substituents at both C-3 and C-4 was connected to C-5 of the pentasubstituted benzoyl unit. Signal enhancement of H_2-11 and H_3-13 (δ_H 2.56) upon irradiation of H_2-12 in the NOEDIFF experiment confirmed the assignment. The remaining one degree of unsaturation together with the chemical shifts of C-1 and C-3 established a lactone linkage between C-1 and C-3 to form an isocoumarin skeleton. Therefore, acremonone F had the structure **6**.

Acremonone F (**7**), with the molecular formula $C_{12}H_{12}O_6$ from HREIMS, was obtained as a pale yellow gum. The IR spectrum exhibited absorption bands similar to those of **6**. The ^{1}H NMR data (Table 2) indicated that the methoxyl group in **6** was replaced by a hydroxyl group. The chemical shift of C-7 (δ_C 165.4) confirmed the attachment of the hydroxyl group at this carbon. Thus, acremonone F (**7**) was identified as a demethylated derivative of **6**.

Acremonone G (**8**) was obtained as a pale yellow gum and had the molecular formula $C_{11}H_{10}O_5$. The IR spectrum exhibited absorption bands similar to those of **7**. The ^{1}H

dx.doi.org/10.1021/np200885e | J. Nat. Prod. 2012, 75, 853–858

NMR data (Table 2) differed from those of **7** in the presence of signals for two *meta*-coupled aromatic protons (δ_H 6.56 and 6.46, both as d, J = 2.0 Hz) and one hydroxymethyl group (δ_H 4.51, d, J = 5.5 Hz, 2H and 4.65, br s, 1H). The *meta*-coupled aromatic protons resonating at δ_H 6.56 and 6.46 were attributed to H-6 and H-8, respectively, on the basis of their HMBC correlations (see Supporting Information), thus revealing that the methyl group at C-6 in **7** was replaced by the *meta*-coupled aromatic proton in **8**. The methyl group in **8**, resonating at δ_H 2.19, was placed at C-4 (δ_C 111.8) on the basis of the HMBC correlations of H_3-12 with C-3 (δ_C 153.0) and C-5 (δ_C 142.0). Signal enhancement of H-6 and H_2-11 (δ_H 4.51) in the NOEDIFF experiment upon irradiation of H_3-12 confirmed the location of the methyl and the hydroxymethyl groups. Thus, acremonone G possessed the structure **8**.

Acremonone H (**9**), with the molecular formula $C_{20}H_{26}O_{10}$ from HREIMS, was obtained as a white solid. The IR spectrum exhibited absorption bands similar to those of **6**. The ^{1}H NMR data (Table 2) were almost identical to those of **6**. The differences were the replacement of signals for one hydroxymethyl group in **6** with a methyl signal (δ_H 2.41, s) in **9** and additional signals for a β-glucopyranose unit [δ_H 4.43 (d, J = 8.0 Hz), 3.92 (m), 3.76 (br d, J = 12.0 Hz), 3.66 (t, J = 9.0 Hz), 3.41 (dd, J = 9.0 and 8.0 Hz), 3.36 (ddd, J = 9.0, 4.5, 3.0 Hz), and 3.24 (t, J = 9.0 Hz), each 1H][16,17] and a methoxyl group (δ_H 3.60, s). The large coupling constants ($J_{1',2'}$ = 8.0 Hz and $J_{2',3'} = J_{3',4'} = J_{4',5'}$ = 9.0 Hz) as well as signal enhancement of H-3′ (δ_H 3.66) and H-5′ (δ_H 3.36) upon irradiation of H-1′ (δ_H 4.43) (Figure 1) supported the presence of the β-glucopyranose unit. The methoxyl protons (δ_H 3.60) exhibited an HMBC correlation with C-4′ (δ_C 79.1) (Figure 1), thus linking the methoxyl group at C-4′ of the sugar moiety. The attachment of the sugar moiety at C-11 (δ_C 66.5) was established on the basis of the HMBC correlation from H-1′ to C-11. The methyl group (H_3-12, δ_H 2.41) was located at C-4 (δ_C 115.3) according to the HMBC correlations of H_3-12 with C-3 (δ_C 147.0), C-4, and C-5 (δ_C 137.5) as well as signal enhancement of H_2-11 (δ_H 4.68 and 4.62, each 1H, d, J = 12.5 Hz) and H_3-13 (δ_H 2.38) upon irradiation of H_3-12. The specific rotation of **9**, $[\alpha]^{26}_D$ −46 (c 0.05, MeOH), was similar to that of 6,8-dihydroxy-5-methoxy-3-methylisocoumarin 6-*O*-(4-*O*-methyl-β-D-glucopyranoside, $[\alpha]^{23}_D$ −55 (c 0.17, MeOH).[17] The absolute configuration of the sugar moiety was not determined due to lack of sample. Consequently, acremonone H (**9**) was identified as a new isocoumarin 4-*O*-methyl-β-D-glucopyranoside.

The crude extract displayed weak antifungal activity against *C. albicans* NCPF3153 and *C. neoformans* ATCC90113 with equal MIC values of 128 μg/mL. The isolated compounds **1**−**2**, **6**, (+)-brefeldin A, guangomide A, guangomide B, 8-deoxytrichothecin, trichodermol, 4-methyl-1-phenyl-2,3-hexanediol, 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide, and Sch 54796, which were obtained in sufficient amounts, were then tested against both fungi. For *C. albicans*, (+)-brefeldin A and 8-deoxytrichothecin exhibited moderate activity with MIC values of 32 and 16 μg/mL, respectively. These compounds were much less active against *C. neoformans*. Trichodermol gave a moderate effect against *C. neoformans* (MIC 32 μg/mL) and *C. albicans* (MIC 64 μg/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-1600 spectrophotometer. Infrared spectra (IR) were recorded on a Perkin-Elmer 783 FTS 165 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 500 MHz Bruker FTNMR Ultra Shield spectrometers. Mass spectra were measured on a MAT 95 XL mass spectrometer (Thermo Finnigan). Thin-layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{256} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70−230 mesh ASTM) with a gradient system of MeOH−CH_2Cl_2, on Sephadex LH-20 with MeOH, or as otherwise stated.

Fungal Material. The mangrove-derived fungus *Acremonium* sp. PSU-MA70 was isolated from a branch of *R. apiculata*, collected from Satun Province, Thailand, in 2007. This fungus was deposited as PSU-MA70 (GenBank accession number GU592000 and GU592010) at the Department of Microbiology, Faculty of Science, Prince of Songkla University, and as BCC 35914 at the National Center for Generic Engineering and Biotechnology (BIOTEC) Culture Collection, Thailand.

Fungal Identification. The fungus was identified on the basis of the analyses of the LSU and ITS regions of its rDNA gene. Its LSU sequence (GU592010) matched with the closely related sequence of *Acremonium alternatum* FJ176883 with 82% bootstrap support. Moreover, its ITS sequence (GU592000) is well placed with Hypocreales species (EU164804) *Acremonium crotocinigenum* DQ882846 and AJ621773 as sister taxa with high statistical support (98%) and sequence similarity between 91% and 100%. The fungus PSU-MA70 was then identified as *Acremonium* sp.

Fermentation, Extraction, and Isolation. The crude EtOAc extract (2.11 g, dark brown gum) from the culture broth of the fungus PSU-MA70 (15 L) was prepared using the same procedure as described previously.[18] It was dissolved with methanol to afford (+)-brefeldin A (890 mg) and a methanol-soluble fraction (1.2 g). The methanol-soluble fraction was separated by CC over Sephadex-LH 20 to give six fractions (A−F). Fraction A (90.2 mg) was purified by CC over silica gel with 20% ethyl acetate in dichloromethane to yield three subfractions (A1−A3). Subfraction A3 (11.1 mg) was further subjected to CC over silica gel with 2% methanol in dichloromethane to afford guangomide A (5.4 mg). Fraction B (199.6 mg) was purified using the same procedure as subfraction A3 to afford four subfractions (B1−B4). Purification of subfraction B2 by CC over Sephadex-LH 20 afforded guangomide B (8.5 mg). Fraction C (794.7 mg) was separated by CC over silica gel using 1% methanol in dichloromethane to yield six subfractions (C1−C6). Purification of subfraction C3 (13.2 mg) by CC over silica gel with a solvent mixture of ethyl acetate, dichloromethane, and petroleum ether in a ratio of 1:2:7 furnished 8-deoxytrichothecin (4.0 mg). Subfraction C4 (156.4 mg) was purified by CC over Sephadex-LH 20 to give four subfractions (C41−C44). Subfraction C42 (37.7 mg) was purified using the same procedure as fraction A to afford 4-methyl-1-phenyl-2,3-hexanediol (17.0 mg). Subfraction C43 (39.7 mg) was subjected to CC over silica gel using 20% acetone in hexane followed by PTLC with 2% acetone in dichloromethane to give (2*R*,3*R*)-4-methyl-1-phenyl-2,3-pentanediol (2.5 mg). Trichodermol (6.1 mg) was obtained from subfraction C5 (28.0 mg) upon purification on CC over silica gel with 30% ethyl acetate in petroleum ether. Subfraction C6 (258.6 mg) was purified by CC over silica gel with 40% acetone in hexane as a mobile phase to afford four subfractions (C61−C64). Subfraction C62 (115.0 mg) was further separated by CC over reversed-phase silica gel using 50% methanol in water followed by PTLC with 40% ethyl acetate in petroleum ether to give 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide (3.7 mg). Subfraction C63 (14.1 mg) was further purified using the same procedure as subfraction A3 to yield Sch 54794 (1.5 mg) and Sch 54796 (5.7 mg). Fraction D (68.2 mg) was separated using the same procedure as subfraction C62 to give six subfractions (D1−D6). Subfraction D3 (15.3 mg) was purified using the same procedure as fraction C to yield five subfractions (D31−D35). Subfractions D31 and D32 were further purified by PTLC with 1% methanol in dichloromethane to give **3** (2.2 mg) and **4** (1.2 mg), respectively. Subfraction D34 was further separated by PTLC with 60% ethyl

acetate in dichloromethane to furnish **9** (2.0 mg). Subfraction D5 (9.0 mg) was further purified by PTLC with 2% methanol in dichloromethane to give **1** (2.7 mg) and **2** (3.1 mg). Fraction E (48.7 mg) was separated using the same procedure as fraction C to yield six subfractions (E1−E6). Subfraction E4 (8.0 mg) was further purified by PTLC with 10% ethyl acetate in dichloromethane to afford **5** (0.7 mg). Subfraction E5 (9.7 mg) was further separated by PTLC with 30% ethyl acetate in petroleum ether to give **6** (3.7 mg). Fraction F (7.1 mg) was further purified using the same procedure as subfraction D5 to yield **7** (0.8 mg) and **8** (0.8 mg).

Acremonide (1): white solid; mp 167−169 °C; UV (MeOH) λ_{max} (log ε) 202 (3.55), 240 (3.63), 278 (3.06), 331 (2.93) nm; IR (neat) ν_{max} 1760 cm^{-1}; HREIMS m/z [M]$^+$ 220.0738 (calcd for $C_{12}H_{12}O_4$, 220.0736); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

Acremonone A (2): white solid; mp 184−186 °C; UV (MeOH) λ_{max} (log ε) 212 (3.67), 254 (3.08), 296 (2.93) nm; IR (neat) ν_{max} 1745 cm^{-1}; HREIMS m/z [M]$^+$ 262.0840 (calcd for $C_{14}H_{14}O_5$, 262.0841); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

Acremonone B (3): pale yellow gum; $[\alpha]^{25}_D$ −68 (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.84), 268 (3.45), 315 (3.26) nm; IR (neat) ν_{max} 3432, 1716, 1673 cm^{-1}; HREIMS m/z [M]$^+$ 266.0787 (calcd for $C_{13}H_{14}O_6$, 266.0790); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

Acremonone C (4): pale yellow gum; $[\alpha]^{25}_D$ −25 (c 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.84), 217 (3.81), 268 (3.39), 312 (3.18) nm; IR (neat) ν_{max} 3394, 1670 cm^{-1}; HREIMS m/z [M]$^+$ 268.0958 (calcd for $C_{13}H_{16}O_6$, 268.0947); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

Acremonone D (5): pale yellow gum; $[\alpha]^{25}_D$ −33 (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.91), 217 (3.77), 230 (3.47), 270 (3.35), 314 (3.14) nm; IR (neat) ν_{max} 3370, 1665 cm^{-1}; HREIMS m/z [M]$^+$ 268.0934 (calcd for $C_{13}H_{16}O_6$, 268.0947); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

Acremonone E (6): pale yellow gum; UV (MeOH) λ_{max} (log ε) 202 (3.80), 249 (3.84), 333 (2.73) nm; IR (neat) ν_{max} 3352, 1666 cm^{-1}; HREIMS m/z [M]$^+$ 266.0789 (calcd for $C_{13}H_{14}O_6$, 266.0790); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 2; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2.

Acremonone F (7): pale yellow gum; UV (MeOH) λ_{max} (log ε) 200 (3.86), 247 (3.47) nm; IR (neat) ν_{max} 3356, 1674 cm^{-1}; HREIMS m/z [M]$^+$ 252.0642 (calcd for $C_{12}H_{12}O_6$, 252.0634); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 2; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2.

Acremonone G (8): pale yellow gum; UV (MeOH) λ_{max} (log ε) 201 (3.84), 219 (3.41), 246 (3.72), 328 (2.25) nm; IR (neat) ν_{max} 3356, 1674 cm^{-1}; HREIMS m/z [M]$^+$ 222.0539 (calcd for $C_{11}H_{10}O_5$, 222.0528); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 2; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2.

Acremonone H (9): white solid; mp 180−182 °C; $[\alpha]^{26}_D$ −46 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.86), 249 (3.75), 305 (2.33), 335 (2.85) nm; IR (neat) ν_{max} 3370, 1665 cm^{-1}; HREIMS m/z [M]$^+$ 426.1529 (calcd for $C_{20}H_{26}O_{10}$, 426.1526); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Antifungal Assays. Crude extracts (200 μg/mL) were preliminarily tested against all the test microorganisms by a colorimetric broth microdilution test.[19−21] The crude extract stock solutions (10 mg/mL) were diluted with MHB to 400 μg/mL, and 50 μL of each extract solution was pipetted into three wells of a 96-well plate. Then 50 μL of each inoculum was added to the test solution and incubated at 35 °C for 15 h (*C. albicans* NCPF3153) and 25 °C for 45 h (*C. neoformans* ATCC90113). A 10 μL amount of 0.18% resazurin was added into each well and further incubated for another 2−3 h. The color change was then observed visually. Any color changes from purple to pink or colorless were recorded as positive. The crude extracts that showed antifungal activity at 200 μg/mL were further assessed for their minimum inhibitory concentrations (MICs). The MICs of extracts were tested over the concentration range 0.25−128 μg/mL by the above colorimetric broth microdilution test. The lowest concentration at which color change occurred (2 to 3 wells) was taken as the MIC value. Pure compounds (400 μg/mL) from the active extracts were prepared in DMSO and initially checked for their inhibition activity against the susceptible microorganisms at a concentration of 200 μg/mL by the same methods. If active, the MICs over the concentration range 0.25−128 μg/mL were determined. Amphotericin B was used as a positive control and exhibited MIC values of 0.25 and 0.5 μg/mL against *C. albicans* and *C. neoformans*, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for new compounds (**1**−**9**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +66-74-288-435. Fax: +66-74-558-841. E-mail: vatcharin.r@psu.ac.th.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

V.R. thanks the Office of Higher Education Commission and the Thailand Research Fund for the research grant (grant no. DBG5280020). A.R. is grateful to the Center for Innovation in Chemistry (PERCH−CIC) for a scholarship. We thank Dr. K. Tadpetch and Mr. S. Klaiklay for the hydrogenation experiment. Finally, the Graduate School, Prince of Songkla University, is gratefully acknowledged for partial support.

■ REFERENCES

(1) Isaka, M.; Palasarn, S.; Auncharoen, P.; Komwijit, S.; Jones, E. B. G. *Tetrahedron Lett.* **2009**, *50*, 284−287.
(2) Poling, S. M.; Wicklow, D. T.; Rogers, K. D.; Gloer, J. B. *J. Agric. Food Chem.* **2008**, *56*, 3006−3009.
(3) Abdel-Lateff, A.; König, G. M.; Fisch, K. M.; Höller, U.; Jones, P. G.; Wright, A. D. *J. Nat. Prod.* **2002**, *65*, 1605−1611.
(4) Jang, J.-H.; Kanoh, K.; Adachi, K.; Shizuri, Y. *J. Antibiot.* **2006**, *59*, 428−431.
(5) Zhang, P.; Bao, B.; Dang, H. T.; Hong, J.; Lee, H. J.; Yoo, E. S.; Bae, K. S.; Jung, J. H. *J. Nat. Prod.* **2009**, *72*, 270−275.
(6) Trisuwan, K.; Rukachaisirikul, V.; Sukpondma, Y.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J. *Chem. Pharm. Bull.* **2009**, *57*, 1100−1102.
(7) Amagata, T.; Morinaka, B. I.; Amagata, A.; Tenney, K.; Valeriote, F. A.; Lobkovsky, E.; Clardy, J.; Crews, P. *J. Nat. Prod.* **2006**, *69*, 1560−1565.
(8) Chinworrungsee, M.; Wiyakrutta, S.; Sriubolmas, N.; Chuailua, P.; Suksamrarn, A. *Arch. Pharm. Res.* **2008**, *31*, 611−616.
(9) Ayer, W. A.; Miao, S. *Can. J. Chem.* **1993**, *71*, 487−493.
(10) Kashiyama, Y.; Yoshikuni, Y.; Baker, D.; Siegel, J. B. WO 2009046370 A2, 2009.
(11) Jiao, P.; Kawasaki, M.; Yamamoto, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 3333−3336.
(12) Shim, S. H.; Sy, A. A.; Gloer, J. B.; Wicklow, D. T. *Bull. Korean Chem. Soc.* **2008**, *29*, 863−865.
(13) Chu, M.; Mierzwa, R.; Truumees, I.; Gentile, F.; Patel, M.; Gullo, V.; Chan, T.-M.; Puar, M. S. *Tetrahedron Lett.* **1993**, *34*, 7537−7540.
(14) Chou, T.-H.; Chen, I.-S.; Hwang, T.-L.; Wang, T.-C.; Lee, T.-H.; Cheng, L.-Y.; Chang, Y.-C.; Cho, J.-Y.; Chen, J.-J. *J. Nat. Prod.* **2008**, *71*, 1692−1695.
(15) Shen, Z.; Dornan, P. K.; khan, H. A.; Woo, T. K.; Dong, V. M. *J. Am. Chem. Soc.* **2009**, *131*, 1077−1091.

(16) Bunyapaiboonsri, T.; Yoiprommarat, S.; Khonsanit, A.; Komwijit, S. *J. Nat. Prod.* **2008**, *71*, 891−894.
(17) Isaka, M.; Palasarn, S.; Supothina, S.; Komwijit, S.; Luangsa-ard, J. J. *J. Nat. Prod.* **2011**, *74*, 782−789.
(18) Rukachaisirikul, V.; Arunpanichlert, J.; Sukpondma, Y.; Phongpaichit, S.; Sakayaroj, J. *Tetrahedron* **2009**, *65*, 10590−10595.
(19) Clinical and Laboratory Standards Institute (CLSI). Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard M7-A4; Clinical and Laboratory Standards Institute: Wayne, PA, 2002.
(20) Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, 2nd ed, M27-A4; Clinical and Laboratory Standards Institute: Wayne, PA, 2002.
(21) Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard. CLSI document M38-A; Clinical and Laboratory Standards Institute: Wayne, PA, 2002.