Ambuic Acid and Torreyanic Acid Derivatives from the Endolichenic Fungus *Pestalotiopsis* sp.

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Six new ambuic acid (**1**) derivatives (**2**−**7**) and a new torreyanic acid analogue (**8**) have been isolated from the crude extract of endophytic fungus *Pestalotiopsis* sp. inhabiting the lichen *Clavaroids* sp. The structures of these compounds were elucidated primarily by NMR and MS methods, and their absolute configurations were assigned by application of the CD excitation chirality method. Compounds **1** and **2** displayed antimicrobial activity against the Gram-positive bacterium *Staphylococcus aureus*.

Endolichenic fungi are microorganisms living in the thalli of lichens that are analogous to the plant endophytic fungi inhabiting the intercellular spaces of the hosts.[1] Although several secondary metabolites have been isolated from lichens and their fungal mycobionts,[2−7] only three heptaketides, corynesporol, herbarin, and 1-hydroxydehydroherbarin, have been previously reported from an endolichenic fungus *Corynespora* sp. inhabiting the cavern beard lichen *Usnea cavernosa*.[8] In a search for new bioactive natural products from fungal species of unique niches, chemical investigations of fungi living with lichens were recently initiated in our laboratory. In this study, the fungus *Pestalotiopsis* sp. was isolated from the lichen *Clavaroids* sp. collected from Bawang Mountain, Hainan Province, People's Republic of China. The fungus was grown in a solid-substrate fermentation culture. Its organic solvent extract showed antimicrobial activity against the Gram-positive bacterium *Staphylococcus aureus* (ATCC 6538). Bioassay-directed fractionation of the extract led to the isolation of ambuic acid (**1**)[9] and seven new metabolites (**2**−**8**). Compounds **2**−**7** are ambuic acid derivatives, and **8** is closely related to the known dimeric quinone torreyanic acid (**9**).[10] Details of the isolation, structure elucidation, and biological activity of these compounds are presented herein.

Ambuic acid (**1**) was a major component of the crude extract, and its structure was identified by comparison of the NMR and MS data with those reported.[9] Ambuic acid is a highly functionalized cyclohexenone and was initially isolated from the rainforest plant endophytic fungi *Pestalotiopsis* spp. and *Monochaetia* sp. as an antifungal agent.

The molecular formula of compound **2** was established as $C_{21}H_{28}O_7$ (eight degrees of unsaturation) on the basis of its HRESIMS (*m/z* 415.1723 $[M + Na]^+$; Δ −0.4 mmu). The extra 42 mass units, compared to that of **1**, suggested the presence of an acetyl group. Analysis of the ^1H and ^{13}C NMR spectroscopic data of **2** revealed structural similarity to those of **1**, except that the oxygenated methylene protons (H_2-18) were shifted downfield to δ_H 4.86 and 4.92, respectively in **2**. In addition, NMR resonances corresponding to an acetyl group (δ_H 2.00; δ_C 20.7 and 172.3) were observed, indicating that the C-18 oxygen of **2** was acylated. It was confirmed by an HMBC correlation from H_2-18 to the carboxyl carbon at δ_C 172.3. The gross structure of compound **2** was established as shown.

Compound **3** gave a molecular formula of $C_{19}H_{24}O_7$ (eight degrees of unsaturation) by analysis of its HRESIMS (*m/z* 387.1415 $[M + Na]^+$; Δ −0.1 mmu). Comparison of the ^1H and ^{13}C NMR spectroscopic data of **3** with those of **1** revealed a resonance for one more ketone functionality (δ_C 213.4) and the absence of signals for a methylene unit (δ_H 1.27; δ_C 32.5), suggesting that the C-15 methylene carbon in **1** was oxidized to a ketone in **3**. HMBC correlations from H_2-13, H_2-14, H_2-16, and H_3-17 to C-15 were observed to support this structure as shown.

The molecular formula of compound **4** was determined to be $C_{19}H_{28}O_6$ (six degrees of unsaturation) on the basis of HRESIMS analysis (*m/z* 375.1788 $[M + Na]^+$; Δ −1.0 mmu). The ^1H and ^{13}C NMR spectra of **4** displayed the resonances for structural fragments similar to those presented in the spectra of **1**, except that the C-11/C-12 olefin was reduced to two methylene units. This observation was confirmed by analysis of relevant $^1H-^1H$ COSY and HMBC data. Interpretation of the HRESIMS and NMR data (Table 1) of **5** enabled assignment of its molecular formula as $C_{19}H_{24}O_6$ (*m/z* 371.1465 $[M + Na]^+$; Δ −0.2 mmu), with two less hydrogens than that of **1**. Detailed comparison of the NMR spectroscopic data between **5** and **1** revealed that the two mutually coupled methylene units (C-13−C-14) in **1** were oxidized to an olefin moiety in **5**. Further analysis of the COSY spectral data of

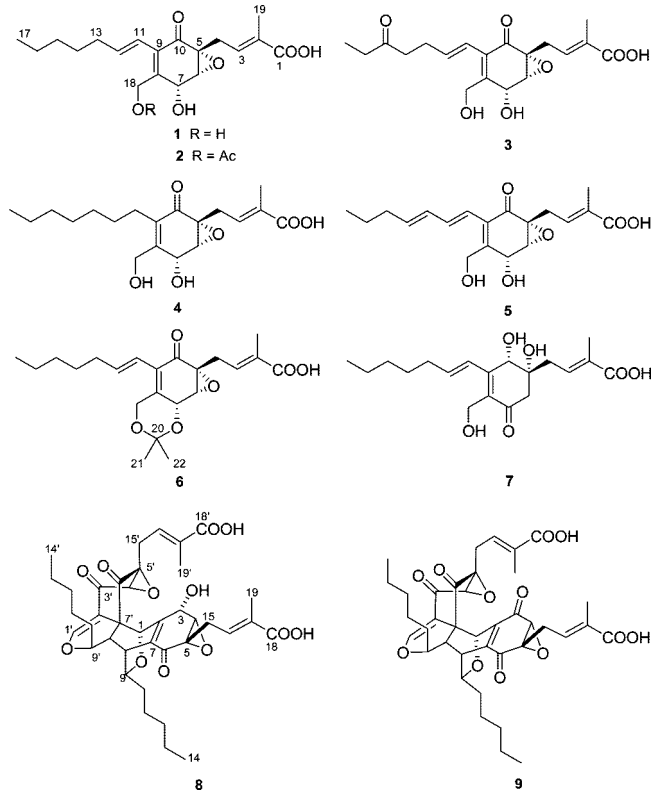

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Table 1. NMR Spectroscopic Data for **2** and **3** (in CD_3OD) and **4** and **5** (in acetone-d_6)

	compound **2**		compound **3**		compound **4**		compound **5**	
position	δ_H[a] (*J* in Hz)	δ_C[b], mult.	δ_H[a] (*J* in Hz)	δ_C[c], mult.	δ_H[a] (*J* in Hz)	δ_C[b] mult.	δ_H[a] (*J* in Hz)	δ_C[d], mult.
1		171.2, qC		167.8, qC		168.5, qC		168.7, qC
2		132.0, qC		131.6, qC		131.0, qC		130.1, qC
3	6.62, t (7.0)	136.4, CH	6.61, t (7.0)	136.8, CH	6.71, t (7.0)	136.2, CH	6.72, t (7.5)	136.2, CH
4	2.77, dd (16, 7.0)	28.6, CH_2	2.76, dd (16, 7.0)	28.7, CH_2	2.86, m	28.7, CH_2	2.84, dd (16, 7.5)	28.5, CH_2
	2.72, dd (16, 7.0)		2.69, dd (16, 7.0)				2.75, dd (16, 7.5)	
5		61.4, qC		61.3, qC		59.9, qC		60.7, qC
6	3.69, d (2.5)	60.9, CH	3.70, d (2.5)	61.1, CH	3.80, d (2.5)	60.2, CH	3.83, d (2.5)	60.5, CH
7	4.66, br s	65.8, CH	4.76, br s	65.9, CH	4.89, br s	65.8, CH	4.96, br s	66.4, CH
8		145.4, qC		151.2, qC		151.6, qC		151.0, qC
9		134.3, qC		131.6, qC		133.0, qC		131.1, qC
10		195.7, qC		195.9, qC		195.2, qC		195.3, qC
11	6.10, d (16)	122.8 CH	6.12, d (16)	123.5, CH	2.46, m; 2.17, m	26.3, CH_2	6.27, d (16)	122.8, CH
12	5.80, td (16, 6.5)	140.8, CH	5.79, td (16, 6.5)	138.4, CH	1.26, m	30.5, CH_2	6.57, dd (16, 11)	137.2, CH
13	2.13, m	34.4, CH_2	2.34, m	28.8, CH_2	1.26, m	30.5, CH_2	6.15, dd (16, 11)	132.1, CH
14	1.38, m	29.8, CH_2	2.54, t (7.5)	42.2, CH_2	1.26, m	29.8, CH_2	5.80, td (16, 7.0)	137.4, CH
15	1.27, m	32.5, CH_2		213.4, qC	1.26, m	32.4, CH_2	2.08, m	35.5, CH_2
16	1.27, m	23.6, CH_2	2.44, m	36.7, CH_2	1.26, m	23.0, CH_2	1.42, m	23.0, CH_2
17	0.85, t (7.0)	14.4, CH_3	0.96, t (7.5)	8.0, CH_3	0.86, t (6.5)	14.2, CH_3	0.89, t (7.5)	13.9, CH_3
18	4.92, d (13),	62.4, CH_2	4.44, d (13)	60.3, CH_2	4.47, d (14)	60.2, CH_2	4.53, d (13)	60.3, CH_3
	4.86, d (13)		4.34, d (13)		4.44, d (14)		4.90, d (13)	
19	1.81, s	12.9, CH_3	1.86, s	12.8, CH_3	1.86, s	12.7, CH_3	1.86, s	12.7, CH_3
AcO		172.3, qC						
	2.00, s	20.7, CH_3						

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz. [c] Recorded at 125 MHz. [d] Recorded at 100 MHz.

Table 2. NMR Spectroscopic Data for **6** (in $CDCl_3$) and **7** (in CD_3OD)

	compound **6**		compound **7**		
position	δ_H[a] (*J* in Hz)	δ_C[b], mult.	δ_H[a] (*J* in Hz)	δ_C[c], mult.	key HMBC (H → C#)
1		169.8, qC		172.0, qC	
2		130.2, qC		133.0, qC	
3	6.77, t (7.5)	136.7, CH	6.92, t (7.5)	136.8, CH	1, 2, 4, 5, 19
4	3.00, dd (16, 7.5)	28.8, CH_2	2.56, d (7.5)	38.6, CH_2	2, 3, 5, 6, 10
	2.79, dd (16, 7.5)				
5		59.4, qC		75.5, qC	
6	3.67, d (2.0)	60.8, CH	2.72, d (17)	48.5, CH_2	4, 5, 7, 8
			2.38, d (17)		
7	4.79, br s	65.0, CH		199.3, qC	
8		146.7, qC		133.0, qC	
9		125.5, qC		153.0, qC	
10		193.6, qC	4.31, s	69.9, CH	4, 5, 6, 8, 9, 11
11	5.94, m	119.7, CH	6.73, d (16)	128.0, CH	8, 9, 10, 13
12	5.94, m	139.2, CH	6.51, td (16, 7.0)	142.9, CH	9, 13, 14
13	2.13, m	33.8, CH_2	2.25, m	35.0, CH_2	11, 12, 14, 15
14	1.41, m	28.8, CH_2	1.46, m	29.8, CH_2	12, 13, 15, 16
15	1.29, m	31.4, CH_2	1.31, m	32.6, CH_2	13, 14, 16, 17
16	1.29, m	22.5, CH	1.31, m	23.5, CH_2	14, 15, 17
17	0.89, t (7.0)	14.0, CH_3	0.87, t (7.0)	13.1, CH_3	15, 16
18	4.55, s	55.5, CH_2	4.40, d (12)	54.5, CH_2	7, 8, 9
			4.36, d (12)		
19	1.91, s	12.5, CH_3	1.82, s	14.3, CH_3	1, 2, 3
20		101.0, qC			
21	1.49, s	24.1, CH_3			
22	1.47, s	24.0, CH_3			

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz. [c] Recorded at 125 MHz.

5 confirmed this observation, leading to the assignment of its gross structure as shown.

The molecular formula of compound **6** was established as $C_{22}H_{30}O_6$ (eight degrees of unsaturation) on the basis of HRESIMS analysis (*m*/*z* 413.1970 $[M + Na]^+$; Δ −3.5 mmu), which was consistent with its NMR data (Table 2). Interpretation of the ^1H, ^{13}C, and HMQC spectroscopic data of **6** revealed the presence of the same highly functionalized cyclohexenone core structure as that found in **1**, but additional resonances corresponding to two methyl singlets (δ_C 1.47 and 1.49, respectively) and one quaternary carbon at δ_C 101.0 were observed in the NMR spectra of **6**, implying that **6** could be an acetonide of **1** originating from the addition of an acetone unit with the OH groups attached to C-7 and C-18. HMBC correlations from H-7, H_2-18 and from H_3-21/22 to C-20 were observed, and therefore, the gross structure of **6** was depicted. Since acetone was never used as a solvent in the isolation and purification of these compounds, compound **6** should be a naturally occurring metabolite rather than an artifact resulting from the isolation process.

The relative configurations of **2**−**6** were determined by analysis of the $^1H-^1H$ coupling constants and NOESY data. The C-11/C-12 double bond in compounds **2**, **3**, **5**, and **6** and the C-13/C-14 olefin in **5** were all assigned *E*-geometry on the basis of the large coupling constant observed for corresponding olefinic protons (16 Hz), and the same assignment was made for the C-2/C-3 olefin in **2**−**6** by NOESY correlation of H_2-4 with H_3-19. The small vicinal coupling constant of 2.0 Hz between H-6 and H-7 in compounds **2**−**6** suggested a *cis* relationship between these two protons, and the NOESY correlation of H_2-4 with H-6 indicated that these

protons have the same orientation with respect to the cyclohexenone ring. The absolute configurations of **2**−**6** were assigned by application of the CD excitation chirality method. The CD spectra of **2**−**6** all showed positive and negative Cotton effects at near 340 and 240 nm, respectively, which closely resembled those of macrophorin A[11] and (+)-epoxydon.[12] The coupling constants observed between H-6 and H-7 in **2**−**6** (2.5 Hz in **2**−**5** and 2.0 Hz in **6**) were also similar to those of the corresponding protons in macrophorin A and (+)-epoxydon, suggesting the 5*R*, 6*R*, and 7*R* absolute configuration for **2**−**6**.

Compound **7** was assigned the molecular formula $C_{19}H_{28}O_6$ (six degrees of unsaturation) by analysis of its HRESIMS (*m*/*z* 375.1779 $[M + Na]^+$; Δ −0.1 mmu). The ^{1}H, ^{13}C, and HMQC NMR spectroscopic data for **7** revealed the presence of the same side chains as those attached to C-5, C-8, and C-9 in **1**, but significant structural changes were observed for the remaining portion of the molecule. Therefore, the HMQC and HMBC for those unaccounted resonances in **7** were analyzed in order to establish its gross structure. HMBC correlations from H-3 to C-5 and from H_2-4 to C-5, C-6, and C-10 led to the connection of C-5 to C-4, C-6, and C-10, whereas those from H_2-6 to C-7 and C-8 indicated that both C-6 and C-8 were connected to the ketone carbon C-7. Additional HMBC cross-peaks from H-10 to C-8 and C-9 permitted completion of the cyclohexenone moiety of **7**. The side chains that were identical to those in compounds **1**−**6** were located at C-5, C-8, and C-9 by relevant HMBC correlations. Collectively, these data allowed assignment of structure **7** as shown. The relative configurations of the C-2/C-3 and C-11/C-12 olefins in **7** were determined on the basis of the ^{1}H−^{1}H *J*-values and NOED data and by analogy to those in **1**. In NOED experiments, upon irradiation of H-10, enhancement was observed for H_2-4, indicating that those protons are on the same face of the cyclohexenone ring. Although the absolute configuration of **7** could not be directly assigned by analysis of its CD spectrum, C-5 was presumed to have the *R*-configuration based on biogenetic considerations, whereas the absolute configuration of the stereogenic center C-10 was deduced to be *S* by NOED results.

The molecular formula of compound **8** was determined to be $C_{38}H_{46}O_{12}$ (16 degrees of unsaturation) on the basis of HRESIMS analysis (*m*/*z* 693.2910 $[M - H]^-$; Δ +0.7 mmu). Analysis of the ^{1}H, ^{13}C, and HMQC NMR data of **8** revealed four methyl groups, 10 methylene units, eight methines (six oxygenated), three quaternary carbons (two oxygenated), eight aromatic/olefinic carbons (three of which were protonated), and five carbonyl carbons. These data, together with three unobserved exchangeable protons, accounted for all the ^{1}H and ^{13}C NMR resonances for **1**. Interpretation of the COSY NMR data of **8** identified four isolated proton spin systems, which were C-3−C-4, C-15−C-16, C-15′−C-16′, and C-14−C-14′. Further analysis of the 2D NMR data, especially HMBC, revealed the presence of two units of 2-methyl-2-butenoic acid. In addition, the correlations in the HMBC spectrum from H-3 to C-2, C-5, and C-7; from H-4 to C-2, C-6, and C-15; and from H_2-15 to C-4 and C-6 established the cyclohexenone ring, with one 2-methyl-2-butenoic acid moiety attached to C-5. Those from H-1 to C-3 and from H-8 to C-6 led to the connection of C-1 to C-2 and C-7 to C-8, respectively. In turn, correlations from H-8 to C-7′ and from H-8′ to C-1 and C-7′ indicated that C-7′ was connected to both C-1 and C-8′, completing the cyclohexane ring fused to the cyclohexenone ring at C2/C7. Key HMBC correlations from H-1 to C-9 and from H-9 to C-1 established the ether linkage between these two carbons. Further correlations from H-1 to C-2′ and C-6′; from H-1′ to C-2′ and C-3′; from H-1′ to C-9′; and from H-9′ to C-1′ allowed assignment of the 3,4-dihydro-2*H*-pyran moiety fused to the cyclohexane ring at C7′/C8′, with one ketone carbon at δ_C 189.8 (C-3′) attached to C-2′, and the remaining carbon at δ_C 201.7 (C-6′) attached to C-7′. HMBC from H-4′ to C-2′, C-3′, C-5′, and C-15′ and from H_2-15′ to C-4′ and C-6′ completed the cyclohexane-1,4-dione substructure with C-15′ attached to C-5′. Since two exchangeable protons in **8** were already accounted for by the presence of two carboxylic acid units, the remaining exchangeable proton was assigned to OH-3 to complete the gross structure of **8**.

The identified structure was similar to the dimeric quinone torreyanic acid (**9**), isolated from the plant endophytic fungus *Pestalotiopsis macrospore* with selective cytotoxicity against human cancer cell lines.[10] Therefore, the relative configuration for the majority of structure **8** was deduced by analogy to torreyanic acid (**9**), except for H-3 and H-4, which were assigned on the basis of their coupling constant value (2.0 Hz) and NOESY data. From the CD spectrum of **8**, the inverse octant rule could not be applied to assign the absolute configuration of C-3 due to the unclear data seen in the range 330−350 nm. Considering the absolute stereochemistry established for **9** by synthesis,[13] the absolute configuration of C-3 was assigned as *S*.

Compounds **1**−**8** were evaluated for antimicrobial activity against a panel of bacteria and fungi, including the bacteria *Staphylococcus aureus* (ATCC 6538), *Streptococcus pneumoniae* (CGMCC 1.1692), and *Escherichia coli* (CGMCC 1.2340), the yeasts *Candida albicans* (ATCC 10231) and *Geotrichum candidum* (AS2.498), and the fungus *Aspergillus fumigatus* (ATCC 10894). Only compounds **1** and **2** showed activity against the Gram-positive bacterium *S. aureus* (ATCC 6538), with IC_{50} values of 43.9 and 27.8 μM, respectively (the positive control AMP showed an IC_{50} value of 1.40 μM), whereas compounds **3**−**8** did not show noticeable *in vitro* antibacterial or antifungal activities against the above-mentioned organisms (IC_{50} > 50 μM). Even though ambuic acid (**1**) was initially isolated as an antifungal agent with moderate activities against several plant pathogenic fungi,[9] compounds **1**−**8** did not display activity against *Aspergillus fumigatus* (ATCC 10894) in our assays due to the selection of different fungal species as targets.

Compounds **2**−**7** are new analogues of the known compound ambuic acid (**1**), but differ from **1** by the presence of different aliphatic side chains at C-9 (in **2**−**5**) and substitution pattern (in **7**), as well as the presence of an unit of acetonide (in **6**). Compound **8** is closely related to the known dimeric quinone torreyanic acid (**9**),[10] with the only difference being an OH group at C-3 instead of the ketone functionality. The known compounds ambuic acid (**1**) and torreyanic acid (**9**) have been synthesized, and the possible biosynthetic pathways, as well as their biogenetic relationships, have been described.[13] The biosynthesis of **2**−**7** probably proceeds in a manner similar to that of **1**, whereas **8** might be a reduction product (ketone functionality C-3) of **9**.

The isolation of ambuic acid (**1**) and its heterodimer **8** further corroborates the biogenetic hypothesis that terroyanic acid (**9**) could be generated through the oxidation, cyclization, and Diels−Alder dimerization of **1**.[13] Compounds **2**−**8** are the first secondary metabolites to be reported from the endolichenic fungus *Pestalotiopsis* sp. and the second examples of natural products discovered from endolichenic fungal sources, implying that endolichenic fungi could prove to be valuable sources of new bioactive natural products.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using solvent signals ($CDCl_3$; δ_H 7.26/δ_C 77.7; CD_3OD; δ_H 3.35/δ_C 49.9; acetone-d_6; δ_H 2.05/δ_C 29.8, 206.0) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using a Bruker APEX III 7.0 T spectrometer.

Table 3. NMR Spectroscopic Data for **8** in CD_3OD

position	δ_H[a] (J in Hz)	δ_C[b], mult.	key HMBC (H → C#)
1	5.34, s	73.4, CH	2, 3, 7, 9, 2′, 7′, 8′
2		153.9, qC	
3	4.29, d (2.0)	64.8, CH	1, 2, 5, 7
4	3.61, d (2.0)	60.8, CH	2, 6, 15
5		61.2, qC	
6		191.1, qC	
7		133.1, qC	
8	3.28, m	38.9, CH	2, 6, 7, 7′, 9′
9	3.98, t (6.0)	73.1, CH	1, 7, 8′, 11
10	1.20, m; 1.40, m	35.3, CH_2	8, 9, 11, 12
11	1.20, m	25.8, CH_2	9, 10, 12, 13
12	1.20, m	32.8, CH_2	10, 11, 13, 14
13	1.12, m; 0.98, m	23.5, CH_2	11, 12, 14
14	0.80, t (6.0)	14.3, CH_3	12, 13
15	2.82, dd (13, 6.5); 2.70, dd (13, 6.0)	28.6, CH_2	4, 6, 16, 17
16	6.62, dd (6.5, 6.0)	135.9, CH	5, 15, 18, 19
17		133.1, qC	
18		171.0, qC	
19	1.83, s	8.0, CH_3	16, 17, 18
1′	7.79, s	159.6, CH	2′, 3′, 7′, 9′
2′		114.7, qC	
3′		189.8, qC	
4′	3.75, s	67.4, CH	2′, 3′, 6′, 15′
5′		65.3, qC	
6′		201.7, qC	
7′		51.7, qC	
8′	2.67, d (5.0)	37.5, qC	1, 7, 9, 2′, 6′, 7′
9′	4.39, dd (8.5, 4.5)	83.1, CH	1′, 7′, 8
10′	1.20, m	34.3, CH_2	
11′	1.20, m	26.6, CH_2	
12′	1.20, m	32.3, CH_2	
13′	1.20, m	23.5, CH_2	
14′	0.84, t (6.0)	14.2, CH_3	12′, 13′
15′	3.08, dd (14, 6.5); 2.39, dd (14, 6.0)	29.6, CH_2	4′, 6′, 16,′ 17′
16′	6.62, dd (6.5, 6.0)	135.1, CH	15′, 17′, 18′, 19′,
17′		133.2, qC	
18′		171.0, qC	
19′	1.82, s	12.8, CH_3	16′, 17′, 18′

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz.

Fungal Material. The culture of *Pestalotiopsis* sp. was isolated by one of authors (S.F.) from samples of the lichen *Clavaroids* sp. collected from Bawang Mountain, Hainan Province, in May 2007. The isolate was characterized as an unidentified species of *Pestalotiopsis* by one of authors (J.W.) based on sequence analysis of the ITS region of the rDNA and assigned the accession number WN1 in J.W.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. The agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. Fermentation was carried out in four 500 mL Fernbach flasks each containing 75 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min.[14] After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was freeze-dried and extracted with EtOAc (3×500 mL), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (10.0 g), which was fractionated by silica column chromatography (CC) (5×25 cm) using $CH_2Cl_2-CH_3OH$ gradient elution. The fraction (50 mg) eluted with 99:1 $CH_2Cl_2-CH_3OH$ was separated by semipreparative reversed-phase HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4×250 mm; 2 mL/min; 60% MeCN in H_2O over 5 min, 60−90% MeCN over 85 min) to afford **2** (2.5 mg; t_R 21.5 min) and **8** (1.8 mg; t_R 30.3 min). Another fraction eluted with 99:1 $CH_2Cl_2-CH_3OH$ was separated by Sephadex LH-20 CC using MeOH as eluent, and one subfraction (30 mg) was further purified by reversed-phase HPLC (60% MeOH in H_2O for 5 min, followed by 60−85% MeOH for 40 min) to afford **4** (2.0 mg; t_R 18.0 min), **5** (2.5 mg; t_R 25.6 min), and **1** (8.0 mg; t_R 28.2 min). The fraction (380 mg) eluted with 98:2 $CH_2Cl_2-CH_3OH$ was chromatographed on a Sephadex LH-20 column using CH_3OH as solvent, and one subfraction (30 mg) was purified by HPLC (29% MeCN in H_2O as eluent) to afford **3** (2.3 mg, t_R 28.0 min) and **6** (1.9 mg, t_R 23.0 min). The fraction (200 mg) eluted with 97:3 $CH_2Cl_2-CH_3OH$ was again separated by Sephadex LH-20 CC eluted with CH_3OH, and one subfraction (35 mg) was purified by reversed-phase HPLC (25% MeOH in H_2O for 2 min, followed by 25−40% for 42 min) to afford **7** (2.0 mg, t_R 27.1 min).

Ambuic acid (1): ^{1}H NMR, ^{13}C NMR, and the ESIMS data were consistent with the literature.[9]

Compound 2: colorless oil; $[\alpha]_D$ +106 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (19 800), 239 (20 300), 294 (17 900) nm; IR (neat) ν_{max} 3395 (br), 2932, 1739, 1685, 1650, 1379, 1233 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 415.1723 (calcd for $C_{21}H_{28}O_7Na$, 415.1719).

Compound 3: colorless oil; $[\alpha]_D$ +124 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 214 (23 500), 234 (23 700), 294 (20 400) nm; IR (neat) ν_{max} 3426 (br), 2933, 1739, 1686, 1650, 1378, 1232 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 387.1415 (calcd for $C_{19}H_{24}O_7Na$, 387.1414).

Compound 4: colorless oil; $[\alpha]_D$ +105 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (14 700), 231 (18 200), 291 (7600) nm; IR (neat) ν_{max} 3400 (br), 2929, 1686, 1649, 1379, 1235 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 375.1788 (calcd for $C_{19}H_{28}O_6Na$, 375.1778).

Compound 5: colorless oil; $[\alpha]_D$ +154 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (20 800), 232 (21 400), 298 (17 400) nm; IR (neat) ν_{max} 3390 (br), 3187 (br), 2920, 2851, 1685, 1642, 1464, 1418 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 371.1465 (calcd for $C_{19}H_{24}O_6Na$, 371.1463).

Compound 6: colorless oil; $[\alpha]_D$ +116 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (14 400), 227 (15 800), 285 (5100) nm; IR (neat) ν_{max} 3379 (br), 2929, 1685, 1649, 1379, 1237 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 413.1970 (calcd for $C_{22}H_{30}O_6Na$, 413.1935).

Compound 7: colorless oil; $[\alpha]_D$ +135 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (14 100), 231 (18 300), 295 (6600) nm; IR (neat) ν_{max} 3352 (br), 2928, 1685, 1650, 1382, 1266 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data see Table 2; HRESIMS *m/z* 375.1779 (calcd for $C_{19}H_{28}O_6Na$, 375.1778).

Compound 8: white, amorphous powder; $[\alpha]_D$ +154 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (20 800), 232 (21 400), 298 (17 400) nm; IR (neat) ν_{max} 3370 (br), 2932, 1738, 1685, 1651, 1378, 1232 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data see Table 3; HRESIMS *m/z* 693.2910 (calcd for $C_{38}H_{45}O_{12}$, 693.2917).

Antimicrobial and Antifungal Bioassays. Antimicrobial and antifungal bioassays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCCLS) recommendations.[15] The bacterial strains *Staphylococcus aureus* (ATCC 6538), *Streptococcus pneumoniae* (CGMCC 1.1692), and *Escherichia coli* (CGMCC 1.2340) were grown on Mueller-Hinton agar, the yeasts, *Candida albicans* (ATCC 10231) and *Geotrichum candidum* (AS2.498), were grown on Sabouraud dextrose agar, and the fungus, *Aspergillus fumigatus* (ATCC 10894), was grown on potato dextrose agar. Targeted microbes (3−4 colonies) were prepared from broth culture (bacteria: 37 °C for 24 h; fungus: 28 °C for 48 h), and the final spore suspensions of bacteria (in MHB medium), yeasts (in SDB medium), and *Aspergillus fumigatus* (in PDB medium) were 10^6 and 10^5 cells/mL and 10^4 mycelial fragments/mL, respectively. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL (antimicrobial peptide AMP, streptomycin, and fluconazole were used as positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (TECAN), and the inhibition rate was calculated and plotted versus test concentrations to afford the IC_{50}.

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Supporting Information Available: ^1H NMR, ^{13}C NMR, and CD spectra of compounds **2**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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