β-Resorcylic Acid Lactones from a *Paecilomyces* Fungus

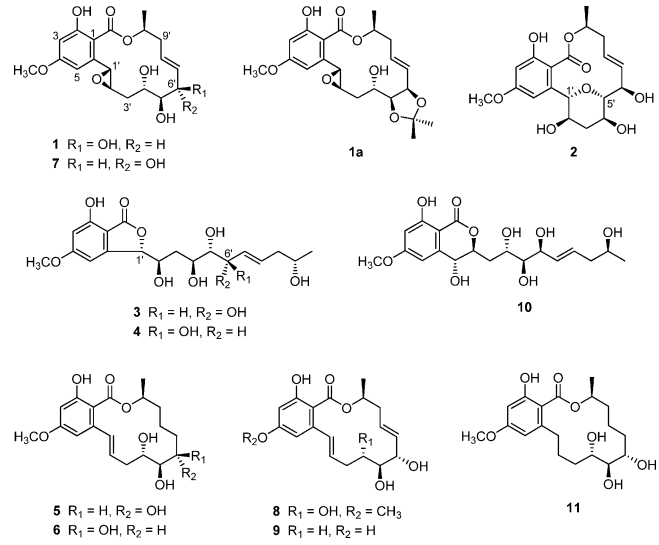
Liangxiong Xu,[†] Zhengxiang He,[‡] Jinghua Xue,[†] Xiaoping Chen,*[,‡] and Xiaoyi Wei*[,†]

Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, People's Republic of China, and Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Kai Yuan Avenue 190, Science Park, Luogang District, Guangzhou 510530, People's Republic of China

Received January 4, 2010

Six new *β*-resorcylic acid lactones (**1**−**6**), named paecilomycins A−F, and five known compounds, aigilomycin B (**7**), zeaenol (**8**), aigialomycin D (**9**), aigialomycin F (**10**), and aigialospirol, were isolated from the mycelial solid culture of *Paecilomyces* sp. SC0924. Their structures were elucidated by extensive NMR analysis, single-crystal X-ray study, and chemical correlations. Compounds **5** and **10** exhibited antiplasmodial activity against *Plasmodium falciparum* line 3D7 with IC_{50} values of 20.0 and 10.9 nM, respectively, and compounds **5**−**7** showed moderate activity against the *P. falciparum* line Dd2.

β-Resorcylic acid lactones (RALs), a group of fungal polyketide metabolites, are *β*-resorcylic acid derivatives possessing a C_{11} side chain that is closed to form a 14-membered lactone ring.[1] Since the first isolation of radicicol in 1953,[2] more than 30 naturally occurring RALs have been reported. They have been shown to have estrogenic, antifungal,[3−5] cytotoxic, antimalarial,[6,7] and nematicidal[8] properties and inhibitory activities against ATPases and kinases.[9−13] Because of their attractive biological activities, in particular the potent and selective inhibition against protein kinases, this group of natural products has provoked much interest.[1] During our search for bioactive natural products produced by filamentous fungi collected in South China,[14−16] we found that the MeOH extract from a mycelial solid culture of *Paecilomyces* sp. SC0924 showed antifungal activity against *Peronophythora litchii*, one of the main pathogens causing litchi (*Litchi chinensis* Sonn.) fruit rot. We therefore investigated the secondary metabolites of this fungus and isolated six new *β*-resorcylic acid lactone derivatives (**1**−**6**) and five known compounds, aigilomycin B (**7**), zeaenol (**8**), aigialomycin D (**9**), aigialomycin F (**10**), and aigialospirol. Compounds **1**−**6** were given the names paecilomycins A−F. Herein, we report the isolation, structure elucidation, and antiplasmodial activity of these compounds.



Results and Discussion

The fermentation and extraction of the fungus were performed by the method previously reported.[16] The $CHCl_3$-, EtOAc-, and n-BuOH-soluble extracts were fractionated by repeated column chromatography (CC) over silica gel, ODS, Sephadex LH-20, and HPLC to furnish the new compounds **1**−**6** and five known compounds, aigilomycin B (**7**),[6] zeaenol (**8**),[17] aigialomycin D (**9**),[6] aigialomycin F (**10**),[7] and aigialospirol.[18] The structures of the known compounds were determined by interpretation of their spectroscopic data as well as by comparison with reported data.

Paecilomycin A (**1**), colorless, columnar crystals (MeOH), was obtained as a major metabolite of this fungus. It had the molecular formula $C_{19}H_{24}O_8$, as determined from the HRESIMS ion at *m*/*z* 379.1414 $[M - H]^-$. The ^1H NMR, ^{13}C NMR (Tables 1 and 2), and DEPT spectra indicated the presence of an ester carbonyl group, a *trans*-disubstituted double bond, a 1,2,3,5-tetrasubstituted benzene ring, and 10 sp^3 carbons including an aromatic OCH_3 group, a methyl, two methylenes, and six oxygenated methines. Analysis of the $^1H-^1H$ COSY spectrum in combination with the HSQC spectrum showed the presence of a (*E*)-undec-7-ene-1,2-epoxy-4,5,6-triol moiety. These findings revealed that paecilomycin A (**1**) had the same planar structure as aigialomycin B (**7**),[6] a known RAL obtained as the second major metabolite of this fungus in the present study. However, compounds **1** and **7** were indicated to be different compounds by their TLC behaviors and optical rotations. Careful examination of the ^1H NMR data revealed that the coupling constant between H-5′ and H-6′ ($J_{5',6'}$ = 3.0 Hz) in **1** was different from that ($J_{5',6'}$ = 8.5 Hz) in **7**, suggesting that **1** was the 6′-epimer of **7**. Then, the acetonide of **1** was prepared. Treatment of **1** with 2,2-dimethoxypropane in the presence of TsOH afforded compound **1a** as the only acetonide product rather than two acetonide isomers as reported for the reaction of **7**.[6] In the NOESY spectrum of **1a**, both H-5′ and H-6′ were correlated with one acetonide methyl at δ_H 1.32, while neither of them was correlated with the other methyl at δ_H 1.47. This revealed that OH groups at C-5′ and C-6′ in **1** oriented to the same side of the macrolide ring. In order to confirm the relative configuration of **1**, a single crystal was obtained by recrystallization in MeOH and subjected to X-ray diffraction analysis using Mo Kα radiation. The result (Figure 1) confirmed that the only difference between **1** and **7** was the relative configuration at C-6′. On the basis of these experimental data and by biogenetic correlation to aigialomycin B (**7**), whose absolute configuration was known to be 1′*R*,2′*R*,4′*S*,5′*S*,6′*S*,10′*S*,[6] as discussed below, the absolute configuration of **1** was assigned as

* To whom correspondence should be addressed. Tel: +86-20-3725-2538. Fax: +86-20-3725-2537. E-mail: wxy@scbg.ac.cn (X.W.) and chen_xiaoping@gibh.ac.cn (X.C.).

[†] South China Botanical Garden.

[‡] Guangzhou Institutes of Biomedicine and Health.

10.1021/np900853n
Published on Web 04/29/2010

Table 1. ^{1}H NMR (400 MHz) Data of **1**−**6**[a]

position	1	2	3	4	5	6
3	6.41 d (2.4)	6.67 d (2.3)	6.71 br s	6.70 br s	6.38 s	6.38 s
5	6.25 d (2.4)	6.68 d (2.3)	7.00 br s	7.06 br s	6.38 s	6.38 s
1′	4.12 d (2.0)	4.44 d (9.0)	5.74 d (4.3)	5.78 d (4.4)	7.20 dd (15.4, 1.6)	7.11 dd (15.4, 1.7)
2′	2.85 dt (10.0, 2.0)	5.11 ddd (11.2, 9.0, 4.4)	4.83 dt (8.8, 4.3)	4.88−4.94 m	5.75 ddd (15.4, 10.6, 3.0)	5.68 ddd (15.4, 11.2, 3.3)
3′	1.85 ddd (13.4, 10.4, 2.0)	3.03 dt (11.5, 4.4)	2.46−2.56 m	2.62 dt (14.2, 8.6)	2.75 dt (14.6, 10.8)	2.47 dt (14.4, 11.2)
	1.50 br dd (13.4, 10.0)	2.24 dt (11.5, 11.2)	2.84 dt (13.8, 3.2)	2.88 dt (14.2, 3.3)	2.63 m	2.67 m
4′	3.48 br dd (10.4, 4.2)	4.16 ddd (11.2, 10.8, 4.4)	4.74 td (8.2, 3.2)	4.74 ddd (8.6, 6.3, 3.3)	4.17 m	4.12−4.18 m
5′	3.52 dd (4.2, 3.0)	4.08 br d (10.8)	4.00−4.08 m	4.26 t (6.3)	3.67 br d (3.5)	3.52 br s
6′	3.99 dd (5.4, 3.0)	5.50 br d (4.4)	5.05 br s	4.88−4.94 m	3.93 dd (10.7, 6.4)	4.12−4.18 m
7′	5.26−5.34 m	6.28 dd (16.0, 4.4)	6.15−6.28 m	6.33 dd (15.6, 6.1)	1.71−1.86 m	1.90 m
						1.33 m
8′	5.26−5.34 m	6.14 ddd (15.4, 7.1, 4.6)	6.15−6.28 m	6.26 dt (15.6, 6.5)	1.66 m	1.44 m
					1.42 m	1.28 m
9′	2.58 dt (15.3, 3.6)	2.38 m	2.46−2.56 m	2.50 dt (12.4 6.5)	1.71−1.86 m	1.81 m
	2.33 dt (15.3, 6.3)	2.36 m	2.38 dt (12.3, 5.4)	2.39 dt (12.4, 6.5)		1.61 m
10′	5.30 m	5.77 m	4.00−4.08 m	4.05 m	5.00 m	4.94 m
11′	1.32 d (6.3)	1.42 d (6.1)	1.30 d (6.3)	1.30 d (6.1)	1.40 d (6.1)	1.39 d (6.1)
4-OMe	3.73 s	3.56 s	3.64 s	3.64 s	3.79 s	3.79 s
2-OH	11.40 br s				12.00 br s	12.26 br s

[a] Solvents: DMSO-d_6 for **1**, pyridine-d_5 for **2**−**4**, $CDCl_3$ for **5** and **6**.

Table 2. ^{13}C NMR (100 MHz) Data (δ) of **1**−**6**[a]

position	1	2	3	4	5	6
1	106.9	116.5	107.3	107.5	103.9	103.3
2	162.5	157.9	159.6	159.5	164.0	164.0
3	100.5	101.6	103.0	103.0	100.2	100.2
4	163.6	160.3	166.6	166.6	165.9	165.9
5	102.0	107.6	100.8	100.8	108.6	109.0
6	141.8	141.5	152.3	152.2	143.0	142.9
7	169.5	169.6	169.9	170.0	171.3	171.4
1′	55.7	87.1	83.4	83.3	128.3	127.3
2′	63.8	68.9	72.1	72.1	134.5	134.1
3′	34.8	44.7	37.0	36.4	39.0	38.7
4′	69.5	66.6	71.6	71.7	77.3	76.1
5′	77.1	89.2	78.2	78.2	71.3	68.9
6′	73.2	71.3	72.7	74.8	76.0	66.9
7′	133.3	134.8	134.8	134.4	33.8	30.9
8′	125.3	123.5	128.8	129.4	21.1	20.9
9′	36.1	37.8	43.4	43.5	35.8	35.2
10′	73.1	70.0	67.2	67.1	74.1	73.7
11′	19.1	21.0	23.8	23.7	20.5	21.2
4-OMe	55.4	55.1	55.8	55.7	55.4	55.4

[a] Solvents: DMSO-d_6 for **1**, pyridine-d_5 for **2**−**4**, $CDCl_3$ for **5** and **6**.

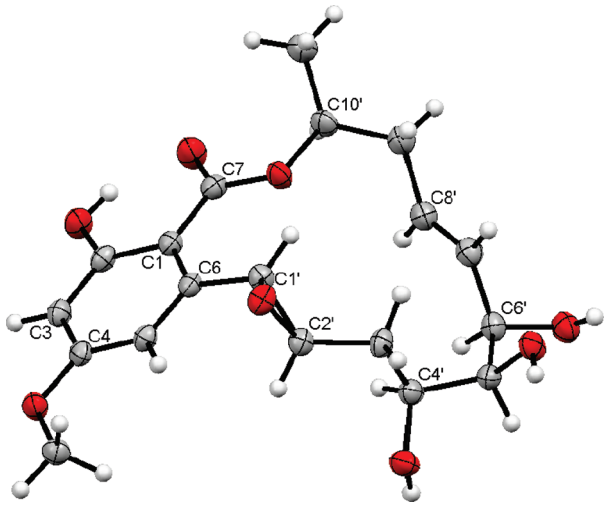


Figure 1. X-ray crystallographic structure of **1**.

1′*R*,2′*R*,4′*S*,5′*S*,6′*R*,10′*S*. Therefore, paecilomycin A (**1**) was determined to be 6′-*epi*-aigialomycin B.

Paecilomycin B (**2**) was a yellowish, amorphous solid and had the same molecular formula as **1** on the basis of the HRESIMS, which gave a quasi-molecular ion at *m*/*z* 379.1378 [M − H]$^-$. Its ^{1}H and ^{13}C NMR data (Tables 1 and 2), assigned by combined analysis of the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra, indicated a structure closely related to compound **1**. The downfield shifts of H-2′ (δ 5.11), C-1′ (δ 87.1), C-2′ (δ 68.9), and C-5′ (δ 89.2) relative to those in **7**, as well as long-range correlations from H-1′ to C-5′ and from H-5′ to C-1′ in the HMBC spectrum, indicated that C-1′ was connected to C-5′ through an oxygen bridge to form a pyran ring rather than a 1′,2′-epoxide as in **1**. The relative configuration of **2** was determined by the NOESY spectrum and ^{1}H NMR coupling constants. In the NOESY spectrum, strong mutual NOE correlations were observed between H-1′/H-3′β (δ 2.24), H-1′/H-5′, H-2′/H-3′α (δ 3.03), and H-2′/H-4′. The ^{1}H NMR spectrum (Table 1) revealed axial−axial coupling constants (J = 9.0−11.2 Hz) between H-1′/H-2′, H-2′/H-3′β, H-3′β/H-4′, and H-4′/H-5′. These correlations and values were consistent with a chair conformation for the pyran ring in which H-1′, H-2′, H-3′β, H-4′, and H-5′ were all in axial positions, while 2′-OH, 4′-OH, and H-3′α were in equatorial positions. Mutual NOE correlations between H-4′/H-6′, H-6′/H-7′, and H-7′/H-10′ were also observed in the NOESY spectrum, suggesting the α-orientation of H-6′. Treatment of compound **1** with 20% H_2SO_4 yielded **2** as a major product. Since this conversion proceeds through the epoxide ring cleavage via intramolecular nucleophilic substitution generated by attack of the 5′-OH oxygen, leading to an inversion of stereochemistry of C-1′,[19] the absolute configuration of **2** was deduced to be 1′*S*,2′*R*,4′*S*,5′*S*,6′*R*,10′*S*. Therefore, the structure of compound **2** was unambiguously assigned as shown.

Paecilomycin C (**3**), a yellowish, amorphous solid, was determined to have the molecular formula $C_{19}H_{26}O_9$ from the ^{1}H and ^{13}C NMR data (Tables 1 and 2) and the HRESIMS ion at *m*/*z* 397.1478 [M − H]$^-$. Analysis of its 1D and 2D NMR spectra, particularly ^{1}H−^{1}H COSY and HMBC, indicated that **3** was also a RAL derivative, with a structure similar to that of aigialomycin F (**10**),[7] except for the lactone ring. The chemical shifts of H-1′ and C-1′ in **3** (Tables 1 and 2) were downfield shifted, while H-2′ and C-2′ were upfield shifted, relative to those in **10**, suggesting that **3** possessed a dihydroisobenzofuranone structure. This was supported by the HMBC spectrum, in which the correlation from H-1′ to the ester carbonyl carbon (C-7) was observed. Therefore, the planar structure of compound **3** was determined to be 7-hydroxy-5-methoxy-3-(1,3,4,5,9-pentahydroxydec-6-enyl)dihydroisobenzofuranone. Alkaline hydrolysis of aigialomycin B (**7**) gave compound **3** as a major product. Since the configuration of **7** is known as 1′*R*,2′*R*,4′*S*,5′*S*,6′*S*,10′*S* and the hydrolysis proceeds through the ester linkage cleavage followed by stereospecific epoxide ring-opening via S_N2-type nucleophilic substitution generated by attack of the carboxylic anion nucleophile leading to an inversion of

stereochemistry of C-1′, the absolute configuration of **3** was assigned as 1′*S*,2′*R*,4′*S*,5′*S*,6′*S*,10′*S*.

Paecilomycin D (**4**) had the same molecular formula as **3** on the basis of the ESIMS and HRESIMS, and its ^{1}H and ^{13}C NMR spectra were very similar to those of **3**. The only significant difference between the spectra of **3** and **4** was in the chemical shift of C-6′ (δ 74.8 in **4** vs δ 72.7 in **3**), suggesting that **4** was the 6′-epimer of **3**. This structure was confirmed by acid hydrolysis of **1**, which yielded compound **4** as a minor product and **2** as the major product. Thus, the configuration of **4** was assigned as 1′*S*,2′*R*,4′*S*,5′*S*,6′*R*,10′*S*.

Paecilomycin E (**5**), colorless needles, had a molecular formula of $C_{19}H_{26}O_9$. The ^{1}H and ^{13}C NMR (Tables 1 and 2) and DEPT spectra indicated that **5** was closely related to zeaenol (**8**),[17] a known RAL also obtained in this study. Analysis of the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra led to a planar structure of 7′,8′-dihydrozeaenol, which was identical to that of antibiotic LL-Z1640-3, a previously reported RAL of undesignated relative configuration.[20] In order to establish its configuration, compounds **5** and zeaenol (**8**) were separately hydrogenated (H_2, Pd/C, $CHCl_3$, rt, 6 h), and both reactions gave the same product, 1′,2′,7′,8′-tetrahydrozeaenol (**11**), which was identified on the basis of its HRESIMS, ^{1}H NMR, and ^{1}H−^{1}H COSY data. Therefore, the structure of **5** was determined to be (4′*S*,5′*R*,6′*S*,10′*S*)-7′,8′-dihydrozeaenol.

Paecilomycin F (**6**) had the same molecular formula as **5** on the basis of the HRESIMS. Its ^{1}H and ^{13}C NMR data, assigned by analysis of the 2D NMR (^{1}H−^{1}H COSY, HSQC, and HMBC) spectra, were very similar to those of **5**, but there was a significant difference in the chemical shift of C-6′ (δ 66.9 in **6** vs δ 76.0 in **5**). These findings showed that this compound was the 6′-epimer of **5**. Considering that compounds **5** and **6** must be biogenetically derived from a common 6′-ketone macrolide, the absolute configuration of **6** was tentatively assigned as 4′*S*,5′*R*,6′*R*,10′*S*.

Among these new RAL derivatives, paecilomycins B−D (**2**−**4**) were structurally noticeable. Compound **2** is the first RAL possessing a 14-membered macrolide ring in which C-1′ and C-5′ are connected via an oxygen bridge to form a pyran ring. Paecilomycins C (**3**) and D (**4**), both having a dihydroisobenzofuranone ring and a polyhydroxylated linear C_{10} side chain, are also unusual for RALs. It is noted that these compounds are unlikely to be isolation artifacts because **2**, **3**, and **10** were detected in the crude extract by HPLC-UV or HPLC-MS/MS analysis (see Supporting Information). As described above, compounds **2**−**4** were transformed from paecilomycin A (**1**) or aigialomycin B (**7**) by acid or alkaline hydrolysis through the epoxide ring cleavage via intramolecular nucleophilic substitution (S_N2). Compounds **1** and **7** could be derived from aigialomycin A by reduction, although this compound was not obtained in the present study. These chemical correlations also suggested a possible biogenetic pathway from aigialomycin A to **1**−**4**. A pathway with similar biogenetic mechanisms was previously proposed for the production of dihydroisobenzofuranone-spiroacetal and dihydroisocoumarin-type RALs by Isaka and co-workers.[7,18]

Compounds **1**−**10** were evaluated for their antiplasmodial activity against *Plasmodium falciparum* lines 3D7 (a chloroquine-susceptive line) and Dd2 (a chloroquine-resistant line) using the protocol described previously.[21] Chloroquine and artemisinin were synchronously tested as positive controls. In the assay against the *P. falciparum* line 3D7, compounds **5** and **10** exhibited remarkable activity with IC_{50} values of 20.0 and 10.9 nM, respectively, which were comparable with those of artemisinin (IC_{50} 12.5 nM) and chloroquine (IC_{50} 23.5 nM). Compounds **1**, **2**, **6**, **7**, and **9** showed moderate activity with IC_{50} values of 0.78, 3.8, 1.1, 0.65, and 3.1 μM, respectively. Dihydroisobenzofuranone derivatives **3** and **4** and the 1′*E*,7′*E*-diene macrolide **8** were all inactive (IC_{50} > 50 μM). However, in the assay against the *P. falciparum* line Dd2, only macrolides **5**−**7** and **9** exhibited activity with IC_{50} values of 8.8, 1.7, 13.5, and 10.5 μM, respectively. They were, however, much less potent than artemisinin (IC_{50} 13.5 nM) and chloroquine (IC_{50} 112.6 nM), and the other compounds were inactive (IC_{50} > 50 μM). The results indicated that antiplasmodial activity of these compounds is structure dependent and that the chloroquine-resistant parasite Dd2 line is also resistant to RALs. It was interesting to note that **5** and **6**, a pair of 6′-epimers closely related to **8**, were active against both parasite lines, whereas **8** was inactive toward both lines. The antiplasmodial activity of RALs was reported to be related to their cytotoxicity.[6,7] However, in our cytotoxicity assay using the MTT method with cisplatin as a positive control, the above antiplasmodial compounds (**1**, **2**, **5**−**7**, **9**, and **10**) were all found to be relatively noncytotoxic (IC_{50} > 50 μM) against the mammalian Vero cell line (for experimental details, see Supporting Information). Thus, these RALs merit further investigation as models for the discovery of new antimalarial molecules.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter. Melting points were determined with a Yanagimoto micro melting point apparatus (Yanagimoto, Kyoto, Japan). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument with residual solvent peaks as references. HRESIMS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer in negative-ion mode. ESIMS data were collected on a MDS SCIEX API2000 LC/MS/MS instrument. Preparative HPLC was performed with a Shimadzu LC-6AD pump and a Shimadzu RID-10A refractive index detector using a YMC-pack ODS-A C_{18} column (5 μm, 250 × 20 mm). For column chromatography, silica gel 60 (100−200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 were used.

Producing Fungus and Fermentation. The producing fungus, *Paecilomyces* sp. SC0924, was isolated from a soil sample collected in the Dinghu Mountain Biosphere Reserve, Guangdong, China, in March 2003. It was authenticated by Prof. Tai-hui Li, Guangdong Institute of Microbiology, Guangzhou, China. This fungal strain (CGMCC No. 2900) has been deposited in the culture collection of China General Microbiological Culture Collection Center (CGMCC), Beijing, China. The seed culture (2500 mL) was prepared by growing the fungus in YMG broth on a rotary shaker for 5 days in the dark at 28 °C, 120 rpm. The culture was transferred into 25 × 5 L Erlenmeyer flasks containing 1 L of YMG medium and 550 g of wheat grains, and the flasks were incubated for 8 days in the stationary phase in the dark at 28 °C.

Extraction and Isolation. The mycelial culture was extracted with 95% EtOH, and the resultant extract was sequentially partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$-soluble extract (19.23 g) was separated by silica gel CC and eluted with $CHCl_3$−MeOH mixtures of increasing polarity (95:5−80:20) to afford five fractions (1−5). Fraction 3, obtained on elution with $CHCl_3$−MeOH (95:5), was subjected to silica gel CC using petroleum ether−acetone (85:15−60:40) to afford five subfractions (3-1−3-5). Subfraction 3-2 was separated by HPLC using 66% MeOH to afford **5** (60 mg) (t_R = 42.00 min) and **6** (25 mg) (t_R = 47.14 min). Subfraction 3-3 was separated by Sephadex LH-20 CC using MeOH followed by HPLC purification using 50% MeOH to afford aigialospirol (17 mg) (t_R = 28.15 min). Fraction 4, obtained on elution with $CHCl_3$−MeOH (85:15), was further subjected to silica gel CC and eluted with petroleum ether−acetone (85:15−60:40) to afford six subfractions (4-1−4-6). Subfraction 4-4 was further separated by Sephadex LH-20 CC using MeOH followed by ODS CC using aqueous MeOH (66−80%) to afford **9** (91 mg). Subfraction 4-5 was recrystallized from MeOH to afford **1** (320 mg), and the mother liquor of subfraction 4-5 was further separated by Sephadex LH-20 CC using MeOH followed by ODS CC using aqueous MeOH (66−80%) to afford **7** (110 mg) and **8** (25 mg).

The EtOAc-soluble extract (39.31 g) was separated by silica gel CC and eluted with $CHCl_3$−MeOH mixtures (95:5−50:50) to afford eight fractions (6−13). Fraction 11, obtained on elution with $CHCl_3$−MeOH (90:10), was further separated by silica gel CC using petroleum ether−acetone (80:20−50:50) followed by purification with HPLC using 33% MeOH to afford **2** (56 mg) (t_R = 41.38 min).

The *n*-BuOH-soluble extract (67.44 g) was subjected to silica gel CC and eluted with $CHCl_3$−MeOH mixtures (95:5−50:50) to afford 10 fractions (14−23). Fraction 21, obtained on elution with $CHCl_3$−MeOH (80:20), was separated by Sephadex LH-20 CC using

MeOH to afford four subfractions (21-1−21-4). Subfraction 21-2 was recrystallized from MeOH to afford **10** (48 mg). Subfraction 21-3 was further separated by ODS CC using 33% MeOH to afford **3** (14 mg) and **4** (9 mg).

Paecilomycin A (1): colorless, columnar crystals (MeOH); mp 184−186 °C; $[\alpha]^{24}_D$ −58.5 (*c* 0.27, MeOH); UV(MeOH) λ_{max} (log ε) 222(4.31), 265(4.05), 306(3.71) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 381 $[M + H]^+$, 403 $[M + Na]^+$; negative ESIMS *m/z* 379 $[M - H]^-$, 415 $[M + Cl]^-$; HRESIMS *m/z* 379.1414 $[M - H]^-$ (calcd for $C_{19}H_{23}O_8$, 379.1393).

Crystal data for **1** at 173(2) K: $C_{19}H_{24}O_8$, $M = 380.38$, orthorhombic, space group, $P2(1)2(1)2(1)$ with $a = 6.7386(3)$ Å, $b = 9.6557(5)$ Å, $c = 27.6093(13)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 1796.42(15)$ Å^3, $Z = 2$, D_{calcd} 1.406 Mg/m^3. $F_{000} = 808$, λ(Mo Kα) = 0.71073 Å, $\mu = 0.110$ mm^{-1}. Data collection and reduction: crystal size 0.49 × 0.42 × 0.22 mm^3, θ range 1.48 to 27.01°, 9192 reflections collected, 2264 independent reflections ($R_{int} = 0.0259$), final *R* indices $I > 2\sigma(I)$: 0.0346, $wR_2 = 0.0946$ for 250 parameters, GOF = 1.079. Flack parameter = 0.9772(11). Intensity data were measured on a Bruker Smart 1000 CCD diffractometer. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 753356). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Paecilomycin B (2): yellowish, amorphous solid, $[\alpha]^{24}_D$ +40.4 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.04), 283 (3.34) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 403 $[M + Na]^+$; negative ESIMS *m/z* 379 $[M - H]^-$, 415 $[M + Cl]^-$, 759 $[2\,M - H]^-$; HRESIMS *m/z* 379.1378 $[M - H]^-$ (calcd for $C_{19}H_{23}O_8$, 379.1393).

Paecilomycin C (3): yellowish, amorphous solid; $[\alpha]^{24}_D$ +12.6 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.28), 256 (3.92), 292 (3.44) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 399 $[M + H]^+$, 421 $[M + Na]^+$; negative ESIMS *m/z* 397 $[M - H]^-$, 433 $[M + Cl]^-$,795 $[2\,M - H]^-$, 831 $[2\,M + Cl]^-$; HRESIMS *m/z* 397.1478 $[M - H]^-$ (calcd for $C_{19}H_{25}O_9$, 397.1499).

Paecilomycin D (4): yellowish, amorphous solid; $[\alpha]^{24}_D$ +9.26 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.21), 256 (3.82), 292 (3.36) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 399 $[M + H]^+$, 421 $[M + Na]^+$; negative ESIMS *m/z* 397 $[M - H]^-$, 433 $[M + Cl]^-$, 795 $[2\,M - H]^-$, 831 $[2\,M + Cl]^-$; HRESIMS *m/z* 397.1520 $[M - H]^-$ (calcd for $C_{19}H_{25}O_9$, 397.1499).

Paecilomycin E (5): colorless needles; mp 104−106 °C; $[\alpha]^{24}_D$ −96.4 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.31), 271 (3.95), 311 (3.64) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 367 $[M + H]^+$, 389 $[M + Na]^+$, 755 $[2\,M + Na]^+$; negative ESIMS *m/z* 365$[M - H]^-$, 401 $[M + Cl]^-$, 731 $[2\,M - H]^-$; HRESIMS *m/z* 365.1617 $[M - H]^-$ (calcd for $C_{19}H_{25}O_7$, 365.1600).

Paecilomycin F (6): colorless needles; mp 175−178 °C; $[\alpha]^{24}_D$ −106.4 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.36), 271 (4.01), 314 (3.70) nm; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; positive ESIMS *m/z* 367 $[M + H]^+$, 389 $[M + Na]^+$, 755 $[2\,M + Na]^+$; negative ESIMS *m/z* 365 $[M - H]^-$, 401 $[M + Cl]^-$, 731 $[2\,M - H]^-$; HRESIMS *m/z* 365.1627 $[M - H]^-$ (calcd for $C_{19}H_{25}O_7$, 365.1600).

Preparation of Acetonide 1a. A mixture of **1** (10.0 mg), 2,2-dimethoxypropane (1.8 mL), and *p*-TsOH (2.0 mg) was stirred at room temperature for 3 h. Saturated aqueous $NaHCO_3$ (5 mL) was then added, and the reaction mixture was extracted with EtOAc (5 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to silica gel CC using $CHCl_3$−MeOH (95:5) to afford **1a** (4.5 mg).

Acetonide 1a: white, amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.80 (1H, br s, 2-O*H*), 6.46 (1H, d, $J = 2.7$ Hz, H-5), 6.35 (1H, d, $J = 2.7$ Hz, H-3), 5.73 (1H, dt, $J = 15.7$, 6.5 Hz, H-8′), 5.57 (1H, dd, $J = 15.7$, 6.0 Hz, H-7′), 5.33 (1H, m, H-10′), 4.72 (1H, dd, $J = 7.8$, 6.0 Hz, H-6′), 4.62 (1H, d, $J = 1.8$ Hz, H-1′), 4.38 (1H, d, $J = 7.8$ Hz, H-5′), 3.77 (3H, s, 4-OC*H*$_3$), 3.64 (1H, d, $J = 10.5$ Hz, H-4′), 2.83 (1H, dt, $J = 10.0$, 1.8 Hz, H-2′), 2.44−2.53 (2H, m, H$_2$-9′), 2.11 (1H, ddd, $J = 14.4$, 10.5, 1.8 Hz, H-3′a), 1.48 (1H, m, H-3′b), 1.47 (3H, s, H$_3$-2″), 1.35 (1H, d, $J = 6.2$ Hz, 10′-C*H*$_3$), 1.32 (3H, s, H$_3$-3″); positive ESIMS *m/z* 421 $[M + H]^+$, 443 $[M + Na]^+$; negative ESIMS *m/z* 419 $[M - H]^-$, 455 $[M + Cl]^-$.

Acid Hydrolysis of 1. A mixture of **1** (80.0 mg) and 20% H_2SO_4 (6 mL) was stirred at 25 °C for 1.5 h, and then 10 mL water was added. The reaction mixture was neutralized with K_2CO_3, and extracted successively with EtOAc and n-BuOH. The n-BuOH extract was subjected to silica gel CC using $CHCl_3$−MeOH (95:5−80:20) to yield **2** (9.0 mg) and **4** (3.5 mg).

Alkaline Hydrolysis of Aigialomycin B (7). A mixture of **7** (10 mg) and 4% NaOH in 90% MeOH (3 mL) was stirred at 25 °C for 4 h, and then 10 mL of water was added. The reaction mixture was neutralized with 0.05 M HCl and extracted with EtOAc and n-BuOH. The n-BuOH extract was subjected to silica gel CC using $CHCl_3$−MeOH (80:20) to yield **3** (3.0 mg).

Hydrogenation of Paecilomycin E (5) and Zeaenol (8). To a solution of paecilomycin E (**5**) (5.0 mg) in $CHCl_3$ (4 mL) was added 10% Pd/C (20 mg), and the mixture was stirred under H_2 for 6 h. The suspension was filtered by suction, and the filtrate was concentrated and subjected to silica gel CC using petroleum ether−acetone (3:2) to obtain **11** (3.5 mg). Zeaenol (**8**) (5.0 mg) was hydrogenated under the same reaction conditions and also gave compound **11** (3.5 mg).

1′,2′,7′,8′-Tetrahydrozeaenol (11): white, amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.32 (1H, br s, 2-O*H*), 6.33 (1H, d, $J = 2.6$ Hz, H-3 or H-5), 6.24 (1H, d, $J = 2.60$ Hz, H-5 or H-3), 5.15 (1H, m, H-10′), 4.10 (1H, br d, $J = 11.5$ Hz, H-6′), 3.91 (1H, dd, $J = 10.6$, 3.5 Hz, H-4′), 3.78 (3H, s, 4-OC*H*$_3$), 3.60 (1H, br s, H-5′), 3.26 (1H, td, $J = 12.4$, 3.8 Hz, H-1′a), 2.36 (1H, td, $J = 12.4$, 4.5 Hz, H-1′b), 1.92−1.36 (total 10H, m, H$_2$-2′, H$_2$-3′, H$_2$-7′, H$_2$-8′, H$_2$-9′), 1.35 (1H, d, $J = 6.0$ Hz, 10′-C*H*$_3$); HRESIMS *m/z* 367.1762 $[M - H]^-$ (calcd for $C_{19}H_{27}O_7$, 367.1757).

Antiplasmodial Activity Evaluation. *P. falciparum* lines 3D7 and Dd2 were maintained continuously in blood group O^+ human erythrocytes and 10% human serum in a gas mixture consisting of 7% CO_2, 5% O_2, and 88% N_2. Antiplasmodial activity was determined by a malaria SYBR Green I-based fluorescence (MSF) method.[21] Compounds **1**−**10** were prepared as 100 mM stock solutions in DMSO. Artemisinin and chloroquine (Sigma-Aldrich) were prepared as 1 mM stock solutions in DMSO and water, respectively. The drug solutions were serially diluted with culture medium and distributed to asynchronous parasite cultures on 96-well plates in quadruplicate in a total volume of 100 μL to achieve 0.2% parasitemia with a 2% hematocrit in a total volume of 100 μL. The plates were then incubated for 72 h at 37 °C. After incubation, 100 μL of lysis buffer with 0.2 μL/mL SYBR Green I was added to each well. The plates were incubated at 37 °C for an hour in the dark and then placed in a 96-well fluorescence plate reader (Multilabel HTS Counter, PerkinElmer) with excitation and emission wavelengths of 497 and 520 nm, respectively, for measurement of fluorescence. The 50% inhibitory concentration (IC_{50}) was determined by nonlinear regression analysis of logistic dose−response curves (GraphPad Prism software).

Acknowledgment. We thank Prof. Tai-hui Li, Guangdong Institute of Microbiology, for microbiological authentication. This work was supported by NSFC grants (nos. 20672114 and 30901856) and the Knowledge Innovation Program of the Chinese Academy of Sciences (grant no. KSCX2-YW-N-036).

Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**6**, **1a**, and **11**, HRESIMS of compounds **1**−**6** and **11**, spectroscopic data of the known compounds, interpretation of key 2D NMR data of **1a** and **2**−**4**, possible biogenetic pathway from aigialomycin A to **1**−**4**, detection of compounds **2**, **3**, and **10** from the crude extract, cytotoxicity of antiplasmodial compounds against the mammalian Vero cell line, and a crystallographic information (cif) file. These data are available free of charge via the Internet at http://pubs.acs.org.

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NP900853N