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Article

Three Bianthraquinone Derivatives from the Mangrove Endophytic Fungus *Alternaria* sp. ZJ9-6B from the South China Sea

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Abstract: Three new bianthraquinone derivatives, alterporriol K (**1**), L (**2**) and M (**3**), along with six known compounds were obtained from extracts of the endophytic fungus *Alternaria* sp. ZJ9-6B, isolated from the mangrove *Aegiceras corniculatum* collected in the South China Sea. Their structures were elucidated by one- and two-dimensional NMR spectroscopy, MS data analysis and circular dichroism measurements. Compounds **1**, **2** and **3** were first isolated alterporriols with a C-2–C-2′ linkage. The crystallographic data of tetrahydroaltersolanol B (**7**) was reported for the first time. In the primary bioassays, alterporriol K and L exhibited moderate cytotoxic activity towards MDA-MB-435 and MCF-7 cells with IC_{50} values ranging from 13.1 to 29.1 μM.

Keywords: endophytic fungus; *Alternaria* sp.; bianthraquinone; alterporriol; cytotoxicity

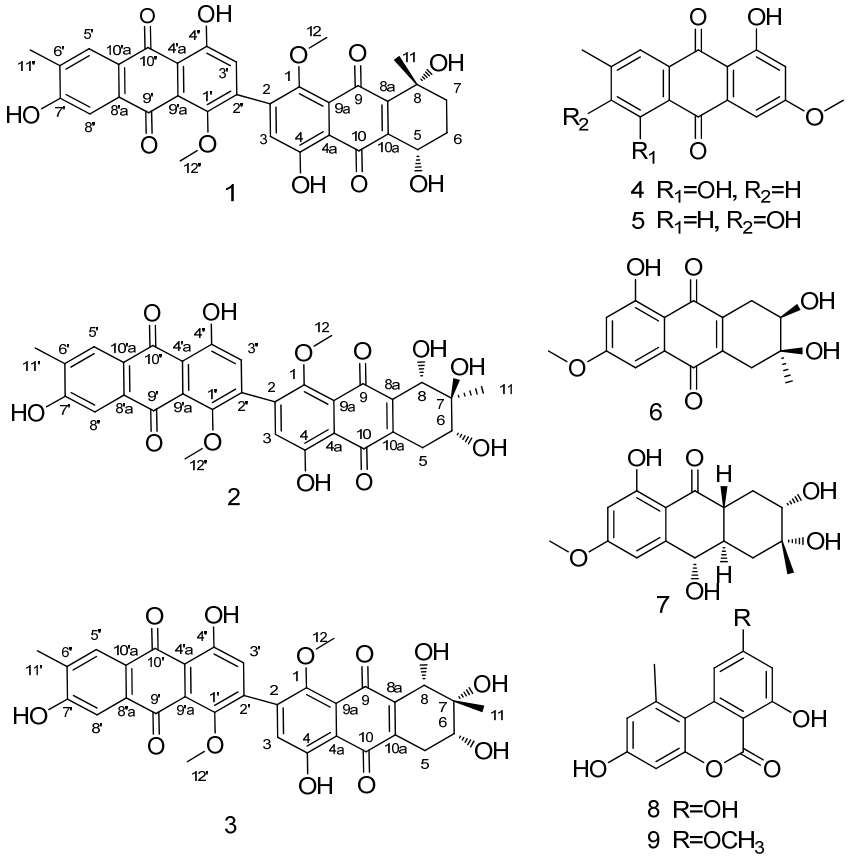
1. Introduction

A wide variety of anthraquinone derivatives isolated from plants, animals and marine fungi have served as candidates for various therapeutic uses [1–3]. Anthraquinones inhibit the proliferation of human breast, colon and lung cancer cells [4]. They also displayed inhibitory ability towards protein kinase, NADH oxidase, quinone reductase and calmodulin [5–8]. Several laboratories have investigated anthraquinones as antibacterial agents [9].

The alterporriol family of bianthraquinone derivatives were first reported from *Alternaria porri* by Suemitsu *et al.* in 1984 [10]. Over the last 27 years, nine additional alterporriols have been reported from fungi. All the alterporriols except alterporriols G-J were described from *Alternaria* sp. [5,11–13]. In terms of the underlying monomers, alterporriols can occur as either homodimers or heterodimers. With regard to the coupling positions of the monomers, alterporriols A, B, D, E, I and J feature a C-5–C-5′ linkage, alterporriol C shows a C-1–C-7′ connection, and G and H possess a C-7–C-5′ linkage [5,11–13].

As part of our ongoing program to search for new bioactive natural products from the South China Sea [14–16], an endophytic fungus *Alternaria* sp. ZJ9-6B has been isolated from the fruit of the marine mangrove *Aegiceras corniculatum* in Zhanjiang, Guangdong, China. Chemical investigation of this fungus led to the isolation of nine metabolites, including three new anthraquinone derivatives **1**–**3** and six known compounds **4**–**9** (Figure 1). It is interesting that compounds **1**–**3** all possess dimeric structures with a C-2–C-2′ linkage. In this report, we describe the isolation, structural elucidation and biological activity of these new metabolites.

Figure 1. Structures of **1**–**9** isolated from *Alternaria* sp. ZJ9-6B.



2. Results and Discussion

The methanol extract of the dried mycelium was subjected to a combination of column chromatography on silica gel, Sephadex LH-20 and C_{18} reversed phase silica gel.

Compound **1** was isolated as a red amorphous powder. HR-EIMS at *m/z* = 586.1471 $[M]^+$ indicated the molecular formula $C_{32}H_{26}O_{11}$ (calcd. for $C_{32}H_{26}O_{11}$, 586.1470). Compound **1** exhibited strong optical rotation $[\alpha]_D^{20}$ +690 (*c* = 1.0, MeOH) which indicated the possibility of an asymmetric centre and/or axial chirality (Figure 2). The IR spectrum (KBr) exhibited a weak shoulder at 1652 cm^{-1} and an intense band at 1638 cm^{-1} for carbonyl groups. The UV spectrum displayed bands at 224, 280 and 437 nm, suggesting a quinonoid chromophore. The ^1H NMR spectrum (Table 1) showed a pair of chelated hydroxyl resonances (δ_H = 13.61 and 13.15 ppm), four aromatic protons (δ_H = 7.67, 7.55, 6.92 and 6.88 ppm), two methoxyl protons (δ_H = 3.68 and 3.66 ppm), two singlet methyls (δ_H = 2.18 and 1.07 ppm), two methylene protons (δ_H = 2.53 and 2.72 ppm, δ_H = 2.20 and 2.34 ppm), and oxygenated methine (δ_H = 3.51 ppm). The ^{13}C NMR spectrum displayed four carbonyl signals (δ_C = 183.6, 187.8, 181.1 and 186.7 ppm), twenty signs of aromatic carbons, one quaternary carbon (δ_C = 69.0 ppm), one methine (δ_C = 70.1 ppm) and two methylenes (δ_C = 29.1 and 36.1 ppm). These data implied that compound **1** possessed a bianthranquinone scaffold, including an anthraquinone unit and a tetrahydroanthraquinone unit (Figure 1) [5,13]. The unsubstituted carbons for two aromatic rings of the anthraquinone unit were located at C-8′ (δ_C = 130.3 ppm; δ_H = 7.67 ppm, d, *J* = 0.8 Hz), C-5′ (δ_C = 110.5 ppm; δ_H = 7.55 ppm, d, *J* = 0.8 Hz) and C-3′ (δ_C = 103.8 ppm; δ_H = 6.921 ppm, s) by the HMBC correlations (Figure 3). In the tetrahydroanthraquinone unit, one aromatic proton at H-3 (δ_H = 6.88 ppm, s) and the protons in the alicyclic ring, including one oxygenated methine H-5 (δ_H = 3.51 ppm, ddd, *J* = 5.4, 5.5, 12.5 Hz) and two methylene protons H-6 (δ_H = 2.53 and 2.72 ppm) and H-7 (δ_H = 2.20 and 2.34 ppm) were observed.

Figure 2. CD Spectra of **1**. Recorded in MeOH at amibient temperature.

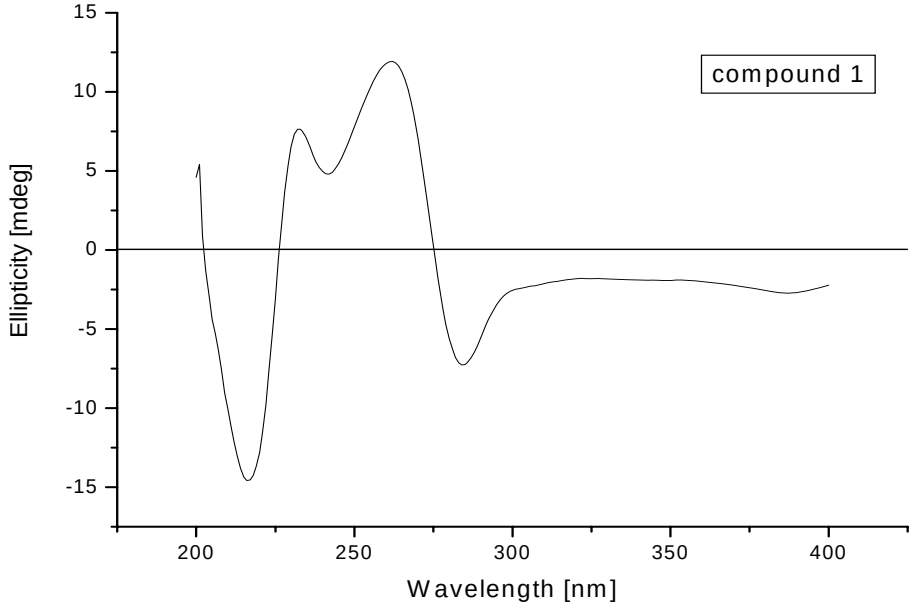


Table 1. NMR spectroscopic data (DMSO-d_6) of **1** [a,b].

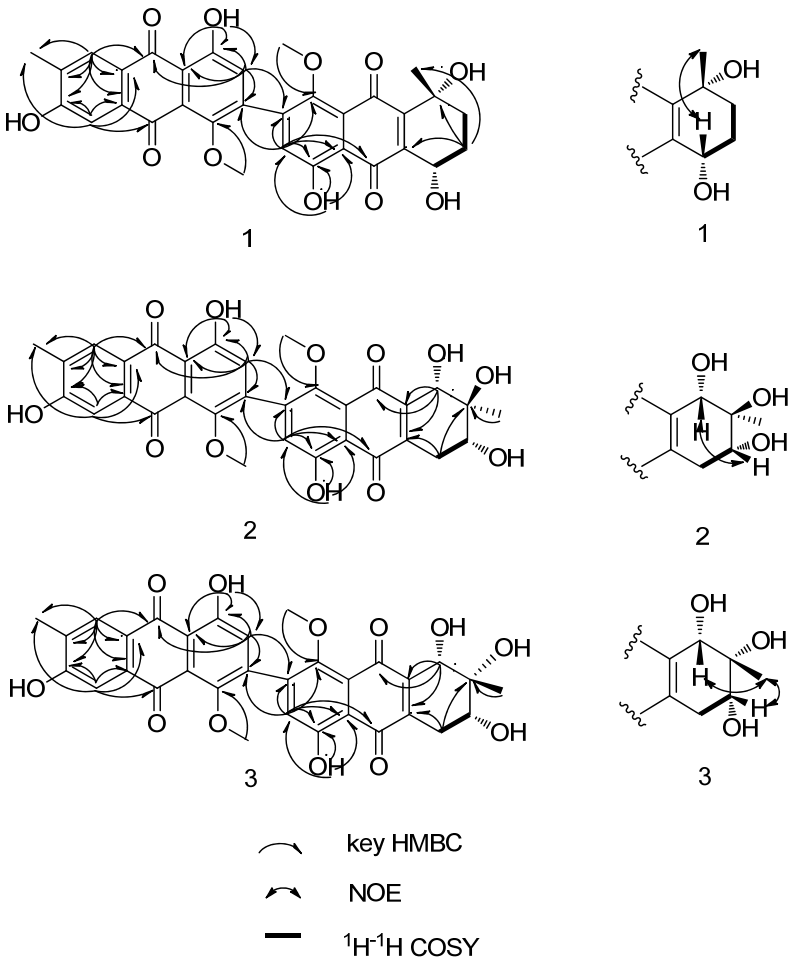
Atom	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* (Hz))	COSY [b]	HMBC [b]	NOESY
1	164.1				
2	123.3				
3	103.7	6.88 (s)		C-1, 2, 2′, 4, 4a, 10	
4	163.8				
4a	108.6				
5	70.1	3.51 (ddd, 5.5, 5.5, 12.5)	H-6a, 6b, 5-OH	C-6, 7, 8a, 11	H-11
6	29.1	a: 2.53 (m)	H-5, 7a, 7b	C-5, 8, 10a	H-11
		b: 2.72 (m)	H-5, 6a, 7a, 7b	C-5, 8, 10a, 11	
7	36.1	a: 2.31 (m)	H-6a, 6b, 7b	C-5, 8, 8a, 11	
		b: 2.35 (m)	H-6a, 6b, 7a	C-5, 8, 8a, 11	H-11
8	69.0				
8a	141.6				
9	183.6				
9a	128.9				
10	187.8				
10a	143.2				
11	25.2	1.07 (s)		C-5, 7, 8	
12	56.7	3.66 (s)		C-1, 3	
4-OH		13.15 (s)		C-3, 4, 4a	
5-OH		4.69 (d, 5.5)	H-5	C-5, 6, 8	
8-OH		4.30 (s)		C-5, 7, 8, 11	
1′	164.7				
2′	122.5				
3′	103.8	6.92 (s)		C-2, 2′, 4′, 4′a, 10′	
4′	165.0				
4′a	110.0				
5′	110.5	7.55 (d, 0.8)		C-6′, 7′, 8′a, 10′, 10′a, 11′	
6′	125.2				
7′	161.3				
8′	130.3	7.67 (d, 0.8)		C-7′, 8′a, 9′, 10′a, 11′	
8′a	132.4				
9′	181.1				
9′a	130.7				
10′	186.7				
10′a	132.2				
11′	16.1	2.18 (s)		C-5′, 7′, 8′	
12′	56.8	3.69 (s)		C-1′	
4′-OH		13.63 (s)		C-3′, 4′, 4′a	
7′-OH		8.11 (s)			

[a] Measured at 500 MHz (for ^{1}H) and 125 MHz (for ^{13}C); [b] For the HMBC and COSY spectra, see the Supporting Information.

A contiguous sequence of coupled signals from H-5 to H-7 in the ^{1}H-^{1}H COSY spectrum combined with the HMBC correlations from H-5 to C-6, C-7 and C-8a, from H-6 to C-5, C-8, and C-10a, and

from H-7 to C-5, C-8, C-8a, C-9 and C-11, established the substructure of the cyclohexene ring (Figure 3).

Figure 3. Key HMBC, NOE and ^{1}H-^{1}H COSY correlations of **1**–**3**.



HMBC correlations from each of 4-OH (δ_H = 13.15 ppm) and 4′-OH (δ_H = 13.63 ppm) to C-4, C-3, C-4a and to C-4′, C-3′, C-4′a, respectively, indicated their location at C-4 and C-4′ of the bianthraquinone scaffold.

The presence of a bond connecting C-2 and C-2′ was suggested by the absence of two sets of *ortho*-coupled doublets and the presence of two singlets H-3 (δ_H = 6.88 ppm, s) and H-3′ (δ_H = 6.92 ppm, s). Moreover, HMBC correlations of H-3 with C-1, C-2, C-2′, C-4, C-4a and C-10, and of H-3′ with C-1′, C-2, C-2′, C-4′, C-4′a and C-10′, respectively (Figure 3) provided evidence for C-2–C-2′ linkage of **1**. The HMBC spectrum showed correlations of the methoxyl proton (δ_H = 3.66 ppm, s, 3H, H-12) with C-1 and C-3. Likewise, correlations were observed from methoxyl proton (δ_H = 3.69 ppm, s, 3H, H-12′) to C-1′. These allowed us to assign two methoxyl groups to C-1 and C-1′.

The relative configuration of the chiral centers of C-5 and C-8 were deduced by 2D ^{1}H-^{1}H NOESY experiments (Figure 3) and the analysis of ^{1}H-^{1}H coupling constants (Table 1). A NOESY correlation between CH_3-11 (δ_H = 1.07 ppm, s) and H-5 suggested that they were on the same side of the cyclohexene ring. The axial position of H-5 was confirmed by coupling constant $J_{H\text{-}5,H\text{-}6a}$ = 12.5 Hz, indicating CH_3-11 to also have an axial orientation. Therefore, compound **1** was determined as

(5*S**,8*R**)-4,4′,5,7′,8-pentahydroxy-1,1′-dimethoxy-6′,8-dimethyl-5,6,7,8-tetrahydro-[2,2′-bianthracene]-9,9′,10,10′-tetraone. We propose the trivial name alterporriol K.

Compounds **2** and **3** were obtained as a mixture after separating with Sephadex LH-20 chromatography. Upon HPLC analysis, the mixture exhibited two partially overlapped peaks (area ratio *ca.* 4:1). Then compounds **2** and **3** were isolated with re-separation by preparative HPLC, respectively. Compound **2** was a red amorphous powder, $[\alpha]_D^{25}$ = +30 (*c* = 1.0, MeOH). The HR-ESI-TOF-MS exhibited a peak at *m/z* = 601.1340 $[M - H]^-$ indicating a molecular formula of $C_{32}H_{26}O_{12}$ (calcd. for $C_{32}H_{25}O_{12}$, 601.1346). Comparison of the ^1H and ^{13}C NMR spectral data of **2** (Table 2) with that of **1** showed a close structural relationship between both compounds, except for the presence of an additional oxymethine group proton (δ_H = 4.03, d, *J* = 6.7 Hz, H-8) and an additional hydroxyl group signal (δ_H = 2.17 ppm, s, 6-OH) and the absence of the methylene signals corresponding to H-7 in the ^1H NMR spectrum of **2**. The substructure of the cyclohexene ring was established by HMBC correlations from H-8 to C-6, C-7, C-8a, C-9, C-10a and C-11 and ^1H-^1H COSY correlations between H-5 and H-6 (Figure 3). NOE difference and analysis of ^1H-^1H coupling constants (Table 2) enabled the relative of configuration of **2** to be deduced. In the NOE experiment, when the methyl signal CH_3-11 (δ_H = 1.16, s) was irradiated, no enhancement of signals H-6 and H-8 was observed. Meanwhile, irradiation of H-8 caused an enhancement of H-6. These data suggested that CH_3-11 with H-6 and H-8 was *trans*-configuration in the cyclohexene ring (Figure 3). A large coupling constant for H-5a/H-6 ($J_{H\text{-}5a,H\text{-}6}$ = 10.0 Hz) indicated an axial location for H-6, likewise suggesting an axial location for H-8. Thus, the relative configuration at C-6, C-7, and C-8 was 6*S**, 7*R** and 8*R**. Compound **2** was finally defined as (6*S**,7*R**,8*R**)-4,4′,6,7,7′,8-hexahydroxy-1,1′-dimethoxy-6′,7-dimethyl-5,6,7,8-tetrahydro-[2,2′-bianthracene]-9,9′,10,10′-tetraone, named as alterporriol L.

Table 2. NMR spectroscopic data (DMSO-d_6) of **2** and **3** [a,b].

Actom	**2**			**3**		
	δ_C (ppm)	**δ_H (ppm) (multiplicity, *J* (Hz))**	**HMBC**	**δ_C (ppm)**	**δ_H (ppm) (multiplicity, *J* (Hz))**	**HMBC**
1	164.3			164.5		
2	123.2			123.5		
3	103.6	6.90 (s)	C-1, 2, 2′, 4, 4a, 10	103.4	6.92 (s)	C-1, 2, 2′, 4, 4a, 10
4	163.8			163.9		
4a	108.8			108.8		
5	28.8	a: 2.33 (dd, 19.5, 10.0)	C-6, 8a, 10a	28.8	a: 2.33 (dd, 19.3, 9.9)	C-6, 8a, 10a
		b: 2.79 (dd, 19.5, 6.0)	C-6, 7, 8a, 10a		b: 2.78 (dd, 19.3, 5.9)	C-6, 7, 8a, 10, 10a
6	66.7	3.70 [c]		66.6	3.69 [c]	
7	71.9			71.9		
8	69.0	4.03 (d, 6.7)	C-6, 7, 9, 8a, 10a	69.0	4.06 (d, 5.7)	
8a	142.6			142.6		
9	183.3			183.1		
9a	128.9			128.3		
10	188.4			188.3		
10a	143.7			143.6		
11	21.8	1.16 (3H, s)	C-6, 7, 8	21.8	1.16 (3H, s)	C-6, 7, 8
12	56.7	3.68 (3H, s)	C-1	56.7	3.68 (3H, s)	C-1

Table 2. *Cont.*

4-OH		13.11 (s)	C-3, 4, 4a		13.13 (s)	C-3, 4, 4a
6-OH		2.17 (s)			2.16 (s)	
7-OH		4.25 (s)	C-7, 8		4.48 (s)	
8-OH		5.42 (d, 6.7)	C-6, 7, 8		5.27 (d, 5.7)	
1′	164.4			164.9		
2′	122.3			122.5		
3′	104.0	6.92(s)	C-2, 2′, 4′, 4′a, 10′	103.5	6.89 (s)	C-1′, 2, 2′, 4′, 4′a,10′
4′	164.9			165.1		
4′a	110.0			110.0		
5′	110.5	7.55 (s)	C-6′, 7′, 8′a, 10′, 10′a, 11′	110.7	7.51 (s)	C-6′, 7′, 8′a, 10′, 10′a, 11′
6′	125.0			125.2		
7′	161.6			162.8		
8′	130.2	7.68 (s)	C-7′, 8′a, 9′, 10′a, 11′	130.1	7.65 (s)	C-7′, 8′a, 9′, 10′a, 11′
8′a	132.4			132.4		
9′	181.1			180.6		
9′a	131.2			130.7		
10′	186.8			186.9		
10′a	132.3			132.4		
11′	16.1	2.19 (s)	C-5′, 6′, 7′, 8′, 10′a	16.3	2.18 (s)	C-5′, 6′, 7′, 8′
12′	56.7	3.68 (s)	C-1′	56.8	3.71 (s)	C-1′
4′-OH		13.61 (s)	C-3′, 4′, 9′a		13.70 (s)	C-3′, 4′, 9′a
7′-OH		7.65 (s)	C-7′, 8′a, 9′, 10′, 11′		7.67 (s)	C-5′, 7′, 8′a, 11′

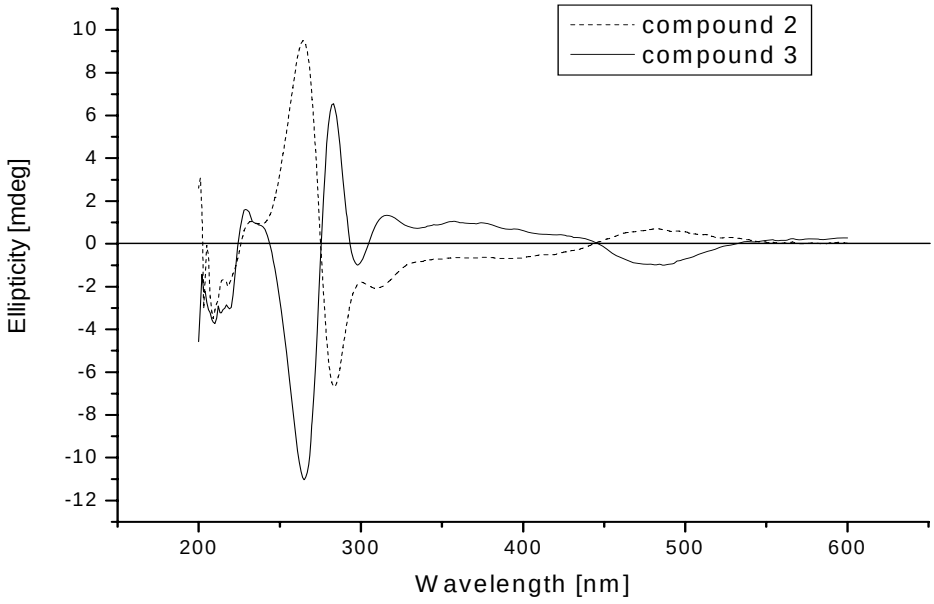
[a] Measured at 500 MHz (for 1H) and 125 MHz (for 13C); [b] For the HMBC and COSY spectra, see the Supporting Information; [c] Overlapped by methoxyl signal.

Compound **3**, obtained as a red amorphous power, $[\alpha]_D^{25} = -90$ (*c* = 1.0, MeOH), had the molecular formula $C_{32}H_{26}O_{12}$, as determined by HR-ESI-TOF-MS at *m/z* = 601.1340 $[M - H]^-$ (calcd. for $C_{32}H_{25}O_{12}$, 601.1346). The MS analysis revealed **3** to be an isomer of **2**. The UV and IR absorptions, ^1H and ^{13}C NMR data, HMBC and ^1H-^1H COSY correlations (Figure 3 and Table 2) of **3** were almost identical with those of **2**, suggesting the two compounds to have identical carbon skeletons. However, in contrast with 2 in the NOE experiment of 3, irradiation of the methyl signal CH_3-11 (δ_H = 1.16 ppm, s) resulted in obvious enhancements of the signals for both H-6 and H-8. The coupling constant for H-5a/H-6 was measured to be $J_{H\text{-}5a,H\text{-}6}$ = 9.9 Hz suggesting axial locations for H-6 and, by extension, H-8. These data suggested that 2 and 3 are epimers at C-7. Therefore compound 3 was elucidated as (6*S**,7*S**,8*R**)-4,4′,6,7,7′,8-hexahydroxy-1,1′-dimethoxy-6′,7-dimethyl-5,6,7,8-tetrahydro-[2,2′-bianthracene]-9,9′,10,10′-tetraone, named alterporriol M.

An interesting feature of the isolated bianthquinones is their optical activity. *Ab initio* calculations of CD spectra for several phenylanthraquinones with chiral axes have recently been reported [17,18]. Specific optical rotation of compounds **2** and **3** showed $[\alpha]_D^{20}$ +60 and −30, respectively. CD spectra of **2** and **3** recorded in methanol showed a near quasi-mirror image pattern (Figure 4). In the case of **2**, the CD spectrum consists of a small positive band at 480 nm, followed by two moderate negative bands in the 300–400 nm regions, and a stronger negative band centered at 220 nm, then a stronger positive

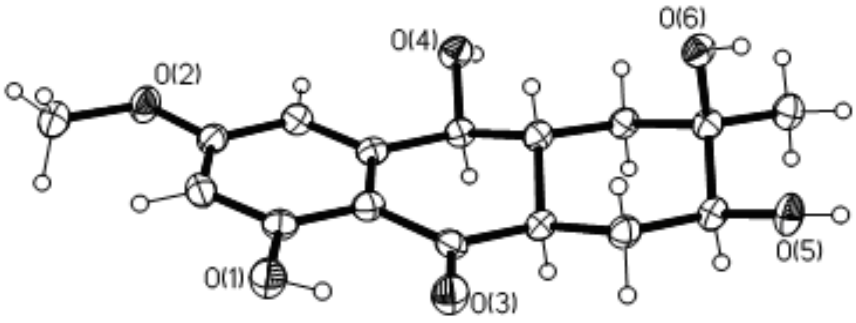
band at 264 nm. For **3,** the sequence of bands is similar but their signs are inverted. The CD spectra of **2** and **3** reflect contributions from a chiral axis and three chiral centers. The chiral axis occurring within the chromophore is expected to dominate the observed CD spectrum [18,19] (Figure 4).The chiral center C-7 is expected to have a much smaller contribution to the observed CD spectrum. With these results, compounds **2** and **3** were deduced as two diastereomers and atropisomers [20].

Figure 4. CD Spectra of **2** and **3**. Recorded in MeOH at amibient temperature.



The known compounds were identified as physcion (**4**), marcrospin (**5**), dactylariol (**6**), tetrahydroaltersolanol B (**7**), alternariol (AOH) (**8**) and alternariol methyl ether (AME) (**9**) by spectral analyses and comparison with reported literature data, respectively [21–24]. The structure of **7** was confirmed by X-ray diffraction analysis (Figure 5) and its crystallographic data was reported for the first time.

Figure 5. X-ray crystal structure of **7**.



Compounds **1** and **2** were evaluated for their cytotoxicity against human breast cancer cell lines MDA-MB-435 and MCF-7 by MTT assay. Compound **1** had an IC_{50} value of 26.97 μM against MDA-MB-435 and 29.11 μM against MCF-7 cells, respectively. Compound **2** showed activities against MDA-MB-435 (IC_{50} = 13.11 μM) and MCF-7 (IC_{50} = 20.04 μM). No biological studies were performed for compound **3** due to the limited yield.

3. Experimental Section

3.1. General

Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao marine Chemical). The HPLC system consisted of a Waters 2010 series. A mini ODS column (250 × 10 mm, 10 μm particle size) was used. Melting points were determined on an X-4 micro-melting point apparatus and were uncorrected. Circular dichroism was measured on a Schmidt Haensch Polartronic HH W5 polarimeter and was uncorrected. UV spectra were measured on a Shimadzu UV-2501 PC spectrophotometer. IR spectra were measured on a Bruker EQUINOX55 spectrophotometer. ^{1}H and ^{13}C NMR data were recorded on a Varian Inova 500 NB and a Burker AVANCE 400 spectrometer, respectively (TMS as internal standard). EIMS were on a Thermo DSQ EI-mass spectrometer. LC/MS data were acquired using an Applied Biosystems/MDS Sciex and ESI source. HR-EIMS were measured on a Thermo MAT95XP High Resolution mass spectrometer. HR-ESIMS were measured on a Schimadzu LCMS-IT-TOF.

3.2. Strain Isolation, Taxonomic Classification and Endophyte Fermentation

The fungus *Alternaria* sp. ZJ9-6B was isolated from the fruit of a mangrove tree *Aegiceras Corniculatum* collected in Zhanjiang Mangrove, Guangdong province, P.R. China, in 2008. A voucher specimen (registration number: ZJ9-6B) has been deposited in the Natural Products Laboratory and the Department of Applied Chemistry, Sun Yat-sen University, China. It was identified according to a molecular biological protocol by DNA amplification and sequencing of the ITS region as described previously with an ITS sequence GenBank ID: HM 754629. The fungal strain was cultivated in potato dextrose broth (PDB) medium (20 g of dextrose and 3 g of crude sea salt in 1 L of potato infusion). Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelia growth were cut and transferred aseptically into a 500 mL Erlenmeyer flask containing 250 mL of liquid medium, and incubated at 28 °C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred into 1000 mL Erlenmeyer flasks containing 500 mL PDB medium and incubated at 28 ± 1 °C for 30 days under stationary conditions.

3.3. Extraction and Separation of Metabolites

The cultures (200 L) were separated into mycelium and filtrate. The dried mycelium (724 g) was extracted with methanol (6 L × 4) to give 151.6 g of a crude extract. The crude extract was subjected to silica gel CC using gradient elution with petroleum-ether (PE) and ethyl acetate (EA) mixture (v/v, 95:5–0:100) to give six fractions (A–F). Fraction C (27.1 g) was purified by silica CC with a PE-EA mixture (v/v, 85/15, 4 L) to give three subfractions C-1 (8.7 g), C-2 (5.1 g) and C-3 (3.5 g). These three subfractions were further purified by silica gel CC with a PE-EA mixture to give **4** (161 mg), **5** (67 mg), **8** (19 mg) and **9** (28 mg). Fraction D (13.5 g) was isolated by silica gel CC with PE-EA (v/v, 75:25, 3 L) to give two subfractions D-1 (2.7 g) and D-2 (3.6 g). These two subfractions D-1 and D-2 were further purified by Sephadex LH-20 gel CC with $CHCl_3$-MeOH (65:35, v/v) as a mobile

phase and by preparative HPLC with an ODS column (10 × 250 mm), eluting with MeOH-H_2O (68:32, v/v) to give **1** (11 mg), **2** (18.4 mg), **3** (3 mg), **6** (13 mg) and **7** (21 mg).

Alterporriol K: Red powder. $[\alpha]_D^{25}$ = +690 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 279.80 (1.52), 223.80 (1.96) nm. IR (KBr): ν_{max} = 3448, 2966, 2855, 1652, 1638, 1595, 1464, 1430, 1388, 1289, 1208, 1111, 1072, 973, 933, 842, 621 cm^{-1}. For ^1H, ^{13}C and 2D NMR spectroscopic data, see Table 1. HR-EIMS: calcd. for $C_{32}H_{26}O_{10}$, 586.1470; found *m/z* = 586.1471 $[M]^+$.

Alterporriol L: Red powder. $[\alpha]_D^{25}$ = +30 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 280.20 (1.24), 225.40 (1.61) nm. IR (KBr): ν_{max} = 3440, 2926, 2857, 1592, 1462, 1430, 1389, 1288, 1208, 1109, 1070, 974, 927, 846, 793, 605 cm^{-1}. For ^1H, ^{13}C and 2D NMR spectroscopic data, see Table 2. HR-ESIMS: calcd. for $C_{32}H_{25}O_{12}$, 601.1346; found *m/z* = 601.1340 $[M - H]^-$.

Alterporriol M: Red powder. $[\alpha]_D^{25}$ = −90 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 279.00 (1.33), 225.60 (1.77) nm. IR (KBr): ν_{max} = 3450, 2928, 2027, 1638, 1463, 1389, 1280, 1207, 1111, 1046, 977, 929, 858, 618 cm^{-1}. For ^1H, ^{13}C and 2D NMR spectroscopic data, see Table 2. ESIMS *m/z* = 601.1 $[M - H]^-$. HR-ESIMS calcd. for $C_{32}H_{25}O_{12}$, 601.1346; found *m/z* = 601.1340 $[M - H]^-$.

Crystallographic data of 7: The X-ray diffraction data for **7** (Figure 5) was measured on an Xcalibur Nova 1000 CCD diffractometer (Mo Kα-radiation, graphite monochromator). Solvent for crystallization: methanol; Wavelength: 0.71073 Å; Temperature: 173 K; Empirical formula: $C_{16}H_{20}O_6$; Crystal system: monoclinic; Space group C2 with *a* = 37.306(6) Å, *b* = 5.8251(10) Å, *c* = 7.9087(13) Å, α = 90.00°, β = 102.210(3)°, γ = 90.00°; *V* = 1679.8(5) $Å^3$; Density: 1.397 g/cm^3; *Z* = 2, *F* (000) = 756.0; Goodness-of-fit on F^2: 1.087; *R* Indices (all data): R_1 = 0.0399, wR_2 = 0.1208. CCDC-751689 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44-1223-336033; E-Mail: deposit@ccdc.cam.ac.uk).

3.4. Cytotoxic Assays

The cytotoxicity of compounds **1** and **2** were evaluated using human breast cancer cell lines MDA-MB-435 and MCF-7 by MTT assay. Cell viability was measured using the CellTiter 96 aqueous nonradioactive cell proliferation assay. Results were expressed as the mean value of triplicate data points.

4. Conclusions

Alternaria sp. ZJ9-6B is a prolific producer of bioactive metabolites. Nine compounds have been isolated from this fungal strain, including three new alterporriols. These three alterporriols all possess dimeric structures with a C-2–C-2′ linkage. In the primary bioassay, compounds **1** and **2** showed moderate cytotoxic activity against human breast cancer cell lines.

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Samples Availability: Available from the authors.