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Bioactive Hydroanthraquinones and Anthraquinone Dimers from a Soft Coral-Derived *Alternaria* sp. Fungus

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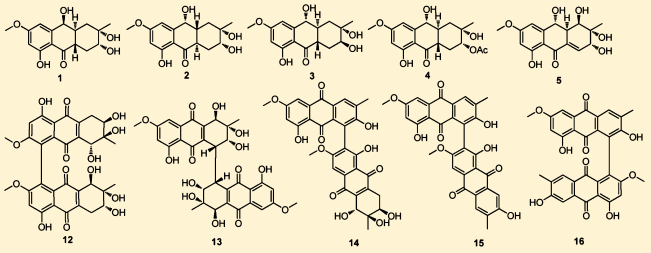
Supporting Information

ABSTRACT: Five new hydroanthraquinone derivatives, tetrahydroaltersolanols C−F (**1**−**4**) and dihydroaltersolanol A (**5**), and five new alterporriol-type anthranoid dimers, alterporriols N−R (**12**−**16**), along with seven known analogues (**6**−**11** and **17**), were isolated from the culture broth and the mycelia of *Alternaria* sp. ZJ-2008003, a fungus obtained from a *Sarcophyton* sp. soft coral collected from the South China Sea. Their structures and the relative configurations were elucidated using comprehensive spectroscopic methods including 1D and 2D NOE spectra as well as single-crystal X-ray crystallography. Compound **13** represents the first isolated alterporriol dimer with a C-4−C-4′ linkage, and the absolute configuration of **4** was determined using the modified Mosher's method. Compounds **1** and **15** exhibited antiviral activity against the porcine reproductive and respiratory syndrome virus (PRRSV), with IC_{50} values of 65 and 39 μM, respectively. Compound **14** showed cytotoxic activity against PC-3 and HCT-116 cell lines, with IC_{50} values of 6.4 and 8.6 μM, respectively.



Fungi in the genus *Alternaria* produce various bioactive metabolites, which have drawn the attention of many chemists. The anthraquinone derivatives especially possess a wide range of biological activities. For example, the tetrahydroanthraquinone altersolanol A was shown to be a potent inhibitor of plant respiration, therefore blocking the uptake of essential metabolites required for photosynthesis.[1] Another compound, anthranoid dimer alterporriol F, had potent cytotoxic activity against HeLa and KB cells.[2] As part of our ongoing investigation on natural antibacterial and cytotoxic products from marine fungi in the South China Sea,[3−6] a soft coral-derived *Alternaria* sp. attracted our attention because an EtOAc extract of the fungal culture exhibited significant antibacterial activity against the pathogenic bacteria *Escherichia coli* and *Vibrio parahemolyticus*. Bioassay-guided fractionation of the EtOAc extract led to the isolation of five new hydroanthraquinones, tetrahydroaltersolanols C−F (**1**−**4**) and dihydroaltersolanol A (**5**), and five new alterporriol-type anthranoid dimers, alterporriols N−R (**12**−**16**), together with seven known analogues, tetrahydroaltersolanol B (**6**),[7,8] altersolanol B (**7**),[9] altersolanol C (**8**),[10] altersolanol L (**9**),[11] ampelanol (**10**),[12] macrosporin (**11**),[13] and alterporriol C (**17**).[10,14] Herein we report the isolation, structure elucidation, relative configurations, and biological activities of these compounds.

RESULTS AND DISCUSSION

The marine-derived fungus ZJ-2008003, isolated from a *Sarcophyton* sp. soft coral, was identified on the basis of molecular characteristics combined with morphologic traits as an *Alternaria* sp. All compounds were isolated using chromatographic techniques including column chromatography and semipreparative HPLC, and their structures were elucidated by spectroscopic data (IR, UV, NMR, and MS). Their relative configurations were assigned according to 1D and 2D NOE experiments and single-crystal X-ray crystallography, and the absolute configuration of **4** was determined using the modified Mosher's method.

Tetrahydroaltersolanol C (**1**) was obtained as colorless crystals. Its molecular formula of $C_{16}H_{20}O_6$ (seven degrees of unsaturation) was determined by HRESIMS. This molecular formula was also corroborated by ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2). In the ^{1}H NMR spectrum, two *meta*-coupled aromatic proton groups at δ_H 6.44 (d, J = 2.5 Hz) and 6.40 (d, J = 2.5 Hz), one methoxy group at δ_H 3.80 (s), one singlet methyl group at δ_H 1.14 (s), and three hydroxy groups at δ_H 5.31 (d, J = 5.0 Hz), 4.46 (d, J = 6.0 Hz), and 3.85 (s) as well as one hydrogen-bonded hydroxy group at δ_H 12.89 (s) were

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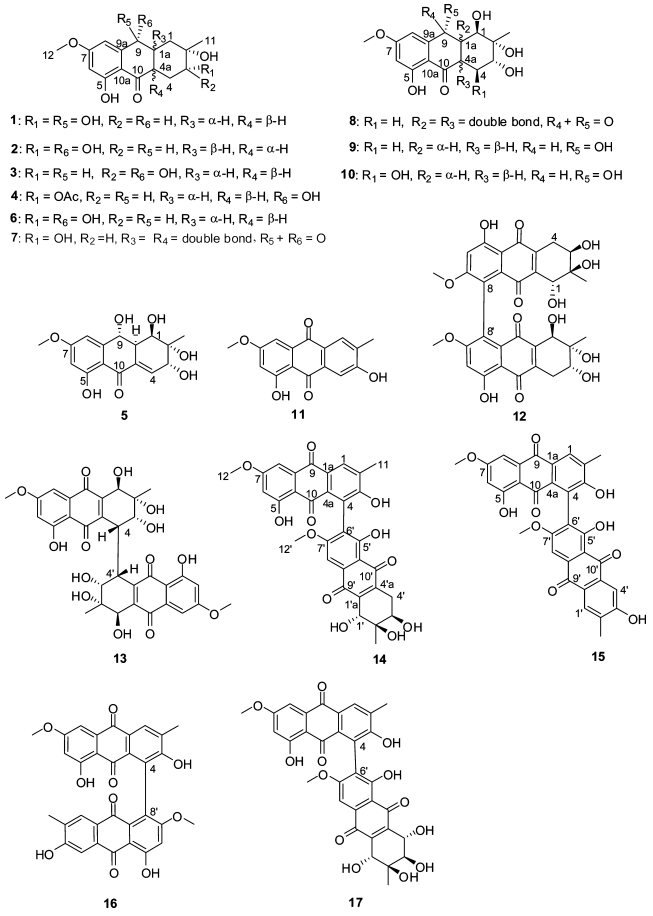

observed. The splitting patterns of the four hydroxy groups indicated that two (δ_H 4.46 and 5.31) of them were attached to methines and the rest (δ_H 3.85 and 12.89) to quaternary carbons. In the ^{13}C NMR spectrum, one carbonyl carbon (δ_C 204.9) and six carbons (δ_C 100.6, 108.2, 109.2, 148.7, 165.0, and 166.1) were observed. These spectroscopic features suggested that compound **1** was similar to tetrahydroaltersolanol B (**6**).[7,8] The ^1H NMR spectrum of **1** was markedly similar to that of **6**, with differences only in the chemical shifts of H-1 (**1**: δ_H 1.53 and 1.64 vs **6**: δ_H 1.21 and 2.17) and the small coupling constant J = 3.0 Hz between H-1a (δ_H 1.97) and H-9 (δ_H 4.28) in **1**, instead of the large coupling constant J = 11.0 Hz between H-1a (δ_H 1.96) and H-9 (δ_H 4.29) in **6**. These features established an equatorial H-9 in **1** in contrast to an axial H-9 in **6**.

In the selective 1D NOE experiments, the irradiation of H-9 resulted in the enhancement of H-1a and H-1eq, which required that they should be on the same face of the molecule. The irradiation of H-3 resulted in the enhancement of H-1ax, H-4eq, H-4a, and H_3-11, indicating that they should be placed on the opposite side of H-9 and H-1a (Figure 1). Thus, the relative configurations of all asymmetric carbons of **1** were confirmed as 1aα, 3β, 4aβ, 9α, and 11β, identical to those of **6** except C-9. Therefore, tetrahydroaltersolanol C (**1**) was determined to be 9-*epi*-tetrahydroaltersolanol B.

Tetrahydroaltersolanol D (**2**) was also obtained as colorless crystals and had the same molecular formula as **6** on the basis of the HRESIMS data. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) also closely resembled those of **6**. The ^1H NMR data (Table 1) were very similar to those of **6**, except for the signals of H-1, H-1a, H-4a, and H-9. The large coupling constant of 13.0 Hz between H-1a (δ_H 2.65) and H-4a (δ_H 2.97) established that H-1a and H-4a were both axial. The small coupling constant of 5.0 Hz between H-1a and H-9 (δ_H 4.90) established that H-9 was equatorial. In the selective 1D NOE experiments, the irradiation of H-3 affected the intensities of H-1eq, H-1a, H-4ax, and H_3-11, and the irradiation of axial H-1a affected the intensities of H-1eq, H-3, H-4ax, and H-9, indicating that H-1eq, H-1a, H-3, H-4ax, H-9, and H_3-11 should be placed on the same face (Figure 1). Therefore, the

Table 1. ^1H NMR Data (δ) for 1 (500 MHz), 2 (500 MHz), and 3−5 (600 MHz) (δ in ppm, J in Hz)

position	1[a]	2[a]	3[a]	4[b]	5[a]
1	1.53, dd (13.5, 3.5 H-1eq)	0.74, ddd (13.5, 13.5, 12.0, H-1ax)	1.36, brd (13.2 H-1eq)	1.40, dd (13.8, 12.6, H-1ax)	3.89, ddd (6.0, 6.0, 3.0)
	1.64, dd (13.5, 12.5, H-1ax)	1.68, m (H-1eq)	1.62, ddd (13.8, 13.2, 4.2 H-1ax)	2.48, dt (13.8, 3.6, H-1eq)	
1a	2.07, dddd (12.5, 12.0, 3.5, 3.0)	2.65, ddd (13.0, 12.0, 5.0)	2.54, ddd (13.8, 12.0, 4.2)	2.21, dddd (12.6, 12.0, 10.8, 3.6)	2.89, ddd (12.0, 6.0, 3.0)
3	3.24, ddd (12.0, 6.0, 3.5)	2.95, ddd (6.5, 6.0, 3.0)	3.68, ddd (5.4, 4.2, 3.0)	4.79, dd (12.0, 4.8)	4.11, ddd (7.8, 6.0, 2.4)
4	1.41, ddd (12.5, 12.5, 12.0, H-4ax)	1.60, ddd (13.5, 12.0, 6.0, H-4ax)	1.47, ddd (15.6, 13.2, 4.2 H-4ax)	1.77, ddd (12.6, 12.0, 12.0, H-4ax)	6.86, dd (6.0, 2.4)
	2.16, ddd (12.5, 3.5, 3.5, H-4eq)	2.33, ddd (12.0, 4.5, 3.0, H-4eq)	1.96, ddd (15.6, 4.2, 3.0 H-4eq)	2.36, ddd (12.6, 4.8, 3.6, H-4eq)	
4a	2.69, ddd (12.5, 12.0, 3.5)	2.97, m	2.23, ddd (13.2, 12.0, 4.2)	2.45, ddd (12.0, 12.0, 3.6)	
6	6.40, d (2.5)	6.32, d (2.5)	6.35, d (2.4)	6.31, d (2.4)	6.38, d (2.4)
8	6.44, d (2.5)	6.62, dd (2.5, 1.0)	6.71, dd (2.4, 1.2)	6.73, brs	6.76, dd (2.4, 1.2)
9	4.31, dd (5.0, 3.0)	4.90, dd (5.5, 5.0)	4.66, dd (12.0, 7.2)	4.39, d (10.8)	4.69, dd (12.0, 7.8)
11	1.14, s	0.99, s	1.14, s	1.26, s	1.29, s
12	3.80, s	3.80, s	3.82, s	3.82, s	3.89, s
13				2.14, s	
1-OH					5.07, d (6.0)
2-OH	3.85, s	3.87, s	4.20, s		4.34, s
3-OH	4.46, d (6.0)	4.47, d (6.5)	4.89, d (5.4)		4.89, d (7.8)
5-OH	12.89, s	13.05, s	13.02, s	12.70, s	13.31, s
9-OH	5.31, d (5.0)	5.74, d (5.5)	5.52, d (7.2)		5.51, d (7.8)

[a]DMSO-d_6. [b]$CDCl_3$.

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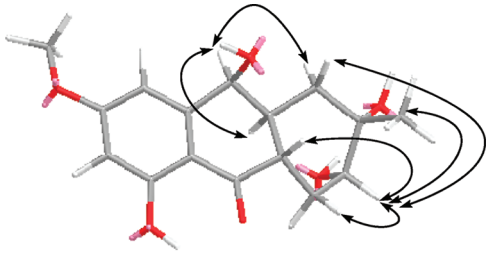
Table 2. ^{13}C NMR Data (δ) for 1 (125 MHz), 2 (125 MHz), and 3–5 (150 MHz) (δ in ppm)

position	1[a]	2[a]	3[a]	4[b]	5[a]
1	41.1, CH_2	34.0, CH_2	32.4, CH_2	40.7, CH_2	71.9, CH
1a	38.8, CH	38.8, CH	42.1, CH	41.9, CH	44.4, CH
2	70.6, C	70.2, C	70.8, C	70.2, C	72.3, C
3	74.1, CH	71.7, CH	70.0, CH	76.9, CH	69.7, CH
4	29.9, CH_2	29.5, CH_2	21.3, CH_2	25.9, CH_2	141.7, CH
4a	42.0, CH	46.2, CH	46.7, CH	46.9, CH	131.7, C
5	165.0, C	165.0, C	164.9, C	165.5, C	166.2, C
6	100.6, CH	99.4, CH	99.5, CH	99.9, CH	99.4, CH
7	166.1, C	166.6, C	166.3, C	166.5, C	166.9, C
8	108.2, CH	104.8, CH	104.7, CH	104.3, CH	104.7, CH
9	68.8, CH	68.6, CH	66.6, CH	72.6, CH	65.2, CH
9a	148.7, C	150.0, C	152.7, C	149.0, C	152.1, C
10	204.9, C	203.9, C	205.5, C	200.7, C	188.8, C
10a	109.2, C	109.6, C	109.3, C	109.4, C	109.6, C
11	27.5, CH_3	27.9, CH_3	28.5, CH_3	27.0, CH_3	23.9, CH_3
12	56.2, CH_3	56.2, CH_3	56.1, CH_3	55.7, CH_3	56.2, CH_3
13				170.2, C	
14				21.1, CH_3	

[a]DMSO-d_6. [b]$CDCl_3$.



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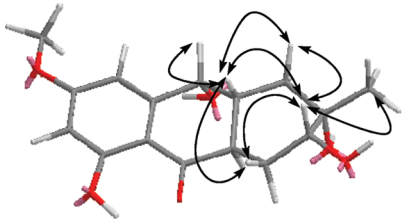

2

Figure 1. Key NOE correlations for **1** and **2**.

relative configurations of all asymmetric carbons in **2** were confirmed as 1aβ, 3β, 4aα, 9β, and 11β, identical to those of tetrahydroaltersolanol B (**6**) except at C-1a and C-4a.

Tetrahydroaltersolanol E (**3**) was also obtained as colorless crystals, with the molecular formula $C_{16}H_{20}O_6$, identical to **6**. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) also very closely resembled those of **6**. The ^1H NMR data (Table 1) differed from those of **6** for signals of H-1, H-1a, H-3, and H-4a. The large coupling constants of J_{1a-4a} (12.0 Hz), J_{1a-9} (12.0 Hz), and J_{4ax-4a} (13.2 Hz) could be explained only by a series of mutual transdiaxial relationships, indicating that H-4a and H-9 should be placed on the same side of the molecule, and therefore H-1a and H-4ax should be placed on the opposite side of H-4a and H-9. The small coupling constant of 4.2 Hz between H-3 and H-4ax indicated an equatorial orientation of H-3. These results indicated that H-1a and H-3 should be on the same face of the molecule. Therefore, the relative configurations of all asymmetric carbons in **3** were confirmed as 1aα, 3α, 4aβ, 9β, and 11β, identical to those of **6** except at C-3. Tetrahydroaltersolanol E was consequently determined to be 3-*epi*-tetrahydroaltersolanol B.

Tetrahydroaltersolanol F (**4**) was obtained as a pink, amorphous powder. Its molecular formula of $C_{18}H_{22}O_7$ (eight degrees of unsaturation) was determined by HRESIMS. Careful comparison of the ^1H NMR and ^{13}C NMR spectra of **4** (Tables 1 and 2) with those of **6** showed a close structural relationship. The obvious differences in the ^1H NMR spectrum were the presence of a singlet methyl signal at δ_H 2.14 and the chemical shift of H-3 was moved downfield significantly (δ_H 4.79 in **4** vs 3.28 in **6**). The presence of a carbonyl group at C-13 (δ_C 170.2) in the ^{13}C NMR spectrum was observed, and the C-4 signal moved upfield (δ_C 25.9 CH_2 in **4** vs 29.8 CH_2 in **6**). These spectroscopic features indicated that the hydroxy group at C-3 was acetylated in **4**. The location of the acetoxy group at C-3 was further confirmed by the HMBC correlation between H-3 and C-13.

The relative configuration of **4** was determined on the basis of the coupling constants and a series of ROESY experiments. The H_3-11 signal showed correlations to H-3 and H-4, and the H-4a correlated to H-3, H-4, and H-9, indicating that they possessed a β-orientation, respectively, and consequently H-1a had to be α-orientated. Consequently, tetrahydroaltersolanol F (**4**) was determined to be 3-acetyltetrahydroaltersolanol B.

Dihydroaltersolanol A (**5**) was obtained as colorless crystals. Its molecular formula of $C_{16}H_{18}O_7$ (eight degrees of unsaturation) was determined by HRESIMS combined with ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2). Comparison of the ^1H NMR spectrum of **5** (Table 1) with that of **9** (altersolanol L)[11] showed a similar skeleton structure between both compounds. The only significant difference in the ^1H NMR spectrum of **5** in comparison with **9** was the presence of an olefinic proton signal for H-4 that was shifted downfield to δ_H 6.86 (instead of a methylene signal at δ_H 1.42 and 2.10 in **9**). The downfield shifts observed for C-4 (δ_C 141.7 vs δ_C 29.5) and C-4a (131.7 vs 41.5) in the ^{13}C NMR spectrum also reflected the presence of a double bond at C-4 and C-4a rather than a methylene group connected to C-4 and a methine group to C-4a.

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Thus the gross structure of **5** was assigned as dihydroaltersolanol A (**5**). The large coupling constant of J_{1a-9} (12.0 Hz) as well as the small coupling constant of J_{1-1a} (3.0 Hz) supported the relative configurations of all asymmetric carbons in **5** as 1α, 1aα, 3β, 9β, and 11β, which corresponded to those of **9**.

Alterporriol N (**12**) was obtained as a red, amorphous powder. The ^{1}H NMR spectrum revealed the presence of one hydrogen-bonded phenolic proton at δ_H 13.18 (5-OH), one aromatic proton at δ_H 6.90 (H-6), three hydroxy groups at δ_H 5.39 (1-OH), 4.69 (3-OH), and 4.42 (2-OH), two oxygenated methine groups at δ_H 4.06 (H-1) and 3.61 (H-3), one methoxy group at δ_H 3.68 (H-12), one methylene group at δ_H 2.79 (H-4eq) and 2.33 (H-4ax), and one singlet methyl group at δ_H 1.17 (H-11) (Table 3). These spectroscopic features suggested that

Table 3. ^{1}H NMR Data (δ) for 12−16 (600 MHz) (δ in ppm, J in Hz)

position	12[a]	13[b]	14[b]	15[b]	16[b]
1	4.06, d (7.2)	4. 63, brs	8.14, s	8.18, s	8.15, s
3	3.61, ddd (9.6, 6.0, 4.2)	4.12, d (6.6)			
4	2.33, dd (19.8, 9.6, H-4ax)	4.07, d (6.6)			
	2.79, dd (19.8, 6.0, H-4eq)				
6	6.90, s	6.71, d (2.4)	6.66, d (2.4)	6.67, d (2.4)	6.64, d (2.4)
8		7.06, d (2.4)	7.26, d (2.4)	7.24, d (2.4)	7.28, d (2.4)
11	1.17, s	1.29, s	2.40, s	2.45, s	2.41, s
12	3.68, s	3.95, s	3.96, s	3.93, s	3.95, s
1′			4.29, s	8.01, s	7.67, s
3′			3.93, dd (9.0, 5.4)		
4′			2.54, dd (19.8, 9.0, H-4′ax),		
			2.98, dd (19.8, 5.4, H-4′eq)	7.65, s	7.65, s
6′					6.91, s
8′			6.88, s	7.49, s	
11′			1.36, s	2.38, s	2.25, s
12′			3.78, s	3.96, s	3.79, s
1-OH	5.39, d (7.2)				
2-OH	4.42, s				
3-OH	4.69, d (4.2)				
5-OH	13.18, s	12.41, s			

[a]DMSO-d_6. [b]Acetone-d_6.

compound **12** was similar to altersolanol C (**8**)[10] except for the absence of the aromatic proton at δ_H 7.02 (H-8) in **12** and the chemical shift of C-8 was moved downfield significantly (δ_C 122.6, C in **12** vs 107.5, CH in **8**). The molecular formula of **12** was determined to be $C_{32}H_{30}O_{14}$ (18 degrees of unsaturation) by HRESIMS. The presence of only 16 signals in the ^{13}C NMR spectrum (Table 4) confirmed that compound **12** was a symmetrical dimer of altersolanol C. In the HMBC spectrum, the correlations from 5-OH to C-5, C-6, and C-10a indicated that the two units were joined via a C-8−C-8′ linkage. On the basis of the results obtained, the structure of alterporriol N (**12**) was identified as an alterporriol-type dimer with a C-8 and C-8′ linkage.

Alterporriol O (**13**) was also obtained as a red, amorphous powder and was determined to be another symmetrical dimer of altersolanol C (**8**)[10] and an isomer of **12** on the basis of HRESIMS data combined with 1D and 2D NMR spectra. In the ^{1}H NMR spectrum, the main difference of **13** and **8** was that two methylene protons at δ_H 2.80 (H-4eq) and 2.30 (H-4ax) in **8** were replaced by a methine proton signal at δ_H 4.07 (H-4) in **13** (Table 3). In a consistent fashion, the ^{13}C NMR spectrum showed a methine carbon for C-4 (δ_C 43.3) in **13** (Table 4), rather than a methylene carbon for C-4 (δ_C 28.6) in **8**. These spectroscopic features indicated that **13** is a symmetrical dimer of **8** connected at C-4−C-4′. The linkage positions were further confirmed by the HMBC correlations from the methine proton H-4 to two quaternary carbons, C-1a and C-4a, and the tertiary carbon C-3. Therefore, alterporriol O (**13**) represents the first alterporriol-type anthranoid dimer with the C-4 and C-4′ linkage.

Alterporriol P (**14**), with the molecular formula $C_{32}H_{26}O_{12}$ from HRESIMS data, was also obtained as a red, amorphous powder. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 3 and 4) indicated that **14** was comprised of one altersolanol C (**8**) subunit and one macrosporin (**11**) subunit.[10,13] In the ^{1}H NMR spectrum of **14**, four aromatic proton signals were observed, among which three were due to C-1, C-6, and C-8 in the macrosporin moiety at δ_H 8.14 (s), 6.66 (d, J = 2.4 Hz), and 7.26 (d, J = 2.4 Hz) and one to C-8′ in the altersolanol C moiety at δ_H 6.88 (s). Therefore, **14** was lacking a proton signal at C-4 in the aromatic macrosporin moiety and at C-6′ in the altersolanol C moiety. Furthermore, the HMBC spectrum showed that both H-1 and H-8 correlated to C-9, H-1′ correlated to C-9′, and H-8′ correlated to C-6′, C-7′, and C-10′a. These results indicated that the two monomers were joined via a C-4−C-6′ linkage. Thus, alterporriol P (**14**) was identified as a new anthranoid dimer with a C-4 and C-6′ linkage.

Alterporriol Q (**15**) was isolated as a yellow, amorphous powder. Its molecular formula of $C_{32}H_{22}O_{10}$ (22 degrees of unsaturation) was determined by HRESIMS, combined with ^{1}H and ^{13}C NMR spectroscopic data (Tables 3 and 4). In the ^{1}H NMR spectrum, six aromatic protons at δ_H 8.18 (s, H-1), 8.01(s, H-1′), 7.65 (s, H-4′), 7.49 (s, H-8′), 7.24 (d, J = 2.4 Hz, H-8), and 6.67 (d, J = 2.4 Hz, H-6), two singlet methoxy protons at δ_H 3.96 (s, H-12′) and 3.93 (s, H-12), and two singlet methyl protons at δ_H 2.45 (s, H-11) and 2.38 (s, H-11′) were observed. These data combined with ^{13}C NMR data suggested that **15** was a dimeric anthraquinone containing two macrosporin (**11**) subunits.[13] Compound **15** lacked the H-4 aromatic proton in one macrosporin subunit and the H-6 aromatic proton in the second macrosporin subunit. Furthermore, the HMBC spectrum showed correlations from both H-1 and H-8 to C-9 (δ_C 181.0) and correlations from H-8′ to C-6′, C-9′, and C-10′a, which indicated the two monomers were linked between C-4 and C-6′.

Alterporriol R (**16**) was determined to be an isomer of **15** on the basis of HRESIMS data combined with 1D and 2D NMR spectra. The obvious difference in the ^{1}H NMR spectrum (Table 3) was that instead of an aromatic proton at δ_H 7.49 (s, H-8′) in **15**, there was a signal with a significant upfield shift at δ_H 6.91 (s, H-6′) in **16**. This can be explained by the δ_H 6.91 proton being connected to C-6′ in **16**, rather than to C-8′ in **15**. In the HMBC spectrum, correlations were observed for H-6′ to C-5′, C-7′, C-8′, and C-10′a, establishing the linkage between the two monomers as C-4 to C-8′ in **16** rather than C-4−C-6′ as in **15**.

The structures of known compounds **6**−**11** and **17** were identified by comparison of their spectroscopic data with those

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Table 4. ^{13}C NMR Data (δ) for 12−14 (150 MHz) and 15 and 16 (125 MHz) (δ in ppm)

position	12[a]	13[b]	14[b]	15[b]	16[b]
1	68.9, CH	68.5, CH	131.4, CH	130.3, CH	129.6, CH
1a	143.5, C	141.3, C	127.1, C	125.8, C	130.4,[c] C
2	71.9, C	72.1, C	133.0, C	132.5,[c] C	131.2, C
3	66.5, CH	70.6, CH	160.2, C	159.4 C	158.6, C
4	28.7, CH_2	43.3, CH	129.1, C	119.5, C	129.6, C
4a	142.7, C	149.5, C	132.4, C	131.2, C	132.0,[c], C
5	164.5, C	164.1, C	168.0,[c] C	165.8 C	165.3, C
6	103.4, CH	105.5, CH	107.6, CH	105.8, CH	105.7, CH
7	163.9, C	165.5, C	166.8, C	163.9, C	165.3, C
8	122.6, C	105.9, CH	108.7, CH	106.6, CH	106.5, CH
9	182.9, C	183.3, C	183.1, C	181.0, C	181.3, C
9a	128.9, C	134.1, C	133.3, C	134.9, C	135.1, C
10	188.2, C	189.2, C	191.0, C	188.0, C	188.3, C
10a	108.7, C	110.3, C	112.7, C	111.0, C	110.6, C
11	21.8, CH_3	21.9, CH_3	18.1, CH_3	16.5, CH_3	16.5, CH_3
12	56.7, CH_3	55.7, CH_3	58.1, CH_3	56.1, CH_3	56.4, CH_3
1′			71.5, CH	130.5, CH	130.3, CH
1′a			144.7, C	135.1,[c] C	126.4,[c] C
2′			74.3 C	132.1,[c] C	131.2, C
3′			69.6, CH	161.6, C	161.2, C
4′			28.5, CH_2	111.4, CH	110.3, CH
4′a			145.5, C	133.6,[c] C	126.1,[c] C
5′			168.0,[c] C	159.6, C	166.1, C
6′			123.8, C	118.3, C	103.9, CH
7′			166.8, C	166.1, C	166.4, C
8′			105.6, CH	103.0, CH	122.0, C
9′			185.8, C	181.1, C	181.7, C
9′a			137.0, C	133.5, C	133.0, C
10′			189.0, C	187.5, C	187.6, C
10′a			111.9, C	111.7, C	111.0, C
11′			23.1, CH_3	15.8, CH_3	15.6, CH_3
12′			57.6, CH_3	55.7, CH_3	55.7, CH_3

[a]DMSO-d_6. [b]Acetone-d_6. [c]Assignments bearing the same superscript may be interchanged.

in the literature. The structures and relative configurations of **9** and **10** were confirmed by single-crystal X-ray crystallography for the first time (Figure 2).

In order to establish the absolute configuration of the isolated compounds, tetrahydroaltersolanol F (**4**) was subjected to the modified Mosher's method.[15] When reacted with (*R*)- and (*S*)-MTPA chloride, **4** gave the corresponding (*S*)- and (*R*)-MTPA esters **4s** and **4r**, respectively. The observed chemical shift differences $\Delta\delta_{S-R}$ (Figure 3) clearly defined the *R* configuration at C-9. Therefore, the configuration of **4** should be assigned as 1a*S*,2*S*,3*R*,4a*S*,9*R*. Consequently, the absolute configurations of the other compounds could be tentatively assigned on the basis of the absolute configuration of **4** according to a shared biogenesis for these compounds. Because the relative configurations of **1**−**3**, **5**, and **13** have been established, the configurations of these compounds can be proposed as 1a*S*,2*S*,3*R*,4a*S*,9*S* for tetrahydroaltersolanol C (**1**), 1a*R*,2*S*,3*R*,4a*R*,9*R* for tetrahydroaltersolanol D (**2**), 1a*S*,2*S*,3*S*,4a*S*,9*R* for tetrahydroaltersolanol E (**3**), 1*R*,1a*S*,2*R*,3*R*,9*R* for dihydroaltersolanol A (**5**), and 1*R*,2*R*,3*R*,4*R*,1′*R*,2′*R*,3′*R*,4′*R* for alterporriol O (**13**), respectively. Recently the absolute configuration of the hydroanthraquinone derivative altersolanol A was published.[16]

Fungi are well-known producers of both anthraquinones such as macrosporin and hydrogenated anthranoid congeners such as altersolanols A and C. Only three fungal-derived hexahydroanthranol compounds with an oxidized C-10 and reduced C-9 have been previously reported, including tetrahydroaltersolanol B,[7,8] altersolanol L,[11] and ampelanol.[12] In the present study, tetrahydroaltersolanols C−F (**1**−**4**) represent four additional hexahydroanthranol congeners.

Most of the alterporriol anthraquinone dimers were isolated from *A. porri*, *A. solani*, and uncharacterized *Alternaria* spp.,[10,14,17−23] while alterporriol G and its atropisomer alterporriol H were isolated as a mixture from the fungus *Stemphylium globuliferum*.[11] In terms of the underlying monomers, alterporriols are either homodimers made of two altersolanol A units (i.e., alterporriols D and E) or, more commonly, heterodimers incorporating one macrosporin unit and one altersolanol A unit (i.e., alterporriols A, B, C, G, and H).[10,11,14,17,21] The new alterporriols N and O (**12** and **13**) are homodimers comprised of two altersolanol C units, while alterporriol P (**14**) is a heterodimer containing one altersolanol C unit and one macrosporin unit, the same as for alterporriol F and its atropisomer.[2] Alterporriols Q and R (**15** and **16**) are the first homodimers comprised of two macrosporin units.

With regard to the connecting positions of the monomers in the dimers, the known compounds alterporriols A, B, D, E, and F and alterporriol N (**12**) feature a C-8−C-8′ linkage. Alterporriols C, P (**14**), and Q (**15**) show a C-4−C-6′ connection, alterporriols K−M have a C-6−C-7′ connection, and alterporriols G and H possess a C-6−C-8′ linkage.

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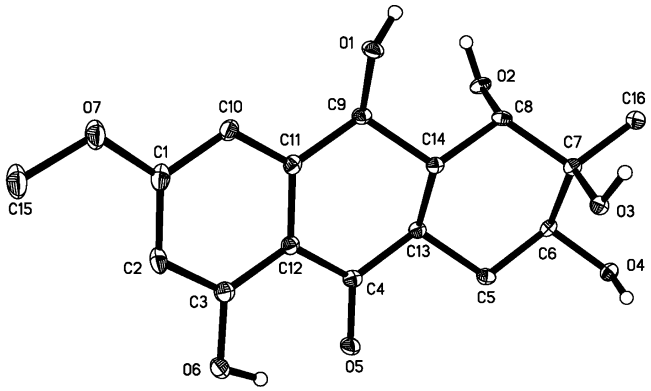
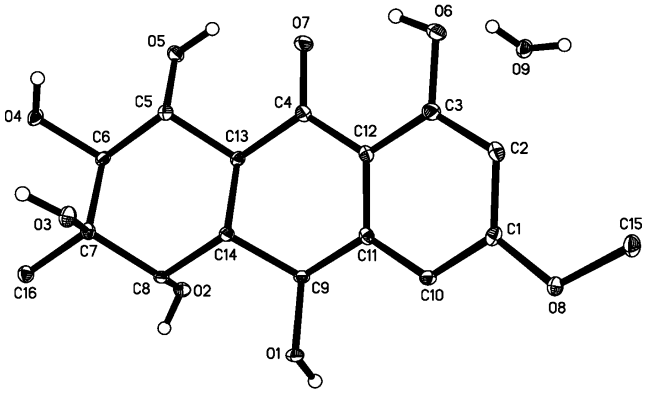


9



10

Figure 2. Perspective ORTEP drawings for **9** and **10**.

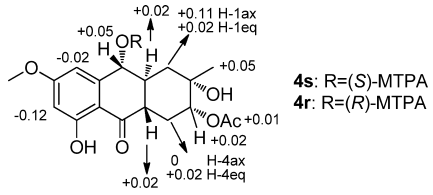


Figure 3. $\Delta\delta$ ($=\delta_S - \delta_R$) values for (*S*)- and (*R*)-MTPA esters of **4**.

Alterporriol O (**13**) represents the first isolated alterporriol dimer with a C-4−C-4′ linkage.

Several alterporriols have been isolated as atropisomers (alterporriols A and B, alterporriols D and E, alterporriols G and H).[11,17,21] It has been reported that some biphenyl-like compounds with *ortho*-substituted methoxy groups exhibit atropisomerism with different degrees of stability, because the steric size of the methoxy groups is large enough to restrict rotation.[17,21] Compounds **12**, **14**, **15**, and **16** have *ortho* methoxy groups and therefore might be expected to exist as atropisomers. The minor atropisomers of compounds **12** and **14** can be observed in the NMR spectra but with a very low ratios, and due to overlap, the NMR spectra of the minor atropisomers cannot be separately assigned. However, the atropisomers for compounds **15** and **16** are enantiomers, and the optical activity of the two compounds confirms that there is restricted rotation about the single bond joining the two monomers.

Compounds **1**−**10**, **12**−**14**, and **17** were evaluated for cytotoxic activity against human colon carcinoma HCT-116, human breast cancer MCF-7/ADR, human prostatic cancer PC-3, and human hepatoma HepG2 and Hep3B cell lines (Table 5). Among the monomeric anthranoids, the anthraquinone derivative **8**, possessing the paraquinone moiety, showed cytotoxic activity toward these five cancer cell lines with IC_{50} values between 2.2 and 8.9 μM, while the hydro-anthraquinone derivatives **1**−**7**, **9**, and **10**, with an oxidized C-10 and a reduced C-9 fragment, were inactive (IC_{50} > 100 μM). These results indicated that the paraquinone moiety was important for cytotoxic activity. Furthermore, among the alterporriol-type dimers, **14** was found to possess cytotoxic activity against PC-3 and HCT-116 with IC_{50} values of 6.4 and 8.6 μM, respectively, while **17** was inactive (IC_{50} > 20 μM).

Table 5. Cytotoxicity Assay for Isolated Compounds 8, 14, and 17[a]

	IC_{50} (μM)				
compound	HCT-116	MCF-7/ADR	PC-3	HepG2	Hep3B
8	2.2	3.2	7.6	8.9	8.2
14	8.6	23	6.4	20	21
17	24	98	27	53	51
EPI[b]	0.82	1.65	0.46	1.65	0.96

[a]Data are expressed in IC_{50} values (μM). HCT-116, human colon carcinoma cell line; MCF-7/ADR, human breast cancer cell line; PC-3, human prostatic cancer cell line; HepG2 and Hep3B, human hepatoma cell lines. [b]EPI (epirubicin) was used as a positive control.

The monomeric anthranoids **1**−**10** and alterporriol-type dimers **12**−**15** as well as **17** were evaluated for antiviral activity against porcine reproductive and respiratory syndrome virus (PRRSV). The monomeric anthranoid **1** and alterporriol-type dimers **15** and **17** showed activity against PRRSV with IC_{50} values of 65, 22, and 39 μM, respectively.

The antibacterial activities of all compounds were determined against seven terrestrial pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus tetragenus*, and *Micrococcus luteus*, and two marine pathogenic bacteria, *Vibrio parahemolyticus* and *Vibrio anguillarum* (Table 6). Only compounds **8**, **11**, and **17** exhibited potent antibacterial activity against *E. coli* and *V. parahemolyticus*, with MIC values between 0.6 and 2.5 μM.

Table 6. Antibacterial Activities of Compounds 8, 11, and 17[a]

	MIC (μM)		
compound	*E. coli*	*V. parahemolyticus*	*S. albus*
8	0.62	1.25	12
11	2.30	5.0	15
17	2.50	2.5	>20
ciprofloxacin[b]	0.62	0.16	0.31

[a]Data are expressed in MIC values (μM). [b]Ciprofloxacin was used as a positive control.

In the present paper, 10 new anthraquinone derivatives, including five new hydroanthraquinone derivatives, tetrahydroaltersolanols C−F (**1**−**4**) and dihydroaltersolanol A (**5**), and five new alterporriol-type anthranoid dimers, alterporriols N−R (**12**−**16**), were isolated from the culture broth and the mycelia of *Alternaria* sp. ZJ-2008003. Alterporriol P (**14**) showed cytotoxic activity against PC-3 and HCT-116 cell lines,

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and tetrahydroaltersolanol C (**1**) and alterporriol Q (**15**) exhibited antiviral activities against PRRSV. Among these new compounds, tetrahydroaltersolanols C–F (**1**–**4**) add to the three previous hexahydroanthranol congeners, and alterporriol O (**13**) represents the first isolated alterporriol dimer with a C-4–C-4′ linkage. The absolute configuration of tetrahydroaltersolanol F (**4**) was determined by the modified Mosher's method, and the configurations of the related compounds **1**–**3**, **5**, and **13** are proposed on the basis of the same biogenesis.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an X-6 micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Nicolet-Nexus-470 spectrometer using KBr pellets. NMR spectra were recorded on a JEOL JEM-ECP NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using TMS as internal standard, and on an AVANCE NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), using TMS as internal standard. ESIMS spectra were obtained from a Micromass Q-TOF spectrometer. HPLC separation was performed using a Hitachi L-2000 prep-HPLC system coupled with a Hitachi L-2455 photodiode array detector. A Kromasil C18 preparative HPLC column (250 × 10 mm, 5 μm) was used. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecylsilyl silica gel (Unicorn; 45–60 μm), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography. Single-crystal data were measured on a Bruker Smart 1000 CCD diffractometer.

Fungal Material. The fungal strain *Alternaria* sp. ZJ-2008003 was isolated from a piece of tissue from the inner part of the fresh soft coral *Sarcophyton* sp. (GX-WZ-20080011), which was collected from the Weizhou coral reef in the South China Sea in September 2008. The strain was deposited in the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, PR China, with the access code ZJ-2008003.

Identification of Fungal Cultures. The fungus was identified as an *Alternaria* sp. according to morphologic traits and a molecular biological protocol by DNA amplification and sequencing as described below. About 100 mg of fresh fungal mycelium was collected in a microcentrifuge tube (1.5 mL) to extract genomic DNA from the fungus using the Fungal DNA Midi kit (E.Z.N.A., Omega) according to the manufacturer's protocol. Consequently, PCR was performed using rTaq polymerase (TaKaRa Taq) and the Invitrogen primer pair ITS1F and ITS4 in a SensoQuest LabCyler thermal cycler according to the following protocol: (1) initial denaturation, 94 °C, 5 min; (2) denaturation, 94 °C, 40 s; (3) annealing, 54 °C, 40 s; (4) extension, 72 °C, 1 min; (5) final extension, 72 °C, 10 min. Steps 2–4 were repeated 30 times. Each sample consisted of 0.25 μL of Taq polymerase; 5 μL of 10× buffer; 4 μL of dNTP, 2 μL of primer mix (20 μmol/mL each), 1 μL of template DNA, and 37.75 μL of H_2O. Then, 5 μL of PCR product was loaded onto an agarose gel (1% agarose in 0.5× TAE, 5 μL of ethidium bromide 1% m/V solution per 100 mL of gel). After electrophoresis at 120 V for 40 min, the band due to the PCR product (approximate size 600 bp) was isolated from the gel slice using the Gel Extraction Kit (E.Z.N.A., Omega) according to the manufacturer's protocol. The PCR product was then submitted for sequencing (Invitrogen, Shanghai) with the primer ITS1F. The sequence data have been submitted to GenBank, accession number JF694938, and the strain was identified as an *Alternaria* sp. Its 592 base pair ITS sequence had 99% sequence identity to that of *Alternaria* sp. SK4YW1P (EU807934).

Fermentation, Extraction, and Isolation. The fungal strain was cultivated in potato glucose liquid medium (15 g of glucose and 30 g of sea salt in 1 L of potato infusion) in 1 L Erlenmeyer flasks each containing 300 mL of culture broth at 25 °C without shaking for 4 weeks. The culture (70 L) was filtered to separate the culture broth from the mycelia. The culture broth was extracted three times with an equal volume of EtOAc. The combined EtOAc layers were evaporated to dryness under reduced pressure to give an EtOAc extract (30.2 g), which was subjected to silica gel column chromatography (CC) (petroleum ether, EtOAc v/v, gradient) to generate nine fractions (Fr. 1–9). Fr. 2 was isolated by CC on silica gel eluted with petroleum ether–EtOAc (4:1) and then subjected to Sephadex LH-20 CC eluting with mixtures of petroleum ether–$CHCl_3$–MeOH (2:1:1) to obtain macrosporin (**11**) (600.0 mg). Fr. 6 was subjected to repeated Sephadex LH-20 CC ($CHCl_3$–MeOH, v/v, 1:1) and further purified by using HPLC on an ODS semipreparative column (Kromasil C_{18}, 10 × 250 mm, 5 μm, 2 mL/min) eluted with 45% MeOH–H_2O to obtain **1** (10.0 mg), **2** (11.0 mg), **3** (6.0 mg), and **6** (3.0 mg). Fr. 7 was subjected to repeated Sephadex LH-20 CC ($CHCl_3$–MeOH, v/v, 1:1) and further purified on HPLC (42% MeOH–H_2O) to afford **4** (3.8 mg), **7** (3.2 mg), **8** (60.0 mg), **9** (5.8 mg), and **10** (4.0 mg). Fr. 8 was subjected to repeated Sephadex LH-20 CC (MeOH) and further purified on HPLC (38% MeOH–H_2O) to afford **12** (12.0 mg) and **13** (6.0 mg). The mycelia cake was extracted with MeOH three times. The MeOH layer was evaporated under vacuum; then the combined residue was suspended in H_2O and partitioned with EtOAc to provide the EtOAc extract (18.0 g), which was chromatographed on a silica gel column using a stepwise gradient of petroleum ether–EtOAc to afford six fractions (Fr. 1–6). Fr. 3 was subjected to CC over Sephadex LH-20 eluting with MeOH to give **5** (3.2 mg). Fr. 5 and Fr. 6 were purified by Sephadex LH-20 CC with MeOH and further purified on HPLC (30% MeOH–H_2O) to give **14** (6.0 mg), **15** (5.6 mg), **16** (3.1 mg), and **17** (6.4 mg), respectively.

Tetrahydroaltersolanol C (1): colorless crystals (MeOH); mp 200.8–201.9 °C; $[\alpha]^{24}_D$ −5.4 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 214 (2.90), 280 (2.50), 314 (1.20) nm; IR (KBr) ν_{max} 3448, 1712, 1624 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS *m*/*z* 307.0 [M − H]$^-$; HRESIMS *m*/*z* 307.1178 (calcd for $C_{16}H_{19}O_6$, 307.1182).

Tetrahydroaltersolanol D (2): colorless crystals (MeOH); mp 192.1–193.0 °C; $[\alpha]^{24}_D$ −6.2 (*c* 0.92, MeOH); UV (MeOH) λ_{max} (log ε) 213 (2.60), 278 (2.40), 310 (1.00) nm; IR (KBr) ν_{max} 3281, 1607 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS *m*/*z* 307.0 [M−H]$^-$; HRESIMS *m*/*z* 307.1191 (calcd for $C_{16}H_{19}O_6$, 307.1182).

Tetrahydroaltersolanol E (3): colorless crystals (MeOH); mp 191.2–192.3 °C; $[\alpha]^{24}_D$ +6.1 (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 216 (2.80), 278 (2.50), 312 (1.10) nm; IR (KBr) ν_{max} 3430, 1713, 1621 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 2; ESIMS *m*/*z* 307.0 [M − H]$^-$; HRESIMS *m*/*z* 307.1195 (calcd for $C_{16}H_{19}O_6$, 307.1182).

Tetrahydroaltersolanol F (4): pink, amorphous powder; $[\alpha]^{24}_D$ −33.1 (*c* 0.45, acetone); UV (MeOH) λ_{max} (log ε) 278 (2.40), 316 (2.00) nm; IR (KBr) ν_{max} 3430, 1713, 1621 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; ESIMS *m*/*z* 349.2 [M − H]$^-$; HRESIMS *m*/*z* 349.1287 (calcd for $C_{18}H_{21}O_7$, 349.1287).

Dihydroaltersolanol (5): colorless crystals (MeOH); mp 201.2–201.9 °C; $[\alpha]^{24}_D$ −27.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 208 (2.90), 276 (2.20), 307 (1.60) nm; IR (KBr) ν_{max} 3417, 1713, 1649 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 2; ESIMS *m*/*z* 323.1 [M − H]$^+$; HRESIMS *m*/*z* 323.1099 (calcd for $C_{16}H_{19}O_7$, 323.1131).

Alterporriol N (12): red, amorphous powder; $[\alpha]^{24}_D$ −17.0 (*c* 0.75, acetone); UV (MeOH) λ_{max} (log ε) 223 (1.40), 279 (2.00), 434 (1.00) nm; IR (KBr) ν_{max} 3445, 1714, 1634 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 3; ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 4; ESIMS *m*/*z* 637.0 [M − H]$^-$; HRESIMS *m*/*z* 637.1541 (calcd for $C_{32}H_{29}O_{14}$, 637.1557).

Alterporriol O (13): red, amorphous powder; $[\alpha]^{24}_D$ −39.0 (*c* 0.96, acetone); UV (MeOH) λ_{max} (log ε) 218 (1.20), 278 (1.80), 434 (0.80) nm; IR (KBr) ν_{max} 3447, 1648 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 150 MHz), see Table 4;

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ESIMS m/z 637.0 $[M - H]^-$; HRESIMS m/z 637.1574 (calcd for $C_{32}H_{29}O_{14}$, 637.1557).

Alterporriol P (14): red, amorphous powder; $[\alpha]^{24}_D$ −28.0 (c 0.35, acetone); UV (MeOH) λ_{max} (log ε) 223 (0.80), 280 (1.40), 400 (0.60) nm; IR (KBr) ν_{max} 3441, 1605 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 150 MHz), see Table 4; ESIMS m/z 601.1 $[M - H]^-$; HRESIMS m/z 601.1337 (calcd for $C_{32}H_{25}O_{12}$, 601.1346).

Alterporriol Q (15): red, amorphous powder; $[\alpha]^{24}_D$ +172 (c 0.54, acetone); UV (MeOH) λ_{max} (log ε) 213 (3.00), 278 (4.20), 310 (2.00), 395 (0.80) nm; IR (KBr) ν_{max} 3505, 1713, 1648 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 4; ESIMS m/z 565.0 $[M - H]^-$; HRESIMS m/z 565.1156 (calcd for $C_{32}H_{21}O_{10}$, 565.1135).

Alterporriol R (16): red, amorphous powder; $[\alpha]^{24}_D$ +186 (c 0.60, acetone); UV (MeOH) λ_{max} (log ε) 214 (2.40), 279 (3.20), 312 (1.60), 394 (1.40) nm; IR (KBr) ν_{max} 3453, 1714, 1642 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 4; ESIMS m/z 565.0 $[M - H]^-$; HRESIMS m/z 565.1151 (calcd for $C_{32}H_{21}O_{10}$, 565.1135).

Tetrahydroaltersolanol B (6): colorless crystals (MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) 12.90 (1H, s, OH-5), 6.68 (1H, dd, 2.5, 1.0, H-8), 6.34 (1H, d, 2.5, H-6), 5.64 (1H, d, 7.5, OH-9), 4.70 (1H, d, 6.0, OH-3), 4.29 (1H, dd, 11.0, 7.5, H-9), 3.81(1H, s, OH-2), 3.80 (3H, s, H-12), 3.28 (1H, ddd, 12.0, 6.0, 4.5, H-3), 2.43 (1H, ddd, 12.5, 12.0, 3.5, H-4a), 2.17 (1H, dd, 13.0, 3.5, H-1eq), 2.10 (1H, ddd, 12.5, 4.5, 3.5, H-4eq), 1.96 (1H, dddd, 12.5, 12.0, 11.0, 3.5, H-1a), 1.48 (1H, ddd, 12.5, 12.0, 12.0, H-4ax), 1.21 (1H, dd, 13.0, 12.0, H-1ax), 1.14 (3H, s, H-11); ^{13}C NMR (DMSO-d_6, 125 MHz) 203.1 (C, C-10), 166.3 (C, C-7), 164.9 (C, C-5), 152.1 (C, C-9a), 109.7 (C, C-10a), 104.5 (CH, C-8), 99.5 (CH, C-6), 74.0 (CH, C-3), 71.3 (CH, C-9), 70.0 (C, C-2), 56.1 (CH_3, 7-OMe), 47.5 (CH, C-4a), 42.2 (CH, C-1a), 41.7 (CH_2, C-1), 29.8 (CH_2, C-4), 27.5 (CH_3, 2-Me); ESIMS m/z 307.0 $[M - H]^-$.

Preparation of the (*S*)-and (*R*)-MTPA Esters of 4 by the Modified Mosher's Method. Compound **4** (1.5 mg) was dissolved in 500 μL of pyridine, and dimethylaminopyridine (2.0 mg) and (*R*)-MTPACl (8 μL) were then added in sequence. The reaction mixture was stirred for 24 h at room temperature, and 1 mL of H_2O was then added. The solution was extracted with 5 mL of CH_2Cl_2, and the organic phase was concentrated under reduced pressure. Then the residue was purified by semipreparative HPLC (90% MeOH−H_2O) to yield (*S*)-MTPA ester **4s** (1.0 mg, t_R 9.47 min). By the same procedure, the (*R*)-MTPA ester **4r** (1.4 mg, t_R = 9.21 min) was obtained from the reaction of **4** (1.5 mg) with (*S*)-MTPACl (8 μL). (*S*)-MTPA ester (**4s**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.33 (1H, d, J = 2.4 Hz, H-8), 6.07 (1H, d, J = 10.8 Hz, H-9), 5.99 (1H, dd, J = 2.4, 1.2 Hz, H-6), 4.75 (1H, dd, J = 12.0, 4.2 Hz, H-3), 3.64 (3H, s, H-12), 2.52 (1H, m, H-4a), 2.50 (1H, m, H-1eq), 2.49 (1H, overlap, H-4eq), 2.47 (1H, m, H-1a), 2.12 (3H, s, H-13), 1.73 (1H, ddd, J = 13.2, 12.0, 12.0 Hz, H-4ax), 1.35 (1H, dd, J = 13.2, 12.0 Hz, H-1ax), 1.12 (3H, s, H-11); ESIMS m/z 567 $[M + H]^+$. (*R*)-MTPA ester (**4r**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.35 (1H, d, J = 2.4 Hz, H-8), 6.11 (1H, dd, J = 2.4, 1.2 Hz, H-6), 6.02 (1H, d, J = 10.8 Hz, H-9), 4.73 (1H, dd, J = 12.0, 4.8 Hz, H-3), 3.73 (3H, s, H-12), 2.50 (1H, m, H-4a), 2.48 (1H, m, H-1eq), 2.47 (1H, overlap, H-4eq), 2.45 (1H, m, H-1a), 2.11 (3H, s, H-13), 1.73 (1H, ddd, J = 12.0, 12.0, 11.4 Hz, H-4ax), 1.24 (1H, dd, J = 13.2, 12.0 Hz, H-1ax), 1.07 (3H, s, H-11); ESIMS m/z 567 $[M + H]^+$.

X-ray Crystallographic Analysis of 9 and 10. Colorless crystals of **9** and **10** were obtained from EtOAc and MeOH, respectively. Crystal data of **9** and **10** were recorded on a Bruker Smart 1000 CCD single-crystal diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms on oxygen were located in difference Fourier maps and refined isotropically. Hydrogen atoms bound to carbon atoms were placed in calculated positions and refined isotropically with a riding model. Crystallographic data for **9** and **10** have been deposited in the Cambridge Crystallographic Data Centre with the deposition numbers 842871 and 842870, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal data for ***9****:* orthorhombic, $C_{16}H_{20}O_7$, space group P_{212121}, a = 5.6703(10) Å, b = 10.858(2) Å, c = 23.955(4) Å, V = 1474.9(5) Å^3, Z = 4, D_{calcd} = 1.461 g/cm^3, μ = l 0.115 mm^{-1}, and F(000) = 688. Crystal size: 0.41 × 0.28 × 0.15 mm^3. Reflections collected/unique: 7898/2716 [R_{int} = 0.0294]. The final indices were R_1 = 0.0357, wR_2 = 0.0813 [$I > 2\sigma(I)$].

Crystal data for ***10****:* monoclinic, $C_{16}H_{20}O_8$, space group P_{21}, a = 5.3587(11) Å, b = 13.167(3) Å, c = 11.052(2) Å, β = 99.957(4)°, V = 768.1(3) Å^3, Z = 2, D_{calcd} = 1.549 g/cm^3, μ = 0.128 mm^{-1}, and F(000) = 380. Crystal size: 0.42 × 0.25 × 0.13 mm^3. Reflections collected/unique: 3874/2913 [R_{int} = 0.0153]. The final indices were R_1 = 0.0327, wR_2 = 0.0833 [$I > 2\sigma(I)$].

Biological Assays. Cytotoxic activity was evaluated by the MTT method as described previously.[24] Five human cancer cell lines, human colon carcinoma HCT-116, human breast cancer MCF-7/ADR, human prostatic cancer PC-3, and human hepatoma HepG2 and Hep3B cell lines, were used. Epirubicin was used as a positive control. Antiviral activity was tested against PRRSV according to established procedures.[25] Antibacterial activity was determined by the conventional broth dilution assay.[26] Nine bacterial strains, *E. coli*, *S. aureus*, *S. albus*, *B. subtilis*, *B. cereus*, *M. tetragenus*, *M. luteus*, *V. parahemolyticus*, and *V. anguillarum*, were used, and ciprofloxacin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, COSY, HMQC, HMBC, and MS spectra of the new compounds (**1**−**5**, **12**−**16**) and 1D NOE spectra of **1** and **2**; NOESY spectra of **4**; CIF files and X-ray crystallographic data for **9** and **10**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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