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Sesquiterpenoids Isolated from an Endophyte Fungus *Diaporthe* sp.

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Ten new sesquiterpenoids including six brasilane-type sesquiterpenoids, diaporols J-O (**1-6**), a 3,6-cycloprecapnellane sesquiterpenoid , diaporol P (**7**), and three drimane sesquiterpenoids, diaporols Q-S (**8-10**) were isolated from *Diaporthe* sp., an endophytic fungus associated with the leaves of *Rhizophora stylosa* collected in Hainan Province, China. The structures of these compounds were elucidated by extensive analysis of NMR, MS, CD spectra and single crystal X-ray diffraction. Among them, compound **9** exhibited moderate cytotoxicity against SW480 cell line with its IC_{50} value of 8.72±1.32 μM.

Introduction

The choice of the cultivation parameters has proved to be critical to the number and type of secondary metabolites produced by microorganisms.[1] Even small changes in the culture medium can impact the quantity of a certain compound as well as the general metabolic profile of an organism.[2] It may activate some cryptic biosynthetic gene clusters of secondary metabolites and then facilitate the discovery of new natural products by manipulating nutritional environmental factors.[3] Therefore, we can isolate hitherto unknown natural products from various fungi by altering easily accessible cultivation parameters, such as media composition, aeration, temperature or shape of culturing flask.[4, 5]

Previous investigation on a fungus *Diaporthe* sp. isolated from the leaves of *Rhizophora stylosa* collected in Hainan Province of China resulted in the discovery of nine new sesquiterpenoids in the solid-substrate fermentation cultures with a mixed medium (7.5 g of grain, 7.5 g of bran, 0.5 g of yeast extract, 0.1 g of sodium tartrate, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.1 g of sodium glutamate, and 30 mL of H_2O).[6] In order to identify more bioactive and/or new components, this strain was fermented on another medium only containing rice, which finally led us to isolate ten new sesquiterpenoids (**1-10**) (Fig. 1). Herein, we reported the isolation, structure elucidation and bioactivities of diaporols J-S (**1-10**).

Results and Discussion

The crude extract of the fungus *Diaporthe* sp., obtained after extracting the mycelia and culture medium of the fungus with ethanol, was subjected to repeated column chromatography followed by semi-preparative HPLC separation to yield ten new sesquiterpenoids diaporols J-S (**1-10**).

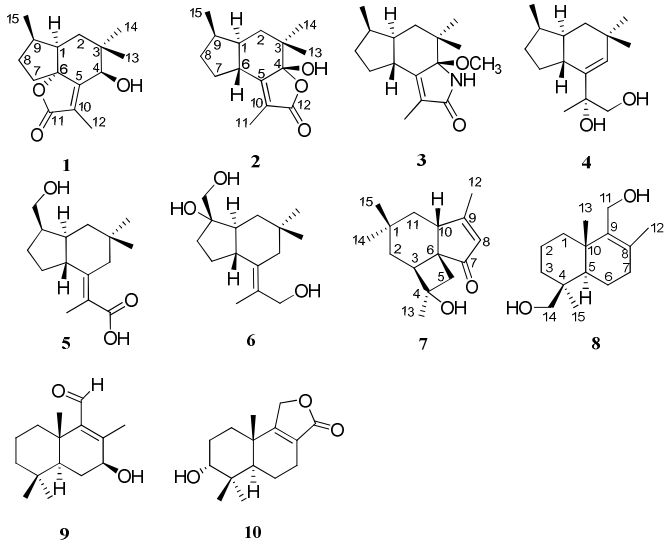

Fig. 1 Structures of compounds **1-10**.

Diaporol J (**1**) was obtained as colorless crystals and its molecular formula was determined as $C_{15}H_{22}O_3$ based on analysis of the positive ion HRESIMS data (*m/z* 251.1640 $[M + H]^+$, calcd 251.1642) and on interpretation ^{13}C NMR data. In the IR spectrum, absorptions for an α,β-unsaturated-γ-lactone (1717 cm^{-1}) moiety were observed. The ^{13}C NMR (Table 1) and HSQC data revealed the presence of 15 carbon resonances including four methyls, three methylene, three methine (one oxygenated) and five quaternary carbons (one carbonyl, two olefinic, and one oxygenated). One γ-lactone ring, one double bond, and one carbonyls accounted for three indicies of hydrogen defiency, the remaining two requiring **1** to be bicyclic. The above data suggested that **1** could be a brasilane-type sesquiterpenoid.[7, 8] The presence of the only hydroxyl group at C-4 was indicated by the chemical shifts of H-4 (δ_H 4.32) and C-4 (δ_C 73.1). The HMBC correlations between H-12 and C-5, C-10, and C-11, together with chemical shifts of C-6 (δ_C 96.1) and C-11 (δ_C 173.4) verified the existence of the α,β-unsaturated-γ-lactone moiety. The X-ray analysis with Cu Kα radiation confirmed the planar structure and further established its relative configuration (Fig. 2). Based on the CD rule for α,β-unsaturated-γ-lactone[9, 10], the positive Cotton effects for n→π* (210-250 nm) and negative π→π* (200-210 nm) of **1** (Fig. 3) indicated 6*R* configuration. Therefore, the stereogenic centers of **1** were assigned as 1*R*, 4*R*, 6*R*, 9*R* configurations.

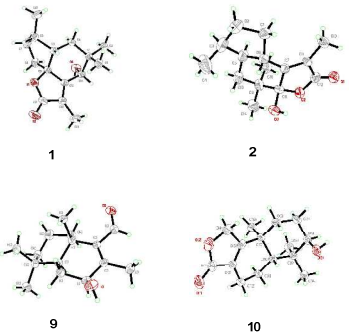

Fig. 2. X-ray crystallographic analysis of **1-2** and **9-10**.

Diaporol K (**2**) was isolated as colorless crystals and its molecular was established as $C_{15}H_{22}O_3$, the same with that of **1**. An α,β-unsaturated-γ-lactone (1752 cm^{-1}) moiety was also observed in the IR spectrum, consistent with the signals in the ^{13}C NMR spectrum (Table 1) (δ_C 120.7, 161.6, 174.0). Comparison of the NMR data of **2** with those of **1** indicated that the oxygen atom at α,β-unsaturated-γ-lactone moiety in **2** was anchored in C-4 instead of C-6 in **1**. Key HMBC correlations from H-13 to C-2 and C-4, from H-14 to C-2 and C-4, from H-11 to C-5, C-10, and C-12 and from H-6 to C-5 and C-10 confirmed the location of the α,β-unsaturated-γ-lactone moiety. Then the gross structure of diaporol K was established. X-ray diffraction analysis of crystals confirmed the gross structure of **2** and established its relative configuration (Fig. 2). Correspondingly, the Cotton effects for the transitions of the α,β-unsaturated γ-lactone group around 223 nm (positive, π–π* transition), and 245 nm (negative, n–π* transition)[9, 10] determined the absolute configuration of **2** was 1*R*, 4*S*, 6*R*, 9*R* (Fig. 3).

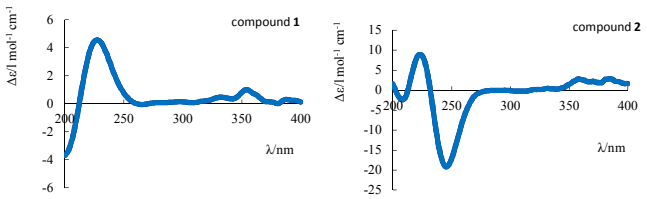

Fig. 3 CD spectrum of **1** and **2**

Diaporol L (**3**) was isolated as yellow amorphous powder, and its molecular formula was determined as $C_{16}H_{25}NO_2$ by the ^{13}C NMR and HRESIMS ion at *m/z* 286.1798 $[M + Na]^+$ (calcd 286.1778). The NMR data for **3** (Table 1) were similar to those for **2**, except for the α,β-unsaturated-γ-lactone in **2** was replaced by an α,β-unsaturated-γ-lactam in **3**, consistent with a characteristic IR

absorption at 1691 cm^{-1}; and a hydroxy group at C-4 in **2** was substituted by a methoxy group in **3**. These observations were further supported by the HMBC correlations (Fig. 3) from the exchangeable proton (δ_{NH} 5.96) to C-5 and C-10, and from the methoxy proton ($\delta_{H\text{-}16}$ 2.97) to C-4. The relative configuration of **3** was proposed on the basis of NOESY data. The NOE correlations (Fig. 4) between H-1/H-9, H-14 and H-6/H-15, H-16, H-13 indicated that β-orientation of H-6, H-15 and H-16 and α-orientation of H-1, respectively.

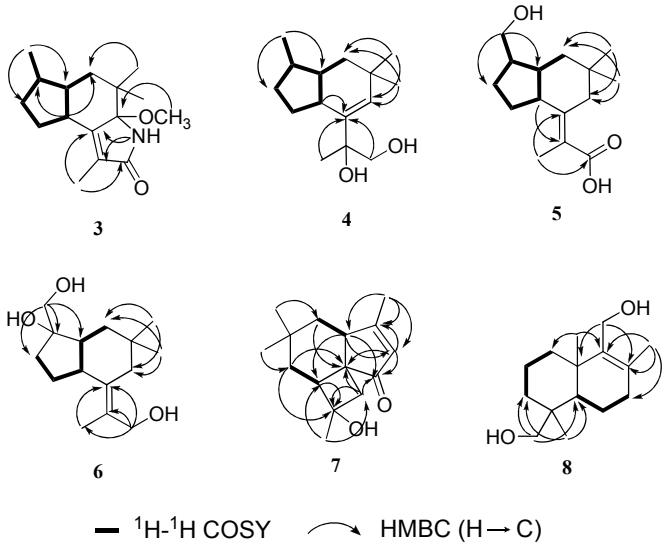

Fig. 3. Key ^{1}H-^{1}H COSY, HMBC correlations of **3**–**8**.

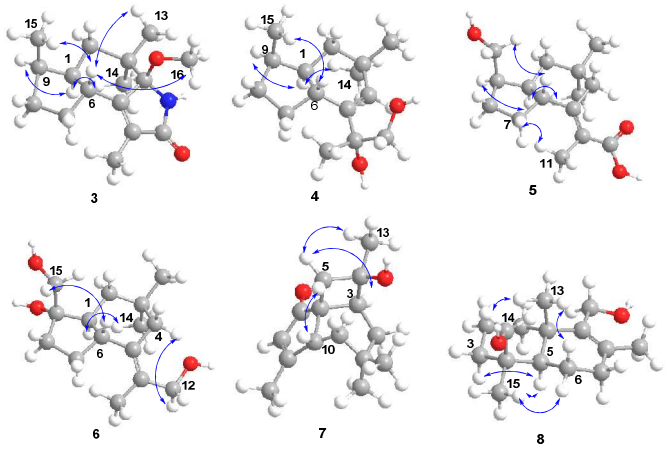

Fig. 4. Key ROESY correlations of **3**–**8.**

Diaporol M (**4**) was isolated as a white amorphous powder. The ^{13}C NMR (Table 1) and HRESIMS ion at *m/z* 239.2046 $[M + H]^+$ (calcd 239.2006) established a molecular formula of $C_{15}H_{26}O_2$ with three indices of hydrogen deficiency. The ^{13}C NMR and HSQC spectra of **4** displayed resonances for 15 carbons comprising four methyl, four methylene (one oxygenated), four methine (one olefinic), and three quaternary (one olefinic, one oxygenated) carbons. The aforementioned data suggested that **4** was a brasilane-type sesquiterpenoid.[7,8] Furthermore the HSQC and ^{1}H–^{1}H COSY data revealed the connectivity sequence of the protons $-C(2)H_2-C(1)H-C(6)H-[C(9)H]C(7)H_2-C(8)H_2-C(9)H-C(15)H_3$. The HMBC correlations of H-6/C-5; H-11, H-12/C-5; H-13, H-14/C-2, C-4; and H-15/C-1, C-8 revealed the planar structure of **4** was determined as shown in Figure 3. The relative configuration of compound **4** was established by the NOESY spectrum. The NOE correlations (Fig. 4) of H-1/H-9, H-14 and H-6/H-15 indicated that Me-15, H-1 and H-6 are β-, α- and β-oriented respectively. According to the rule proposed by Snatzke,[11] the sign of the diagnostic band at about 310 nm is correlated to the absolute configuration of the chiral centers in the 1,2-diol moiety. Thus, the negative sign observed in the spectra shown in Figure 5 allowed us to assign the *R*-configuration to C-10.

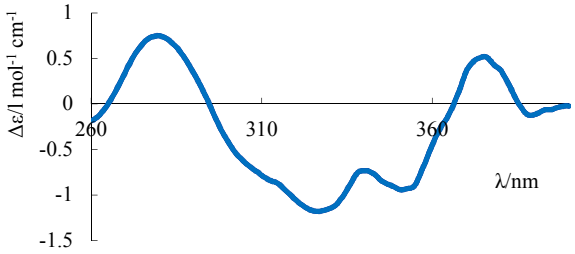

Fig. 5. Circular dichroism spectra of **4** in a DMSO solution of dimolybdenum tetracetate.

Diaporol N (**5**) was isolated as a white amorphous powder, and its molecular formula was established as $C_{15}H_{24}O_3$ based on the ^{13}C NMR (Table 1) and HRESIMS ion at *m/z* 253.1754 $[M + H]^+$ (calcd 253.1758) data. The ^{13}C NMR spectrum of **5** exhibited 15 carbon signals consisting of three methyls, five methylenes (one oxygenated), three methines and four quaternary carbons (two olefinic, one carbonyl) (Table 1). The above data suggested that compound **5** was also a brasilane-type sesquiterpenoid.[7,8] Analysis of the ^{1}H–^{1}H COSY data revealed that **5** had the similar proton coupling sequence with **4** $[-C(2)H_2-C(1)H-C(6)H-[C(9)H]C(7)H_2-C(8)H_2-C(9)H-C(15)H_3]$. The planar structure of **5** was established by the HMBC correlations (Fig. 3) from H-6 to C-10, from H-11 to C-5 and C-12, from H-13 to C-2 and C-4, from H-14 to C-2 and C-4, and from H-15 to C-1 and C-8. The relative configuration of **5** was elucidated based on the observed NOE correlations and by the analysis of its ^{1}H

NMR *J*-values. The significant NOE correlations (Fig. 4) between H-1/H-9, H-14 and H-6/H-15 indicated that the relative configurations of the stereocenters in **5** are the same as those in **4**. Furthermore, the large coupling constant ($J_{H\text{-}1/H\text{-}2\beta}$=13.0 Hz and $J_{H\text{-}6/H\text{-}7\alpha}$=12.0) suggested a trans-junction of the two ring system. The geometry of the double bond was assigned as *E* based on the NOE correlation of H-7/H-11.

Diaporol O (**6**) was isolated as a white amorphous powder. Its molecular formular was determined as $C_{15}H_{26}O_3$ by HRESIMS (*m/z* 255.1960 $[M + H]^+$, calcd 255.1954). Analysis of its NMR data (Table 2) revealed that the structure of **6** resembled that of **5**, except for the presence of one hydroxylated quaternary carbon ($\delta_{C\text{-}9}$ 79.2) and one hydroxlated methylene group ($\delta_{H\text{-}12}$ 3.87 and 4.17, $\delta_{C\text{-}12}$ 64.4) and the concomitant absence of one methine ($\delta_{H\text{-}9}$ 1.97, $\delta_{C\text{-}9}$ 48.2) and one carboxylic acid group ($\delta_{C\text{-}12}$ 172.7). The HMBC correlations (Fig. 3) of H-12/C-5, C-10, C-11 and H-15/ C-1, C-8, C-9 revealed the location of three hydroxy groups in **6** was at C-9, C-12 and C-15. The NOE correlations of H-1/H-14 and H-6/H-15 and the *J* value (td, 13.0, 3.6 Hz) of H-1 suggested a 15β-hydroxymethyl orientation and a *trans*-junction of the two ring system. The NOE correlation of H-4/H-12 indicated the geometry of the double bond was *E* (Fig. 4).

Diaporol P (**7**) was obtained as a white powder. Its molecular formula was established as $C_{15}H_{22}O_2$ by analysis of its HRESIMS (*m/z* 235.1670 $[M + H]^+$, calcd 235.1693) and ^{13}C NMR data, indicating five indices of hydrogen deficiency. The ^{13}C NMR (Table 2) and HSQC data revealed the presence of 15 carbon signals, including four methyls, three methylenes, three methine (one olefinic), and five quaternary carbons (one olefinic carbon, one carbonyl and one oxygenated). One double bond and one carbonyl group accounted for two indices of hydrogen deficiency, the remaining three thus requiring **7** to be tricyclic. The aforementioned data suggested that **7** was a 3,6-cycloprecapnellane-type sesquiterpenoid,[12] which was supported by the HMBC correlations of H-2, H-5, H-8, H-11/C-6 (δ_C 43.6 shifted to high field because of γ-effect). The HMBC correlations of H-2, H-3, H-5/C-4 (δ_C 70.9) revealed the location of hydroxy group was at C-4. The HMBC correlations of H-8/C-7 (δ_C 213.7), C-9 (δ_C 181.6); H-10/C-7, C-8 (δ_C 129.0); and H-12/C-8, C-9, C-10 revealed the location of the α,β-unsaturated ketone moiety. Thus, the planar structure of **7** was established as shown in Figure 3**.** The relative stereochemistry of **7** was established by NOE correlations (Fig. 4) of H-5α/H-3, H-5α/H-13, and H-5β/H-10. Furthermore, no correlation between H-13/H-15 indicated the hydroxy group at C-4 was assigned a β-orientation. According to the octant rule for cyclopentenones,[13] the positive Cotton effect at 331 nm for n–π* transition and the negative Cotton effect at 243 nm for π–π* transition reflected 10*R* configuration (Fig. 6). Therefore, the 3*S*, 4*R*, 6*S*, 10*R* absolute configuration was proposed for **7**. The biosynthetic origin of compound **7** may be the condensation product by cyclizing the carbons 6 and 11 of brasilane sesquiterpenoid.

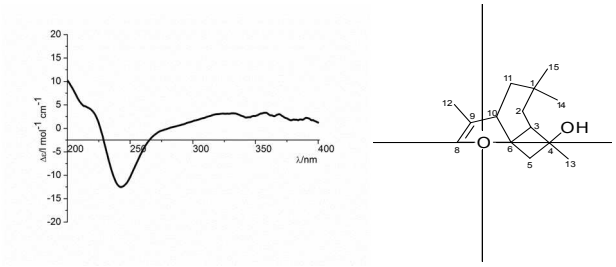

Fig. 6. CD spectrum of **7**

Diaporol Q (**8**) was obtained as white amorphous powder and its molecular formula was established as $C_{15}H_{26}O_2$ by analysis of the HRESIMS data (*m/z* 239.2014 $[M + H]^+$, calcd 239.2006), which accounted for three indices of hydrogen deficiency. The ^1H NMR data (Table 2) indicated the presence of three tertiary methyl groups at δ_H 0.95 (3H, s), 1.00 (3H, s), and 1.72 (3H, s), two oxygenated methylenes [δ_H 3.48 d (*J* = 10.8 Hz), 3.77 d (*J* = 10.8 Hz); and δ_H 4.04 d (*J* = 11.6 Hz), 4.20 d (*J* = 11.6 Hz)]. The ^{13}C NMR spectrum (Table 2) showed and two olefinic carbons (δ_C 133.1 and 141.3) accounting for a double-bond. The remaining indices required **8** to contain two saturated rings. The ^1H and ^{13}C NMR data suggested that compound **8** was a drimane-type sesquiterpenoid.[6] The $^1H–^1H$ COSY spectrum established the proton sequence of −C(1)H−C(2)H_2−C(3)H_2− and −C(5)H −C(6)H_2−C(7)H−. HMBC correlations of H-11/C-8, C-10; H-12/C-7, C-8, C-9; H-13/C-1, C-9; H-14/C-3, C-5; and H-15/C-3, C-5 established the planar structure of **8**. The relative configuration of **8** was proposed on the basis of NOESY data and by the analysis of its ^1H NMR *J*-values. NOESY correlations of H-13 with H-14 and H-6β indicated that these protons adopt the same orientation, whereas those of H-6α with H-15, and H-5 with H-3α and H-15 placed these protons on the opposite face of the ring system. Furthermore, the *J* value (dd, 13.0, 2.0 Hz) of H-5 suggested a *trans*-junction of the two ring system.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1-5**.

	1[a]		**2**[a]		**3**[a]		**4**[b]		**5**[c]	
No.	δ_C	δ_H (J in Hz)	δ_C,	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	50.5	1.21, dt (13.2,6.4)	46.2	1.75, m	45.8	1.67, m	45.3	1.65, m	44.2	1.71 m
2	30.4	1.15, dd (13.6,5.2) 1.52, dd (13.6,13.2)	37.9	1.37, dd (13.0,3.6) 1.64, t (13.0)	36.7	1.24, m 1.68, m	39.2	1.21, m 1.50, m	40.3	1.25, t (13.0) 1.51, dd (13.0,3.6)
3	38.5		41.4		41.1		35.7		33.3	
4	73.1	4.32, s	107.6		93.6		135.0	5.46, s	45.5	1.73, d (13.2) 2.27, d (13.2)
5	160.1		161.6		153.9		141.0		140.1	
6	96.1		42.2	2.52, m	41.1	2.12, m	41.9	2.02, m	39.6	1.94, m
7	34.2	1.77, ddd (5.6, 12.0, 14.8) 2.77, ddd (5.6, 9.6, 14.8)	27.0	1.73, m 2.07, m	27.0	1.76, m 2.09, m	31.5	1.21, m 2.03, m	30.1	1.57, qd (12.0,7.2) 2.07, dq (12.0,7.2)
8	30.2	1.49, m 2.06, m	33.4	1.29, m 2.16, m	32.8	1.25, m 2.14, m	33.7	1.22, m 2.08, m	27.8	1.32, m 1.85, m
9	36.0	2.62, m	32.8	2.15, m	32.2	2.11, m	32.6	2.09, m	48.2	1.97, m
10	124.0		120.7		125.7		76.4		123.4	
11	173.4		9.3	1.89, d (2.0),	8.26	1.89, d (2.4)	25.0	1.32, s	15.9	1.86, s
12	8.6	1.84, s	174.0		174.5		69.9	3.45, d (11.0) 3.59, d (11.0)	172.7	
13	23.8	1.13, s	24.1	1.22, s	24.6	1.08, s	32.5	1.05, s	32.2	0.90, s
14	26.4	0.77, s	22.2	0.83, s	22.6	0.77, s	30.4	1.01, s	26.0	0.79, s
15	14.8	0.97, d (6.8)	18.4	0.87, d (7.2)	17.9	0.86, d (7.0)	18.5	0.81, d (7.0)	62.7	3.19, dd (7.2, 10.2) 3.40, dd (7.2, 10.2)
16					48.9	2.97, s				

[a] ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data in $CDCl_3$
[b] ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data in $CDCl_3$
[c] ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in DMSO-d_6

Diaporol R (**9**) was isolated as colorless crystals and the molecular formula $C_{15}H_{24}O_2$ was determined by analysis of its HRESIMS ion at *m/z* 237.1860 $[M + H]^+$ (calcd 237.1849) and ^{13}C NMR (Table 2). Its ^{1}H, ^{13}C NMR and HSQC data showed **9** had four methyls, four methylenes, three methines (one oxygenated and one aldehyde), and four quaternary carbons (two olefinic), suggesting **9** was a drimane-type sesquiterpenoid.[14] The presence and location of an α,β-unsaturated aldehyde group was indicated by their relevant chemical shifts ($\delta_{H\text{-}11}$ 10.1 , $\delta_{C\text{-}11}$ 194.6, $\delta_{C\text{-}8}$ 151.8, and $\delta_{C\text{-}9}$ 145.4) and HMBC correlations of H-11/C-8, C-9; H-12/C-8, C-9; and H-13/C-9. Further HMBC correlations of an oxygenated methine proton ($\delta_{H\text{-}7}$ 4.18) with C-8 and C-9 revealed hydroxyl group was located at C-7. The relative configuration of **9** was unambiguously determined by single-crystal X-ray diffraction analysis (Fig. 2).

Diaporol S (**10**) was obtained as colorless crystals and its molecular formula was deternined as $C_{15}H_{22}O_3$ by its HRESIMS ion at *m/z* 251.1654 $[M + H]^+$ (calcd. 251.1642). The analysis of the ^{1}H and ^{13}C NMR data of **10** (Table 2) showed high similarities to those of 3β-hydroxyconfertifolin[15] except for the coupling constant of the H-3 [δ_H 3.47 (t, J = 2.0 Hz) for **10**, and δ_H 3.27 (dd, J = 4.9, 9.9 Hz) for 3β-hydroxyconfertifolin], indicating the hydroxyl group was α-orientation in **10**. X-ray diffraction analysis confirmed the structure and established the relative configuration of **10** (Fig. 2).

The isolated compounds (**1**-**10**) were evaluated for their cytotoxic activities against five human cancer cell lines (HCT116, MDA-MB-231, SMMC-7721, SW480 and HepG2) using the MTT method with doxorubicin as a positive control.[16] Among them, only diaporol R (**9**) showed moderate cytotoxic activity against SW480 cell line with its IC_{50} value of 8.72±1.32 μM, whereas the other compounds are almost inactive (IC_{50} > 30 μM) against these cell lines.

Table 2. ^{1}H and ^{13}C NMR data for compounds for **6-10**.

	6[d]		**7**[a]		**8**[a]		**9**[a]		**10**[a]	
No.	δ_C	δ_H (J in Hz)	δ_C,	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	54.0	1.78, td (13.0,3.6)	30.6		37.4	1.32,td (13.0,4.0) 1.96, d (13.0)	36.7	0.96, d (13.5) 2.42, td (13.5, 4.0)	29.0	1.34, td (13.5, 4.0) 1.85, d (13.5)
2	39.6	1.21, t (13.0) 1.60, dd (13.0,3.6)	35.1	1.38, dd (13.6, 8.8) 1.63, dd (13.6, 8.8)	19.2	1.57, m 1.63, m	19.3	1.50, m 1.65, m	25.1	1.66, m 1.98, m
3	33.6		45.7	2.62, t (8.8)	35.9	0.97, m 1.81, m	41.8	1.16, m 1.45, m	75.2	3.47, t (2.0)
4	44.3	1.55, d (13.6) 2.41, dd (2.4, 13.6)	70.9		39.2		33.6		37.5	
5	135.3	1.39, m	45.9	2.08, d (12.0) 2.41, dd (12.0, 2.8)	53.0	1.30,dd(13.0,2.0)	49.7	1.14, dd (14.0, 4.0)	44.0	1.71, dd (2.4, 12.5)
6	50.8	2.03, m	43.6		19.8	1.45,m 1.79, m	29.6	1.43, m 2.43, m	17.6	1.53, m 1.76, m
7	30.2	1.85, m 2.05, m	213.7		34.6	2.04, m 2.07, m	74.3	4.18, t (8.0)	21.3	2.10, m 2.32, m
8	37.8	1.69, m 1.79, m	129.0	5.81, s	133.1		151.8		123.4	
9	79.2		181.6		141.3		145.4		170.7	
10	128.4		51.1	2.60, dd (6.0, 7.6)	38.6		39.1		36.2	
11	17.4	1.85, s	40.5	1.26, dd (12.0, 6.0) 1.73, dd (12.0, 7.6)	59.0	4.04, d (11.6) 4.20, d (11.6)	194.6	10.1, s	68.3	4.62, ddd (17.2,3.9,1.6) 4.69, dt (17.2, 2.8)
12	64.4	3.87, d (12.0) 4.17, d (12.0)	18.0	2.09, s	19.9	1.72, s	15.0	2.10, s	174.7	
13	32.6	0.94, s	28.8	1.65, s	21.8	0.95, s	21.1	1.26, s	20.7	1.12, s
14	26.2	0.82, s	28.1	0.72, s	66.1	3.48, d (10.8) 3.77, d (10.8)	22.3	0.88, s	21.8	0.87, s
15	67.7	3.38, d (10.8) 3.48, d (10.8)	32.2	1.02, s	27.4	1.00, s	33.9	0.91, s	28.1	0.97, s

[a] ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data in $CDCl_3$
[d] ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data in acetone-d_6

Conclusions

In summary, ten new sesquiterpenoids (Diaporols J-S) were isolated from the detailed investigation of endophytic *Diaporthe* sp. in rice solid-substrate fermentation. All the structurally diverse sesquiterpenoids were endured the biological activity screening resulting in diaporol R showed the moderate cytotoxic activity against SW480 with IC_{50} values of 8.72±1.32μM. None of the diaporols A-I[6] was isolated in this study, indicating the change of culture conditions was likely to influence the secondary metabolism by directly regulation of the enzyme activity or by the variation of complicated regulatory networks involved in natural product biosynthesis.[17,18] The study also indicated that the strain may produce new active natural products by variation of the cultivation cluture, which may induce or promote the biosynthesis of metabolites.

Experimental Section

General Experimental Procedures.

Optical rotations were obtained on a Rudolph Autopol III automatic polarimeter. Melting points (mp) were determined with a Boetius micromelting apparatus and are uncorrected. The UV spectra were recorded on a Hitachi U-3000 spectrophotometer, and the IR spectra (KBr) were measured on a Nexus 870 FT-IR spectrometer. NMR spectroscopic data were acquired on BRUKER DRX500, BRUKER AVANCE Ⅲ 600, or BRUKER AVANCE Ⅲ 400 NMR spectrometer with tetramethylsilane (TMS) and solvent signals as internal references. HRESIMS spectra were recorded on an Agilent 6210 TOF LC-MS spectrometer. Single-crystal X-ray diffraction data were collected on a Bruker APEX-II CCD diffractometer with an Atlas detector (Cu Kα radiation, λ = 1.54178 Å). Silica gel (200-300 mesh) for column chromatography (CC) was produced by Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Sephadex LH-20 was purchased from Pharmacia Biotech, Uppsala, Sweden. ODS-A GEL(AA12S50) was purchased from YMC Co., Ltd, Japan. The semi-preparative HPLC was accomplished over a Hypersil ODS column (5 μm, 250 mm×10 mm, Thermo Fisher Scientific, USA) on a Hitachi HPLC system consisted of a L-7110 pump (Hitachi) and a L-7420 UV-VIS Detector (Hitachi).

Fungal Material.

The culture of *Diaporthe* sp. was isolated from the healthy leaves of *Rhizophora stylosa* from the mangrove forest of Hainan Province of China, in April, 2005. A voucher specimen has been deposited at the

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University. The fungal strain *Diaporthe* sp. IFB-3lp-10 was cultured on slants of potato dextrose agar (PDA) at 28°C for 5 days. After that the fungus was inoculated into Erlenmeyer flasks (1 L) containing 400 mL of Czapek's medium (consisting of 20 g/L malt extract, 20 g/L sucrose, 1 g/L peptone, 20 g/L agar, and deionized water) for 4 d at 28 °C on a rotary shaker at 150 rpm. Scale-up fermentation was carried out in twenty 500-mL flasks each containing 80 g of rice (japonica rice) and 100 mL distilled H_2O which were soaked overnight before autoclaving. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 28 °C for 40 days with humidity in the range 60–70 %.

Extraction and Isolation.

The fermented material was extracted with 95 % EtOH and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (100g). The crude extract (100 g) was separated into six fractions(Fr1- Fr6) by silica column chromatography eluted with a gradient of CH_2Cl_2/MeOH (v/v 100:0, 100:1, 100:2, 100:4, 100:8, 100:20, 0:100) based on TLC monitoring.Fraction 2 was further separated on a reversed phase ODS column with a gradient of MeOH/H_2O (v/v 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) into nine subfractions (Fr2.1- Fr2.9). Fr2.3 was subjected to repeated Sephadex LH20 column chromatography (100 % MeOH) to yield compounds **9** (6 mg), compounds **10** (4 mg) and **8** (7 mg). Chromatography of Fr 2.5 over Sephadex LH-20 (100% MeOH) followed by the semi-preparative HPLC (MeCN/H_2O, 40:60, 2 mL/min) gave **3** (1.2 mg, t_R = 20.1 min) and **4** (1.1 mg, t_R = 31.7 min). The fourth fraction was also subjected to a reversed phase ODS column (4 cm×40 cm) with a gradient of MeOH/H_2O (v/v 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) to give subfractions Fr4.1- Fr4.9. Compound **1** (1.0 mg, t_R = 25.2 min) and **2** (11.5 mg, t_R = 32.1 min) were obtained from HPLC (MeOH/H_2O, 50:50, 2mL/min) over Fr4.4 was chromatographed over Sephadex LH-20 (100% MeOH). Fraction 4.5 was chromatographed on silica gel (petroleum ether/EtOAc 5:1) and Sephadex LH-20 (100% MeOH) followed by the semi-preparative HPLC (MeOH/H_2O, 40:60, 2mL/min) to yield compounds **5** (2mg), **6** (4mg) and **7** (8 mg).

*Compound (**1**)*: colorless crystals; m.p. 143 °C; $[\alpha]^{28}_D$ = -40 (*c* = 0.160, MeOH). UV (MeOH): λ_{max} (log ε) = 221 (2.87), 272 (2.06) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =202 (-3.52), 228 (4.54) nm; IR (KBr): ν_{max} = 3428, 2960, 1717, 1634, 1400, 1276, 1132, 1021, 897 cm^{-1}. HRESIMS: *m/z* 251.1640 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1642). For 1D and 2D NMR data, see Table 1 and Supporting Information.

*Compound (**2**)*: colorless crystals; m.p. 172 °C; $[\alpha]^{28}_D$ = -300 (*c* = 0.165, MeOH). UV (MeOH): λ_{max} (log ε) = 220 (3.06), 348 (1.00) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =208 (-2.41), 223 (8.96), 245 (-19.17) nm; IR (KBr): ν_{max} =3408, 2961, 2934, 2868, 1752, 1633, 1384, 1077, 895 cm^{-1}. HRESIMS: *m/z* 251.1638 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1642). For 1D and 2D NMR data, see Table 1 and Supporting Information.

*Compound (**3**)*: yellow amorphous powder; $[\alpha]^{28}_D$ = -80 (*c* = 0.175, MeOH). UV (MeOH): λ_{max} (log ε) = 210 (3.31), 332 (0.98) nm. IR (KBr): ν_{max} = 3420, 2955, 1691, 1634, 1384, 1083, 1054 cm^{-1}. HRESIMS: *m/z* 286.1798 $[M + Na]^+$ (calcd for $C_{16}H_{25}NO_2Na$, 286.1778). For 1D and 2D NMR data, see Table 1 and Supporting Information.

*Compound (**4**)*: white amorphous powder; $[\alpha]^{28}_D$ = 44.5 (*c* = 0.160, MeOH). UV (MeOH): λ_{max} (log ε) = 205 (3.13) nm. IR (KBr): ν_{max} = 3405, 2956, 2871, 1638, 1461, 1400, 1385, 1250, 1122, 1042, 892 cm^{-1}. HRESIMS: *m/z* 239.2046 $[M + H]^+$ (calcd for $C_{15}H_{27}O_2$, 239.2006). For 1D and 2D NMR data, see Table 1 and Supporting Information.

*Compound (**5**)*: white amorphous powder; $[\alpha]^{28}_D$ = 400 (*c* = 0.125, MeOH). UV (MeOH): λ_{max} (log ε) = 206 (3.11), 332 (1.26) nm. IR (KBr): ν_{max} = 3397, 2952, 2926, 2867, 1653, 1575, 1455, 1384, 1299, 1273, 1156, 1055, 800 cm^{-1}. HRESIMS: *m/z* 253.1754 $[M + H]^+$ (calcd for $C_{15}H_{25}O_3$, 253.1758). For 1D and 2D NMR data, see Table 1 and Supporting Information.

*Compound (**6**)*: Yellow amorphous powder; $[\alpha]^{28}_D$ = -32 (*c* = 0.140, MeOH). UV (MeOH): λ_{max} (log ε) = 205 (3.09) nm. IR (KBr): ν_{max} = 3401, 2955, 2925, 2856, 1654, 1461, 1384, 1258, 1113, 1045, 876 cm^{-1}. HRESIMS: *m/z* 255.1960 $[M + H]^+$ (calcd for $C_{15}H_{27}O_3$, 255.1954). For 1D and 2D NMR data, see Table 2 and Supporting Information.

*Compound (**7**)*: white amorphous powder; $[\alpha]^{28}_D$ = -50 (*c* = 0.135, MeOH). UV (MeOH): λ_{max} (log ε) = 222 (3.43) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =210 (5.66), 243 (-12.52), 331 (3.20) nm; IR (KBr): ν_{max} = 3415, 2928, 2866, 1693, 1632, 1384, 1255, 1171, 1102, 961, 870 cm^{-1}. HRESIMS: *m/z* 235.1670 $[M + H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1693). For 1D and 2D NMR data, see Table 2 and Supporting Information.

*Compound (**8**)*: white amorphous powder; $[\alpha]^{28}_D$ = -120 (*c* = 0.155, MeOH). UV (MeOH): λ_{max} (log ε) = 205 (2.79), 332 (1.53) nm. IR

(KBr): ν_{max} = 3407, 2925, 2854, 1623, 1546, 1385, 1243, 1162, 1111, 1034 cm^{-1}. HRESIMS: *m/z* 239.2014 $[M + H]^+$ (calcd for $C_{15}H_{27}O_2$, 239.2006). For 1D and 2D NMR data, see Table 2 and Supporting Information.

*Compound (**9**)*: colorless crystals; m.p. 184 °C; $[\alpha]^{28}_D$ = -17.4 (*c* = 0.147, MeOH). UV (MeOH): λ_{max} (log ε) = 205 (3.11), 249 (2.98) nm. IR (KBr): ν_{max} = 3418, 2955, 2925, 2854, 1635, 1384, 1261, 1205, 1122, 1044, 836 cm^{-1}. HRESIMS: *m/z* 237.1860 $[M + H]^+$ (calcd for $C_{15}H_{25}O_2$, 237.1849). For 1D and 2D NMR data, see Table 2 and Supporting Information.

*Compound (**10**)*: colorless crystals; m.p. 192 °C; $[\alpha]^{28}_D$ = 36.2 (*c* = 0.126, MeOH). UV (MeOH): λ_{max} (log ε) = 218 (2.68), 333 (0.78) nm. IR (KBr): ν_{max} = 3453, 2959, 2871, 1740, 1669, 1454, 1389, 1238, 1059, 964 cm^{-1}. HRESIMS: *m/z* 251.1654 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1642). For 1D and 2D NMR data, see Table 2 and Supporting Information.

X-ray Crystallographic Analysis of **1–2, 9-10**:Colorless crystals of **1–2, 9-10** were obtained by crystallization of the compounds from a 1:1 solution of MeOH and CH_2Cl_2. Room-temperature diffraction measurements were carried a Bruker APEX DUO diffractometer (Cu *K*α radiation, λ = 1.54178 Å).The crystal structures were refined by *SHELXL*-97; Friedel pairs were merged. CCDC-1014738, CCDC-1014740, CCDC-1014741 and CCDC-1014751 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

*Crystal Data of **1**:* $C_{15}H_{22}O_3$, M_r = 250.33, orthorhombic, space group $P2_12_12_1$, *a* = 8.064(1) Å, *b* = 11.542(1)Å, *c* =14.904(1) Å, *V* = 1387.2(2) $Å^3$, *Z* = 4, *Dx* = 1.199 g/cm^3, μ(Cu Kα) = 0.656 mm^{-1}, *F*(000) = 544. Crystal dimensions: 0.27×0.25×0.22 mm^3. Independent reflections: 1347(R_{int} = 0.026).The final R_1 values are 0.035, wR_2 = 0.105[$I > 2\sigma(I)$]. CCDC 1014751.

*Crystal Data of **2**:* $C_{15}H_{22}O_3$, M_r = 250.33, monoclinic, space group $P2_1$, *a* = 6.896(1) Å, *b* = 7.706(1)Å, *c* = 13.516(1) Å, □= 96.435 (5)°, *V* = 713.7(2) $Å^3$, *Z* = 2, *Dx* = 1.165 g/cm^3, μ(Cu Kα) = 0.637 mm^{-1}, *F*(000) = 272. Crystal dimensions: 0.28×0.19×0.17 mm^3. Independent reflections: 1232 (R_{int} = 0.090). The final R_1 values are 0.089, wR_2= 0.220[$I > 2\sigma(I)$]. CCDC 1014738.

*Crystal Data of **9**:* $C_{15}H_{24}O_2$, M_r = 236.34, orthorhombic, space group $P2_12_12_1$, *a* = 7.5653(2) Å, *b* = 10.7212(4)Å, *c* = 16.5230(4)Å, *V* = 1340.17(8)$Å^3$, *Z* = 4, *Dx*= 1.171 g/cm^3, μ(Cu Kα) = 0.589 mm^{-1}, *F*(000)= 520. Crystal dimensions: 0.27×0.25×0.22 mm^3. Independent reflections: 1323 (R_{int} = 0.026). The final R_1 values were 0.043, wR_2= 0.121[$I > 2\sigma(I)$]. CCDC 1014740.

*Crystal Data of **10**:* $C_{15}H_{22}O_3$, M_r = 250.33, orthorhombic, space group$P2_12_12_1$, *a* = 7.3041(2) Å, *b* = 11.6752(3) Å, *c* = 15.6101(4) Å, *V* = 1331.18(6) $Å^3$, *Z* = 4, *Dx*= 1.249 g/cm^3, μ(Cu Kα) = 0.684 mm^{-1}, *F*(000) = 544. Crystal dimensions: 0.27×0.25×0.09 mm^3. Independent reflections: 1292 (R_{int} = 0.049). The final R_1 values were 0.038, wR_2= 0.085[$I > 2\sigma(I)$]. CCDC 1014741.

Cytotoxic activity assay.

Compound **1-10** were evaluated for cytotoxicity against four cell lines, HCT116 (human colon cancer), MDA-MB-231 (human breast cancer), SMMC-7721 (human hepatic carcinoma), HepG2 (human hepatic carcinoma), SW480 (Human colon adenocarcinoma cell line) (all from the Jiangsu Provincial Center for Disease Prevention and Control), using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[16] Doxorubicin HCl (Sigma-Aldrich) was used as a positive control, and the medium without compounds as a negative control in the bioassay.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [1D and 2D NMR spectra of compounds **1-10**]. See DOI: 10.1039/b000000x/

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Graphic abstract

Diaporthe sp.