Published in final edited form as:

Phytochemistry. 2017 August ; 140: 77–82. doi:10.1016/j.phytochem.2017.04.017.

Anti-proliferative ambuic acid derivatives from Hawaiian endophytic fungus *Pestalotiopsis* sp. FT172

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Abstract

Five previously undescribed ambuic acid derivatives, pestallic acids A–E and three known analogs were isolated from the cultured broth of *Pestalotiopsis* sp. FT172. The structures of the new compounds were determined through the analysis of HRMS and NMR spectroscopic data. The absolute configurations (ACs) of pestallic acids B–E were assigned by comparison of the experimental electric circular dichroism (ECD) spectra or the optical rotations with those in the literature. All compounds were tested against A2780 and cisplatin resistant A2780 (A2780CisR) cell lines. Pestallic acid E and (+)-ambuic acid showed potent activities with IC₅₀ values from 3.3 to 17.0 μ M.

Graphical Abstract

Bioassay-guided fractionation of an extract from a Hawaiian endophytic fungus *Pestalotiopsis* sp. FT172 led to the isolation of eight compounds, including five previously undescribed ambuic acid derivatives pestallic acids A–E. Their chemical structures were determined by HRMS and NMR spectroscopy. All isolates were evaluated for their anti-proliferative activity against A2780 and cisplatin-resistant A2780 (A2780CisR) cancer cell lines. Pestallic acid E showed potent anti-proliferative activity against A2780 and A2780CisR cell lines with IC₅₀ values of 3.3 and 5.1 μ M, respectively, and (+)-ambuic acid demonstrated inhibitory activity against the above two cancer cell lines with IC₅₀ values of 10.1 and 17.0 μ M, respectively.

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Notes

The authors declare no competing financial interest.

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pestallic acid E $R_1 + R_2 = O$
(+)-ambuic acid $R_1 = OH, R_2 = H$

Keywords

Myrsine sandwicensis; Myrsinaceae; endophytic fungi; *Pestalotiopsis*; ambuic acid; antiproliferative

1. Introduction

Hawaiian islands are at the middle of the Pacific Ocean, which are separated far from any other land in the world. The specific location and environment built a unique kingdom with diverse natural organisms (Eldredge and Miller, 1995). Endophytic fungi have been proved to be important sources for producing structurally unique and biologically active secondary metabolites (Cao et al., 2010; Cao and Clardy 2011; Cao, Cryan et al., 2012; Cao, McMillin et al., 2012; Khaarwar et al., 2011; Zhang et al., 2006). A study conducted in 2012 on endophytic fungal communities in leaves of a single tree species, *Metrosideros polymorpha*, across wide environmental gradients in the Mauna Loa volcano indicated that foliar endophytic fungi in Hawaii are very diverse (Zimmerman et al., 2012). But endophytic fungi in Hawaii are underexplored. In the past few years, we have isolated about 3,000 endophytic strains from Hawaiian plants, established a natural product library with more than 5,000 semi-pure fractions, and identified different types of compounds with anti-proliferative activity (Fei-Zhang et al., 2016; Huang et al., 2017; Li, Ding, Yang, Miklossy et al., 2015; Li, Ding, Yang, Hoffman et al., 2015; Li, Ren et al., 2016; Li, Yang et al., 2015).

During an ongoing investigation of bioactive compounds from Hawaiian plants-derived fungi (Li, Ding et al., 2015; Li, Yang et al., 2015), our natural product library was screened against A2780 (human ovarian cancer cell line) and A2780CisR (cisplatin-resistant A2780 human ovarian cancer cell line). Some samples including a semi-pure fraction from an endophytic strain FT172, *Pestalotiopsis* sp. isolated from a Hawaiian indigenous plant, *Myrsine sandwicensis* A. DC. (Myrsinaceae), showed inhibitory activity against A2780 and A2780CisR at 20 $\mu\text{g/mL}$. Fungi in the genus *Pestalotiopsis* are rich sources of many interesting compounds, including alkaloids, polyketides, terpenoids, flavonoids, coumarins, xanthonoids, quinones, cyclohexenones, peptides, phenols, phenolic acids, and lactones (Chen et al., 2016; Xu et al, 2014; Wang et al, 2012; Yang et al, 2012). Some compounds from the genus *Pestalotiopsis* showed antibacterial, antifungal, antiviral, anticancer, and antioxidant activities (Chen et al., 2016; Xu et al, 2014; Wan, et al, 2012; Yang et al, 2012).

Bioassay-guided separation of the active fraction from *Pestalotiopsis* sp. FT172 led to the isolation of eight ambuic acid derivatives (**1–8**) including five undescribed compounds, pestallic acids A–E (**1–5**). Here, we describe the isolation, structure determination, and anti-proliferative activity of these eight compounds, among which compounds **5** and **7** showed potent inhibitory activity against A2780 and A2780CisR cell lines.

2. Results and discussion

The fermented whole broth (5 L) of FT172 was filtered through filter paper. The supernatant solution was passed through HP-20, and then eluted with aqueous MeOH-H₂O (10%, 30%, 50%, 70%, 90% MeOH in H₂O) to afford five fractions (Frs. A–E). The active fraction (Fr. E) was further separated by preparative and semi-preparative HPLC to afford compounds **1–8** (Figure 1).

Compound **1** was isolated as yellow gum. Its molecular formula was determined by HRESIMS (m/z 431.1660 [M + Na]⁺, calcd 431.1676) to be C₂₁H₂₈O₈, indicating eight degrees of unsaturation. Comprehensive analysis of the 1D and 2D NMR spectra indicated the presence of four methyls (including two methoxy groups), five methylenes, four methines (one olefinic, three oxygenated), and eight other carbons with no hydrogen attached, including two conjugated ketones (δ_C 186.2, 186.4) and one carboxyl group (δ_C 172.5) (Table 1). Two spin systems, C-3–C-4 and C-11–C-17, were established by the ¹H-¹H COSY spectrum as shown in Figure 2, which was also verified by the corresponding HMBC correlations. HMBC correlations from the olefinic proton (δ_H 6.71, H-3) and the methyl (δ_H 1.92, H₃-18) to the carboxylic carbon (δ_C 172.5, C-1), from H₃-18 to the olefinic carbons C-2 (δ_C 129.4), and C-3 (δ_C 141.6), and from H₂-4 (δ_H 3.30) to C-2 suggested the presence of a 2-methylbut-2-enoic acid moiety. Similarly, the spin system C-11–C-17 was determined to be a 1,2-dioxygenated heptane moiety. These two chains (C-1–C-4, and C-11–C-17) were characteristic moieties of the ambuic acid derivatives (Ding et al., 2009; Jung et al., 2012; Qi et al., 2015; Li et al., 2001; Li et al., 2003; Xie et al., 2014). The HMBC correlations from H₂-4 to the shielded olefinic carbon (δ_C 118.0, C-5) and the conjugated carbonyl carbon C-10 (δ_C 186.4), from the oxygenated proton H-11 (δ_H 4.03) to C-8 (δ_C 141.9), C-9 (δ_C 135.3), and C-10, as well as from the acetal proton H-19 (δ_H 5.28) to C-8 and C-9 suggested the presence of a benzoquinone ring system. Seven out of eight degrees of unsaturation were established, indicating the presence of one more ring system. The HMBC correlations from the oxygenated proton H-11 to C-8 and C-9, from H-19 to the oxygenated carbon C-12 (δ_C 72.0) established the 3,6-dihydro-2H-pyran ring with an acetal group. The HMBC correlations from the two methoxy groups to C-11 and C-19 established their positions on the ring system. However, no correlation from any proton to C-6 and C-7 was observed. The up-fielded chemical shift of C-5 (δ_C 118.0) implied that C-6 should be oxygenated. Analysis of the molecular formula of compound **1** and comparison of the NMR data of **1** with those of ambuic acid analogs (Ding et al., 2009; Jung et al., 2012; Qi et al., 2015; Li et al., 2001; Li et al., 2003; Xie et al., 2014) suggested a hydroxyl group at C-6. Hence, the planar structure of compound **1** was established as shown. The relative configuration of **1** was determined by analysis of the coupling constant and NOE correlations (Figure 3).

Compound **2** was isolated as a colorless solid. The molecular formula of **2** was established by HRESIMS (m/z 345.1666 $[M + Na]^+$, calcd 345.1672) to be $C_{18}H_{26}O_5$, with six degrees of unsaturation. Detailed comparison of the 1H and ^{13}C NMR data of **2** with those of compound **8** (Qi et al., 2015) suggested that the planar structure of **2** was very similar to that of **8**, both of which had a 2-methylbut-2-enoic acid moiety and a 1-substituted hept-1-ene. The hydroxymethyl unit at 8-position in **8** was absent in **2**, which had one more olefinic methine (δ_H 5.55, δ_C 123.8) than **8** instead. Therefore, the only difference between **2** and **8** was at 8-position, which was confirmed by the COSY correlation (Figure 2) between H-7 and the olefinic proton H-8, and also by the HMBC correlations (Figure 2) from H-8 to C-6, and from H-10 and H-11 to C-8 in **2**. The relative configurations were determined by analysis of the coupling constants and ROESY data. The C-11/C-12 double bond was assigned as *E*-geometry according to the coupling constant ($J = 15$ Hz) between H-11 and H-12 (1H in pyridine- d_5). The C-2/C-3 double bond was also determined to be *E* due to the NOE correlation (Figure 3) with H₂-4 to H₃-18. Meanwhile, the small vicinal coupling constant ($J = 1.3$) between H-6 and H-7 indicated that they were on *cis* orientation. The observed NOE correlations of H-6 with H_b-4, and H_a-4 with H-10 implied they were on the same side of the ring system. Hence, the relative configuration of **2** was determined as shown.

Compound **3** was obtained as a colorless solid. The molecular formula of **3** was determined by HRESIMS (m/z 361.1638 $[M + Na]^+$, calcd 361.1622) to be $C_{18}H_{26}O_6$, with 18 units (H_2O) more than that of compound **6** (Xie et al., 2014). The 1D and HSQC NMR spectra of **3** showed that it had the same number of methyl, methylene, methine and other carbons with no hydrogen attached (Table 2) as **6**, both of which had a 2-methylbut-2-enoic acid moiety and a 1-substituted hept-1-ene. However, the much down-fielded chemical shifts of C-5 and C-6 in compound **3** suggested that the epoxide ring was open to form a 5,6-diol, which was confirmed by the HMBC correlations (Figure 2) from the de-shielded proton H-6 (δ_H 3.85) to C-4 (δ_C 33.2), C-7 (δ_C 200.3), and C-8 (δ_C 122.6), and also from H₂-4 (δ_H 2.72 and 2.74) to C-5 (δ_C 77.7) and C-6 (δ_C 78.6). The NOE correlations (Figure 2) from H_b-4 to H-6, and from H_a-4 to H-10 suggested that they were on the same side of the ring. Hence, the planar structure and the relative configuration of **3** were determined as shown, a 3,5-disubstituted 4,5,6-trihydroxycyclohex-2-en-1-one.

Compound **4** was isolated as a colorless solid. Its molecular formula was established by HRESIMS (m/z 345.1659 $[M + Na]^+$, calcd 345.1672) to be $C_{18}H_{26}O_5$, suggesting one oxygen atom less than that of **3**. Comprehensive comparison of the NMR data (Table 2) of compounds **3** and **4** revealed that one oxygenated methine in **3** was replaced by one methylene group (δ_C 45.6) in **4**, which could be at either 6- or 10-positions. HMBC correlation from the olefinic proton H-11 (δ_H 6.25) to the oxygenated methine (δ_C 68.5) suggested that there was a hydroxyl group at 1-position, indicating a methylene at position in **4**, which was confirmed by the HMBC correlations from H₂-4 (δ_H 2.48 and 2.59) and H-8 (δ_H 5.85) to C-6 (δ_C 45.6). The NOE correlation from H_a-4 to H-10 suggested both of the hydroxyl groups at C-5 and C-10 should be co-facial. Hence, the planar structure and relative configuration of **4** were determined as shown.

Compound **5**, a colorless solid, was determined to have the molecular formula $C_{19}H_{26}O_7$ by HRESIMS (m/z 385.1585 $[M + Na]^+$, calcd 385.1571). The 1H , ^{13}C , as well as HSQC NMR spectra indicated that the structure of **5** was similar to that of ambuic acid (**7**) (Li et al., 2001), both of which had a 2-methylbut-2-enoic acid moiety and a 1-substituted hept-1-ene. The only difference between **5** and **7** was that one oxygenated methine in **7** was replaced by a ketone moiety in **5** at C-7, which was confirmed by the HMBC correlations from H-6 and H₂-19 to C-7. Hence, the planar structure of **5** was determined as shown, a 4,6-disubstituted 3-(hydroxymethyl)-7-oxabicyclo[4.1.0]hept-3-ene-2,5-dione.

The structures of the known compounds **6** (Xie et al., 2014), **7** (Li et al., 2001) and **8** (Qi et al., 2015) were identified by comparison of their physical data with reported values in the literature.

The relative configuration of compound **1** was determined to be 11*S*',12*R*',19*S*' as shown, but we couldn't determine its absolute configuration. Compound **2**, a tetra-oxygenated cyclohexene, is structurally similar to the tetra-oxygenated cyclohexenes **9a** [(1*R*,2*R*,3*S*,4*S*)-5-(hydroxymethyl)-2-methyl-6-((*E*)-prop-1-en-1-yl)cyclohex-5-ene-1,2,3,4-tetra ol] and **9b** [(*E*)-4-((1*S*,2*S*,5*R*,6*R*)-3-((*E*)-hept-1-en-1-yl)-2,5-dihydroxy-4-(hydroxymethyl)-7-oxabicyclo[4.1.0]hept-3-en-1-yl)-2-methylbut-2-enoic acid (5*S*,6*R*,7*R*,10*S*)] (Qi et al., 2015) (Fig. 4). Compound **2** had a positive Cotton effect at 239 nm, which was like that of **9a**, but opposite to that of **9b**. Hence, the absolute configuration of compound **2** was determined to be 5*R*,6*S*,7*S*,10*R*. Compound **3** is a 4,5,6-trihydroxy α,β -unsaturated cyclohexenone derivative. Similar to (4*R*,5*S*,6*R*)-6-ethyl-4,5-dihydroxy-3-methylcyclohex-2-en-1-one (**10**) (Fig. 4) (Kwit et al, 2010) that is also a α,β -unsaturated cyclohexenone with substitutes at 4-, 5-, and 6-positions, **3** has a negative Cotton effect at about 220 nm followed by a positive Cotton effect. Therefore, the absolute configuration of **3** was determined to be 5*R*,6*R*,10*R*. Compound **4** is a 4,5-dihydroxycyclohex-2-en-1-one derivative. The CD spectrum of compound **4** had a negative Cotton effect at 235 nm, which was similar to that of the known 4,5-dihydroxycyclohex-2-en-1-one derivative **11** (Fig. 4), (4*R*,5*S*)-3-ethyl-4,5-dihydroxycyclohex-2-en-1-one (Kwit et al, 2010). Hence, the absolute configuration of compound **4** was determined to be 5*S*,10*R*. The epoxide in compound **5** was in a *cis* relationship, indicating that the configuration was either 5*R*,6*S*, or 5*S*,6*R*. Like its *t*-Bu ester (a quinone epoxide, $[\alpha]_D^{23} + 63.5$ ($c = 1.0$, $CHCl_3$)) (Li et al., 2003), compound **5** had a positive sign of optical rotation ($[\alpha]_D^{25} + 41$ ($c = 0.48$, $CHCl_3$)), indicating that both should have the same orientation for the epoxide. Therefore, the absolute configuration of **5** was determined to be 5*R*, 6*S*.

All the compounds were tested against A2780S and A2780CisR. Compound **5** showed potent anti-proliferative activity with IC₅₀ values of 3.3 and 5.1 μM , respectively. Meanwhile, compound **7** ((+)-ambuic acid) also exhibited inhibition on these two cancer cell lines with IC₅₀ values of 10.1 and 17.0 μM , respectively.

3. Conclusions

The bioassay (A2780 and A2780CisR) guided separation of the secondary metabolites of the endophytic fungus *Pestalotiopsis* sp. FT172, obtained from a plant of *Myrsine sandwicensis* at Mokuleia Forest Reserve on the Oahu Island, Hawai'i, led to the isolation of eight ambuic acid derivatives. Their structures and configurations were elucidated on the basis of spectroscopic data as well as optical rotation and CD analysis. The structure of **1** had a unique core ring system, 7-hydroxy-1,4-dimethoxy-3,4-dihydro-1*H*-isochromene-5,8-dione with a heptyl group and a 2-methylbut-2-enoic acid moiety at 3- and 6-positions of the ring system, respectively. All the compounds except **1** each had a six-membered ring system together with a 2-methylbut-2-enoic acid moiety and a 1-substituted hept-1-ene, which were at the 1,3-positions of the six-membered ring system. All the compounds were tested against A2780S and A2780CisR. Compound **5**, showed potent anti-proliferative activity with IC₅₀ values of 3.3 and 5.1 μ M, respectively. Meanwhile, the known compound **7** also exhibited inhibition on the two cancer cell lines with IC₅₀ values of 10.1 and 17.0 μ M, respectively. Both compounds **5** and **7** have a α,β -unsaturated ketone at 10-position flanked by a 2-methylbut-2-enoic acid moiety at 5-position and a 1-substituted hept-1-ene at 9-position, which is different from all the other molecules (**1–4**, **6**, and **8**). When the ketone at 10-position of **7** was reduced to an alcohol, it could generate compound **8**, which was tested inactive, indicating that the ketone at 10-position is essential for the activity.

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured with a Rudolph Research Analytical AutoPol IV Automatic Polarimeter. UV and IR spectra were obtained with Shimadzu UV-1800 spectrophotometer and Thermo scientific Nicolet iS50FT-IR spectrometer, respectively. CD spectrum was recorded on Jasco J-815 circular dichroism spectrophotometer in methanol. NMR spectra including 1D and 2D experiments were recorded in methanol-*d*₄ on a Varian Unity Inova 500 MHz and Bruker 400 MHz. High resolution mass spectra were obtained on a Waters Micromass Q-ToF Ultima ESI-TOF mass spectrometer. HPLC was carried out on Agilent 1100 LC system, and all solvents were HPLC grade. Diaion HP-20 was used for open column chromatography.

4.2. Fungal material

The fungal strain *Pestalotiopsis* sp. FT172 was isolated on PDA medium from a healthy leaf of Hawaiian indigenous plant, *Myrsine sandwicensis* A. DC. (Myrsinaceae), at Mokuleia Forest Reserve on the Oahu Island in 2014. The leaf was collected on the trail, which is from Mokuleia Access Road at the north of the Oahu Island to the Peacock Flats area. The fungus (Code number: UHCCFT172) has been deposited at the strain bank of Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo. Mycelia were retrieved by filtration and ground to a fine powder in liquid N₂. Genomic DNA was extracted using the SurePrep RNA/DNA/protein purification kit (Fisher Bioreagents), and large subunit rDNA was amplified by PCR using primers ITS1 and ITS4. PCR products were sequenced at Genewiz

(<http://www.genewiz.com/>). The DNA sequence data obtained from the fungal strain UHCCFT172 have been deposited at GenBank with accession number KU318714.

4.3. Fermentation

The fungus was grown under static conditions at room temperature for 30 days in one 1L conical flask containing the liquid medium (300 mL/flask) composed of mannitol (20 g), glucose (10 g), monosodium glutamate (5 g), KH_2PO_4 (0.5 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.3 g), yeast extract (3 g), corn steep liquor (2 mL), for 1L distilled water; pH 6.5 prior sterilization.

4.4. Extraction and isolation

The fermented whole broth (5 L) was filtered through filter paper to separate the supernatant from the mycelia. The filtered supernatant was passed through HP-20 eluted with $\text{MeOH}-\text{H}_2\text{O}$ (10%, 30%, 50%, 70%, 90% methanol in H_2O) to afford five fractions (Fr. A–E). Fraction E was active at 20 $\mu\text{g/mL}$ (>50% inhibition). Fraction E (720.0 mg) was separated by preparative HPLC (C18 column, 5 μ , 100.0×21.2 mm; 15 mL/min; 20–100% $\text{MeOH}/\text{H}_2\text{O}$ in 30 min, followed by 100% MeOH for 10 min) to generate 40 sub-fractions (SF. 1340). SF. 17331 was active at 20 $\mu\text{g/mL}$ (>50% inhibition). SF.17 (5.27 mg) was subjected to the semi-preparative HPLC (C18 column, 5 μ , 250.0×10.0 mm; 3 mL/min; with 0.1% formic acid in 32% $\text{MeCN}/\text{H}_2\text{O}$ (v/v)) to afford compounds **4** (3.16 mg, t_R 33.2 min) and **8** (1.42 mg, t_R 36.7). SF. 20 (20.70 mg) was purified by semi-preparative HPLC (Phenyl-Hexyl column, 5 μ , 250.0×10.0 mm; 3 mL/min; with 0.1% formic acid in 45% $\text{MeCN}/\text{H}_2\text{O}$ (v/v)) to yield compound **3** (1.68 mg, t_R 35.9 min). SF. 21 (14.21 mg) was subjected to semi-preparative HPLC (C18 column, 5 μ , 250.0×10.0 mm; 3 mL/min; 30% to 65% $\text{MeCN}/\text{H}_2\text{O}$ (v/v) in 30 min with 0.1% formic acid in the eluent) to afford compound **5** (3.67 mg, t_R 21.9 min). SF. 22 (91.11 mg) was separated by preparative HPLC (C18 column, 5 μ , 100.0×21.2 mm; 10 mL/min; 20% to 60% $\text{MeCN}/\text{H}_2\text{O}$ in 30 min) to obtain compound **2** (3.49 mg, t_R 22.6 min) and **7** (2.38 mg, t_R 24.2min). SF. 23 (19.97 mg) was separated by HPLC (C18 column, 5 μ , 250.0×10.0 mm; 3 mL/min; with 0.1% formic acid in 42% $\text{MeCN}/\text{H}_2\text{O}$ (v/v)) to yield compound **6** (1.18 mg, t_R 28.7 min). SF. 25 (18.21 mg) was separated by semi-preparative HPLC (Phenyl-Hexyl column, 5 μ , 250.0×10.0 mm; 3 mL/min; with 0.1% formic acid in 45% $\text{MeCN}/\text{H}_2\text{O}$ (v/v)) to afford compound **1** (1.64 mg, t_R 31.4 min).

4.4.1. Pestalic acid A(1)—Yellow gum; $[\alpha]_D^{25} -10.7$ ($c = 0.03$, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 217 (4.18), 282 (3.77) nm; IR $\nu_{\max}$ 3369, 2954, 2930, 2858, 2161, 1645, 1557, 1457, 1361, 1316, 1270, 1212, 1161, 1061 cm^{-1} ; ^1H (methanol- d_4 , 500 MHz) and ^{13}C NMR (methanol- d_4 , 125 MHz) data, see Table 1; positive HR-ESIMS m/z 431.1660 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{28}\text{O}_8\text{Na}$, 431.1676).

4.4.2. Pestalic acid B(2)—Colorless solid; $[\alpha]_D^{25} +6.3$ ($c = 0.11$, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 229 (4.25) nm; IR $\nu_{\max}$ 3334, 2957, 2927, 2857, 1689, 1646, 1416, 1258, 1130, 1019, 966 cm^{-1} ; ^1H (methanol- d_4 , 500 MHz) and ^{13}C NMR (methanol- d_4 , 125 MHz) data, see Table 1; positive HR-ESIMS m/z 345.1666 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{26}\text{O}_5\text{Na}$, 345.1672).

4.4.3. Pestalic acid C(3)—Colorless solid; $[\alpha]_{\text{D}}^{25} + 4.5$ ($c = 0.07$, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.43), 283 (3.17) nm; IR ν_{max} 3360, 2957, 2928, 2866, 1599, 1381, 1360, 1115, 1089, 1020, 974 cm^{-1} ; ^1H (methanol- d_4 , 500 MHz) and ^{13}C NMR (methanol- d_4 , 125 MHz) data, see Table 2; positive HR-ESIMS m/z 361.1638 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{26}\text{O}_6\text{Na}$, 361.1622).

4.4.4. Pestalic acid D(4)—Colorless solid; $[\alpha]_{\text{D}}^{25} - 7.14$ ($c = 0.13$, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.54), 282 (3.48) nm; IR ν_{max} 3417, 2958, 2927, 2816, 2777, 2682, 1663, 1626, 1594, 1559, 1361, 1261, 1095, 1.33, 802, 772 cm^{-1} ; ^1H (methanol- d_4 , 500 MHz) and ^{13}C NMR (methanol- d_4 , 125 MHz) data, see Table 2; positive HR-ESIMS m/z 345.1659 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{26}\text{O}_5\text{Na}$, 345.1672).

4.4.5. Pestalic acid E(5)—Colorless solid; $[\alpha]_{\text{D}}^{25} + 41.0$ ($c = 0.48$, CHCl_3); $[\alpha]_{\text{D}}^{25} + 41.0$ ($c = 0.48$, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 213 (4.03), 304 (3.49) nm; IR ν_{max} 3367, 2957, 2928, 2829, 1667, 1597, 1355, 1260, 1089 cm^{-1} ; ^1H (methanol- d_4 , 400 MHz) and ^{13}C NMR (methanol- d_4 , 100 MHz) data, see Table 2; positive HR-ESIMS m/z 385.1585 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{26}\text{O}_7\text{Na}$, 385.1571).

4.5. Cytotoxic Assays

Viability of A2780 and A2780CisR, was determined using the CyQuant cell proliferation assay kit, according to the manufacturer's instructions (Life Technologies, CA, USA) (Delazar et al., 2004; Sridhar et al., 2004). Briefly, cells were cultured in 96-well plates at 6000 cells per well for 24 h and subsequently treated with compounds (20 $\mu\text{g/mL}$) for 72 h and analyzed. Relative viability of the treated cells was normalized to the DMSO-treated control cells (Delazar et al., 2004; Sridhar et al., 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was financially supported by a start-up funding from University of Hawaii Cancer Center, the Victoria S. and Bradley L. Geist Foundation (15ADVC-74420) (SC) and the National Institutes of Health (NIH)/National Cancer Institute (NCI) Grant CA128865 (JT). We are grateful to Mr. Wesley Yoshida and Dr. Walter Niemczura at the Chemistry Department, University of Hawaii at Manoa for collecting the NMR data.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.phytochem>.

Highlights

- *Pestalotiopsis* sp. FT172 is the first fungus isolated from *Myrsine sandwicensis*.
- Pestallic acids A–E from *Pestalotiopsis* sp. FT172 have not been reported before.
- The configurations of pestallic acids B–E were determined by using CD and polarimetry.
- Pestallic acid E had an IC₅₀ value of 3.3 μ M against A2780 cancer cell line.

Fig. 1.
Structures of Compounds **1–8**

Fig. 2.
Key HMBC (arrow) and ^1H - ^1H COSY (bold) Correlations of Compounds **1**, **2**, and **3**.

Fig. 3.
Key NOESY (dash arrow) Correlations of Compounds **1**, **2**, and **3**.

Fig. 4.
Structures of Compounds **9a**, **9b**, **10**, and **11**

Table 1¹H and ¹³C NMR spectroscopic data for compounds **1** and **2** in methanol-*d*₄

position	1		2	
	δ_{H}, J (Hz)	$\delta_{\text{C}}, ^a$	δ_{H}, J (Hz)	$\delta_{\text{C}}, ^a$
1		172.5		171.4
2		129.4		132.0
3	6.71, t, 7.3 3.30, m	141.6 23.5	6.85, td, 7.4, 1.2 3.01, dd, 15.7, 7.5	137.1 32.1
4			2.61, dd, 15.7, 7.5	2.93, dd, 15.6, 7.5
5		118.0		61.5
6		<i>b</i>	3.15, d, 1.3	59.2
7		<i>b</i>	4.38, br. d, 5.4	64.6
8		141.9	5.55, dd, 5.5, 1.2	123.8
9		135.3		137.7
10		186.4	4.45 br. s	65.4
11	4.03, d, 2.0	70.3	5.97, m	131.3
12	3.91, ddd, 9.3, 4.1, 2.0	72.0	5.98, m	133.2
13	1.79, m 1.68, m	31.0	2.12, m	34.1
14	1.45, m	26.6	1.42, m	30.1
15	1.37, m	32.9	1.32, m	32.6
16	1.37, m	23.7	1.32, m	23.6
17	0.93, t, 7.0	14.4	0.90, t, 7.0	14.4
18	1.92, s	12.7	1.86, s	12.9
19	5.28, s	94.2		2.10, s
MeO-11	3.50, s	60.4		
MeO-19	3.45, s	56.4		

^adata were deduced by combined analysis of HSQC and HMBC^bdata were not found

Table 2

¹H and ¹³C NMR spectroscopic data for compounds **3–5** in methanol-*d*₄

position	3		4		5 ^b	
	$\delta_{\text{H}}, J \text{ (Hz)}$	δ_{C}^a	$\delta_{\text{H}}, J \text{ (Hz)}$	δ_{C}^a	$\delta_{\text{H}}, J \text{ (Hz)}$	δ_{C}
1		175.9		178.2		170.2
2		130.0		129.9		131.9
3	6.83, t, 7.7	134.5	6.55, t, 7.3	138.3	6.67, td, 7.4, 1.6	133.2
4	2.74, dd, 15.2, 7.5	33.2	2.59, dd, 14.9, 7.2	38.2	3.00, dd, 15.8, 7.0	26.5
	2.72, dd, 15.1, 7.8		2.48, dd, 14.9, 7.9		2.89, dd, 15.9, 7.8	
5		77.7		76.4		61.0
6	3.85, s	78.6	2.70, d, 16.8	45.6	3.82, s	58.2
			2.38, d, 17.4			
7		200.3		201.3		192.8
8	5.92, s	122.6	5.85, s	125.8		136.9
9		158.4		131.1		141.7
10	4.40, s	72.6	4.33, s	68.5		193.8
11	6.29, d, 15.9	129.8	6.25, d, 15.8	131.1	6.47, dt, 15.9, 1.4	121.1
12	6.50, dt, 15.8, 7.0	142.0	6.54, dt, 15.9, 7.1	141.8	6.61, dt, 15.9, 7.0	145.5
13	2.24, dd, 14.4, 7.1	34.6	2.25, dd, 14.7, 7.3	34.6	2.28, dd, 14.0, 7.3	33.9
14	1.46, m	29.6	1.49, m	29.7	1.52, m	28.2
15	1.33, m	32.6	1.34, m	32.6	1.36, m	31.2
16	1.33, m	23.6	1.34, m	23.6	1.36, m	22.1
17	0.91, t, 6.9	14.4	0.91, t, 6.7	14.3	0.94, t, 6.9	12.9
18	1.84, s	13.6			1.90, s	11.5
19					4.57, d, 11.7	54.6
					4.29, d, 11.6	

^adata were obtained by combined analysis of HSQC and HMBC^bdata were collected on 400 MHz for ¹H and 100 MHz for ¹³C