Depsidones from the Endophytic Fungus BCC 8616

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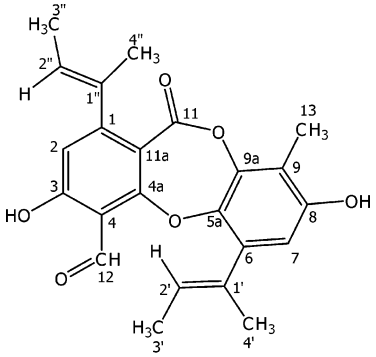
Three new depsidones (**1**−**3**) have been isolated from the endophytic fungus BCC 8616 and their structures analyzed on the basis of spectroscopic data interpretation. Compound **1** exhibited weak cytotoxic activity against breast and epidermoid carcinoma cell lines.

In a continuing search for bioactive substances from microorganisms,[1−3] the crude extract of an endophyte deposited at the BIOTEC culture collection as BCC 8616 and classified as a member of the order Pleosporales (class Ascomycetes, subclass Dothideomycetidae; see the Fungus Identification section) exhibited cytotoxic activity against human epidermoid carcinoma (KB) and human breast cancer (BC) cell lines. We have therefore investigated this extract for its active constituents and have identified three new depsidones (**1**−**3**), of which one exhibited weak cytotoxic activity. Depsidones have been isolated commonly from various lichens.[4−8] However, many depsidones are constituents of non-lichen sources, for example, auranticins from the mangrove fungus *Preussia aurantica*,[9] emeguisins from the ascomycete *Emericella unguis*,[10] and garcidepsidones from the leaves of *Garcinia* spp.[11,12] The present finding represents the first example of depsidones being isolated from an endophytic fungus.



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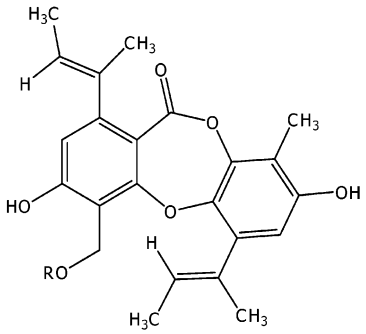

2 R = H
3 R = CH_3

Compound **1** revealed a molecular formula of $C_{23}H_{22}O_6$ by HRMS, showing a protonated molecular ion peak at *m*/*z* 395.1497 $[M + H]^+$. Analysis of the ^{13}C NMR and DEPT spectra established 23 carbons in the molecule, consisting of five methyls, five methines, and 13 quaternary carbons. The proton at δ_H 10.27 on the carbon at δ_C 194.1 together with a strong absorption at ν_{max} 1649 cm^{-1} indicated an aldehyde group. The aldehyde correlated in the HMBC spectrum to oxygen-bearing carbons at δ_C 165.2 (C-3) and 110.5 (C-4), while a chelated hydroxyl proton at δ_H 12.16 correlated to C-2, C-3, and C-4. The presence of a 1-methylprop-1-enyl moiety was revealed by the resonances of a methine at δ_H 5.68, which exhibited coupling constants of 6.85 and 1.26 Hz, and of two methyls at δ_H 1.80 (δ_C 14.4) and 1.96 (δ_C 18.0) ppm, respectively. This methine proton also correlated to C-1 and C-3″ in the HMBC spectrum. The aromatic methine at δ_H 6.70 (2-H) attached to the carbon at δ_C 115.0 (C-2) correlated in the HMBC spectrum with C-1 and C-3. The above information suggested the partial structure A. Likewise, the methine at δ_H 5.51, which coupled to two methyls at δ_H 1.80 (δ_C 13.8) and 2.04 (δ_C 18.1), with coupling constants of 6.85 and 1.40 Hz, respectively, indicated the presence of another methylpropenyl unit in the molecule. The HMBC spectrum showed correlations from H-13 to C-9, C-9a, and C-8; from H-7 to C-8, C-6, and C-5a; from H-2′ to C-3′, C-4′, and C-6; from H-3′ to C-2′ and C-1′; and from H-4′ to C-6, C-1′, and C-2′. Three low-field resonances at δ_C 142.3 (C-5a), 151.5 (C-8), and 143.4 (C-9a) suggested that these three carbons are attached to oxygen atoms, and hence, subunit B was inferred. The remaining carbon at δ_C 166.0 (C-11) indicated a carbonyl ester due to the observation of a strong absorption at ν_{max} 1721 cm^{-1} and could be adjacent to C-11a.

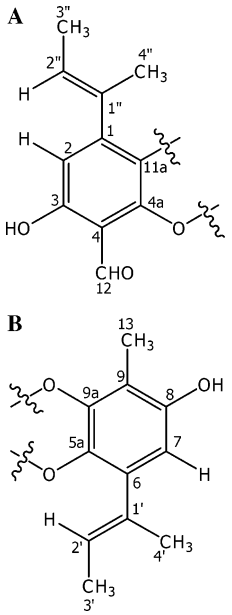

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**3**

position	**1**[a]	**2**[b]	**3**[a]
2	6.70 (s)	6.57 (s)	6.60 (s)
7	6.43 (s)	6.49 (s)	6.41 (s)
12	10.27 (s)	4.96 (s)	4.86 (s)
13	2.28 (s)	2.17 (s)	2.24 (s)
2′	5.51 (qq, J = 6.9, 1.4 Hz)	5.57 (qq, J = 6.8, 1.3 Hz)	5.60(qq, J = 6.8, 1.4 Hz)
3′	1.80 (m)	1.81 (d, J 6.8 Hz)	1.85 (dq, J = 6.8, 0.9 Hz)
4′	2.04 (s)	2.04 (s)	2.05 (s)
2″	5.68 (qq, J = 6.9, 1.3 Hz)	5.42 (qq, J = 6.8, 1.3 Hz)	5.54 (qq, J = 6.8, 1.3 Hz)
3″	1.80 (m)	1.69 (d, J = 6.8 Hz)	1.74 (dq, J = 6.9, 0.9 Hz)
4″	1.96 (s)	1.88 (s)	1.92 (s)
OH	12.16 (s)	2.99, 8.65 (s, br)	8.69 (s)
OCH_3			3.50 (s)

[a] Data were recorded in $CDCl_3$. [b] Data were recorded in acetone-d_6.

On the basis of strong correlations from H-12 to H-2′, H-3′, and H-4′ in the NOESY spectrum, subunits A and B could be joined. In addition, the NOESY spectrum showed correlations from H-2 to H-2″, H-3″, and H-4″ and from H-7 to H-2′, H-3′, and H-4′ and strong correlations from H-3′ to H-4′ and from H-3″ to H-4″, indicating *E*-geometry for the two external double bonds of **1**. Although not very common, the presence of two methylpropenyl units in **1** is not without precedent, with the reported depsidones, the auranticins and guisiol, from *Emericella unguis* and *Preussia aurantiaca*, respectively, possessing a similar functionality.

Compound **2** gave a molecular formula of $C_{23}H_{24}O_6$ by HRMS showing a protonated molecular ion peak at *m*/*z* 397.1646 $[M + H]^+$. The ^{1}H and ^{13}C NMR spectra were similar to those of compound **1**, except for the absence of an aldehyde carbon at δ_C 194.1 (δ_H 10.3) and the presence of a methylene carbon signal at δ_C 56.5, bearing a proton at δ_H 4.96. This suggested the replacement of an aldehyde by a primary alcohol. The HMBC spectrum showed correlations from H-2 to C-1″, C-3, C-4, and C-11a; H-12 to C-3 and C-4; H-2″ to C-1, C-3″, and C-4; H-7 to C-5a, C-8, and C-9; H-13 to C-8, C-9, and C-9a; and H-2′ to C-1′, C-3′, C-4′, and C-6. These spectroscopic data were also used to form two subunits, one of which was identical to that of compound **1**. Strong HMBC correlations from H-12 to H-3′ and H-4′ supported the structure of compound **2**. Correlations from H-3′ to H-4′ and from H-3″ to H-4″ were also observed in its NOESY spectrum.

Compound **3** revealed a molecular formula of $C_{24}H_{26}O_6$ by HRMS and exhibited a protonated molecular ion peak at *m*/*z* 411.1817 $[M + H]^+$. The ^{13}C NMR spectrum gave signals for 24 carbons, assigned as six methyls, one methylene, four methines, and 13 quaternary carbons. The ^{1}H NMR spectrum showed a pattern similar to that of compound **2** except for an additional methyl signal (δ_H 3.95), of a carbon attached to an oxygen at δ_C 58.8. The HMBC spectrum correlated this methyl to the methylene C-12, and the NOESY spectrum displayed strong correlations from H-12 to H-2′, H-3, and H-4′. Therefore, the structure of compound **3** was determined as shown. The geometry of the external double bonds was identical to compound **2**, as confirmed by NOESY experiments.

Auranticin A was also isolated from this fungus. The spectroscopic data including ^{1}H, ^{13}C, HMQC, HMBC, and NOESY spectra were also identical to those reported in the literature.[9]

Compound **1** exhibited weak cytotoxic activity against KB and BC cell lines with IC_{50} values of 6.5 and 4.1 μg/mL, respectively, while compounds **2**, **3**, and auranticin A were inactive against the KB and BC cell lines ($IC_{50} > 5$ μg/mL).

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a Cary 1E UV−visible spectrophotometer. FT-IR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on a Bruker AV500D spectrometer. ESITOFMS were obtained from a Micromass LCT mass spectrometer; accurate mass was determined by lock mass calibration. Column chromatography was performed on silica gel (Merck; 15 μm) and Sephadex LH-20. Semipreparative HPLC was performed on a C_{18} reversed-phase column (6 μm, 25 × 100 mm, Waters).

Fungal Material. An endophytic fungus was isolated from a leaf collected from the Hala-Bala evergreen forest, Narathiwat Province, Thailand, and registered at the BIOTEC Culture Collection (BCC) as BCC 8616.

Fungus Identification. The fungus was identified based on the ITS1-5.8S-ITS2, 28S rDNA sequences, which were amplified using universal primers ITS1, ITS4, NS1, NS5, NS6, NS23UCBR, LROR, and LR7.[13,14] BLAST was used to search for sequences similar to their ITS and 28S rDNA, with sequence identities of 89−90% for 28S rDNA and 85−90% for the ITS region. ITS1-5.8S-ITS2 and 28S rDNA sequence data have been submitted to GenBank (accession numbers DQ851131, DQ851132). The data were analyzed by the Clustal W[15] and PAUP phylogenetic analysis programs,[16] and the fungus was identified in the order Pleosporales (class Ascomycetes, subclass Dothideomycetidae).

Fermentation, Extraction, and Isolation. The fungus was developed on potato dextrose agar (PDA) at 25 °C for 21 days, and the mycelium was then cut into pieces (1 × 1 cm) to inoculate in two 1 L Erlenmeyer flasks; each contained 250 mL of potato dextrose broth (PDB). After incubation on a shaker at 200 rpm for 8 days (25 °C), the culture was transferred into a malt extract medium (MEB) in 20 1 L Erlenmeyer flasks; each contained 250 mL of MEB and was incubated for 18 days at 25 °C on a rotary shaker at 200 rpm.

The mycelium was filtered, and the broth was then extracted with EtOAc (3 × 1 L). The EtOAc extract was dried with Na_2SO_4 and evaporated under reduced pressure to yield a gum (0.47 g). The gum was purified by passage over Sephadex LH-20 (using MeOH as an eluent), silica gel column chromatography (using 40% of EtOAc in CH_2Cl_2 as eluent), and semipreparative reversed-phase HPLC (using acetonitrile−water (60:40) as an eluent), to yield compound **1** (6.3 mg) and 4,8-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (7.1 mg), respectively.

The cells were macerated in MeOH for 4 days, followed by CH_2Cl_2 for 4 days. The MeOH and CH_2Cl_2 extracts were combined and evaporated under reduced pressure, and then water (30 mL) was added. The mixture was washed with hexane (3 × 150 mL), which was discarded, and then extracted with EtOAc (3 × 150 mL). The EtOAc was dried with Na_2SO_4 and then evaporated under reduced pressure to yield a solid gum (2.90 g). The solid gum was triturated with EtOAc to give a solid (0.11 g) and the residual filtrate (2.77 g). The solid (0.11 g) was further purified by semipreparative reversed-phase HPLC (using acetonitrile−water (65:35) as a mobile phase) to obtain compounds **2** (44.6 mg) and **3** (12.5 mg) at t_R 4.9 and 6.7 min, respectively. In addition, the filtrate (2.77 g) was further purified by passage over Sephadex LH-20 (using MeOH as an eluent) to give two fractions; the first fraction was purified by a silica gel column (employing 20% EtOAc in CH_2Cl_2 as eluent) followed by semipreparative reversed-phase HPLC on a C_{18} reversed-phase column (using acetonitrile−water (65:35) as a mobile phase) to afford auranticin A (0.15 g) and compound **2** (0.35 g), respectively. The second fraction was further purified from Sephadex LH-20 (eluted with MeOH) to yield compounds **2** (15.3 mg), **1** (12.9 mg), and **3** (2.9 mg), respectively.

Table 2. ^{13}C NMR Assignments of Compounds **1**−**3**

position	**1**[a]	**2**[b]	**3**[a]
1	158.7	150.5	151.3
2	115.0	113.4	114.4
3	165.2	160.6	160.4
4	110.5	115.2	111.0
4a	162.8	160.9	160.4
5a	142.3	142.4	143.2
6	136.4	136.2	136.1
7	112.0	111.2	111.8
8	151.5	152.6	150.9
9	115.7	115.3	115.4
9a	143.4	143.8	143.7
11	166.0	163.2	164.0
11a	112.0	112.4	112.4
12	194.1	56.5	68.9
13	9.1	9.1	9.2
1′	136.6	133.6	133.5
2′	126.7	125.7	126.0
3′	13.8	13.2	14.2
4′	18.1	17.1	17.9
1″	135.5	135.9	135.6
2″	127.3	124.3	125.4
3″	14.4	13.4	14.4
4″	17.1	16.8	17.5
OCH_3			58.8

Compound 1: pale yellow solid; mp 179−180 °C; UV (MeOH) λ_{max} (log ϵ) 206 (4.55), 240 (4.25), 296 (3.94) nm; IR ($CHCl_3$) ν_{max} 3407 (br), 2926, 2857, 1721, 1649, 1559, 1422, 1389, 1361, 1264, 1217, 1126, 1105, 1040 (w), 759 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 395.1497 $[M + H]^+$ (calcd for $C_{23}H_{22}O_6$, 395.1494).

Compound 2: colorless needles; mp 210 °C (dec); UV (MeOH) $_{max}$ (log ϵ) 210 (4.56), 234 (4.37), 275 (3.99) nm; IR ($CHCl_3$) ν_{max} 3387, 2971, 2925, 2855, 1702, 1604, 1579, 1420, 1379, 1264, 1210, 1125, 756 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 397.1646 $[M + H]^+$ (calcd for $C_{23}H_{24}O_6$, 397.1651).

Compound 3: colorless solid; mp 153−154 °C; UV (MeOH) λ_{max} (log ϵ) 215 (4.59), 275 (4.05) nm; IR ($CHCl_3$) ν_{max} 3407 (br), 3332, 2925, 2854, 1712, 1607, 1457, 1421, 1378, 1265, 1210, 1125, 759 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 411.1817 $[M + H]^+$ (calcd for $C_{24}H_{26}O_6$, 411.1807).

Biological Testing. Cytotoxicity of the purified compounds against human epidermoid carcinoma cells (KB) and human breast cancer cells (BC) was evaluated using a colorimetric method.[17] Ellipticine and doxorubicin were used as reference compounds. Ellipticine and doxorubicin exhibited IC_{50} values of 0.21 ± 0.06 and 0.14 ± 0.03 μg/mL and 0.27 ± 0.06 and 0.17 ± 0.03 μg/mL against KB and BC cells, respectively.

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Supporting Information Available: NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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