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Antimicrobial metabolites from the aquatic fungus *Delitschia corticola*

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ABSTRACT

Two new metabolites, (3*S**,4*S**,5*S**,6*R**)-4,5,6-trihydroxy-3-methyl-3,4,6,7-tetrahydro-1H-isochromen-8 (5H)-one (**1**) and (3*R**,4*S**)-7-ethyl-3,4,6,8-tetrahydroxy-3,4-dihydronaphthalen-1 (2H)-one (**2**), were isolated from the culture broth of the aquatic fungus *Delitschia corticola* YMF 1.01111, together with four known ones, 6-ethyl-7-hydroxyl-2-methoxyjuglone (**3**), 6-ethyl-2,7-dimethoxyjuglone (**4**), 6-(1-hydroxyethyl)-2,7-dimethoxyjuglone (**5**) and sporidesmin A (**6**). Structures of these compounds were elucidated mainly by NMR spectroscopic and mass spectroscopic methods. Furthermore, the antimicrobial activities of compounds **1**–**6** were evaluated against a panel of bacteria and fungi.

1. Introduction

Fungi are a well-known source of bioactive compounds, and the research for isolation of novel fungal metabolites that blossomed more than 40 years ago is still very active now. At present, the search for new producers of biologically active compounds is actively underway among fungi growing under extreme conditions, because the synthesis of new secondary metabolites and potential biologically active compounds that help them to survive and adapt to these conditions can be expected in these fungi with the greatest probability (Gloer, 1995; Grabley et al., 1999). Due to different habitat from those of terrestrial fungi, the aquatic fungi are of special interest. In fact, aquatic fungi are being accepted as a potentially important source of novel bioactive secondary metabolites that can be lead compounds for the development of novel pharmaceuticals and/or agrochemicals as many novel bioactive compounds have been gradually reported from aquatic fungi such as *Annulatascus triseptatus*, *Dendrospora tenella*, *Decaisnella thyridioides*, *Helicodendron giganteum, Kirschsteiniothelia* sp., *Massarina tunicate*, *Ophioceras venezuelense,* and *Stachybotrys* sp. (Beatriz and Gamboa-Angulo, 2010). Our research group has been interested in studies of bioactive compounds produced by aquatic fungi. Previously, we have identified that aquatic fungi contain some unique biologically active metabolites such as astropaquinones (Wang et al., 2009), caryospomycins (Dong et al., 2007), colelomycerones (Dong et al., 2009), pseudohalonectrins (Dong et al., 2006), and YMF 1029 A-E (Dong et al., 2008). During our continuous characterization of structurally novel metabolites from aquatic fungi, a new isochromenone, (3*S**,4*S**,5*S**,6*R**)-4,5,6-trihydroxy-3-methyl-3,4,6,7-tetrahydro-1H-isochromen-8 (5H)-one (**1**) and a new dihydronaphthalenone, (3*R**,4*S**)-7-ethyl-3,4,6,8-tetrahydroxy-3,4-dihydronaphthalen-1 (2H)-one (**2**) were isolated from the culture extract of the aquatic fungus *Delitschia corticola* YMF 1.01111 together with four known compounds, **3**–**6**. Herein we describe the isolation and structural elucidation of these compounds and their *in vitro* antimicrobial activities against a panel of bacteria and fungi. These studies represent the first reported chemical examination of *D. corticola.*

2. Results and discussion

An isolate of *D. corticola* YMF 1.01111, originally obtained from submerged wood in a freshwater habitat, was grown in liquid shake culture with a potato/glucose/peptone/yeast extract medium. After 10 days, the EtOAc extract of the culture filtrate was

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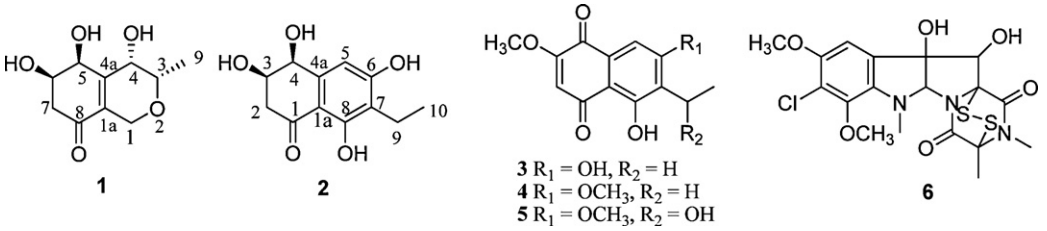

Fig. 1. The structures of compounds **1–6** isolated from *D. corticola*.

repeatedly subjected to column chromatography on Sephadex-LH-20 and silica gel to furnish two new metabolites (**1** and **2**) and four known ones (**3–6**).

Compound **1**, obtained as a yellow oil, displayed a very weak molecular ion peak at *m/z* 214 $[M]^+$ in the EIMS, which in conjunction with NMR data suggested the molecular formula $C_{10}H_{14}O_5$, thus implying four degrees of unsaturation. The molecular formula was subsequently confirmed by the analysis of high-resolution ESIMS (*m/z*: 249.0535 $[M+Cl]^+$, calcd for $C_{10}H_{14}O_5Cl$, 249.0529). The IR spectrum displayed hydroxyl and conjugated carbonyl absorption bands at 3495, 3442, 1674 and 1658 cm^{-1}, respectively. The ^1H NMR spectrum in CD_3COCD_3 solvent showed the presence of a secondary methyl [δ_H 1.14 (3H, d, J = 6.20 Hz, CH_3-9)], an oxymethylene [δ_H 4.10 (1H, dt, J = 3.05, 16.35 Hz, H_β-1) and 4.27 (1H, d, J = 1.60, 16.35 Hz, H_α-1)], an aliphatic methylene [δ_H 2.68 (1H, dd, J = 4.39, 16.13 Hz, H_β-7) and 2.39 (1H, dt, J = 10.20, 16.02 Hz, H_α-7)], four oxymethines [δ_H 3.34 (1H, m, H-3), 4.17 (1H, m, H-4), 4.53 (1H, d, J = 4.45 Hz, H-5), and 4.02 (1H, m, H-6)]. Analysis of ^{13}C NMR and DEPT experiments revealed the presence of one methyl group (δ_C 18.8), two methylene units (one of which was oxygenated) (δ_C 44.3 and 63.9), four oxymethines (δ_C 75.3, 69.1, 71.5, and 72.5), two olefinic carbons (δ_C 155.1 and 132. 5), and one ketone carbon (δ_C 196.1). These data, together with three exchangeable protons indirectly required by the DEPT results, indicated that compound **1** to be bicyclic and must contain three hydroxyl groups and one ether moiety.

Analysis of the ^1H–^1H COSY NMR data led to the identification of two isolated proton spin-systems corresponding to the **H**O-4–C-4–C-3–C-9 and **H**O-5–C-5–C-6–C-7 subunits of structure **1**. These two subunits were connected together through the C-1a/C-4a double bond, as evident from the HMBC correlations of O**H**-5 with C-4a, C-5 and C-6, of **H**O-4 with C-3, C-4 and C-4a, of H-6 with C-5 and C-4a, and of H-4 with C-4a and C-1a. HMBC correlations from H_2-1 to C-8, C-1a and C-4a, and H_2-7 to C-8 and C-1a indicated that the olefinic quaternary carbon at C-1a was connected to the keto carbonyl (C-8) and the oxygenated methylene carbon (C-1), which further clarified the substitution patterns of the C-1a/C-4a double bond. These data, together with the additional HMBC correlations from H_2-1 to C-3 and the chemical shifts of C-1 (δ_C 63.9) and C-3 (δ_C 75.3), also indicated that C-1 is connected to C-3 via an oxygen atom, thereby constructing the dihydropyran moiety of **1**, accounting for one of the rings. Finally, the HMBC correlations of the oxymethine proton at δ_H 4.02 (H-6) and the aliphatic methylene protons at δ_H 2.68 and 2.39 (H_2-7) to the keto carbon at δ_C 196.1 led to the direct connection of the methylene carbon (C-7) to the keto carbon (C-8), which yielded a cyclohexenone ring that was fused to the dihydropyran unit at C-1a/C-4a. The above HMBC correlations also indicated the presence of two hydroxyls at C-4 (δ_C 69.1) and C-5 (δ_C 71.5), respectively, and the remaining hydroxyl group required by the molecular formula must occupy the only available site (C-6), which was consistent with the ^{13}C NMR chemical shift of C-6 (δ_C 72.5). Thus, the planar structure of **1** was constructed (Figs. 1 and 2).

The relative stereochemistry of compound **1** was proposed on the basis of NOESY data and ^1H–^1H coupling constants. Specifically, the fact that the oxymethine proton on C-6 exhibited one larger ($J_{H\text{-}6,\ H\alpha\text{-}7}$ = 10.20 Hz) and one medium ($J_{H\text{-}6,\ H\beta\text{-}7}$ = 4.29 Hz) coupling constant with the vicinal methylene protons on C-7 indicated the hydroxyl group attached to C-6 in a pseudoequatorial orientation. Furthermore, the stereochemistry of the 5-hydroxyl was assigned to be β-oriented because of the observed small coupling constant (4.45 Hz) and the presence of a cross-peak between H-5 and H-6 in the NOESY spectrum. On the basis of a computer-generated 3D structure obtained by CHEM3D ULTRA V 8.0, with MM2 force-field calculations for energy minimization, if H-3 was assigned to be α-oriented, the interatomic distances of H_β-1(δ_H 4.10) with H-3 and C**H**$_3$-9 would be approximately 3.662 and 2.352 Å, respectively, so the NOESY correlations of H_β-1(δ_H 4.10) with H-3 and CH_3-9 may be observed. However, only a cross peak between H_β-1 and H-3 but no between H_β-1 and CH_3-9 in the NOESY spectrum was observed, which indicated that H-3 in **1** is β-oriented. Additionally, the NOESY correlations of H-4 with C**H**$_3$-9 and H-3 suggested the hydroxyl at C-5 had the α-configuration, which was further confirmed by the observed small coupling constant (6.48 Hz) in the ^1H NMR ($CD_3COCD_3 + D_2O$). Finally, according to the above computer-generated 3D structure, the calculated interatomic distances between H-3/C**H**$_3$-9 (2.264), H-3/H-4 (2.588), H-3/H_β-1 (2.603), H-4/C**H**$_3$-9 (2.613), H-5/O**H**-4 (2.963), H-5/H-6 (2.502) are all less than 3.00 Å (Fig. 3). This further supported the well-defined NOESY correlations observed for each of these proton pairs. Thus, the structure of **1** was unambiguously determined as (3*S**,4*S**,5*S**,6*R**)-4,5,6-trihydroxy-3-methyl-3,4,6,7-tetrahydro-1H-isochromen-8 (5H)-one (**1**).

Compound **2** was obtained as optically active yellow solid. The molecular formula was determined as $C_{12}H_{14}O_5$, by HRESIMS, which showed the pseudomolecular ion peak at *m/z* 261.0742 $[M+Na]^+$(calcd 261.0738), in conjunction with NMR data, indicating six degrees of unsaturation. The IR spectrum showed the absorptions for hydroxyl (3418 cm^{-1}), carbonyl (1693 cm^{-1}) and the aromatic ring (1621 cm^{-1}). The UV absorption maxima at λ_{max} 289.6 (3.66), 219.8 (3.84) nm indicated an aromatic nucleus. The

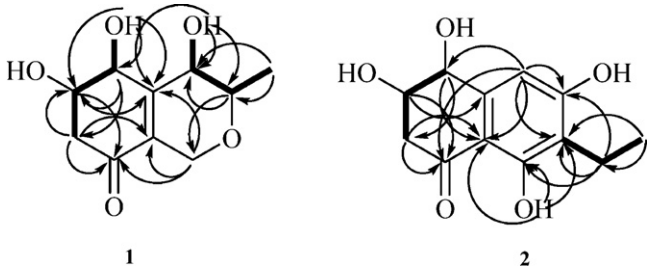

Fig. 2. Key HMBC and COSY correlations for compounds **1** and **2**.

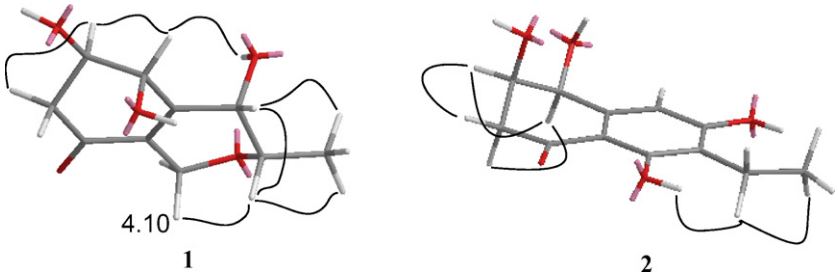

Fig. 3. Key NOESY correlations for compounds **1** and **2** and conformations generated from computer modeling (ChemDraw.9.0 3d).

^{1}H NMR spectrum showed a chelated hydroxyl signal (δ_H 13.21, O**H**-8) and signals for an aromatic proton [δ_H 6.74 (1H, s, H-5)], two oxymethines [δ_H 4.79 (1H, s, H-4) and 4.33 (1H, d, J = 4.68 Hz, H-3)], two methylenes [δ_H 2.86 (2H, d, J = 4.51 Hz, H_2-2) and 2.68 (2H, m, H_2-9)], and a secondary methyl [δ_H 1.12 (3H, t, J = 7.47 Hz, CH_3-10)]. Analysis of ^{13}C NMR and DEPT experiments revealed the presence of a keto carbonyl (δ_C 201.8) strongly hydrogen-bonded with an OH, six aromatic carbons of which two were oxygenated (δ_C 163.0 and 163.3), and one protonated (δ_C 107.5), two oxymethine (δ_C 70.3 and 70.7), two methylene (δ_C 43.2 and 16.1), and a methyl (δ_C 13.5) carbon. These data, together with four exchangeable protons indirectly required by the DEPT results, indicated that compound **1** is bicyclic, with at least one being aromatic.

In the HMBC spectrum, the correlations from the chelated hydroxyl resonance at δ_H 13.21 ppm (O**H**-8) to C-8, C-1a and C-7, and the aromatic proton singlet at δ_H 6.74 ppm (H-5) to C-1, C-4, C-6, C-7 and C-1a permitted the completion of a pentasubstituted benzene ring with two hydroxyls at positions C-6 (δ_C 163.0) and C-8 (δ_C 163.3). The above HMBC correlations also allowed the attachment of the keto carbon (δ_C 201.8) and the oxymethine carbon (δ_C 70.7) to C-1 and C-4, respectively. Furthermore, an ethyl group was placed at C-7 of the pentasubstituted benzene ring because of ^{1}H–^{1}H COSY correlation of H_2-9/C**H**$_3$-9 and HMBC correlations from the methylene proton signal at δ_H 2.86 (H_2-9) to C-7, C-6 and C-8, and the methyl protons at δ_H 1.12 (C**H**$_3$-10) to C-7 and C-9. Further analysis of H–^{1}H COSY NMR data led to the identification of another isolated proton spin-system corresponding to the C-2–C-4 subunit of structure **2**. Finally, this subunit, in conjunction with the HMBC correlations of H-2 to C-1, C-1a and C-4, of H-3 to C-1 and C-4a, and of H-4 to C-1a, C-2 and C-5, illustrated the presence of the α,β-unsaturated cyclohexenone ring with a hydroxyl group, at C-3 (δ_C 70.3) and C-4 (δ_C 70.7) respectively, as was seen for **1**. Thus, the planar structure of **2** was constructed.

The observation of NOESY correlations of H-3 with H-2, and H-4 with H-3 and H-2 indicated that these hydroxyl groups attached to C-4 and C-3 in a pseudoequatorial orientation, while H-3 and H-4 occupied a pseudoaxial orientation with respect to the corresponding six-membered ring and *cis* to each other. The dihedral angles from H_α-2 and H-4 to H-3 must be ca. 90°, since no coupling was observed between H_α-2 and H-3, and between H-4 and H-3. Molecular modeling studies, generating minimum energy structures with the NOESY constraints described above, gave rise to predicted J-values that agreed within 1–1.5 Hz of the actual experimental values. Additionally, a computer-generated 3D structure was obtained by CHEM3D ULTRA V 8.0, with MM2 force-field calculations for energy minimization. The calculated interatomic distances between H_2-2/H-3 (2.509, 2.523 Å), H_β-2/H-4 (2.688 Å) and H-3/H-4 (2.498 Å) are all less than 3.00 Å (Fig. 3). This further supported the well-defined NOESY correlations observed for each of these proton pairs. Thus, the structure of **2** was unambiguously determined as (3*R**,4*S**)-7-ethyl-3, 4, 6, 8-tetrahydroxy-3, 4-dihydronaphthalen-1 (2H)-one.

The structures of the known compounds **3**–**6** were determined as 6-ethyl-7-hydroxyl-2-methoxyjuglone (Otomo et al., 1983), 6-ethyl-2, 7-dimethoxyjuglone (Poch and Gloer, 1989a), 6-(1-hydroxyethyl)-2, 7-dimethoxyjuglone (Poch and Gloer, 1989b) and sporidesmin A (Dingley et al., 1962) by comparison with the corresponding literature data, respectively.

The antimicrobial activities of compounds **1**–**6** were examined together with ciclopirox and ampicillin sodium, a well-known broad-spectrum antifungal and antibacterial agents, respectively. The results of their activities are reported in Table 1. In standard disk assays at 50 μg/disk, compound **6** was active against all tested microbial species and the inhibitions were comparable to those of the control antibiotics, the antibacterial agent ampicillin sodium and the antifungal ciclopirox. Compounds **1**–**5** were found active against three fungal strains, *Alternaria* sp. YMF 1.01991, *Sclerotium* sp. YMF 1.01993 and *Fusarium* sp. YMF 1.01996, and three bacterial strains, *Bacillus cereus* YMF 3.19, *Bacillus laterosporus* YMF 3.08, and *Staphylococcus aureus* YMF 3.17. Compound **1** also exhibited activity against *Gibberella saubinetii* YMF 1.01989 and *Colletotrichum sp.* YMF 1.01994. However, none of compounds **1**–**5** showed any activity in assays against *Exserohilum turcicum* YMF

Table 1
Antimicrobial activities of metabolites **1**–**6** and two reference antibiotics.

Strain	**1**	**2**	**3**	**4**	**5**	**6**	Ciclopirox	Ampicillin sodium
Gibberella saubinetii YMF 1.01989	11	ND	ND	ND	ND	22	25	NT
Exserohilum turcicum YMF 1.01990	ND	ND	ND	ND	ND	18	20	NT
Alternaria sp. YMF 1.01991	12	7	7	8	7	22	26	NT
Rhizoctonia solani YMF 1.01992	ND	ND	ND	ND	ND	17	19	NT
Sclerotium sp. YMF 1.01993	9	8	7	8	8	20	19	NT
Colletotrichum sp. YMF 1.01994	7	ND	ND	ND	ND	16	23	NT
Phyllosticta sp. YMF1.01995	ND	ND	ND	ND	ND	19	21	NT
Fusarium sp. YMF 1.01996	10	7	9	8	7	20	20	NT
Bacillus cereus YMF 3.19	9	12	14	10	11	13	NT	35
Bacillus laterosporus YMF 3.08	10	10	11	10	12	16	NT	30
Escherichia coli YMF 3.16	ND	ND	ND	ND	ND	14	NT	15
Staphylococcus aureus YMF 3.17	11	20	10	11	13	19	NT	18

Diameter of the inhibition areas (mm) using the plate diffusion assay (50 μg of each tested compound soaked in a 6 mm filter disk). ND: not detectable. NT: not tested.

1.01990, *Rhizoctonia solani* YMF 1.01992, *Phyllosticta* sp. YMF1.01995 and *Escherichia coli* YMF 3.16 at the tested concentration. From Table 1, compounds **1**–**5** showed moderate antimicrobial activity respectively, and compound **6** was the most potent. Apparently, the antimicrobial activities of compounds **1**–**6** were significantly influenced by their chemical skeleton.

There is no report on any previous phytochemical investigation of *D. corticola* available to the present. From the reported 83 species of the genus *Delitschia* only one paper on the phytochemical studies of *D. cofertaspora* has been reported (Hensens et al., 1995). But there are big differences between the compounds isolated from *D. corticola* and *D. cofertaspora*. The metabolites found in *D. cofertaspora* mainly belong to pyrazinedione alkaloids whereas the structures of compounds **1**–**6** isolated in *D. corticola* cultures were completely different from them. Notably, this inconsistence indicated that the genus *Delitschia* could be a rich source of structurally diverse natural products. Finally, it was also noted that both genus *Kirschsteiniothelia* (Poch et al., 1992) and genus *Delitschia* are in the order Pleosporales, subclass Dothideomycetidae and, therefore, the similarity of the produced secondary metabolites suggested a close relationship between these two genera.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in a MeOH on a Horiba SEPA-300 digitalpolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. A Tenor 27 spectrophotometer was used for the spectra. IR spectra were measured on a Perkin-Elmer-577 spectrophotometer with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shift (δ) were expressed in ppm with reference to the solvent signals. EI-MS data were taken on a VG Autospec-3000 spectrometer. HR-MS (ESI-TOF) data were recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh; Qing dao Marine Chemical Inc., Qingdao, People's Republic of China) and Seqhadex LH-20 (Pharmacia). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents including petroleum ether (60–90 °C) were distilled prior to use.

3.2. Cultivation and fermentation of the fungal strain YMF1.01111

The fungal strain was initially isolated from a submerged woody substrate collected from the freshwater habitat in Yunnan Province, China and was identified by Dr. J. Luo as *D. corticola*. The culture was deposited in the key laboratory for conservation and utilization of bio-resources, Yunnan University, Yunnan Province, China (culture collection number YMF 1.01111) and subcultured on PDA (potato 200 g, glucose 20 g, agar 18 g and water 1000 mL) agar at 25 °C for a period of 15 days. The mycelium-containing agar was then cut into pieces (1 × 1 cm), and inoculated into 10× 250 mL Erlenmeyer flasks, each containing 25 mL of potato dextrose broth (PDB: potato 200 g, glucose 20 g, and water 1000 mL) (10 pieces for each flask). After incubation at 25 °C for 15 days on a rotary shaker (190 rpm), each primary culture was transferred into a 500 mL Erlenmeyer flasks containing 150 mL of the same liquid medium (PDB), and incubated at 25 °C for 10 days on a rotary shaker (190 rpm). These secondary cultures were pooled and each 15 mL portion was transferred into 100× 500 mL Erlenmeyer flasks containing 150 mL of liquid media (potato 200 g, glucose 20 g, peptone 2 g, yeast extract 4 g, and water 1000 mL), and final fermentation was carried out at 25 °C for 15 days on a rotary shaker (180) rpm.

3.3. Extraction and isolation

The culture broths were filtered through cotton yarn and concentrated under vacuum to 3 L below 60 °C and extracted with an equal volume of EtOAc (3× 3000 mL, 2 h each). The EtOAc extract was collected and then evaporated to dryness to afford 5.11 g of crude extract. This crude extract was subjected to Sephadex LH-20 column chromatography (3.5 cm × 180 cm) and eluted with MeOH to yield two main fractions (A and B) based on TLC behavior. Fraction A (87 mg) was further chromatographed by Sephadex LH-20 column (1.5 cm × 150 cm), eluted with CH_3COCH_3, to give pure **3** (31 mg). Fraction B (954 mg) was further applied to a silica gel column chromatography (200–300 mesh; 2.5 cm × 80 cm; with 50 g of silica gel), eluted with cyclohexane–EtOAc (from 9:1 to 6:4, 5% gradient, 150 mL each eluent) to give five main subfractions [IIa (87 mg), IIb (57 mg), IIc (61 mg), IId (93 mg) and IIe (77 mg)]. Each subfraction was again purified on a Sephdex LH-20 gel column (1.5 cm × 150 cm) eluting with MeOH to yield pure compounds **6** (30 mg), **4** (14 mg), **5** (15 mg), **1** (28 mg), and **2** (21 mg), respectively.

3.3.1. (3S*,4S*,5S*,6R*)-4,5,6-trihydroxy-3-methyl-3,4,6,7-tetrahydro-1H-isochromen-8 (5H)-one (1)

Yellow oil (CH_3COCH_3); $[\alpha]_D^{25.3}$ − 8.2 (CH_3COCH_3; *c* 0.10); UV ($CHCl_3$) λ_{max} (log ε) 231.6 (3.66), 197.6 (3.49), 193.4 (3.48) nm; IR (film) ν_{max} 3495, 3442, 2983, 2947, 2915, 2880, 2851, 2521, 1674, 1658, 1444, 1418, 1401, 1388, 1314, 1260, 1231, 1208, 1145, 1118, 1097, 1071, 1055, 957, and 864 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz, δ): 4.27 (1H, d, *J* = 1.60, 16.35 Hz, H_α-1), 4.10 (1H, dt, *J* = 3.05, 16.35 Hz, H_β-1), 3.34 (1H, m, H-3), 4.17 (1H, m, H-4), 4.41 (1H, d, *J* = 6.48 Hz, OH-4), 4.65 (1H, d, *J* = 5.54 Hz, OH-5), 4.53 (1H, d, *J* = 4.45 Hz, H-5), 4.02 (1H, m, H-6), 2.68 (1H, dd, *J* = 4.39, 16.13 Hz, H_β-7), 2.39 (1H, dt, *J* = 10.20, 16.02 Hz, H_α-7), 1.14 (3H, d, *J* = 6.20 Hz, CH_3-9); ^1H NMR (CD_3COCD_3 + D_2O, 500 MHz, δ): 4.24 (1H, d, *J* = 1.35, 14.81 Hz, H_α-1), 4.11 (1H, dt, *J* = 2.95, 14.81 Hz, H_β-1), 3.38 (1H, dd, 6.22, 6.48 Hz, H-3), 4.52 (1H, d, *J* = 6.48 Hz, H-4), 4.05 (1H, m, H-5), 4.05 (1H, m, H-6), 2.71 (1H, dd, *J* = 4.16, 16.23 Hz, H_β-7), 2.41 (1H, dt, *J* = 9.23, 16.23 Hz, H_α-7), 1.24 (3H, d, *J* = 6.22 Hz, CH_3-9); ^{13}C NMR (CD_3COCD_3, 500 MHz, δ): 63.9 (t, C-1), 75.3 (d, C-3), 69.1 (d, C-4), 71.5 (d, C-5), 72.5 (d, C-6), 44.3 (t, C-7), 196.1 (s, C-8), 132.5 (s, C-1a), 155.1 (s, C-4a), 18.8 (q, C-9); EIMS *m/z* (rel. int) 214 $[M]^+$ (1), 178 $[M-2H_2O]^+$ (2), 170 (55), 152 (100), 135 (5), 124 (48), 110 (12), 92 (21), 81(12), 69 (25); HRMS (ESI-TOF) *m/z*: 249.0535 $[M+Cl]^+$ (calcd for $C_{10}H_{14}O_5Cl$, 249.0529).

3.3.2. (3R*,4S*)-7-ethyl-3,4,6,8-tetrahydroxy-3,4-dihydronaphthalen-1 (2H)-one (2)

Yellow solid (CH_3COCH_3); $[\alpha]_D^{25.6}$ − 2.6 (CH_3OH; *c* 0.11); UV (CH_3OH) λ_{max} (log ε) 289.6 (3.66), 219.8 (3.84) nm; IR (film) ν_{max} 3418, 2962, 2926, 2872, 2853, 1693, 1621, 1492, 1437, 1365, 1305, 1251, 1208, 1163, 1140, 1113, 1098, 1072, 1060, 1015, 994, 930, 820, 788, and 565 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz, δ): 2.86 (2H, d, *J* = 4.51 Hz, H_2-2), 4.33 (1H, d, *J* = 4.68 Hz, H-3), 4.79 (1H, s, H-4), 6.74 (1H, s, H-5), 13.21 (1H, s, OH-8), 2.68 (2H, m, H_2-9) 1.12 (3H, t, *J* = 7.47 Hz, H_3-10); ^{13}C NMR (CD_3COCD_3, 500 MHz, δ): 201.8 (s, C-1), 110.1 (s, C-1a), 43.2 (t, C-2), 70.3 (d, C-3), 70.7 (d, C-4), 145.0 (s, C-4a), 107.5 (d, C-5), 163.0 (s, C-6), 116.7 (s, C-7), 163.3 (s, C-8), 16.1 (t, C-9), 13.5 (q, C-10); EIMS *m/z* (rel. int) 238 $[M]^+$ (93), 223 (71), 205 (21), 194 (24), 177 (6), 166 (34), 151 (100), 123 (15), 91 (10); HRMS (ESI-TOF) *m/z*: 261.0742 $[M+Na]^+$ (calcd for $C_{12}H_{14}O_5Na$, 261.0738).

3.4. Bioassays

Antimicrobial bioassays were conducted using the agar diffusion method according to a literature procedure (Wicklow et al., 1998). The target fungi, including eight plant pathogens *Gibberella saubinetii* YMF 1.01989, *Exserohilum turcicum* YMF 1.01990, *Alternaria sp.* YMF 1.01991, *Rhizoctonia solani* YMF 1.01992, *Sclerotium sp*. YMF 1.01993, *Colletotrichum sp*. YMF 1.01994, *Phyllosticta sp*. YMF1.01995 and *Fusarium sp.* YMF 1.01996 were grown on potato dextrose agar. The target bacterial strains, including *Bacillus cereus* YMF 3.19, *Bacillus laterosporus* YMF 3.08, *Escherichia coli* YMF 3.16, *Staphylococcus aureus* YMF 3.17, were grown on Mueller-Hinton agar. Test compounds were absorbed onto individual paper disks (6 mm diameter) at 50 μg/disk and placed on the surface of agar. The assay plates were incubated at 25 °C for 48 h testing their antifungal activities and at 36 °C for 24 h for their antibacterial activities. The plates were examined for the presence of a zone of inhibition. The results are shown in Table 1.

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