Lasiodiplodins from mangrove endophytic fungus *Lasiodiplodia* sp. 318#

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Four new lasiodiplodins (1–4), together with three known analogues, have been isolated from a mangrove endophytic fungus, *Lasiodiplodia* sp. 318#. Their structures were elucidated by spectroscopic techniques. Cytotoxic activities of compounds 1–7 were evaluated in vitro against human cancer lines THP1, MDA-MB-435, A549, HepG2 and HCT-116. Compound 4 exhibited moderate cytotoxic activities.

**Keywords:** endophytic fungus; lasiodiplodins; cytotoxic activity

1. Introduction

Mangrove forests are inhabited by large number of fungal communities due to unique ecosystem and are biodiversity hotspots for marine fungi (Carol et al. 2007). Mangrove fungi constitute the second largest part of marine-derived fungi (Sridhar 2004). Mangrove fungi not only play an important role in the nutritive cycle, but also possess immense biotechnological potentials (Hrudayanath et al. 2013). Because of the unique and extreme environmental conditions, including extensive salinity, moisture, high temperature, tidal activities and high microbial competition, mangrove endophytic fungi are believed to contribute to mangrove adaptation to the extreme environment (Abdessamad et al. 2013). In addition, these fungi are also a rich source of natural products. Many novel bioactive metabolites from mangrove fungi are used in pharmaceutical industry as lead compounds of antiviral, anticancer, antibiotic and immunosuppressive drugs and so on (John et al. 2014).

In the course of continuing search for novel bioactive natural compounds, our attention has focused on a fungal strain, *Lasiodiplodia* sp. 318#, which was collected from *Excoecaria agallocha* of Mangrove National Nature Reserve in Gaoqiao, Zhanjiang city, Guangdong
Province, China. Lasiodiplodins have been reported as fungal metabolites in 1971 and have also been found in plants (Aldridge et al. 1971; Lee et al. 1982). This class of compound showed cytotoxicity against the P388 murine leukaemia cell line (Sadia et al. 2014). In this paper, four new lasiodiplodins 1–4, together with three known lasiodiplodins were isolated from Lasiodiplodia sp. 318# (Figure 1) and the cytotoxic activities were evaluated in vitro.

2. Results and discussion

The fungus Lasiodiplodia sp. 318# was fermented using rice solid medium. The EtOAc extract of the culture of Lasiodiplodia sp. 318# was fractionated and purified by SiO2 column chromatography (CC), Sephadex LH-20 and HPLC to obtain compounds 1–7 (Figure 1). Known compound 5 was assigned as lasiodiplodin by comparison with spectral data and single-crystal X-ray evidence reported in the literature (Lee et al. 1982) (Figure S1). Compounds 6–7 were also identified by comparison of their spectroscopic data with those of literature (Matsuura et al. 1998).

Compound 1 was isolated as colourless needles. HREIMS established its molecular formula as C13H22O5 with 7 degrees of unsaturation. The 1H NMR and 13C NMR spectrum of compound 1 bore good resemblance to those of compound 5, except for the 1H NMR signals in the δ 1.55–2.72 region and the 13C NMR signal at δC 210.4. The 13C NMR and DEPT analyses revealed that compound 1 contained a ketone moiety (C-7, δC 210.4). This deduction was supported by the observed 1H–1H COSY correlations of H-17/H-3/H-4/H-5/H-6, H-8/H-9/H-10 and HMBC correlations from H-17 to C-4, H-4 to C-5, H-6 to C-7, H-8 to C-7 and C-9 (Figure S6). Accordingly, the structure of compound 1 is proposed as 7-oxolasiodiplodin. The absolute configuration of 1 was established on the basis of the CD spectrum and optical rotation as compared with those of lasiodiplodin (5). The CD spectrum of 1 is very similar to that of 5, which was assigned as R by single-crystal X-ray diffraction analysis (Figure S8). The similar values of optical rotation for 1 and 5 also supported that both compounds possess the same R configuration at C-3.

The molecular formula of compound 2 was determined to be C19H26O5 with 7° of unsaturation. A comparison of the 13C NMR and DEPT spectrum of compound 2 with those of compound 1 showed similarities, but with two additional methylenes in the δ 22.6–46.4 region. Accordingly, the corresponding signals for four additional sp2 hydrogens were also observed in the 1H NMR spectrum. The position of ketone moiety was deduced from the 1H–1H COSY correlations of H-19/H-3/H-4/H-5/H-6/H-7/H-8/H-9/H-10 and HMBC correlations from H-19 to

![Figure 1. Structures of compounds 1–7.](image-url)
C-4, H-5 to C-4, H-6 to C-5, H-7 to C-6, H-8 to C-7, H-9 to C-8, C-10, H-12 to C-11, C-14 and C-18 (Figure S13). Comprehensive analysis of the NMR data revealed that compound 2 was an enantiomer of 8,9-dihydrogreensporone C from a freshwater aquatic fungus Halenospora sp. (El-Elimat et al. 2014). The absolute stereochemistry of 2 was also determined by comparing the optical rotation values. The opposite optical rotation values for 2 and the enantiomer 8,9-dihydrogreensporone C ([α]D20 = +79, c = 0.29, MeOH) supported that compound 2 posses the R configuration at C-3.

Compound 3 was isolated as an orange red solid, and its molecular formula was determined to be C17H22O5 with 7 degrees of unsaturation, also fitted the lasiodiplodin structural class. The gross structure of compound 3 was identified by comparison of its NMR data to those of compound 5. The 1H NMR and 13C NMR data of 3 resembled closely to those of lasiodiplodin (5), the main differences being in the 1H NMR signals of benzene protons (δH 6.20, 6.21 for 5 and δH 5.85 for 3) and the downfield shifts observed for C-12 (δC 180.9) and C-13 (δC 178.0) in the 13C NMR spectrum of 3, indicated that two aromatic carbons in 5 were replaced by two ketone carbonyl carbons in 3. The location of the ketone carbonyl carbons was also demonstrated by the HMBC correlations from H-10 to C-11, C-12, and H-14 to C-12, C-13. The 12-membered lactones moiety in 3 was confirmed by the 1H–1H COSY correlations from H-17 to H-10 and the HMBC correlations from H-17 to C-4, H-4 to C-5, H-5 to C-6, H-6 to C-7, H-9 to C-8, which was the same to 5 (Figure S19). Therefore, the structure of 3 was established. The absolute configuration of 3 was tentatively assigned as 3R on the basis of optical rotation values for 3 and 5.

Compound 4 was isolated as colourless needles and the molecular formula was determined to be C18H23O5 with 5° of unsaturation. Analysis of the 13C NMR and DEPT spectra displayed the presence of 18 carbon resonances, including 2 methyls, 8 methylenes (1 oxygenated), 3 methines (1 oxygenated and 2 olefinic) and 5 quaternary carbon signals (3 oxygenated and 2 olefinic). The 1H NMR (δH 6.22, 6.28) and 13C NMR spectrum (δC 105.1, 115.5, 101.6, 160.8, 111.1, 148.8), with the aid of HMBC, revealed the presence of benzene ring moiety. The 1H NMR, 13C NMR and DEPT analyses revealed that compound 4 was one open-ring structure of lasiodiplodins. This conclusion was supported by the HMBC correlations (Figure S25). The planar structure of compound 4 is proposed as ethyl-2,4-dihydroxy-6-(8′-hydroxynonyl)-benzoate. The absolute configurations at C-8′ has not been determined.

In this study, compounds 1–7 were evaluated for their cytotoxic activities against human leukaemia THP1 cell line, melanoma cell line MDA-MB-435, human alveolar adenocarcinoma cell line A549, hepatocellular carcinoma HepG2 and colonic epithelial cell line HCT-116. Compound 4 showed moderate cytotoxic activities with IC50 values of 10.13 μM against MDA-MB-435, 12.50 μM against HepG2, 11.92 μM against HCT-116, 13.31 μM against A549 and 39.74 μM against leukaemia THP1. However, other compounds showed no notable cytotoxic activities.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined with an ADP 440 + (Bellingham + Stanley, UK) spectrometer. CD spectra were acquired on a Jasco J-810 spectropolarimeter (Jasco Corporation, Japan). NMR spectra were recorded with Bruker Avance 400 and 500 spectrometers (Bruker Corporation, Switzerland). EI-MS spectra were recorded on a Thermo DSQ EI-mass spectrometer, and HREIMS spectra were measured using a MAT95XP High Resolution mass spectrometer (Thermo Corporation, USA). UV spectra were performed on a Shimadzu UV-2501PC spectrophotometer (Shimadzu Corporation, Japan). IR spectra were obtained by an EQUINOX55 spectrometer (Bruker Corporation, Germany) in KBr disks. CC was performed on silica gel (60–100 mesh, 200–300 mesh and 300–400 mesh, Qingdao Marine Chemical Factory, PR China).
3.2 Fermentation, extraction and isolation

The strain of fungus was isolated from *Excoecaria agallocha*. The fungus was identified as *Lasiodiplodia* sp. by the ITS region. *Lasiodiplodia* sp. 318# was fermented on autoclaved rice solid-substrate medium (seventy 500 mL Erlenmeyer flasks, each containing rice 60 g, distilled water 80 mL, sea salt 2.4 g) for 28 days at 28°C. Following incubation, the mycelia and solid rice medium were crushed and extracted with MeOH. The extracts were concentrated and extracted three times with EtOAc. The EtOAc extract (105 g) was subjected to CC (silica gel 200–300 mesh, petroleum ether–EtOAc 1:0 to 1:0 and then with EtOAc–MeOH 4:1 to 1:1) to afford five fractions. Fraction 1 was isolated by CC on silica gel to yield 5 (30 g) and the crystals were grown in petroleum ether–EtOAc at rt. Compound 3 (30 mg) was also obtained from fraction 1 using Sephadex LH-20 with petroleum ether–CH₂Cl₂–MeOH (v/v/v, 2:1:1) and further purified on HPLC with 80% MeOH–H₂O. Fraction 2 was rechromatographed on silica gel and then purified on HPLC with 80% MeOH–H₂O for 2 (38 mg). Fraction 3 was first subjected to repeated silica gel CC and then purified on HPLC with 75% MeOH–H₂O for 1 (5 mg) and 4 (70 mg). Fraction 5 was purified with HPLC with 80% MeOH–H₂O to give 6 (8 mg) and 7 (28 mg).

3.2.1 13-Hydroxy-15-methoxy-3-methyl-3,4,5,6,9,10-hexahydro-1H-benzo[c][1] oxacyclododecene-1,7(8H)-dione

Colourless needles. [α]D\textsubscript{20} = +16 (c 0.32, MeOH). UV λ\textsubscript{max} (nm) MeOH: 226, 282; IR (KBr, ν\textsubscript{max}): 3259, 1711, 1679, 1607, 1499, 1376 cm\textsuperscript{-1}; HREIMS m/z 306.1461 (calcd for C\textsubscript{17}H\textsubscript{22}O\textsubscript{5} 306.1462). \textsuperscript{1}H NMR (400 MHz, Acetone-\textit{d}_6), δ: 4.89 (1H, m, H-3), 1.82 (1H, m, H-4a), 1.63 (1H, m, H-4b), 1.67 (1H, m, H-5a), 2.07 (1H, m, H-5b), 2.28 (1H, m, H-6a), 2.58 (1H, m, H-6b), 2.24 (1H, m, H-8a), 2.28 (1H, m, H-8b), 2.14 (1H, m, H-9a), 1.55 (1H, m, H-9b), 2.55 (1H, m, H-10a), 2.72 (1H, m, H-10b), 6.30 (1H, d, J = 2.1 Hz, H-12), 6.34 (1H, d, J = 2.1 Hz, H-14), 1.26 (3H, d, J = 6.2 Hz, H-17), 3.74 (3H, s, H-18), 8.5 (1H, s, H-OH). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}), δ: 168.4 (C-1), 74.0 (C-3), 34.5 (C-4), 19.0 (C-5), 41.4 (C-6), 210.4 (C-7), 38.9 (C-8), 24.4 (C-9), 30.0 (C-10), 141.4 (C-11), 107.7 (C-12), 158.0 (C-13), 97.3 (C-14), 157.8 (C-15), 118.8 (C-16), 19.8 (C-17), 55.9 (C-18).

3.2.2 15-Hydroxy-17-methoxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[c][1] oxacyclotetradene-1,11(12H)-dione

Colourless needles. [α]D\textsubscript{20} = −51 (c 0.72, MeOH). UV λ\textsubscript{max} (nm) MeOH: 206, 220, 228, 250, 286; IR (KBr, ν\textsubscript{max}): 3427, 1715, 1702, 1607, 1531, 1501 cm\textsuperscript{-1}; HREIMS m/z 334.1775 (calcd for C\textsubscript{19}H\textsubscript{26}O\textsubscript{5} 334.1775). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}), δ: 5.24 (1H, m, H-3), 1.38 (1H, m, H-4a), 1.23 (1H, m, H-4b), 1.62 (2H, m, H-5), 1.34 (2H, m, H-6), 1.34 (1H, m, H-7a), 1.26 (1H, m, H-7b), 1.64 (1H, m, H-8a), 1.54 (1H, m, H-8b), 1.34 (2H, m, H-9), 2.56 (1H, m, H-10a), 2.33 (1H, m, H-10b), 4.28 (1H, d, J = 17.5 Hz, H-12a), 3.49 (1H, d, J = 17.5 Hz, H-12b), 6.19 (1H, d, J = 2.1 Hz, H-14), 6.21 (1H, d, J = 2.1 Hz, H-16), 1.32 (3H, d, J = 6.4 Hz, H-19), 3.72 (3H, s, H-20). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}), δ: 168.6 (C-1), 71.0 (C-3), 22.6 (C-4), 35.0 (C-5), 26.4 (C-6), 35.7 (C-7), 23.4 (C-8), 25.6 (C-9), 41.9 (C-10), 210.5 (C-11), 46.4 (C-12), 116.6 (C-13), 110.3 (C-14), 158.5 (C-15), 98.7 (C-16), 159.1 (C-17), 134.0 (C-18), 20.5 (C-19), 55.9 (C-20).

3.2.3 15-Methoxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[c][1] oxacyclododecene-1,12,13-trione

Orange red needles. [α]D\textsubscript{20} = +84 (c 0.32, MeOH). UV λ\textsubscript{max} (nm) MeOH: 204, 274, 422; IR (KBr, ν\textsubscript{max}): 1719, 1694, 1663, 1630, 1372 cm\textsuperscript{-1}; HREIMS m/z 307.15379 [M + H]\textsuperscript{+} (calcd
for C\textsubscript{17}H\textsubscript{22}O\textsubscript{5} 307.15400). \textsuperscript{1}H NMR (500 MHz, Acetone-\textsubscript{d}\textsubscript{6}), \(\delta\): 5.23 (1H, m, H-3), 1.93 (1H, m, H-4a), 1.68 (1H, m, H-4b), 1.72 (1H, m, H-5a), 1.41 (1H, m, H-5b), 1.45 (2H, m, H-6), 1.40 (1H, m, H-7a), 1.26 (1H, m, H-7b), 1.40 (1H, m, H-8a), 1.26 (1H, m, H-8b), 1.59 (2H, m, H-9), 2.55 (1H, m, H-10a), 2.31 (1H, m, H-10b), 5.85 (1H, s, H-14), 1.32 (3H, d, \(J = 6.4\) Hz, H-17), 3.95 (3H, s, H-18). \textsuperscript{13}C NMR (125 MHz, Acetone-\textsubscript{d}\textsubscript{6}), \(\delta\): 164.5 (C-1), 74.4 (C-3), 33.2 (C-4), 21.4 (C-5), 27.3 (C-6), 25.1 (C-7), 25.3 (C-8), 27.2 (C-9), 26.0 (C-10), 140.4 (C-11), 180.9 (C-12), 178.0 (C-13), 103.0 (C-14), 167.3 (C-15), 140.1 (C-16), 19.5 (C-17), 57.9 (C-18).

3.2.4 Ethyl-2,4-dihydroxy-6-(8'-hydroxynonyl)-benzoate

Colourless needles. \([\alpha]_{D}^{21} = -25\) (c 0.2, MeOH). UV \(\lambda_{\max}\) (nm) MeOH: 212, 264, 302; HREIMS \(m/z\) 324.1930 (calcld for C\textsubscript{18}H\textsubscript{28}O\textsubscript{5} 324.1931). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}), \(\delta\): 4.39 (2H, q, \(J = 7.1\) Hz, H-3\''), 1.40 (3H, t, \(J = 7.1\) Hz, H-4\''), 6.28 (1H, d, \(J = 2.5\) Hz, H-3), 6.22 (1H, d, \(J = 2.5\) Hz, H-5), 2.83 (2H, dd, \(J = 6.6\) Hz, 9.3 Hz, H-1\'), 1.53 (2H, m, H-2'), 1.30 (2H, m, H-3'), 1.30 (2H, m, H-4'), 1.30 (2H, m, H-5'), 1.31 (1H, m, H-6a'), 1.41 (1H, m, H-6b'), 1.46 (2H, m, H-7'), 3.83 (1H, m, H-8'), 1.21 (3H, d, \(J = 6.2\) Hz, H-9'). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}), \(\delta\): 171.7 (C-1\'), 61.4 (C-3\''), 23.6 (C-4\''), 105.1 (C-1), 165.5 (C-2), 101.6 (C-3), 160.8 (C-4), 111.1 (C-5), 148.8 (C-6), 36.9 (C-1'), 32.8 (C-2'), 29.5 (C-3'), 29.6 (C-4'), 29.6 (C-5'), 25.7 (C-6'), 39.3 (C-7'), 68.7 (C-8'), 14.3 (C-9').

3.3 Cytotoxicity assay

Cytotoxic activity was tested by MTS assay (Bliss 1935). The cytotoxicity activities of all the compounds were evaluated. Four human cancer cell lines MDA-MB-435, HepG2, HCT-116, A549 and human leukaemia THP1 cell line were used in the cytotoxicity bioassay. Epirubicin was used as positive control.

4. Conclusion

In summary, a series of lasiodiplodins have been isolated from a mangrove endophytic fungus by culturing the fungus in rice medium. The cytotoxicity activities of all the compounds were tested against THP1, MDA-MB-435, A549, HepG2 and HCT-116. Compound 4 showed moderate cytotoxic activities, which indicated that the open-ring structure contributes to cytotoxicity activities.

Supplementary material

All the spectra of compounds 1–4 (Figures S1–S26) are available online at http://dx.doi.org/10.1080/14786419.2015.1062762.

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