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Acylphloroglucinol derivatives from *Decaspermum gracilentum* and their antiradical and cytotoxic activities

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1. Introduction

*Decaspermum gracilentum* (Myrtaceae) is a shrub or small tree, growing in Southeast mainland China (Provinces of Guangdong and Hainan), Taiwan, and northern Vietnam [1]. The leaves of *D. gracilentum* are used to treat dysentery as well as rheumatism and skin disease, kill insect, and anticorrosion [2]. The chemical components of *D. gracilentum* have not been reported hitherto. In our continuing search for bioactive compounds from Chinese herbs, two new acylphloroglucinol derivatives (1, 2) and two known ones (3, 4) (Figure 1) were obtained from the EtOAc soluble fraction of EtOH extract of *Decaspermum gracilentum*. The structures of the new compounds were determined by extensive spectroscopic analyses including HRESIMS, 1D and 2D NMR data. Interestingly, all of the compounds showed ABTS\(^+\) radical scavenging activity with the IC\(_{50}\) values less than 10 \(\mu\)M. Furthermore, compounds 3 and 4 displayed moderate cytotoxicity on human non-small-cell lung carcinoma cell line A549 (IC\(_{50}\) = 50.9 \(\mu\)M) and human renal carcinoma cell line 786-O (IC\(_{50}\) = 38.6 \(\mu\)M), respectively.
moderate cytotoxicity against human non-small-cell lung cancer A549 and human renal cell carcinoma 786-O cell lines, respectively.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder, and its molecular formula was determined to be C_{17}H_{24}O_{8} by the positive-mode HRESIMS at m/z 379.1353 [M + Na]^+, corresponding to six degrees of unsaturation. The IR spectrum showed the absorption bands due to a phenyl group (1604 cm\(^{-1}\)), a keto carbonyl (1629 cm\(^{-1}\)), and hydroxyl groups (3468, 3289 cm\(^{-1}\)). The \(^1\)H NMR spectral data (Table 1) of 1 showed the presence of two nonequivalent aromatic protons (\(\delta_H 6.21, d, J = 2.4\) Hz, 1H; \(\delta_H 6.00, d, J = 2.4\) Hz, 1H), which indicated the occurrence of an aromatic ring with two meta-coupled protons. Furthermore, the \(^13\)C NMR spectrum suggested three oxygenated aromatic carbons as pointed out by their high chemical shifts (\(\delta_C 162.8, 168.4, 165.7\)). The characteristic NMR data at \(\delta_H 5.05 (d, J = 6.4\) Hz, 1H) and \(\delta_H 102.7, 72.6, 74.7, 69.3, 67.0\) manifested the presence of an arabinopyranose [3]. The remaining signals exhibited one carbonyl group (\(\delta_C 207.8\)), four methylene groups [\(\delta_H 3.20 (td, J = 6.8, 1.2\) Hz, 2H), 1.63–1.68 (m, 2H), 1.33–1.41 (m, 2H), 1.33–1.41 (m, 2H); \(\delta_C 45.5, 33.0, 25.7, 24.1\)], and a methyl group (\(\delta_H 0.87, t, J = 6.8\) Hz; \(\delta_C 15.1\)), which were
ascribable to a \(n\)-hexanoyl moiety. The \(^1\)H-\(^1\)H COSY spectrum also confirmed the spin system of H-6'/H-5'/H-4'/H-3'/H-2'. Then, compound 1 was deduced as an acylphloroglucinol glycoside with a \(n\)-hexanoyl moiety. The locations of the \(n\)-hexanoyl moiety and the sugar unit were determined by the HMBC correlations from H-4 and H-6 to C-2, and from H-1'' (\(\delta^H 5.05\)) to C-1 (\(\delta^C 162.8\)).

The sugar portion of 1 was assigned as an \(\alpha\)-d-arabinopyranose. An \(\alpha\)-arabinopyranose was deduced from the coupling constant between H-1'' and H-2'' (\(J = 6.4\) Hz). Moreover, the presence of d-arabinopyranose substituent was confirmed by acidic hydrolysis of 1 followed by TLC analysis and measurement of the optical rotation value of the sugar obtained from the water-soluble fraction \([\alpha]^{22}_D +60\) (c 0.1, H\(_2\)O) and comparison with reference standard \([\alpha]^{22}_D +75\) (c 0.1, H\(_2\)O). Thus, on the basis of these evidences, the structure of 1 was established as 1-(hexanoyl)phloroglucinol-\(\alpha\)-d-arabinopyranoside.

Compound 2 was isolated as a white amorphous powder. A quasi-molecular ion peak in the HRESIMS of compound 2 at \(m/z\) 409.1481 [M + Na]\(^+\) suggested a molecular formula of C\(_{18}\)H\(_{26}\)O\(_9\). The IR spectrum of 2 showed the absorption bands due to a phenyl group (1599 cm\(^{-1}\)), a keto carbonyl (1626 cm\(^{-1}\)), and hydroxyl groups (3393 cm\(^{-1}\)). Compounds 1 and 2 showed similar \(^1\)H and \(^{13}\)C NMR spectra, except for the signals due to the sugar moiety. The resonance for the anomeric proton at \(\delta^H 5.02\) (d, 8.0 Hz) and the set of signals at \(\delta^C 101.8, 74.7, 78.9, 71.1, 78.5,\) and 62.4 indicated the presence of a \(\beta\)-d-glucopyranose [4]. The HMBC correlation between the anomeric proton (\(\delta^H 5.02\)) and C-1 (\(\delta^C 162.8\)) showed that the sugar unit was located at C-1. The absolute configuration of the sugar moiety was confirmed by comparison with the optical rotation value from its hydrolysis product after acid hydrolysis \([\alpha]^{22}_D +60\) (c 0.1, H\(_2\)O) with an authentic standard \([\alpha]^{22}_D +75\) (c 0.1, H\(_2\)O). Therefore, the structure of 2 was established as 1-(hexanoyl)phloroglucinol-\(\beta\)-d-glucopyranoside.

Table 1. \(^1\)H NMR and \(^{13}\)C NMR spectral data of compounds 1\(^a\) and 2\(^b\).

<table>
<thead>
<tr>
<th>No.</th>
<th>(\delta^H) (J in Hz)</th>
<th>(\delta^C)</th>
<th>(\delta^H) (J in Hz)</th>
<th>(\delta^C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>162.8</td>
<td>–</td>
<td>162.2</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>107.2</td>
<td>–</td>
<td>106.7</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>168.4</td>
<td>–</td>
<td>167.5</td>
</tr>
<tr>
<td>4</td>
<td>6.21 d (2.4)</td>
<td>98.6</td>
<td>6.17 d (2.4)</td>
<td>98.3</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>165.7</td>
<td>–</td>
<td>165.8</td>
</tr>
<tr>
<td>6</td>
<td>6.00 d (2.4)</td>
<td>95.7</td>
<td>5.95 d (2.4)</td>
<td>95.3</td>
</tr>
<tr>
<td>1''</td>
<td>–</td>
<td>207.8</td>
<td>–</td>
<td>207.5</td>
</tr>
<tr>
<td>2''</td>
<td>3.20 td (6.8, 1.2)</td>
<td>45.5</td>
<td>3.31 td (7.2, 17.2)</td>
<td>45.2</td>
</tr>
<tr>
<td>3''</td>
<td>1.33–1.41 m</td>
<td>33.0</td>
<td>1.35–1.37 m</td>
<td>32.7</td>
</tr>
<tr>
<td>4''</td>
<td>1.63–1.68 m</td>
<td>25.7</td>
<td>1.65–1.67 m</td>
<td>25.5</td>
</tr>
<tr>
<td>5''</td>
<td>1.33–1.41 m</td>
<td>24.1</td>
<td>1.35–1.37 m</td>
<td>23.7</td>
</tr>
<tr>
<td>6''</td>
<td>0.87 t (6.8)</td>
<td>15.1</td>
<td>0.92 t (6.8)</td>
<td>14.4</td>
</tr>
<tr>
<td>1''</td>
<td>5.05 d (6.4)</td>
<td>102.7</td>
<td>5.02 d (8.0)</td>
<td>101.8</td>
</tr>
<tr>
<td>2''</td>
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<td>72.6</td>
<td>3.34–3.39 m</td>
<td>74.7</td>
</tr>
<tr>
<td>3''</td>
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<td>74.7</td>
<td>3.42–3.47 m</td>
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</tr>
<tr>
<td>4''</td>
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<td>69.3</td>
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<tr>
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<td>67.0</td>
<td>3.43–3.46 m</td>
<td>78.5</td>
</tr>
<tr>
<td>6''</td>
<td>3.74–3.75 m</td>
<td>62.4</td>
<td>3.92 dd (12.0 2.0)</td>
<td>62.4</td>
</tr>
</tbody>
</table>

\(^a\)#H NMR (400 MHz) and \(^{13}\)C NMR (100 MHz), \(\delta\) ppm, J in Hz, Measured in CD\(_3\)COCD\(_3\).

\(^b\)#H NMR (400 MHz) and \(^{13}\)C NMR (100 MHz), \(\delta\) ppm, J in Hz, Measured in CD\(_3\)OD.
Besides the new compounds 1 and 2, two known ones were isolated and identified as 1-(acetyl)phloroglucinol-β-D-glucopyranoside (3) [5] and ethyl 2,4,6-trihydroxybenzoate (4) [6] by comparing the 1H and 13C NMR data with the reference data.

Free radicals have been implicated in over a hundred disease conditions in humans, including cardiovascular disease, cancer, inflammation, atherosclerosis, diabetes mellitus, and Alzheimer's disease [7]. Therefore, the great interest has been recently focused on the natural foods, medicinal plants, and phytoconstituents due to their well-known abilities to scavenge free radicals. Our experimental data revealed that all these compounds isolated from D. gracilentum had the effect of scavenging free radical. As shown in Figure 2, a dose–response relationship was found in the ABTS⁺ radical scavenging activity and the IC₅₀ values ranged from 0.5 to 10 μM, the positive control resveratrol gave an IC₅₀ value of 12 μM (data not shown). Within the tested compounds, compound 4 was the most active, displaying potent ABTS⁺ radical scavenging activity with IC₅₀ value of 0.5–1 μM. Moreover, compounds 1–3 showed the similar activity with the IC₅₀ values less than 10 μM.

All of the isolated compounds were also evaluated for their antiproliferative effects by a cytotoxic sulforhodamine B (SRB) assay on human non-small-cell lung carcinoma cell line A549 and human renal carcinoma cell line 786-O as previously described [8], and colchicine was selected as positive control. As shown in Table 2, compounds 1 and 2 did not show any cytotoxicity with IC₅₀ values of more than 70 μM against both human 786-O and A549 cell lines. Compound 3 exhibited moderate cytotoxic activity against A549 cell line with the IC₅₀ value of 50.9 μM and did not show bioactivity against 786-O (IC₅₀ > 70 μM). The IC₅₀ value of compound 4 on 786-O was relatively low (38.6 μM). However, compound 4 showed weak inhibition of cell replication in A549 cell lines (IC₅₀ > 70 μM) (Table 2).

In summary, from the EtOAc soluble fraction of EtOH extract of D. gracilentum, two new acylphloroglucinol derivatives (1 and 2) and two known ones (3 and 4) were isolated. Compounds 1 and 2 have an unusual n-hexanoyl side chain [5,6,9,10]. Moreover, compound 1 has an α-D-arabinopyranose unit which was obviously different from those known acylphloroglucinol glycosides [5,6,9,10]. Though the isolated compounds from D. gracilentum showed weak cytotoxic activity, compounds 1–4 showed strong ABTS⁺ radical scavenging activity. And we expect that our findings will provide a foundation for researchers to find more compounds of antiradical in Decaspernum genus.

3. Experimental

3.1. General experimental procedures

Human non-small-cell lung carcinoma cell line A549 and human renal carcinoma cell line 786-O were obtained from the American Type Culture Collection (Manassas, VA, USA).
Roswell Park Memorial Institute (RPMI) 1640 and Fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT, USA). The absorbance of the resulting formazan product was recorded on a TECAN Infinite M200 (Tecan, Austria). 2,2′-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and Sulforhodamine B (SRB) were obtained from Sigma-Aldrich (St. Louis, MO). Infrared spectra (IR) were recorded on a Nicolet FTIR-360 spectrometer (Thermo Nicolet Corporation, WI, USA). Optical rotations were carried out using a 1.5-mL cell on a Rudolph Research Analytical Perkin-Elmer 341 polarimeter set to the Na 589 nm wavelength (USA). 1H NMR, 13C NMR, and 2D NMR spectra were recorded on a Bruker Avance 400 instrument (Bruker BioSpin AG Facilities, Fällanden, Switzerland) with residual solvent peaks as references. HRESIMS data were collected on a Q-TOF Synapt G2 HDMS system (Waters, Pittsburgh, PA, USA) in positive ion mode. GF254 for TLC and silica gel (200–300 mesh; Qingdao Marine Chemical Co. Ltd, Qingdao, China) and Sephadex LH-20 (25–100 mm; Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). All chemicals used in the study were of analytical grade (Sinopharm Chemical Reagent CO., Ltd, Shanghai, China).

3.2. Plant material

The leaves of Decaspernum gracilentum were collected from Hainan Province of China, in the summer of 2012. The plant was identified by Prof. Guo-Liang Zhang, Lanzhou University, China. A voucher (No. 2012-09) specimen was deposited with Lanzhou University.

3.3. Extraction and isolation

The air-dried and powdered sample (4.6 kg) was extracted with EtOH (3 × 10 L) at room temperature. The EtOH extract was evaporated in vacuum to yield a residue, which was suspended in water and then successively partitioned with EtOAc and n-BuOH. The EtOAc extract (20.3 g) was separated on a D101 macroporous resin column and eluted with a gradient mixture of H2O/EtOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100 v/v) to give six fractions (Fr.1-Fr.6). The Fr.4-Fr.6 (combined, 15.0 g) was further resolved on a silica gel column, eluting in a gradient of CHCl3-MeOH (100:1, 50:1, 20:1, 10:1, 5:1, 1:1, 1:00, v:v) with increasing amounts of MeOH to give seven subfractions (Fr.A.1-A.7). Fr.A.4 (2.3 g) was subjected to silica gel column chromatography eluted with CHCl3-MeOH (5:1, v:v) to yield compound 4 (2.9 mg). Fr.A.5 (1.3 g) was purified by silica gel column chromatography (EtOAc/MeOH 5:1) to yield compounds 1 (10.1 mg) and 2 (6.2 mg). Fr.A.6 (4.5 mg) was purified by semi-preparative HPLC with MeOH-H2O (30:70, v:v) to yield compound 3 (2.3 mg).

3.3.1. 1-(Hexanoyl)phloroglucinol-α-d-arabinopyranoside (1)

White amorphous powder; [α]D22 −15 (c 8.40, methanol); IR (KBr): \( \nu_{\text{max}} \) 1008, 1030, 1076, 1182, 1196, 1629, 1604, 3289, 3468 cm\(^{-1}\). 1H NMR and 13C NMR spectral data (see Table 1). HRESIMS: m/z 379.1363 [M + Na]+ (calcd for C17H24O8Na, 379.1327).

3.3.2. 1-(Hexanoyl)phloroglucinol-β-d-glucopyranoside (2)

White amorphous powder; [α]D22 −139 (c 76.40, methanol); IR (KBr): \( \nu_{\text{max}} \) 1008, 1030, 1076, 1182, 1196, 1629, 1604, 3289, 3468 cm\(^{-1}\). 1H NMR and 13C NMR spectral data (see Table 1). HRESIMS: m/z 409.1481 [M + Na]+ (calcd for C18H26O9Na, 409.1469).
3.4. Acid hydrolysis of compounds 1 and 2

Compound 1 (5.0 mg) was refluxed with 0.04 M H₂SO₄ (2 ml) in MeOH (2 ml) at 60 °C for 12 h. After cooling, the reaction mixture was extracted with EtOAc three times (3 × 5 ml). The aqueous phase was neutralized with saturated NaHCO₃ aqueous solution and evaporated. This fraction was purified via silica gel column chromatography [CH₂Cl₂/MeOH/H₂O (128:80:16)], and the sugar was obtained. The specific rotation value was measured to confirm D or L configuration for arabinose and compared with those of the standards [11]. In a similar manner, the presence of the sugar was also verified for 2.

3.5. In vitro antioxidant activity of compounds 1–4

The ABTS⁺ scavenging capacity was determined according to the published method [12]. Resveratrol was selected as positive control. The ABTS⁺ solution, which was prepared from oxidation of ABTS (7 mM) by potassium persulfate (2.5 mM) for 12–16 h in the dark at room temperature, was diluted to an absorbance of 0.700 ± 0.020 at 734 nm. The ABTS⁺ solution (2 ml) and the antioxidant solution (100 μl) were mixed and incubated at room temperature, and the absorbance at 517 nm was recorded after 30 min.

3.6. In vitro anti-proliferation bioassay

A549 and 786-O human cancer cell lines were cultured in RPMI-1640 medium containing 10% FBS (Sigma, Logan, USA), 100 U/ml penicillin (Sigma, Logan, USA), and 100 μg/ml streptomycin sulfate (Sigma, USA). Briefly, cells were maintained at 37 °C under 5% CO₂. A549 (5 × 10^3 cells/well) and 786-O (3 × 10^3 cells/well) were plated in 96-well plate and allowed to adhere. After incubation for 4 h at 37 °C, fresh medium (100 μl) containing various concentrations of the tested compounds were added to the cells. Colchicine was selected as positive control. 48 h later, the cultures were fixed by addition of ice-cold 50% trichloroacetic acid (50 μl) at 4 °C. Fixed cells were rinsed with deionized H₂O and stained with 0.4% sulforhodamine B (70 μl). The wells were washed with 0.1% HOAc and left to dry. The absorbed sulforhodamine B was dissolved in 100 μl unbuffered 1% Tris-base [tris-(hydroxymethyl) aminomethane]. The absorbance of extracted sulforhodamine B was measured at 570 nm using an automated microplate reader.

Disclosure statement

No potential conflict of interest was reported by the authors.

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