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Susan Matthew\(^a\), Kuo-Chin Kao\(^b\), Yuan-Shiun Chang\(^b\), Pedro Abreu \(^a\)

\(^a\) CQFB/REQUIMTE, FCT-UNL, 2829-516 Caparica, Portugal

\(^b\) Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan

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Ellagic acid glycosides from *Turpinia ternata*

SUSAN MATTHEW*†, KUO-CHING KAO‡, YUAN-SHIUN CHANG‡ and PEDRO ABREU†

†CQFB/REQUIMTE, FCT-UNL, 2829-516 Caparica, Portugal  
‡Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan

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Bioassay-guided fractionation of the methanolic extract of *Turpinia ternata* stems, has led to the isolation of the new ellagic acid derivative 3,4°-di-O-methylellagic acid-4-O-α-L-arabinofuranoside (1), and the known compounds ellagic acid (2), 3-O-methyl ellagic acid (3), 3-O-methylellagic acid-3’-O-α-L-rhamnopyranoside (4), and 3,3’-di-O-methylellagic acid-4’-O-α-D-glucopyranoside (5). Their structures were elucidated by extensive spectroscopic methods. Compounds 1, 3, and 4 showed moderate antioxidant activity against DPPH free radical, whereas compound 1 was found to be moderately cytotoxic against *Artemia salina* larvae.

Keywords: *Turpinia ternata*; Staphyleaceae; Ellagic acid derivatives; Antioxidant activity

1. Introduction

*Turpinia ternata* Nakai (Staphyleaceae) is a rare tree belonging to the bladdernut family, indigenous to southern Japan and central and southern forested regions of Taiwan [1]. About 17 species of the genus *Turpinia* are found in tropical Asia and America. The only previous phytochemical investigation on *T. ternata* resulted in the isolation of megastigmane glucosides from the leaves of plant material collected in Japan [2].

In the course of our studies on medicinal plants from Taiwan [3,4], we have detected in the MeOH extract of *Turpinia ternata* stems, a moderate antioxidant activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Following a bioassay-guided isolation procedure, the new ellagic acid derivative 3,4°-di-O-methylellagic acid-4-O-α-L-arabinofuranoside (1) has been identified, along with the known compounds ellagic acid (2) [5], 3-O-methyl ellagic acid (3) [6], 3-O-methylellagic acid-3’-O-α-L-rhamnopyranoside (4) [7], and 3,3’-di-O-methylellagic acid-4’-O-α-D-glucopyranoside (5) [8], whose structures were elucidated by extensive spectroscopic methods. Compounds 1, 3, and 5 were evaluated for their antioxidant activity against DPPH free radical [9].

*Corresponding author. Tel.: 351-212948354. Fax: 351-212948550. Email: susmatt111@yahoo.com*
whereas 1 was also assayed for its cytotoxicity using the brine shrimp lethality experiment [10].

2. Results and discussion

The MeOH extract of *T. ternata* stems was partitioned successively with CHCl₃, EtOAc, n-BuOH, and water. Successive column chromatography of the n-BuOH extract over Diaion HP-20, silica gel, and Sephadex LH-20 yielded compounds 1–5.

Compound 1 was isolated as a pale yellow amorphous powder, whose molecular formula was established as C₂₁H₁₈O₁₂ on the basis of the pseudomolecular ion peak at *m/z* 463 [M + H]⁺ displayed by positive FABMS, and its NMR spectral data. The IR spectrum displayed characteristic absorptions for hydroxyl and ester functions, at *ν* max 3400 and 1744 cm⁻¹, respectively. The ¹³C NMR and DEPT spectra showed the presence of ten aromatic quaternary carbons, two isolated aromatic methine carbons at δ 112.9 and 113.0, two ester carbons at δ 159.2 and 159.3, two methoxyl carbons at δ 58.0 and 61.7, and the characteristic resonances of a furanose moiety at δ 62.1, 78.1, 83.8, 87.7, and 109.2 (table 1) [11]. ¹H NMR, COSY, and HMBC spectra allowed the identification of the sugar protons, in addition to two methoxy methyl groups at δ 3.88 and 4.21, and two aromatic protons at δ 8.02 (s) and 8.41 (s). These data were in good agreement with those reported for ellagic acid glycosides [5]. The two methoxyl groups were located at C-3 and C-4 on the basis of their ¹³C NMR chemical shifts (δ 58.9 and 61.7) [12], and the NOESY correlation between H-5' and 4'-OCH₃ (figure 1). The presence of one free phenolic group was corroborated by the observed bathochromic shift in the UV spectrum after addition of NaOH [12].

<table>
<thead>
<tr>
<th>Position</th>
<th>¹³C (δ)</th>
<th>¹H (δ)</th>
<th>HMBC (H to C)</th>
<th>COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112.8</td>
<td>–</td>
<td>C-3, C-4, C-5, C-6, C-7</td>
<td>H-1”</td>
</tr>
<tr>
<td>2</td>
<td>142.4</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>141.3</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>151.9</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>113.0</td>
<td>8.41 (s)</td>
<td>C-3, C-4, C-5, C-6, C-7</td>
<td>H-1”</td>
</tr>
<tr>
<td>6</td>
<td>114.7</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>159.3</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1’</td>
<td>113.9</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2’</td>
<td>141.8</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3’</td>
<td>143.1</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4’</td>
<td>152.9</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5’</td>
<td>112.9</td>
<td>8.02 (s)</td>
<td>C-3’, C-4’, C-5’, C-6’, C-7’</td>
<td>H-4’</td>
</tr>
<tr>
<td>6’</td>
<td>111.7</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7’</td>
<td>159.2</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OMe</td>
<td>61.7</td>
<td>4.21 (s)</td>
<td>C-4’, C-3’</td>
<td></td>
</tr>
<tr>
<td>4’-OMe</td>
<td>58.9</td>
<td>3.88 (s)</td>
<td>C-4’, C-3’</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1”</td>
<td>109.2</td>
<td>6.44 (d, 1.8)</td>
<td>C-4, C-2”, C-3”, C-4”</td>
<td>H-5, H-2”</td>
</tr>
<tr>
<td>2”</td>
<td>83.8</td>
<td>5.25 (dd, 1.8, 4.2)</td>
<td>C-1”</td>
<td>H-1”, H-3”</td>
</tr>
<tr>
<td>3”</td>
<td>78.1</td>
<td>5.03 (q, 2.4, 4.2)</td>
<td>C-2”, C-4”</td>
<td>H-2”, H-4”</td>
</tr>
<tr>
<td>4”</td>
<td>87.7</td>
<td>4.89 (m)</td>
<td>C-3”</td>
<td>H-3”, H-5’a, H-5”b</td>
</tr>
<tr>
<td>5”a</td>
<td>62.1</td>
<td>4.38 (m)</td>
<td>C-3”</td>
<td>H-3”, H-4”</td>
</tr>
<tr>
<td>5”b</td>
<td></td>
<td>4.25 (m)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The sugar was identified as L-arabinose by comparison of its optical rotation and TLC properties with those of an authentic sample, after acid hydrolysis of 1. Its configuration was assigned as α from the small coupling constant (1.8 Hz) observed for the anomeric proton at δ 6.44. The observed NOE and HMBC cross peaks of the anomeric proton with H-5 (δ 8.41) and C-4 (δ 151.9), respectively, allowed to establish the attachment position of the arabinose moiety at C-4. Other key NOESY and HMBC correlations (figure 1), led to the identification of compound 1 as the new ellagic acid derivative 3,4-0-methylellagic acid-4-0-L-arabinofuranoside.

Compounds 2–5 (figure 2) were identified as ellagic acid (2) [5], 3-O-methyl ellagic acid (3) [6], 3-O-methylellagic acid-3′-O-α-L-rhamnopyranoside (4) [7], and 3,3′-di-O-methylellagic acid-4′-O-α-D-glucopyranoside (5) [8], by comparison of their spectral data with those reported in literature. All the compounds were isolated for the first time from Turpinia sps.
Free radical scavenging activity of the MeOH and BuOH extracts of *T. ternata*, and that of compounds 1, 3, and 4 were evaluated in the DPPH assay (figure 3) [9]. Compound 3 showed a more effective hydrogen donating capacity (IC$_{50}$ 30.24 µg mL$^{-1}$) than 1 (IC$_{50}$ 59.15 µg mL$^{-1}$) and 4 (IC$_{50}$ 70.13 µg mL$^{-1}$). Compound 1 was further evaluated for cytotoxicity in the brine shrimp lethality assay, where it showed a moderate activity (LC$_{50}$ 45.9 µg mL$^{-1}$).

3. Experimental

3.1. General methods

Melting points were determined on a Yanaco MP-500 micro-melting point apparatus and are uncorrected. Optical rotation was determined using a Perkin-Elmer 241-MC polarimeter, and UV spectra were obtained on a Shimadzu UV-160A-visible spectrophotometer. FTIR spectra were recorded on a Perkin-Elmer spectrum 1000 spectrometer. $^1$H, $^{13}$C NMR and 2D NMR spectra (COSY, NOESY, HMBC, HMQC) were recorded on a Varian VXR-600 and Bruker AMX ($^1$H at 600 MHz; $^{13}$C at 150 MHz), using CDCl$_3$, (CD$_3$)$_2$CO, DMSO-d$_6$, CD$_3$OD, and pyridine-d$_5$ as solvents. FAB and EI Mass spectra were recorded on a JOEL JMS-SX/SX 102A Tandem Mass Spectrometer, and a Finnigan Mass Spectrometer, respectively. Thin layer chromatography (TLC) were performed on normal-phase precoated silica gel F$_{254}$ plates, using anisaldehyde, vanillin, and CeSO$_4$ as spray reagents. Diaion HP-20 (Ion Exchange Resin, Mitsubishi Kasei Corp., Japan), Sephadex LH-20 and silica gel (230–400 mesh) were used for column chromatography.

3.2. Plant material

*Turpinia ternata* was collected at the central forested mountains of Taiwan, in April 2002. A voucher specimen (IPS-T02) has been deposited in the herbarium of the Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung.
3.3. Extraction and isolation

Air-dried and pulverized stems of *T. ternata* (5 kg) were defatted with hexane (60 L), and subsequently extracted with MeOH (60 L × 4) at room temperature for two weeks to yield 145 g of extract after evaporation of the solvent. The MeOH extract, which showed moderate scavenging activity against DPPH radical (IC₅₀ 238.97 μg mL⁻¹), was suspended in water and partitioned successively with CHCl₃, EtOAc, and n-BuOH. The active n-BuOH fraction (30 g, IC₅₀ 91.63 μg mL⁻¹ against DPPH) was eluted on a Diaion HP-20 column with H₂O, MeOH and CHCl₃, to afford 8 fractions (B₁–B₈). Fraction B₃ (6.2 g) obtained from H₂O/MeOH (1:1) elution, was then subjected to successive column chromatography over Diaion HP-20 and Sephadex LH-20, with H₂O/MeOH elution, to afford 51 mg of 1, after crystallization with MeOH. Fraction B₄ (3.9 g), obtained from H₂O/MeOH (25:75) elution, was subjected to Sephadex LH-20 chromatography using the same eluting mixture, followed by silica gel flash chromatography with EtOAc/MeOH gradient elution, to afford compounds 4 (16 mg) and 5 (4 mg). Concentration of fraction B₅ formed a precipitate, which was filtered and washed with MeOH, to yield 13 mg of 2. Compound 3 (76 mg) precipitated from fraction B₆, and was further purified by silica gel flash chromatography, using CHCl₃ and MeOH as eluents.

3.3.1. 3,4-Di-O-methylellagic acid-4-O-α-L-arabinofuranoside (1). Amorphous powder; m.p. 235°C (decomposes); [α]D°²⁵ = −110° (c = 1.03, DMSO); UV (MeOH) λ_max (log ε) 200 (4.13), 246 (4.68), 284 (4.07), 368 (4.1) nm; (MeOH + NaOH) 208, 255, 306, 404 nm; IR (KBr) ν_max 3445, 1743, 1604, 1489, 1411, 1364, 1110, 1066 cm⁻¹; ¹H- and ¹³C-NMR data (see table 1); FABMS: m/z 463 (15.6) [M + H]⁺, 149 (100) [C₅H₉O₃], 150 (98) [C₅H₁₀O₅], 460 (44), 330 (18), 307 (99), 289 (89), 136 (99), 107 (86).

3.4. Acid hydrolysis of 1

Compound 1 (5 mg) was dissolved in 10 mL MeOH–H₂O (1:1) and refluxed at 60°C for 6 h with 2 M HCl (5 mL). After cooling the reaction mixture was neutralized with NaOH, concentrated *in vacuo*, and the aglycon extracted thrice with EtOAc. The aqueous layer was analyzed for its sugar composition on NH₂ TLC plates (CH₃CN/H₂O, 7:3), using naphthol as spray reagent, and sugar standards.

3.5. DPPH assay

DPPH scavenging activity of *T. ternata* extracts and compounds were determined by DPPH method as described by Blois [9] with minor modifications. Test samples (100 μL) of various concentrations (0.015–2.0 mg mL⁻¹) were added to 950 μL of freshly prepared DPPH solution (1 mM in MeOH). The decrease in absorbance at room temperature was determined at 515 nm after 30 min of incubation. All experiments were performed in triplicate. The inhibition percentage of radical-scavenging activity was calculated as (1–Aₛ/Aₒ) × 100, where Aₒ and Aₛ are the absorbance of the control and sample, respectively. Quercetin was used as positive control.
3.6. Brine shrimp assay

The brine shrimp (*Artemia salina*) lethality assay was used as described in literature [10].

Acknowledgement

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References