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Antiprotozoal Activity of Flavonoid Glycosides Isolated from *Clidemia sericea* and *Mosquitoxylum jamaicense*

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Abstract
A new O-galloyl-C-glycosylflavone, 2',6'-O-digalloylvitexin (1), along with four known glycosylflavones (2-5) have been isolated from *Clidemia sericea* D. Don (Melastomataceae), and four other known glycosylflavones (6-9) have been isolated from *Mosquitoxylum jamaicense* Krug & Urb. (Anacardiaceae). Compound 1, 3, and 6 showed mild antimalarial activity (24 ± 1, 38 ± 2, and 44 ± 1 μM, respectively) against a chloroquine-resistant *Plasmodium falciparum* strain. Additionally, tests against leishmaniasis and *Trypanosoma cruzi* were made. These compounds were identified by MS, UV, IR, and ID and 2D NMR data and by comparison with the literature data.

Keywords: *Clidemia sericea*, flavonoid glycosides, ICBG, leishmaniasis, malaria, *Mosquitoxylum jamaicense*, tropical diseases, *Trypanosoma cruzi*.

Introduction
Malaria is one of the tropical diseases with the greatest impact on world health, causing 300 million cases and one million deaths annually (Gelb & Hol, 2002). Strains of *Plasmodium falciparum* that are resistant to the latest drugs, as well as chloroquine, have emerged and spread rapidly (Ridley, 2002a,b). Continuing with our search for antiprotozoal drugs, we report now the bioassay-guided isolation of five flavonoids from *Clidemia sericea* D. Don (Melastomataceae): the new O-galloyl-C-glycosylflavone 1 (Fig. 1) and the known isovitexin 2, 2'-O-galloylvitexin 3, rutin 4 (Lin et al., 2000), and vitexin 5 (Latte et al., 2000), and four known flavonoids from *Mosquitoxylum jamaicense* Krug & Urb. (Anacardiaceae): phloridzin 6 (Hilt et al., 2003), 4-hydroxy benzenepropanal 7 (Ishikawa & Kishi, 2000), trilobatin 8 (Tanaka et al., 1983), and quercetin-3-O-β-D-galactoside 9 (Zhang & Mao, 2001) as part of the ongoing ICBG program based on Panamá (Coley et al., 2003).

Materials and Methods

General experimental procedures
Optical rotations were determined on an Autopol III 6971 Automatic Polarimeter (Rudolph Research Analytical, NJ, USA). Infrared (IR) spectra were measured on a Perkin-Elmer Fourier transformer infrared (FT-IR) Spectrometer Spectrum RXI (Perkin-Elmer, USA). The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz for proton and 75 MHz for carbon) (Bruker BioSpin, MA, USA). The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz for proton and 75 MHz for carbon) (Bruker BioSpin, MA, USA). Low Resolution and High Resolution Mass Spectra (HRMS) were recorded on a Kratos MS50TC instrument using chemical Ionization (Kratos Analytical Instruments, NJ, USA). High Pressure Liquid Chromatography (HPLC) and ultraviolet (UV) spectrometer were carried out on a Waters Liquid Chromatography (LC) system, with a 600 pump and 996 photodiode array detector (Waters, MA, USA).

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Plant material
Young leaves of Clidemia sericea and Mosquitoxylon jamaicense were collected in Alisos de Campana National Park and Chagres National Park, respectively, in the Republic of Panama. The material was identified by Professor Mireya Correa of the University of Panama and the Smithsonian Tropical Research Institute. Vouchers have been deposited at the Herbarium of the University of Panama, numbers PMA53118 (C. sericea) and PMA53117 (M. jamaicense).

Extraction and isolation
The leaf material of C. sericea (300 g) and of M. jamaicense (900 g) was homogenized and processed as previously described (Torres et al., 2003). The organic extracts were concentrated as described (Montenegro et al., 2003), obtaining 15 g (IC(50): 16 μg/mL) and 44 g (IC(50): 7 μg/mL), correspondingly.

The methanol crude extract of C. sericea (15 g) was subjected to liquid-liquid partition with n-hexane and MeOH. The methanol fraction was evaporated and subjected to a second solvent partition using EtOAc and H2O. The EtOAc part (6.6 g, IC(50): 7 μg/mL) was subjected to vacuum-liquid chromatography (VLC) on silica gel (7GF, VWR Scientific) using hexane-AcOEt-MeOH mixtures of increasing polarity.

The fractions were combined according to TLC composition into Frs. 5a–5k. Fraction 5d (132 mg) was filtered on a solid-phase extraction cartridge of RP-18 (Merck) and recrystallized from MeOH. This fraction was purified using RP-HPLC (XTerra 10 μm, 10 × 250 mm) with MeOH:H2O (40:60), flow 2 mL/min, yielding 5 (5 mg).

Galloylglycosylflavone 1
Yellow amorphous powder; [α]D22: −14.25 (MeOH, c 0.25); IR: max cm−1: 3352, 2924, 1624, 1598, 1516, 1452, 1262, 1206, 1174, 1076, 1046, 1024, 994, 828; UV λmax (MeOH) nm 225.0, 269.8, 281.1, 339.9, 343.4; 1H NMR (MeOD; 300 MHz) δ 7.76 (2H, d, J = 8.3 Hz, H-2′ and H-6′), 7.11 (2H, s, H-2′′ and H-6′′), 6.88 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 6.62 (2H, s, H-2′′ and H-6′′), 6.52 (1H, s, H-3′′), 6.42 (1H, s, H-8), 5.50 (1H, t, J = 7.8 Hz, H-2′), 5.33 (H, m, H-3′′), 5.32 (H, d, J = 7.8 Hz, H-1′′), 3.67 (1H, m, H-5′), 3.89 (1H, m, H-6′), 3.83 (3H, m, H-4′′′, H-4′′′′); 13C NMR (MeOD; 75 MHz) δ 184.2 (C-4), 168.5 (C-O 3′′), 167.5 (C-O 2′′), 166.5 (C-2), 164.9 (C-7), 163.0 (C-5 and C-4′), 159.2 (C-9), 146.6 (C-3′′ and C-5′′′′), 146.5 (C-3′′′″ and C-5′′′″), 140.2 (C-4′′ and C-4′′′′), 129.8 (C-2′′′ and C-6′), 123.4 (C-1′′), 121.8 (C-1′′′), 121.3 (C-1′′′′), 117.3 (C-3′ and C-5′), 110.8 (C-2′′′″ and C-6′′′), 110.7 (C-2′″ and C-6′″), 107.8 (C-10), 105.3 (C-6), 104.2 (C-3), 95.4 (C-8), 83.2 (C-5″), 79.4 (C-3″), 73.4 (C-2″), 72.0 (C-4″), 70.5 (C-6′), 69.8 (C-2′), 65.8 (C-5), 65.4 (C-6), 55.4 (C-5′), 42.7 (C-2′′).
OH

63.0 (C-6'); HRFABMS (NBA, positive mode) [M]+ 736.12760 (calculated C_{35}H_{28}O_{18}, 736.12756).

Assays

All assays were based on inhibition of growth of the parasites by added compounds or extracts, as described previously (Molinar-Toribio et al., 2006 and 2004; Corbett et al., 2004; Torres et al., 2003 and 2004; Williams et al., 2003).

Cytotoxicity assay

Vero cells adhering to 96-well plates were used to evaluate the toxicity of the compounds purified on the basis of the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma) (Torres et al., 2004). After treatment with the test compound and 4 h of incubation at 37°, cell viability was evaluated in an ELISA reader at 570 nm.

Results and Discussion

Clidemia sericea

The ethyl acetate partition fraction of the crude extract of C. sericea showed significant antiplasmodial activity (IC_{50}: 5 μg/mL), and was selected for bioguided fractionation, yielding the novel galloylglycosylflavone (I) and four known compounds (2–5).

Compound I was obtained as a yellow powder and showed a pseudomolecular ion from its HRFABMS at m/z 737.12760 (C_{35}H_{28}O_{18}, calcd 736.12756), which was consistent with 35 carbons observed in the 13C NMR spectra, sorted by DEPT experiments into 1-CH oxygenate, 15-CH (5 oxygenate), and 19 quaternary. The UV spectra of compound I exhibited three absorption bands at 222, 272, and 337 nm, consistent with a flavone derivative (Latte et al., 2000). The IR spectrum showed bands consistent with the presence of one or more hydroxyl groups (3352 cm⁻¹), an ester carbonyl (1624 cm⁻¹), a conjugated carbonyl (1598 cm⁻¹), and a phenyl group (1516, 1452 cm⁻¹). The 1H NMR spectrum showed signals due to two galloyl groups at δ 6.82 (2H, s) and 7.11 (2H, s) and 13C NMR signals at δ 110.7, 121.3, 140.2, 146.5, 167.5 (COO), due to one galloyl group, and signals at δ 110.8, 121.8, 140.2, 146.6, 168.5 (COO), due to a second galloyl group.

The 1H NMR spectrum revealed the presence of two protons at δ 6.42 (H-8) and δ 6.52 (H-3) belonging to the flavone skeleton, assigned from HMQC and HMBC correlations. A para-substituted phenol was characterized by aromatic A₂B₂-spin system of the B-ring at δ 7.76 and δ 6.88 (each 2H, d, J = 8.3 Hz). The anomic proton of the β-p-glucopyranosyl moiety (δ 5.32, d, J = 7.8 Hz) had correlations with C-2″ and C-3″ and a long-range correlation with C-6″. A methylene proton at δ 5.50 (t, J = 7.89 Hz) correlated with C-3″ and had a long range correlation with the carbonyl (167.5) of a galloyl group, indicating that one of the galloyl group
**Table 1.** Antimalarial activity and cytotoxicities of flavonoid glycosides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity <strong>IC</strong>&lt;sub&gt;50&lt;/sub&gt; µg/mL</td>
<td>18</td>
<td>&gt; 50</td>
<td>23</td>
<td>33</td>
<td>&gt; 50</td>
<td>19</td>
<td>32</td>
<td>&gt; 50</td>
<td>23</td>
</tr>
<tr>
<td>(µM)</td>
<td>24 ± 1</td>
<td>&gt; 116</td>
<td>38 ± 2</td>
<td>76 ± 3</td>
<td>&gt; 116</td>
<td>44 ± 1</td>
<td>218 ± 1</td>
<td>&gt; 115</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Cytotoxicity** IC**&lt;sub&gt;50&lt;/sub&gt; µg/mL</td>
<td>96</td>
<td>&gt; 100</td>
<td>&gt; 151</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chloroquine-resistant strain of *Plasmodium falciparum* (IC<sub>50</sub> 0.06 µM).
<sup>b</sup>Experiments performed with Vero cells.
<sup>c</sup>ND = not determined.

is attached to C-2" as in 3. A second methines proton at δ 5.33 (m) correlated with C-2" and another carbonyl group (168.5) of a second galloyl group, indicating that this second galloyl group was attached to C-3."" Mosquitoxylon jamaicense

The EtOAc liquid-liquid partition fraction of the crude MeOH-EtOAc extract of *M. jamaicense* with an activity IC<sub>50</sub>: 12 µg/mL against *P. falciparum* was selected to bioassay-guided fractionation; yielding four known compounds (6-9).

From the nine compounds reported here, three (i.e., 1, 3, and 6) showed moderate activity against W2, a *Plasmodium falciparum* strain (chloroquine-resistant), while a further six isolated compounds were with activities that exceeded 50 µg/mL (Table 1). Compounds 1 and 3 are more active than 2, and this suggests that the galloyl moiety is necessary for the activity as previously reported by our group (Corbett et al., 2004). Likewise, none of the isolated compounds demonstrated activity against *Leishmania* and *T. cruzi* parasites at concentrations of 40 and 50 µg/mL, respectively, or significant cytotoxic activity when tested against Vero cells (100 µg/mL).

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