Article

Huberine, a New Canthin-6-One Alkaloid from the Bark of Picrolemma huberi

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Abstract: A new alkaloid, Canthin-6-one, Huberine (1), together with three known compounds including 1-Hydroxy-canthin-6-one (2), Canthin-6-one (3) and stigma sterol (4), were isolated from the stem bark of Picrolemma huberi. The isolation was achieved by chromatographic techniques and the purification was performed on a C18 column using acetonitrile/water (90:10, v/v) with 0.1% formic acid as the mobile phase. The structural elucidation was performed via spectroscopic methods, notably 1D- and 2D-NMR, UV, IR, MS and HRMS. The antiplasmodial activity of the compounds was studied.

Keywords: canthin-6-one; Picrolemma huberi; Simaroubaceae; antiplasmodial activity

1. Introduction

Plants of the family Simaroubaceae are widely used in traditional medicine for the treatment of diseases in different countries around the world. Species belonging to the genus Picrolemma (Simaroubaceae) have long been used in traditional medicine for their antitumoral and antimalarial properties [1]. Previous phytochemical investigations of Picrolemma huberi revealed the presence of terpenoids and alkaloids. Among these compounds, quassinoids and canthin-6-ones are principal constituents of the Picrolemma species [2–5]. Canthin-6-ones are a subclass of tryptophan-derived β-carboline alkaloids, and are characterized by an additional ring, D, giving the 6H-Indolo(3,2,1-de)(1,5) naphthyridin backbone. A general biosynthetic pathway of canthin-6-one alkaloids starts from tryptophan as a precursor and produces tryptamine which condense with acetic or ketoglutarate units, giving rise to a series of β-carboline intermediates, each time more oxidized. Except canthin-6-one itself, which has a simple structure, all the canthin-6-one alkaloids isolated from plants are oxidized at any position from C-1 to C-11 of the skeleton to form hydroxy and/or methoxy derivatives [1,6–9]. Meanwhile, more than 60 canthin-6-one alkaloids have been isolated from natural sources, mainly plants from the Rutaceae and Simaroubaceae families [10]. A broad range of biological activities has been reported for canthin-6-ones, such as antitumor, antibacterial, antifungal, antiparasitic, antiviral, anti-inflammatory, antiproliferative, and aphrodisiacal properties [11]. In this paper, we report the results of an investigation of the stem barks of Picrolemma huberi. Three canthinone alkaloids have been isolated; one of which is new, Figure 1. All of these alkaloids are reported for the first time from the genus Picrolemma.
Identification of compound 1 from the *Picrolemma huberi* bark. Compound 1, named Huberine, was isolated as an amorphous, pale-yellow solid. The HR LCMS spectrum of 1 showed a pseudomolecular ion peak, \([M + H]\) at \(m/z\) 281.0926, corresponding to a molecular formula \(C_{16}H_{12}N_{2}O_{3}\). A positive Dragendorff test was obtained, suggesting that 1 was an alkaloid. IR absorption bands of conjugated carbonyl group were observed at 1664 cm\(^{-1}\) and unsaturation 1630 and 1598 cm\(^{-1}\). The UV spectrum of 1 displayed absorption maxima at 227, 296, 356, and 376 nm, which were similar to those reported for canthin-6-one alkaloids [12]. The \(^{13}\)C-NMR and DEPT-NMR spectra for 1 indicated the presence of 16 carbon signals, including two methoxyls, six methines and eight quaternary carbon signals. All the proton and protonated carbon signals of 1 were assigned unambiguously by an 2D-HSQC (Heteronuclear Single-Quantum Correlation) experiment. In the \(^1\)H-NMR spectrum (Table 1), four mutually coupled aromatic protons at \(\delta 8.67\) (1H, d, \(J = 8.1\) Hz, H-8), \(\delta 7.66\) (1H, t, \(J = 7.6\) Hz, H-9), \(\delta 7.51\) (1H, t, \(J = 7.7\) Hz, H-10) and \(\delta 8.22\) (1H, d, \(J = 7.6\) Hz, H-11) were observed in the \(^1\)H-\(^1\)H COSY spectrum, meaning that the ring A of compound 1 is not substituted.

### Table 1. \(^1\)H-NMR (600 MHz) and \(^{13}\)C-NMR (125 MHz) spectral data of compound 1 in CDCl\(_3\) (\(\delta\), in ppm, \(J\) in Hz).

<table>
<thead>
<tr>
<th>Position</th>
<th>(^1)H (ppm), (J) (Hz)</th>
<th>(^{13})C (ppm)</th>
<th>COSY Coupling</th>
<th>HMBC Coupling((2,3)J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>141.3</td>
<td></td>
<td></td>
<td>OCH(_3)</td>
</tr>
<tr>
<td>2</td>
<td>155.1</td>
<td></td>
<td></td>
<td>OCH(_3)</td>
</tr>
<tr>
<td>4</td>
<td>7.83(d, 9.6)</td>
<td>138.2</td>
<td>H-5</td>
<td>H-5</td>
</tr>
<tr>
<td>5</td>
<td>6.82(d, 9.6)</td>
<td>125.6</td>
<td>H-4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>160.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.67(d, 8.1)</td>
<td>117.4</td>
<td>H-9</td>
<td>H-9</td>
</tr>
<tr>
<td>9</td>
<td>7.66 (t, 7.6)</td>
<td>130.4</td>
<td>H-8, H-10</td>
<td>H-11</td>
</tr>
<tr>
<td>10</td>
<td>7.51 (t, 7.7)</td>
<td>125.8</td>
<td>H-9, H-11</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>8.22(d, 7.6)</td>
<td>124.9</td>
<td>H-10</td>
<td>H-9</td>
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<tr>
<td>12</td>
<td>123.5</td>
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<td>13</td>
<td>140.1</td>
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<td>H-9, H-11</td>
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<td>14</td>
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<td>15</td>
<td>130.3</td>
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<td></td>
<td>H-4</td>
</tr>
<tr>
<td>16</td>
<td>126.4</td>
<td></td>
<td></td>
<td>H-5</td>
</tr>
<tr>
<td>OCH(_3)</td>
<td>4.15 (s)</td>
<td>54.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCH(_3)</td>
<td>4.20 (s)</td>
<td>61.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Isolated vicinal doublets at \(\delta 7.83\) (1H, \(J = 9.6\) Hz, H-4) and \(\delta 6.82\) (1H, \(J = 9.6\) Hz, H-5) were characteristic of *cis*-coupled protons on the conjugated lactam ring of a canthin-6-one. A 2D-HMBC (Heteronuclear Multiple Bond Correlation) experiment further confirmed the structure of alkaloid 1. In the spectrum, cross-peaks were found for H-4 (\(\delta 7.83\)) with C-6 (\(\delta 160.1\)) and C-15 (\(\delta 130.3\)),
H-5 (δ 6.82) with C-16 (δ 126.4), showing that these are the quaternary carbons that join the C and D rings.

The placement of the methoxy groups was deduced from the HMBC experiments. The methoxy signals showed clear HMBC correlations with the C at 141.1 and 154.9, assigned as C-1 and C-2. The assignment of quaternary carbons was established by HSQC and HMBC spectral data. Thus, the structure of Huberine 1 was established as 1,2-dimethoxycanthin-6-one, which is reported here for the first time. The new compound 1 showed no effective antiplasmodial activity at concentrations evaluated (from 100 µg/mL to 1.56 µg/mL) in Plasmodium falciparum strain FCR-3.

Identification of compound 2 and 3 from the Picrolemma huberi bark. The structures of the known compounds 2 and 3 were identified as 1 hydroxycanthin-6-one (2) [13,14] and canthin-6-one (3) [15], by spectroscopic data (1H-NMR, 13C-NMR, 2D-NMR, and MS) and by comparison with published values. Although 1-hydroxycanthin-6-one (3) was isolated from the Simaroubaceae family, it has not been reported from P. huberi. Stigmasterol (4) was also isolated.

2.2. Antiplasmodial Activity In Vitro

The antiplasmodial activity of compounds 1, 2 and 3 was evaluated in vitro against the multi-resistant strain of FCR-3 of P. falciparum. In the concentrations evaluated (from 100 µg/mL to 1.5 µg/mL), they did not show any activity.

3. Materials and Methods

3.1. General Procedures

Spectra were recorded on the following instruments: UV: Shimadzu UV-250 UV-Visible spectrophotometer (Canby, OR, USA); IR: Perkin Elmer 1600 (Waltham, MA, USA); NMR: BRUKER 600 MHz (Silberstreifen, Rheinstetten, DE); HRMS to compound (1) were measured on a Xevo Q-Tof Waters® spectrometer (Milford, MA, USA) and MS of compounds 2, 3 and 4 were measured on a Nermag-Sidar R10-10C spectrometer (Argenteuil, FR) with a quadrupolar filter. All solvents, except those used for bulk extraction, were AR grade. Silica gel 60 F254 was used for column chromatography. Glass and aluminum-supported silica gel 60 F254 plates were used for preparative TLC. TLC spots were visualized under UV light (254 and 365 nm) after spraying with Dragendorff’s reagent for alkaloid detection.

3.2. Plant Material

The stem bark of P. huberi was collected from the village, La Guada Reserve, [coordinates: 06°52′006″ N to 75°08′49.9″ W, (1.662 msnm)], close to Amalfi, Antioquia, Colombia, in January 2017. A voucher specimen (Tobón Juan Pablo 2392) has been deposited in the Herbarium JAUM (Joaquin Antonio Uribe Botanic Garden of Medellín, Antioquia, Colombia).

3.3. Extraction and Isolation

Dried stem bark (1.5 kg) of P. huberi was defatted with n-hexane (3 L). The marc was extracted with MeOH-H2O (90:10) (6 L) by percolation for 72 h and the same material was re-extracted in the same manner. The extract was filtered and concentrated up to 1 L under reduced pressure, and then partitioned with EtOAc (2 L). The EtOAc layer was dried over anhydrous Na2SO4 and then concentrated under reduced pressure (0.5 L). This extract (30 g) was initially subjected to an acid-base extraction [11] to give CHCl3 alkaloid (2.0 g).

The crude alkaloid (2.0 g) was subjected to column chromatography over silica gel using CH2Cl2 gradually enriched with methanol as eluent to yield ten fractions (A–J). Fraction A (102 mg) was chromatographed on a silica gel column and eluted with DCM-AcOEt (1:1) to give six subfractions, A1–A6. Fraction A1 (75 mg) was chromatographed by preparative TLC with CH2Cl2-MeOH (95:5) and further purified by preparative RP-HPLC using the mobile phase
CH₂CN/H₂O (90:10), 0.1% formic acid to yield compound 1 (10 mg). Similar HPLC of fraction A2 (15.0 mg) yielded compound 2 (1.3 mg) eluting at 13.2 min, and HPLC of fraction A3 (38.0 mg) yielded compound 3 (1.0 mg) eluting at 9.5 min.

Antiplasmodial in vitro activity assay of each compound (from 100 µg/mL to 1.5 µg/mL) was evaluated in FCR3 strain. The diphosphate salt of chloroquine (≥98%, SIGMA C6628), evaluated in a range of 2000 nM to 2.3 nM, was used as a treatment control in each assay [16].

3.4. Spectral Data

**Huberine: 1,2-Dimethoxy-canthin-6-one (1).** Yellow amorphous powder; UV (MeOH, max nm): 207, 266, 294, 354, 369. IR (KBr, cm⁻¹): 1664, 1550, 1439, 1214, 1086, 753; ¹H- and ¹³C-NMR data, see Table 1; MS: Waters LCT Premier (ESI-TOF) spectrometer at m/z 281.0926 [M + H]⁺; calcd. for C₁₆H₁₂N₃O₅, 281.0926.

**1-Hydroxy-canthin-6-one (2).** Yellow amorphous powder. UV (MeOH, nm): 210, 249, 256,288, 341, 415. IR (KBr, cm⁻¹): 3276, 1567, 1600, 1629. ¹H-NMR (600 MHz, MeOD-d₄, δ in ppm, J, Hz): 8.60 (d, 1H, 8.4Hz), 8.35 (s, 1H), 8.27 (d, 1H, 7.7Hz), 8.02 (d, 1H, 9.7Hz), 7.70 (t, 1H, 7.6Hz), 7.59 (t, 1H, 9.7Hz), 7.02 (1H, d, J = 7.8 Hz, H-1), 8.86 (1H, d, J = 9.7 Hz, H-2), 8.7 (1H, d, J = 9.7 Hz, H-4), 7.02 (1H, d, J = 9.7 Hz, H-5), 8.55 (1H, d, J = 8.1 Hz, H-8), 7.79 (1H, t, J = 7.6 Hz, H-9), 7.62 (1H, t, J = 7.6 Hz, H-10), 8.42 (1H, d, J = 7.8 Hz, H-11). MS TOF ES⁺ spectrometer at m/z 237.0708 [M + H]⁺.

**Canthin-6-one (3).** Yellow amorphous powder. UV (MeOH, nm): 210, 249, 256,288, 341, 415. IR (KBr, cm⁻¹): 3276, 1567, 1600, 1629. ¹H-NMR (600 MHz, DMSO-d₆, δ, ppm, J/Hz): 8.35 (1H, d, J = 4.8 Hz, H-1), 8.86 (1H, d, J = 4.8 Hz, H-2), 8.7 (1H, d, J = 9.7 Hz, H-4), 7.02 (1H, d, J = 9.7 Hz, H-5), 8.55 (1H, d, J = 8.1 Hz, H-8), 7.79 (1H, t, J = 7.6 Hz, H-9), 7.62 (1H, t, J = 7.6 Hz, H-10), 8.42 (1H, d, J = 7.8 Hz, H-11). MS TOF ES⁺ spectrometer at m/z 221.0715 [M + H]⁺. Supplementary material is available online.

4. Conclusions

Huberine, a new canthin-6-one alkaloid (1) and 3 known compounds (2, 3 and 4) were isolated from the stem bark of *P. huberi*. The structure of the new compound (1) was elucidated by spectroscopic data Huberine (1); it was isolated from this plant for the first time. The isolates were screened for inhibitory activity against *Plasmodium falciparum* strains. Compounds 1, and 2 showed no effective antiplasmodial activity.

**Supplementary Materials:** The following are available online. Figure S1: Huberine: 1,2-Dimethoxy-canthin-6-one (1). ¹H-NMR (CDCl₃, 600 MHz); Huberine: 1,2-Dimethoxy-canthin-6-one (1). ¹³C-NMR (CDCl₃, 150 MHz); Huberine: 1,2-Dimethoxy-canthin-6-one (1). COSY H-H (CDCl₃); Huberine: 1,2-Dimethoxy-canthin-6-one (1). HSQC (CDCl₃); Huberine: 1,2-Dimethoxy-canthin-6-one (1). HMBC (CDCl₃); Huberine: 1,2-Dimethoxy-canthin-6-one (1). HRMS; Huberine: 1,2-Dimethoxy-canthin-6-one (1). FT-IR, 1-Hydroxy-canthin-6-one (2). ¹H-NMR (MeOD-d₄, 600 MHz); 1-Hydroxy-canthin-6-one (2). ¹³C-NMR (DMSO-d₆, 150 MHz); 1-Hydroxy-canthin-6-one (2). COSY H-H (MeOD-d₄); 1-Hydroxy-canthin-6-one (2). HSQC (MeOD-d₄); 1-Hydroxy-canthin-6-one (2). HRMS; Canthin-6-one (3). ¹H-NMR (DMOS-d₆, 600 MHz); Canthin-6-one (3). HRMS.

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**Author Contributions:** C.L.: Identification of the compounds from *P. huberi* and writing of the manuscript; M.P.: Preparation of extracts, isolation and purification of the compounds; A.R.: Conducting biological tests; A.C.: Identification, classification, collection and knowledge of the plant; A.P.: Conception, design, analysis and interpretation of results, and writing of the manuscript

**Conflicts of Interest:** The authors declare no conflict of interest.
References


Sample Availability: Samples of the compounds 1, 2 and 3 are available from the authors.

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