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

**Carlos López [1], Manuel Pastrana [2], Alexandra Ríos [2], Alvaro Cogollo [3] and Adriana Pabón [2,*]**

1 Instituto de Química, Universidad de Antioquia, Medellín 050010, Colombia; carlopez.udea@gmail.com
2 Grupo Malaria, Facultad de Medicina, Universidad de Antioquia, Medellín 050010, Colombia; manuel.pastrana@udea.edu.co (M.P.); alexrioso@hotmail.com (A.R.)
3 Jardín Botánico Joaquín Antonio Uribe, Medellín 050010, Colombia; cogolloi@yahoo.com
* Correspondence: adriana.pabon@udea.edu.co

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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 *Picrolema 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*.

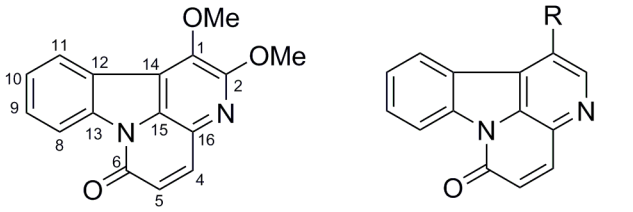


**Figure 1.** Canthin-6-one alkaloids isolated from *Picrolemma huberi* bark.

## 2. Results and Discussion

### *2.1. Identification of Isolated Compounds*

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 $C_{16}H_{12}N_2O_3$. 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 δ 8.67 (1H, d, *J* = 8.1 Hz, H-8), δ 7.66 (1H, t, *J* = 7.6 Hz, H-9), δ 7.51 (1H, t, *J* = 7.7 Hz, H-10) and δ 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 $CDCl_3$ (δ, in ppm, *J* in Hz).

| Position | $^1$H (ppm), *J* (Hz) | $^{13}$C (ppm) | COSY Coupling | HMBC Coupling($^{2,3}J$) |
|---|---|---|---|---|
| 1 | | 141.3 | | $OCH_3$ |
| 2 | | 155.1 | | $OCH_3$ |
| 4 | 7.83(d, 9.6) | 138.2 | H-5 | H-5 |
| 5 | 6.82(d, 9.6) | 125.6 | H-4 | |
| 6 | | 160.1 | | H-4 |
| 8 | 8.67(d, 8.1) | 117.4 | H-9 | H-9 |
| 9 | 7.66 (t, 7.6) | 130.4 | H-8, H-10 | H-11 |
| 10 | 7.51 (t, 7.7) | 125.8 | H-9, H-11 | |
| 11 | 8.22(d, 7.6) | 124.9 | H-10 | H-9 |
| 12 | | 123.5 | | H-10 |
| 13 | | 140.1 | | H-9, H-11 |
| 14 | | 130.3 | | |
| 15 | | 130.3 | | H-4 |
| 16 | | 126.4 | | H-5 |
| $OCH_3$ | 4.15 (s) | 54.7 | | |
| $OCH_3$ | 4.20 (s) | 61.3 | | |

Isolated vicinal doublets at δ 7.83 (1H, *J* = 9.6 Hz, H-4) and δ 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 (δ 7.83) with C-6 (δ 160.1) and C-15 (δ 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 ($^{1}$H-NMR,$^{13}$C-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 (Joaquín 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-$H_2O$ (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 $Na_2SO_4$ and then concentrated under reduced pressure (0.5 L). This extract (30 g) was initially subjected to an acid-base extraction [11] to give $CHCl_3$ alkaloid (2.0 g).

The crude alkaloid (2.0 g) was subjected to column chromatography over silica gel using $CH_2Cl_2$ 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 $CH_2Cl_2$-MeOH (95:5) and further purified by preparative RP-HPLC using the mobile phase

$CH_3CN/H_2O$ (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, n, $cm^{-1}$): 1664, 1550, 1439, 1214, 1086, 753; $^1$H- and $^{13}$C-NMR data, see Table 1; MS: Waters LCT Premier (ESI-TOF) spectrometer at *m*/*z* 281.0926 $[M + H]^+$; calcd. for $C_{16}H_{12}N_2O_3$, 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. $^1$H-NMR (600 MHz, MeOD-$d_4$, δ in ppm, *J*), 8.60 (d, 1H, 8.4Hz), 8.35 (s, 1H), 8.27 (d, 1H, 7.7Hz), 8.02 (d, 1H, 9.7Hz), 7.69 (t, 1H, 8.4Hz), 7.58 (t, 1H, 8.0Hz), 6.80 (d, 1H, 9.7Hz). $^{13}$C-NMR (150 MHz, DMSO-$d_6$, δ in ppm): 151.39 (C-1), 135.55 (C-2), 139.48 (C-4), 123.38 (C-5), 159.66 (C-6), 123.60 (C-8), 125.79 (C-9), 129.16 (C-10), 116.22 (C-11), 137.61 (C-12), 137.5 (C-13), 114.31(C-14), 133.32 (C-15), 128.11 (C-16). 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. $^1$H-NMR (600 MHz, DMSO-$d_6$, 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**). $^1$H-NMR ($CDCl_3$, 600 MHz); Huberine: 1,2-Dimethoxy-canthin-6-one (**1**). $^{13}$C-NMR ($CDCl_3$, 150 MHz); Huberine: 1,2-Dimethoxy-canthin-6-one (**1**). COSY H-H ($CDCl_3$); Huberine: 1,2-Dimethoxy-canthin-6-one (**1**). HSQC ($CDCl_3$); Huberine: 1,2-Dimethoxy-canthin-6-one (**1**). HMBC ($CDCl_3$); 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**). $^1$H-NMR (MeOD-d4, 600 MHz); 1-Hydroxy-canthin-6-one (**2**). $^{13}$C-NMR (DMSO-$d_6$, 150 MHz); 1-Hydroxy-canthin-6-one (2). COSY H-H (MeOD-$d_4$); 1-Hydroxy-canthin-6-one (**2**). HSQC (MeOD-$d_4$); 1-Hydroxy-canthin-6-one (**2**). HRMS; Canthin-6-one (**3**). $^1$H-NMR (DMOS-$d_6$, 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


1. Ohmoto, T.; Koike, K. *The Alkaloids*; Brossi, A., Ed.; Academic: New York, NY, USA, 1989; Volume 36, pp. 135–170.
2. Tischler, M.; Cardellina, J.H., II; Boyd, M.R.; Cragg, G.M. Cytotoxic quassinoids from *Cedronia granatensis*. *J. Nat. Prod.* **1992**, *55*, 667–671. [CrossRef] [PubMed]
3. Fo, E.R.; Fernandes, J.B.; Vieira, P.C.; da Silva, M.F.G.F. Canthin-6-one alkaloids from *Picrolemma granatensis*. *Phytochemistry* **1992**, *31*, 2499–2501. [CrossRef]
4. Rodrigues-Filho, E.; Fernandes, J.B.; Vieira, P.C.; Silva, M.F.G.F. Quassinoids and tetranortriterpenoids from *Picrolemma granatensis*. *Phytochemistry* **1993**, *34*, 501–504. [CrossRef]
5. Fo, E.R.; Fernandes, J.B.; Vieira, P.C.; da Silva, M.F.G.F.; Zukerman-Schpector, J.; Corrêa de Lima, R.M.O.; Nascimento, S.C.; Thomas, W. Protolimonoids and quassinoids from *Picrolemma granatensis*. *Phytochemistry* **1996**, *43*, 857–862. [CrossRef]
6. Haynes, H.F.; Nelson, E.R.; Price, J.R. Alkaloids of the Australian Rutaceae: *Pentaceras australis* Hook. FI Isolation of the alkaloids and identification of canthin-6-one. *Aust. J. Chem.* **1952**, *5*, 387–400. [CrossRef]
7. Crespi-Perellino, N.; Guicciardi, A.; Malyszko, G.; Minghetti, A. Biosynthetic relationship between indole alkaloids produced by cell cultures of *Ailanthus altissima*. *J. Nat. Prod.* **1986**, *49*, 814–822. [CrossRef]
8. Aragozzini, F.; Maconi, E.; Gualandris, R. Evidence for involvement of ketoglutarate in the biosynthesis of canthin-6-one from cell cultures of *Ailanthus altissima*. *Plant Cell Rep.* **1988**, *7*, 213–215. [CrossRef] [PubMed]
9. Anderson, L.A.; Hay, C.A.; Roberts, M.F.; Phillipson, J.D. Studies on *Ailanthus altissima* cell suspension cultures. *Plant Cell Rep.* **1986**, *5*, 387–390. [CrossRef] [PubMed]
10. Showalter, H.D.H. Progress in the synthesis of canthine alkaloids and ring-truncated congeners. *J. Nat. Prod.* **2013**, *76*, 455–467. [CrossRef] [PubMed]
11. Dai, J.; Li, N.; Wang, J.; Schneider, U. Fruitful Decades for Canthin-6-ones from 1952 to 2015: Biosynthesis, Chemistry, and Biological Activities. *Molecules* **2016**, *21*, 493. [CrossRef] [PubMed]
12. Ohmoto, T.; Koike, K.; Sakamoto, Y. Studies on the constituents of *Ailanthus altissima* Swingle. II. Alkaloidal constituents. *Chem. Pharm. Bull.* **1981**, *29*, 390–395. [CrossRef]
13. Suroor, A.K.; Shamsuddin, K.M. 1-Hydroxycanthin-6-one, an alkaloid from *Ailanthus giraldii*. *Phytochemistry* **1981**, *20*, 2062–2063.
14. Ohmoto, T.; Koike, K. Studies on the Constituents of *Ailanthus altissima* SWINGLE.III. The Alkaloidal Constituents. *Chem. Pharm. Bull.* **1984**, *32*, 170–173. [CrossRef]
15. Nafiah, M.A.; Mukhtar, M.R.; Omar, H.; Ahmad, K.; Morita, H.; Litaudon, M.; Awang, K.; Hadi, A.H. *N*-Cyanomethylnorboldine: A New Aporphine Isolated from Alseodaphneperakensis (Lauraceae). *Molecules* **2011**, *16*, 3402–3409. [CrossRef] [PubMed]
16. Pabón, A.; Ramirez, O.; Rios, A.; López, E.; De Las Salas, B.; Cardona, F.; Blair, S. Antiplasmodial and Cytotoxic Activity of Raw Plant extracts as reported by knowledgeable indigenous people of the amazon region (Vaupés Medio in Colombia). *Plant Med.* **2016**, *82*, 717–722. [CrossRef] [PubMed]


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