

# Aporphine Alkaloids from *Guatteria* spp. with Leishmanicidal Activity

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## Abstract

Fractionation of *Guatteria amplifolia* yielded the alkaloids xylopine (**1**), nornuciferine (**4**), lysicamine (**6**), and laudanosine (**5**). Fractionation of *Guatteria dumetorum* yielded the alkaloids cryptodrine (**2**) and nornantenine (**3**). Compounds **1–4** demonstrated significant activity against *Leishmania mexicana* and *L. panamensis*. Xylopine (**1**) was among the most active compounds (LD<sub>50</sub> = 3 μM) and showed a 37-fold higher toxicity towards *L. mexicana* than macrophages, the regular host cells of *Leishmania* spp.

The genus *Guatteria*, from the neotropical Annonaceae, has 279 species including *Guatteria amplifolia* Triana & Planch and *Guatteria dumetorum* R.E. Fr. [1]. Previous studies of *G. amplifolia* yielded aporphine alkaloids [2], [3], and extracts showed activity against *Leishmania* spp. [4]. We carried out fractionation of leaf extracts from *G. amplifolia* and *G. dumetorum* in order to find compounds to treat leishmaniasis, a parasitic disease for which plant natural products represent a potential source of new medicines [5], [6].

Following activity against the promastigote form of *Leishmania mexicana*, the aporphine alkaloids xylopine (**1**) and nornuciferine (**4**), the oxoaporphine alkaloid, lysicamine (**6**) and the tetrahydrobenzylisoquinoline alkaloid, laudanosine (**5**) were isolated from *Guatteria amplifolia* (Fig. 1). From *Guatteria dumetorum*, aporphine alkaloids cryptodrine (**2**) and nornantenine (**3**) were isolated. This is the first report of alkaloids **1**, **5** and **6** from *G. amplifolia* and the first report of **5** from the Annonaceae family. Compounds **1** and **2** showed the greatest leishmanicidal activity (IC<sub>50</sub> = 3 μM against *L. mexicana*) followed by **3** and **4** (24 and 14 μM, respectively). Comparable results were obtained with *L. panamensis* (Table 1). Neither **5** nor **6** showed significant leish-

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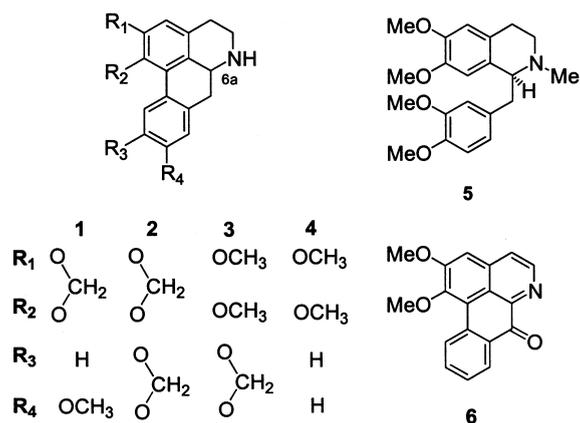


Fig. 1 Structures of xylopine (**1**), cryptodorine (**2**), normantenine (**3**), normuciferine (**4**), laudanosine (**5**) and lysicamine (**6**). The absolute configurations at position 6a for compounds **1–4** are as follows: (**1**) R, (**2**) S, (**3**) S, (**4**) S.

manicidal activity (data not shown). Compounds **1–4** were evaluated in macrophages and in human foreskin fibroblasts [15]. The Selectivity Index in Table 1 indicates a 37-fold higher toxicity of **1** towards *L. mexicana* than macrophages, the regular host cells of *Leishmania*.

## Materials and Methods

IR spectra were measured on a Perkin-Elmer 1699 spectrophotometer. NMR spectra were recorded on 300 and 500 MHz Bruker NMR spectrometers. Low resolution EIMS (70 eV) were collected on a Jeol SX 102A mass spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter.

Leaves of *Guatteria amplifolia* and *G. dumetorum* were collected in the Barro Colorado Natural Monument in the Republic of Panama. The taxonomy was confirmed by Professor Mireya Correa of the Smithsonian Tropical Research Institute. Vouchers were deposited at the University of Panama (*G. amplifolia* and *G. dumetorum* voucher numbers PMA 50979 and PMA 50980, respectively). Fresh young leaves were homogenized in 30 g aliquots with 240 mL of MeOH for 30 s in a Waring blender followed by treatment with a Polytron homogenizer (Brinkmann Instruments). After filtration, the marc was washed with 150 mL

of EtOAc. The crude extract (30 g) from *G. amplifolia* was partitioned between hexane and MeOH. The residue from the MeOH fraction (18.5 g) was partitioned between EtOAc and water. The EtOAc fraction was subjected to acid-base extraction yielding Fraction A (2.3 g). Fr. A was chromatographed on a column of basic alumina (4×40 cm) (Merck type T, 70–230 mesh) and eluted with CHCl<sub>3</sub> (700 mL), CHCl<sub>3</sub>/MeOH (75:25, 400 mL), CHCl<sub>3</sub>/MeOH (67:73, 750 mL), and 100% MeOH (400 mL), and the fractions were combined according to their TLC profile into Frs. 1–5. Fr. 1 yielded **5** (370 mg), tR of **5**: between 240–810 mL, Rf **5**: 0.65, detection of eluates by TLC (see below). Fr. 2 (1.03 g), between 810–1370 mL, was chromatographed by preparative reverse-phase TLC (RP-18, Merck) with CHCl<sub>3</sub>/acetone/NH<sub>4</sub>OH (4:1:0.1), yielding **4** (40 mg), Rf **4**: 0.57, and Fr. 2a (730 mg), Rf Fr. 2a: 0.5. Fr. 2a was subjected to preparative TLC (Whatman, PK5F, 500 μm) with CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH (2:1:1), yielding Fr. 2b (57 mg), Rf Fr. 2b: 0.42, which was purified by reverse-phase HPLC (YMC ODS-A S-5 μm, 4.6×250 mm) with MeOH/water/Et<sub>3</sub>N (70:30:0.1), 1.0 mL/min, yielding **1** (37 mg, tR of **1**: 20 min). Lysicamine (**6**) was obtained from 153 g of Fr. A by column chromatography on silica gel 60 (37–75 microns, 1.5×15 cm), 100% acetone (1300 mL), acetone/MeOH (91:9, 600 mL) yielding 90 mg of **6**, tR of **6**: between 950 and 1550 mL, Rf **6**: 0.22.

The crude extract (20 g) from *G. dumetorum* was subjected to solvent partition and acid-base extraction as described for *G. amplifolia* yielding fraction B (645 mg). Fr. B was chromatographed on a column of silica gel 60 (37–75 μm, 2.5×28 cm) and eluted with 800 mL of CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH (2:1:1), yielding a mixture of **2** and **3** (96 mg, tR of the mixture of **2** and **3**: between 190 mL–230 mL), which was then subjected to preparative TLC (Whatman, PK5F, 500 μm) and developed with CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH (2:1:1), yielding **2** (50 mg) and **3** (15 mg), Rf **2**: 0.25, Rf **3**: 0.20, monitoring of eluates by TLC (silica gel) CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH 2:1:1, detection Dragendorff reagent.

Structures for **1–6** were determined by optical rotation, MS, UV, IR, 1D and 2D <sup>1</sup>H-NMR and <sup>13</sup>C-NMR experiments and by comparisons with the literature data: **1** [7], [8], [9]; **2**, **3** [8], [9]; **4**, **6** [7], [9]; **5** [10], [11]. Compounds **1**: [α]<sub>D</sub><sup>22</sup>: –28.18° (MeOH, c 0.001); **2**: [α]<sub>D</sub><sup>22</sup>: +19.67° (CHCl<sub>3</sub>, c 0.001); **3**: [α]<sub>D</sub><sup>22</sup>: +85° (EtOH, c 0.75); **4**: [α]<sub>D</sub><sup>22</sup>: +138° (EtOH, c 0.22); **5**: [α]<sub>D</sub><sup>22</sup>: +43° (CHCl<sub>3</sub>, c 0.03). Copies of original spectra are obtainable from the author of correspondence.

Table 1 Activity<sup>a</sup> of alkaloids **1–4** and amphotericin B against *Leishmania* spp. and mammalian cell lines

Compound	IC <sub>50</sub> (μM)			HFF <sup>b</sup>	SI <sup>c</sup>
	<i>L. mexicana</i>	<i>L. panamensis</i>	Macrophages		
Xylopine ( <b>1</b> )	3 ± 0.27	6 ± 0.07	112 ± 0.2	115 ± 0.1	37.3, 38.3
Cryptodorine ( <b>2</b> )	3 ± 0.65	6 ± 0.08	64 ± 0.03	58 ± 0.07	21.3, 19.3
Normantenine ( <b>3</b> )	24 ± 0.03	15 ± 0.45	> 40	> 40	ND
Normuciferine ( <b>4</b> )	14 ± 1.0	28 ± 11.3	> 40	> 40	ND
Amphotericin B	0.1 ± 0.004	0.1 ± 0.004	ND	ND	ND

<sup>a</sup> Results are expressed as IC<sub>50</sub> (Inhibitory Concentration), the concentration of compound that inhibited 50% growth of the parasite or cell line. Mean values of the IC<sub>50</sub> (μM ± standard deviation) were determined by testing each concentration of sample in triplicate.

<sup>b</sup> Human foreskin fibroblasts.

<sup>c</sup> SI (Selectivity Index) is a measure of the IC<sub>50</sub> values obtained in macrophages and HFF, respectively, divided by the IC<sub>50</sub> in *L. mexicana*.

*L. mexicana* MOHM/B2/82/BELZ and *L. panamensis* MOHM/PA/71/LS 91 were cultured using established protocols [12]. The study used a colorimetric *Leishmania* spp. promastigote growth assay which is based upon the reduction of sodium 2,3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide (XTT) [13], and amphotericin-B was used as a positive control [14]. Cell toxicity was assessed by the reduction of XTT by either human foreskin fibroblasts or murine macrophages (cell line J774) [15].

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