

# Minor Alkaloids From *Gutteria dumetorum* with Antileishmanial Activity

Jhonny Edmuth Correa<sup>1</sup>, Carlos Hernán Ríos<sup>1</sup>, Amparo del Rosario Castillo<sup>2</sup>, Luz I. Romero<sup>2</sup>, Eduardo Ortega-Barría<sup>2</sup>, Phyllis D. Coley<sup>3,4</sup>, Thomas A. Kursar<sup>3,4</sup>, Maria Verónica Heller<sup>4</sup>, William H. Gerwick<sup>5</sup>, Luis Cubilla Rios<sup>1</sup>

## Abstract

Nine known alkaloids [(+)-isodomeesticine (**1**), (+)-norisodomeesticine (**2**), (+)-nantenine (**3**), (+)-neolitsine (**4**), (+)-lirioferine (**5**), (+)-*N*-methyllaurotetanine (**6**), (+)-norlirioferine (**7**), (+)-isoboldine (**8**) and (+)-reticuline (**9**)] were isolated from young leaves of *Gutteria dumetorum*. Their structures were confirmed by NMR, mass and UV spectral analysis and by comparison to literature data. The growth inhibitory activity of each alkaloid was determined against the parasite *Leishmania mexicana*. Compounds **1–4** all showed significant activity whereby potency increased when a methylenedioxy functionality was present, especially at the 1,2-positions.

**Supporting information** available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

Our previous studies on *G. dumetorum* yielded several aporphine alkaloids that showed significant activity against *Leishmania* spp. [1]. Because these metabolites may be potentially useful in the treatment of leishmaniasis, a pleomorphic disease that affects more than 12 million people in 88 countries worldwide [2], we conducted additional isolation efforts on the minor alkaloids of this species.

A standard alkaloid extraction protocol was used to obtain an extract of young leaves of *G. dumetorum*, which was fractionated by VLC, preparative TLC and HPLC to obtain compounds **1–9**. Their structures were deduced using standard NMR and MS methods and by comparison to literature data. This is the first report on the identification of alkaloids **1–9** from *G. dumetorum* (Fig. 1).

**Affiliation:** <sup>1</sup> Laboratorio de Bioquímica Tropical, Universidad de Panamá, República de Panamá · <sup>2</sup> Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, República de Panamá · <sup>3</sup> Department of Biology, University of Utah, XXX, Utah, USA · <sup>4</sup> Instituto Smithsonian de Investigaciones Tropicales, República de Panamá · <sup>5</sup> College of Pharmacy, Oregon State University, Corvallis, Oregon, USA

**Correspondence:** Dr. Luis Cubilla Rios · Departamento de Química Orgánica · Apdo. 0824–10835 · Universidad de Panamá · Panama City · Republic of Panamá · Phone: +507-6681-5371 · Fax: +507-264-4450 · E-mail: [lucr@ancon.up.ac.pa](mailto:lucr@ancon.up.ac.pa)

**Received:** May 14, 2005 · **Accepted:** July 22, 2005

**Bibliography:** *Planta Med* 2006; 72: 270–272 © Georg Thieme Verlag KG Stuttgart · New York · DOI 10.1055/s-2005-916179 · Published online December 5, 2005 · ISSN 0032-0943

All nine of these metabolites were tested against the promastigote form of *L. mexicana* in an *in vitro* assay. From the activities observed (Table 1) and those reported for cryptodrine (**10**), nor-nantenine (**11**) and xylopine (**12**) [1], we found the highest antileishmanial activity for alkaloids with two methylenedioxy functionalities (**4**: 15  $\mu$ M and **10**: 3  $\mu$ M). Similar trends have been observed previously for other methylenedioxy compounds tested against different human diseases [3], [4], including several camptothecin analogues tested in *Leishmania donovani* [5]. The replacement of methylenedioxy functionalities with either hydroxy or methoxy moieties leads to a significant decrease in activity (**5**: 210  $\mu$ M; **6**: 395  $\mu$ M; **7**: > 916  $\mu$ M and **8**: > 916  $\mu$ M). Furthermore, a 1,2-methylenedioxy function (i.e., in **12**: 3  $\mu$ M) increases the activity more than a 9,10-methylenedioxy functionality (**1**: 73  $\mu$ M; **2**: 48  $\mu$ M; **3**: 41  $\mu$ M and **11**: 15  $\mu$ M). Additionally, an *N*-methyl group appears to moderately decrease the activity of agents in this series (e.g., **1** vs. **2** and **4** vs. **10**).

Compounds **1–9** were also evaluated for toxicity to murine macrophages, the normal host cell type for the *Leishmania* parasite, and to VERO cells, a cell line derived from African green monkey kidney. All of these alkaloids showed low toxicity (> 300  $\mu$ M) in both cell types (Table 1). Indeed, the most potent antileishmanial compound, alkaloid **4**, showed a high selectivity index to *L. mexicana* over murine macrophages (25-fold). The results presented here provide further insights into the structure-activity relationships of aporphine alkaloids against the *Leishmania* parasite and could be useful in designing more potent and selective agents against this devastating disease.

## Materials and Methods

**Instrumentation:** <sup>1</sup>H- (300 MHz), <sup>13</sup>C- (75 MHz) and 2D-NMR spectra were measured on a Bruker Avance 300 spectrometer in CDCl<sub>3</sub> (**3**, **4**, **5** and **6**), MeOD (**1**, **2**, **7** and **9**) and deuterated DMSO (**8**). HR-MS were obtained on a Kratos MS50TC instrument. Optical rotations were determined on an Autopol III 6971 automatic polarimeter.

**Plant material and isolation:** Young leaves of *G. dumetorum* were collected, stored and extracted as described previously [1]. The crude extract (53.5 g) was subjected to acid-base extraction (EtOAc/HCl 5%) to yield the alkaloid fraction (3.7 g). It was subjected to VLC on silica gel 60 (37–75  $\mu$ m, 3.5×6.5 cm) eluting with CHCl<sub>3</sub>/EtOAc/NH<sub>4</sub>OH 75:0:25 (450 mL), 50:25:25 (150 mL) and MeOH (200 mL). Fractions were combined according to their TLC profiles into fractions A–D.

Fraction B (1156 mg), 100–400 mL, was subjected to PTLC (Whatman, PK5F, 500  $\mu$ m) and developed with CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH, 50:25:25, to yield the mixtures B1 (333 mg, R<sub>f</sub> = 0.2) and B2 (57 mg, R<sub>f</sub> = 0.8). Fraction B1 was chromatographed on reversed-phase HPLC (YMC ODS S-5  $\mu$ m, 10×150 mm) with MeOH/H<sub>2</sub>O/Et<sub>3</sub>N, 70:30:0.1, 1.5 mL/min, yielding **1** (8 mg; t<sub>R</sub> = 10 min) and **5** (21 mg; t<sub>R</sub> = 12 min). Fraction B2 was chromatographed on reversed-phase HPLC (YMC ODS S-5  $\mu$ m, 25×200 mm) with MeOH/H<sub>2</sub>O/Et<sub>3</sub>N, 70:30:0.1, 6.0 mL/min, yielding **3** (12 mg; t<sub>R</sub> = 32 min) and **4** (17 mg, t<sub>R</sub> = 44 min).

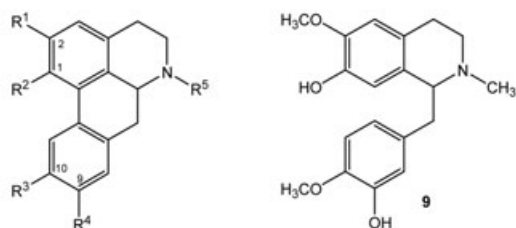


Fig. 1 Structures of alkaloids 1–12.

	1	2	3	4	5	6	7	8	10	11	12
R <sup>1</sup>	OH	OH	OCH <sub>3</sub>	O	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	O	OCH <sub>3</sub>	O
R <sup>2</sup>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	O	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OH	O	OCH <sub>3</sub>	O
R <sup>3</sup>	O	O	O	O	OH	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	O	O	H
R <sup>4</sup>	O	O	O	O	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	OH	O	O	OCH <sub>3</sub>
R <sup>5</sup>	CH <sub>3</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	CH <sub>3</sub>	H	H	H

Table 1 Growth inhibitory activity of alkaloids 1–9 and amphotericin B against *L. mexicana* and mammalian cell lines<sup>a</sup>

Compound	IC <sub>50</sub> (μM)			SI <sup>b</sup>
	<i>L. mexicana</i>	Macrophages	VERO Cells	
Isodomesticine (1)	73.3 ± 0.2	415 ± 46	> 611	5.68
Norisdodomesticine (2)	48.2 ± 2.6	> 642	> 642	> 13.4
Nantenine (3)	41.0 ± 0.4	315 ± 38	345 ± 24	7.68
Neolitsine (4)	15.4 ± 1.6	387 ± 37	> 619	25.1
Lirioferine (5)	210 ± 9.1	401 ± 29	> 586	1.91
N-Methylaurotetanine (6)	395 ± 35	331 ± 12	> 586	0.84
Norlirioferine (7)	> 916	406 ± 9	428 ± 36	ND <sup>c</sup>
Isoboldine (8)	> 916	> 610	538	ND <sup>c</sup>
Reticuline (9)	518 ± 23	> 668	> 668	> 1.23
Amphotericin B	0.1 ± 0.005	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>

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

<sup>b</sup> The SI (= selectivity index) is obtained by dividing the IC<sub>50</sub> value obtained in macrophages by the IC<sub>50</sub> in *L. mexicana*.

<sup>c</sup> ND = not determined.

Fraction C (1841 mg), 400–800 mL, was subjected to VLC on silica gel 60 (37–75 μm, 3.5×15 cm) eluting with CHCl<sub>3</sub>/MeOH, 95:5 (400 mL), 92:8 (100 mL), 90:10 (200 mL), 85:5 (100 mL), 80:20 (300 mL), 75:25 (100 mL), 70:30 (400 mL), 50:50 (400 mL), 40:60 (100 mL), 25:75 (200 mL), 15:85 (100 mL) and 100% MeOH (200 mL). Fractions were combined according to their TLC profiles into fractions C1 – C9. Fractions C2 (84 mg), 400–500 mL, and C3 (44 mg), 500–700 mL, were separately chromatographed on reversed-phase HPLC (YMC ODS S-5 μm, 2×250 mm) eluting with MeOH/H<sub>2</sub>O/Et<sub>3</sub>N, 70:30:0.1, 2 mL/min, yielding from C2 alkaloid 6 (40 mg, t<sub>R</sub> = 32 min) and from C3 fractions C3A – C3C. Fraction C3B (25 mg, t<sub>R</sub> = 11 min) was chromatographed on amine-phase HPLC (YMC NH<sub>2</sub> S-5 μm, 4×150 mm) eluting with CHCl<sub>3</sub>/MeOH, 96:4, 4 mL/min to yield 8 (12 mg, t<sub>R</sub> = 12 min).

Fraction C7 (323 mg), 2000–2100 mL, was submitted to PTLC (Whatman, PK5F, 500 μm) and developed with a mixture of CHCl<sub>3</sub>/hexane/NH<sub>4</sub>OH (50:25:25) with MeOH (93:7) to yield fractions C7A – C7G. Fractions C7E (25 mg; R<sub>f</sub> = 0.24), C7F (24 mg; R<sub>f</sub> = 0.29) and C7G (32 mg; R<sub>f</sub> = 0.53) were separately

submitted to reversed-phase HPLC (YMC ODS S-5 μm, 4×150 mm) eluting with MeOH/H<sub>2</sub>O/Et<sub>3</sub>N, 70:30:0.1, 2 mL/min; to yield 9 (5 mg, t<sub>R</sub> = 9 min), 2 (13 mg, t<sub>R</sub> = 9 min) and 7 (11 mg, t<sub>R</sub> = 7 min), respectively.

The structures for 1–9 were assigned by optical rotation, MS, 1D and 2D <sup>1</sup>H-NMR and <sup>13</sup>C-NMR experiments and by comparison to the literature data (compounds 1, 3, 4, 6, 8 with reference [6]; compound 2 with reference [7]; compound 5 with reference [8]; compounds 7, 9 with references [9], [10]). From the present study, the following rotations were measured: 1, [α]<sub>D</sub><sup>25</sup>: +111.7° (CHCl<sub>3</sub>, c 0.19); 2, [α]<sub>D</sub><sup>25</sup>: +61.0° (CHCl<sub>3</sub>, c 0.39); 3, [α]<sub>D</sub><sup>25</sup>: +72.5° (CHCl<sub>3</sub>, c 0.55); 4, [α]<sub>D</sub><sup>25</sup>: +58.2° (CHCl<sub>3</sub>, c 0.75); 5, [α]<sub>D</sub><sup>25</sup>: +73.6° (CHCl<sub>3</sub>, c 0.54); 6, [α]<sub>D</sub><sup>25</sup>: +56.6° (CHCl<sub>3</sub>, c 0.32); 7, [α]<sub>D</sub><sup>25</sup>: +87.2° (CHCl<sub>3</sub>, c 0.28); 8, [α]<sub>D</sub><sup>25</sup>: +30.6° (CHCl<sub>3</sub>, c 0.60); 9, [α]<sub>D</sub><sup>25</sup>: +84.7° (CHCl<sub>3</sub>, c 0.38). Copies of the original spectra are available from the corresponding author.

**Anti-leishmanial bioassay:** The anti-leishmanial activity of metabolites 1–9 was assessed by their effect on the growth of *Leishmania mexicana* (MOHM/B2/82/BELZ) promastigotes.

Growth was assayed by measuring the reduction of sodium 2,3-bis-[2-methoxy-4-nitro-5-sulphophenyl]-2*H*-tetrazolium-5-carboxanilide (XTT) [11]. Amphotericin-B was used as positive control [12], and the cell toxicity was assessed by measuring the reduction of XTT by murine macrophages (cell line J744) and VERO cells [13], [14].

## Acknowledgements

This work was supported by US NIH grant #1U01 TW00663401 from the International Cooperative Biodiversity Groups Program ICBG-Panama and by funds from the Smithsonian Tropical Research Institute.

## References

- 1 Montenegro H, Gutiérrez M, Romero LI, Ortega-Barría , Capson TL, Cubilla L. Aporphine alkaloids from *Guatteria* spp. with leishmanicidal activity. *Planta Med* 2003; 69: 677–9
- 2 Berman J. Recent developments in leishmaniasis: epidemiology, diagnosis, and treatment. *Curr Infect Dis Rep* 2005; 7: 33–8
- 3 Li D, Zhao B, Sim SP, Li TK, Liu A, Liu LF et al. 2,3-Dimethoxybenzo[*i*]phenanthridines: topoisomerase I-targeting anticancer agents. *Bioorg Med Chem* 2003; 11: 521–8
- 4 Iwasa K, Nishiyama Y, Ichimaru M, Moriyasu M, Kim HS, Wataya Y et al. Structure-activity relationships of quaternary protoberberine alkaloids having an antimalarial activity. *Eur J Med Chem* 1999; 34: 1077–83
- 5 Werbovetz A, Bhattacharjee K, Brendle J, Scovil P. Analysis of stereo-electronic properties of camptothecin analogues in relation to biological activity. *Bioorg Med Chem* 2000; 8: 1741–7
- 6 Guinaudeau H, Leboeuf M, Cave A. Aporphine alkaloids. *J Nat Prod* 1975; 38: 275–335
- 7 Hocquemiller R, Cave A, Raharisololalao A. Alcaloides de *Xylopya buxifolia* et de *Xylopya danguyella*. *J Nat Prod* 1981; 44: 551–6
- 8 Chen CL, Chang HM, Cowling E, Huang CY, Gates R. Aporphine alkaloids and lignans formed in response to injury of sapwood in *Liriodendron tulipifera*. *Phytochemistry* 1976; 15: 1161–7
- 9 Castro O, López J, Vergara A. Aporphine alkaloids from *Phoebe pittieri*. *Phytochemistry* 1985; 24: 203–4
- 10 Gellert E, Summons RE. Alkaloids of the genus *Cinnamomum*. II. Alkaloids of the bark of *Cinnamomum* sp. T.G.H. 13 077. *Aust J Chem* 1970; 23: 2095–9
- 11 Williams C, Espinosa OA, Montenegro H, Cubilla L, Capson TL, Ortega-Barría E et al. Hydrosoluble formazan XTT: its application to natural products drug discovery for *Leishmania*. *J Microbiol Methods* 2003; 55: 813–6
- 12 Golenser J, Frankenburg S, Enrenfreund T, Domb AJ. Efficacious treatment of experimental leishmaniasis with amphotericin B-arabinogalactan water-soluble derivatives. *Antimicrob Agents Chemother* 1999; 43: 2209–14
- 13 Shin IS, Tanifuji H, Arata Y, Morizawa Y, Nakayama T, Wataya Y. 3'-Deoxy-3'-fluorinosine as a potent antileishmanial agent. The metabolism and selective cytotoxic effect of 3'-deoxy-3'-fluorinosine against *Leishmania tropica* and *L. donovani* *in vitro* and *in vivo*. *Parasitol Res* 1995; 81: 622–6
- 14 Sahpaz S, Bories C, Loiseau PM, Cortes D, Hocquemiller R, Laurens A et al. Cytotoxic and antiparasitic activity from *Annona senegalensis* seeds. *Planta Med* 1994; 60: 538–40