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Antimalarial Activity and Cytotoxicity of (−)-Roemrefidine Isolated from the Stem Bark of *Sparattanthelium amazonum*

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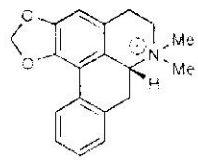
Abstract: (−)-Roemrefidine, an aporphine alkaloid isolated from *Sparattanthelium amazonum* Martius (Hernandiaceae) a vine from Bolivia, has been found to be active against both resistant and sensitive strains of *Plasmodium falciparum in vitro* and against *P. berghei* in mice. The compound demonstrated no cytotoxic activity against three cell lines (KB, HEp-2 and HeLa).

Decoction of the stem bark of *Sparattanthelium amazonum* Martius (Hernandiaceae), a vine of the South American subtropical rain forest, is used traditionally by the Chacobo. This native community lives in the Amazonian part of Bolivia and uses the plant for digestive problems, stomach ache, vomiting and diarrhoea. Extracts from the stem bark of *S. amazonum* exhibited antiplasmodial activity *in vitro* (1). Preliminary *in vitro* antimalarial assessment showed that the activity was concentrated in the crude alkaloid mixture extracted from the stem bark. Using a bioguided fractionation of this extract, we isolated (−)-roemrefidine (**1**), an aporphine alkaloid, isolated previously from species of Papaveraceae (2 - 4) and Menispermaceae (5), as the active compound. Identification of the alkaloid was carried out by comparison of their spectral data with those reported (2, 3).



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In a visual method based test (6), compound **1** possessed an *in vitro* antimalarial activity with an IC_{50} of 0.71 μM against the chloroquine sensitive strain 20/87 and of 0.58 μM against the chloroquine resistant strain INDO of *Plasmodium falciparum* (Table **1**). In order to determine at which stage of the *Plasmodium* erythrocytic cycle the alkaloid acts, parasite cultures of a synchronised resistant chloroquine strain FcB1 were incubated with compound **1** and radio-labelled hypoxanthine for two periods representative of the parasite erythrocytic cycle (7). The IC_{50} of (−)-roemrefidine (**1**) against

Compound	*P. falciparum* IC_{50} (μM)[1]		*P. berghei* ED_{50} (mg/kg/4day)	Cell lines tested (A) IC_{50} (μM)		
	2087 (B)	INDO (B')	NK65	KB (A/B, A/B')	Hep-2 (A/B, A/B')	HeLa (A/B, A/B')
(−)-roemrefidine	0.71	0.58	5.98	39.5 (56, 68)	50.0 (70, 86)	87.1 (123, 150)
chloroquine	0.06	0.22	0.98	> 70 (> 1167, > 318)	> 70 (> 1167, > 318)	50.3 (838, 229)

[1] Measurements were done in triplicate.

Table 1 Antimalarial activity against *P. falciparum in vitro* (strain 20/87 and INDO), *P. berghei in vivo* (strain NK65), cytotoxic activities (KB, Hep-2 and HeLa cells) and cytotoxic: antiplasmodial ratios (IC_{50} against mammalian cell lines / IC_{50} against parasite) of (−)-roemrefidine.

the strain FcB1, measured for these two periods was the same, 0.17 μM. This result shows that (−)-roemrefidine (**1**) acts on the level of parasite maturation, has no effect on the erythrocytic reinvasion and that there is no cumulative influence of the compound on the metabolic pathways of the parasite. Compound **1** demonstrated also an interesting antimalarial activity *in vivo* against a *P. berghei* strain with an ED_{50} value of 5.98 mg kg^{-1} day^{-1}. In addition, compound **1** exhibited a weak cytotoxicity and good cytotoxic:antiplasmodial ratios as defined by Phillipson (8) (Table **1**). Studies on aporphine-type alkaloids as possible antimalarial agents are currently under investigation.

Materials and Methods

Sparattanthelium amazonum was collected in October 1993 during ethnobotanical field work in the north-east region of Bolivia (Alto Ivón). A voucher specimen (Bergeron 819) has been deposited at the National Herbarium of La Paz, Bolivia.

Defatted dried stem bark (700 g) of *S. amazonum* was exhaustively extracted with 2 litres of EtOH (95 %) for 72 hours. The combined solutions were made alkaline with NH_4OH and extracted with dichloromethane to afford 0.8 g of crude alkaloid extract (1.14 %). The active alkaloid extract was chromatographed (CC) over Sephadex LH-20 (35 g, 1 × 92 cm), eluted with CH_2Cl_2 : MeOH (50 : 50) to give 5 fractions, each of 50 ml. The third fraction (523 mg), as the most active fraction, was chromatographed (CC) over Sephadex LH-20 (35 g, 1 × 92 cm), eluted with MeOH to give 12 fractions, each of 50 ml. Combined CC active fractions (3 – 5, 318 mg), were submitted to CC on polyamide (Ac-6) 50 g, 2 × 30 cm), eluted with CH_3OCH_3 : MeOH (90 : 10) to give 16 fractions, each of 50 ml. Active fractions were pooled together (5 – 7, 41 mg) and recrystallized from ethyl acetate : MeOH (70 : 30) leading to white crystals (31 mg) of **1** ($[\alpha]_D^{20}$: −26°, $CHCl_3$, c 0.5).

Antiplasmodial activity assays on extracts, fractions and pure alkaloid were carried out *in vitro*, in triplicate, using the visual method as described by Sauvain et al. (6). Unsynchronised cultures of a sensitive strain /87 and a chloroquine resistant strain INDO of *P. falciparum*, with a dominant trophozoite stage, were used.

The specificity of the (−)-roemrefidine (**1**) activity was measured with a radio-isotopic method using the ringstage form of a chloroquine resistant FcB1-Colombia *P. falciparum* strain. The plasmodia were synchronised by flotation on gelatine and lysed with 5 % *D*-sorbitol. The parasite cultures were incubated with the extract for 24 and 72 hours. These time periods correspond to the half time and the 1.5 time of the parasite erythrocytic life cycle (7).

A four day suppressive *in vivo* assay in mice was executed against *P. berghei* strain NK 65 (9). The cytotoxic potential of (−)-roemrefidine (**1**) was evaluated in three cell lines, utilizing sulforodamine B. Cell lines HeLa (uterine carcinoma), KB (human epidermoid carcinoma) and HEp-2 (human epidermoid carcinoma, larynx) were cultured as previously described (10). Full details of the isolation and biological procedures, including copies of the original spectra, are availalbe on request from the author of the correspondence.

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