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## Antimalarial constituents from *Guatteria amplifolia*

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In our search for natural substances with antimalarial activities from Colombian plants, we investigated the aerial parts of *Guatteria amplifolia* Tr. & Pl. (Annonaceae), a medium-sized tree distributed through the Occidental coast of Northern South America. In preliminary bioassays, we observed promising antimalarial activity with various extracts from this species.

Using chromatographic methods, we isolated four aporphine alkaloids identified as liriodenine, corydine, isocorytuberine and *O*-methyloschatoline. Structural elucidation was performed using IR, UV, MS and <sup>1</sup>H and <sup>13</sup>C NMR mono- and bidimensional spectroscopy. The spectral data for the four alkaloids are in accordance with those reported. Liriodenine, corydine and *O*-methyloschatoline have been previously isolated from various genera of the Annonaceae, and particularly from the genus *Guatteria* [1–3]. Isocorytuberine has been isolated from *Trivalvaria macrophylla* (Annonaceae) [4], but, to our knowledge, it is the first time that its occurrence in the genus *Guatteria* is reported.

The evaluation of the antimalarial activity of the purified alkaloids was performed *in vitro* against F32 (chloroquine sensitive) and D2 (chloroquine resistant) strains of *Plasmodium falciparum*. The results (Table) show good antimalarial activity for isocorytuberine and corydine, whereas liriodenine was notably less active and *O*-methyloschatoline inactive.

## Experimental

### 1. Plant material

A sample of aerial parts was collected during march 1996 in Bajo Calima, Colombia. A voucher was deposited (BW 030) at the Herbarium of the Universidad del Valle (Cali).

### 2. Extraction and isolation

Dried ground aerial parts (250 g) were extracted following the usual work-up procedure [5] to give 1.8 g of an alkaloidal mixture. This mixture was fractionated by CC on silica gel using CH<sub>2</sub>Cl<sub>2</sub>-MeOH (9:1) as eluent. Further purification was achieved by preparative TLC on silica gel using the same eluent, affording 12 mg of liriodenine, R<sub>f</sub> = 0.7, 15 mg of corydine, R<sub>f</sub> = 0.6, 18 mg of isocorytuberine, R<sub>f</sub> = 0.4, and 38 mg of *O*-methyloschatoline, R<sub>f</sub> = 0.8.

**Table: Antimalarial activity (μM) against F32-Tanzania (chloroquine sensitive) and D2 (chloroquine resistant) strains of *Plasmodium falciparum***

Compound	IC <sub>50</sub> μM	
	F32-Tanzania	D2
Isocorytuberine	3.05	4.88
Corydine	5.27	5.27
Liriodenine	54.5	36.3
<i>O</i> -Methyloschatoline	> 155	> 155

### 3. Biological assays

Culture of the D2 and F32-Tanzania strains of *Plasmodium falciparum* was carried out according to Trager and Jensen [6] on glucose-enriched RPMI 1640 medium supplemented with HEPES and 10% human serum at 37 °C. Dimethyl sulphoxide (50 µl) was added to samples of extracts which were then dissolved in RPMI 1640 medium with the aid of mild sonication in a sonicleaner bath, and further diluted as required in medium. The DMSO concentration for tested solution was no greater than 0.1%. 150 µl of total culture medium with the diluted extract and the suspension of human red blood cell in medium (O+, 5% haematocrit) with 1% parasitaemia, were placed into the wells of 96-well microtitre plates. The tests were performed in triplicate. After 24 h of incubation at 37 °C using the candle jar method, the medium was replaced daily by fresh medium with the diluted extract, and incubation was continued for a further 48 h. On the third day of the test, a blood smear was taken from each well and parasitaemia counted. The tests included an untreated control, control with solvent and extract of *Cinchona calisaya* as internal standard. The parasitaemia for each well was obtained and the % inhibition of parasitaemia for each concentration of extract was calculated in relation to the control. Linear regression analysis was used to determine the best fitting straight line from which IC<sub>50</sub> values were determined.

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