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SHORT COMMUNICATION

Antiprotozoal Activity of Aporphine Alkaloids Isolated from *Unonopsis buchtienii* (Annonaceae)

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On a preliminary screening, substantial leishmanicidal activity was observed for the petroleum ether and alkaloidal extracts of the stem bark of *Unonopsis buchtienii,* the alkaloids and sterols isolated from these were studied. Of the alkaloids, liriodenine exhibited the highest activity against *Leishmania major* and *L. donovani* (IC_{100} = 3.12 μg/mL). On the other hand, *O*-methylmoschatoline and the petroleum ether extract without alkaloids showed an interesting *in vitro* activity against *Trypanosoma brucei* with an IC_{100} of 6.25 μg/mL. The highest cytotoxic activities were found with the petroleum ether extracts without alkaloids and with all alkaloids isolated (IC_{50} < 9 μg/mL for Vero cell line).

INTRODUCTION

Cutaneous and mucocutaneous leishmaniasis are endemic diseases in South America, especially in the subandean areas of the humid lowlands of Bolivia. For Latin American countries comprising Amazonian areas, development of their vast wilderness regions has become a national priority. The impact of this disease was increased by the colonization of the subandean tropical regions of Bolivia. In a preliminary screening (Fournet *et al.*, 1994), the petroleum ether and alkaloidal extracts of the stem bark of *Unonopsis buchtienii* R. E. Fries (Annonaceae) displayed activity *in vitro* at 100 and 25 μg/mL, respectively, against three strains of promastigote forms of *Leishmania* species, *L. braziliensis, L. amazonensis* and *L. donovani* and three strains of another Trypanosomatidae, *Trypanosoma cruzi*, responsible for Chagas' disease.

In this paper we describe the isolation by activity-directed fractionation, of four alkaloids, two sterols from the stem bark of *U. buchtienii*, and also the *in vitro* biological activities of these compounds against two strains of *Leishmania* species, *L. major* and *L. donovani* and against *Trypanosoma brucei*, responsible for African trypanosomiasis. The cytotoxic activity of the crude extracts and six compounds isolated from *U. buchtienii* against the Vero cell line are also reported.

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MATERIALS AND METHODS

General experimental procedures. Optical rotations were determined on a Schmidt-Haensch Polartronic I polarimeter. UV spectra were obtained on a Philips PU 8720 spectrometer. IR spectra were measured on a Perkin-Elmer 257 spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra ($CDCl_3$) were obtained with Bruker AC-200 or AC-400 instruments at 200 and 50 MHz or at 400 and 100 MHz, respectively. EIMS and CIMS (methane) were performed on a Nermag R10-10C spectrometer.

Plant material. The stem of *Unonopsis buchtienii* R. E. Fries was collected by Alain Fournet (AF 879) in August 1988, in Bolivia near Fatima de Chimane, Department of Beni and identified by P. J. M. Mass (Institute of Systematic Botany, Utrecht, Netherlands). A voucher specimen has been deposited at the National Herbarium of Bolivia (La Paz).

Extraction and isolation. The air-dried powdered stems of *U. buchtienii* (367 g) were extracted with petroleum ether and then with dichloromethane. The dichloromethane extract (5.75 g) was treated with HCl (5%), and the acidic solution was basified with NH_4OH to pH 9–10 and extracted with CH_2Cl_2. Evaporation of the organic solvent under reduced pressure led to the alkaloid extract (200 mg). This extract was chromatographed on a silica gel column (Kieselgel H, Merck), eluted with $CHCl_3$ and an increasing amount of MeOH to provide four alkaloids, O-methylmoschatoline (**1**), lysicamine (**2**), liriodenine

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Table 1. Leishmanicidal effect of the crude extracts, alkaloids and sterols isolated from stem bark of *Unonopsis buchtienii* (IC_{100} µg/mL)

Extract or compound	*L. major*	*L. donovani*
Petroleum ether extract	50	50
Dichloromethane extract	100	100
O-methylmoschatoline (1)	50	50
Lysicamine (2)	25	25
Liriodenine (3)	3.12	3.12
Fraction containing Unonopsine (4)	25	25
β-Sitosterol (5)	>100	>100
Stigmasterol (6)	>100	>100
Pentamidine	5	5

Table 2. Activity of the crude extracts and alkaloids isolated from the stem bark of *Unonopsis buchtienii* against on *Trypanosoma brucei brucei* (IC_{100} µg/mL)

Extract or compound	After 1h	After 24 h
Petroleum ether extract	6.25	6.25
Dichloromethane extract	6.25	6.25
O-methylmoschatoline (1)	6.25	6.25
Lysicamine (2)	>200	>200
Liriodenine (3)	50	50
Mel W	5	0.05
Pentamidine	20	0.2

(3) and unonopsine (4). The petroleum ether extract was subjected to chromatography on silica gel, eluted with hexane and increasing amounts of EtOAc (5% to 100%), then EtOAC and an increasing amount of MeOH (10%) and furnished stigmasterol and β-sitosterol.

Leishmanicidal activity. Cultures of *Leishmania* ssp were obtained from the laboratory of parasitology of the Faculty of Pharmacy, Chatenay-Malabry, France. Two strains of *Leishmania* were used during these investigations: *L.major* (MHOM/PT/92/CRE26) and *L. donovani* (MHOM/ET/67/L82, LV 9) grown at 27 °C in RPMI 1640 medium containing 20% fetal bovine serum and 20% of Schneider's Drosophila medium. Compounds were dissolved in 5 µL of dimethyl sulphoxide (DMSO), then in medium and placed in microtitre plates in triplicate. The minimal amount (µg) of compound to inhibit the growth of *Leishmania* sp was evaluated after 4 days by observation with an inverted microscope by comparison with control cells and with reference compound (pentamidine) treated cells. The maintenance, cultivation and isolation of promastigote-stage parasites have been described in detail elsewhere (Sahpaz *et al.*, 1994). Pentamidine was used as a positive control (Fournet *et al.*, 1994). The IC_{100} is the dose required to produce no mobile parasites with negative subculture.

Trypanocidal activity. The *Trypanosoma brucei brucei* parasites (CMP strain) were isolated from previously infected CD1 female mice (Loiseau *et al.*, 1992; Kaminsky and Zweygarth, 1989). Cultures of *T. brucei brucei* were axenically performed in RPMI-1640 medium

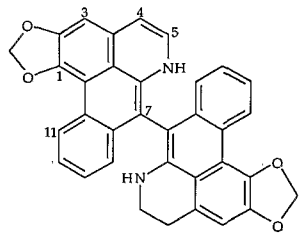

Figure 1. Structure of unonopsine.

with 10% fetal bovine serum containing 2 mM L-glucamine and 25 mM HEPES (Gibco).

Trypanosome cultures were kept at 37°C in 5% CO_2 in air atmosphere. Plant extracts and alkaloids were dissolved in cold DMSO to a final concentration of 200 µg/mL. Aliquots of each extract of different concentrations (100, 50, 25 and 12.5 µg/mL) were mixed in microtitre plates with 100 µL of infected blood containing different parasite concentrations. Infected blood and infected blood containing Mel W and pentamidine at 200 µg/mL were used as controls.

The growth inhibition test to determine the drug concentration which inhibits the trypanosomes by 100% (IC_{100}) value was defined as the minimum inhibitory which kills all parasites.

Cytotoxicity assay. The crude extracts and compounds were evaluated using a range of concentrations in the *in vitro* Vero cell system using the method described by Fleury *et al.*, (1984). Each experiment was carried out in triplicate, IC_{50} was given as the concentration (µg/mL) required for 50% inhibition cell growth.

RESULTS AND DISCUSSION

The petroleum extract of the stem bark of *U. buchtieniii* exhibited protozoal activities against two strains of *Leishmania* and *Trypanosoma brucei* (Tables 1 and 2). Two triterpenes isolated from this extract, β-sitosterol and stigmasterol did not exhibit any activity against *Leishmania* strains at a concentration of 100 µg/mL. Four alkaloids were isolated from the dichloromethane extract; three aporphine alkaloids, lysicamine, liriodenine and *O*-methylmoschatoline and a dimeric aporphine alkaloid, unonopsine (see Fig. 1). The two oxoaporphine alkaloids, *O*-methylmoschatoline and lysicamine had IC_{100} values of 50 and 25 mg/mL, respectively, and liriodenine, the most active alkaloid had an IC_{100} of 3.12 µg/mL. It was

Table 3. *In vitro* cytotoxic activity of crude extracts and alkaloids isolated from the stem bark of *Unonopsis buchtienii* against Vero cell lines

Extract or compound	IC_{50} (µg/mL)
Ether petroleum extract	28
Dichloromethane extract	35
Methanol extract	126
Ether petroleum extract without alkaloid	9
O-methylmoschatoline (1)	7
Lysicamine (2)	8
Liriodenine (3)	1