

Antileishmanial Activity of a Tetralone Isolated from *Ampelocera edentula*, a Bolivian Plant Used as a Treatment for Cutaneous Leishmaniasis

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Abstract

The stem bark of *Ampelocera edentula* Kuhl. (Ulmaceae) is used by the Chimanes Indians from Bolivia for the treatment of cutaneous leishmaniasis caused by the protozoan *Leishmania braziliensis*. A chloroform extract of the stem barks was found to be active against extracellular forms of *Leishmania* spp. and *Trypanosoma cruzi* at 50 µg/ml. Bioassay-guided fractionation of this extract allowed us to isolate one active compound. Its structure was elucidated by spectral and chemical studies as 4-hydroxy-1-tetralone. BALB/c mice infected with *L. amazonensis* (PH8) or *L. venezuelensis* were treated one day after the parasitic infection with 4-hydroxy-1-tetralone (25 mg/kg/day) or with reference drug, Glucantime® (56 mg Sb^v/kg/day) for 14 days. Lesion development was the criteria used to evaluate the disease severity. 4-Hydroxy-1-tetralone was slightly less effective than the reference drug against *L. amazonensis* or *L. venezuelensis*. Single treatment near the site of infection, 14 days after infection with *L. amazonensis*, with 4-hydroxy-1-tetralone (50 mg/kg) was more effective than Glucantime (112 mg/kg). This study is, to our knowledge, the first to show the activity of a tetralone for the experimental treatment of New World cutaneous leishmaniasis.

Key words

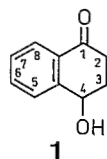
Ampelocera edentula, Ulmaceae, cutaneous leishmaniasis, tetralone, *Leishmania* spp., *Trypanosoma cruzi*, leishmanicidal activity, trypanocidal activity.

Introduction

Cutaneous and mucocutaneous leishmaniasis are protozoan diseases of the tropic and subtropic areas in South America, particularly in the subandean areas of the humid lowlands of Bolivia. In the endemic re-

gions of Bolivia, the classic treatments are too expensive or unavailable for the population suffering from cutaneous leishmaniasis or *espundia*. In Bolivia, the Instituto Boliviano de Biología de Altura (IBBA) and the French Institute of Scientific Research for the Development in Cooperation (ORSTOM) have initiated an original program of investigations on alternative compounds for the treatment of cutaneous leishmaniasis. We have collected and studied medicinal plants used by Chimane Indians as a specific treatment for the cutaneous leishmaniasis. In previous works, we have described the chemical and biological studies of two antileishmanial Chimane-plants, *Pera benensis* Rusby (Euphorbiaceae) (1) and *Galipea longiflora* Krause (Rutaceae) (2). The aim of this study is to evaluate the leishmanicidal activity of another plant identified as *Ampelocera edentula* Kuhl. (Ulmaceae).

The stem bark in the form of a poultice is applied on the cutaneous lesions until complete cicatrization of the wound. In a preliminary screening, a chloroform acid extract of the stem barks of *A. edentula* displayed an *in vitro* activity at 50 µg/ml against five strains of promastigote forms of *Leishmania* species and five strains of epimastigote forms of *Trypanosoma cruzi*. Activity-directed fractionation and purification of this crude extract of the stem barks afforded one active compound identified by its physical and spectral data as 4-hydroxy-1-tetralone (1). This work is devoted to the *in vitro* antileishmanial and trypanocidal activities and the *in vitro* activity of this tetralone. We sought to verify its effect when administered directly in the rear footpad of mice infected with *L. amazonensis*.



Materials and Methods

Isolation and chemistry

General experimental procedures

The UV spectrum was recorded on a Unicam SP 1800 spectrophotometer; IR spectrum was measured in KBr with a Perkin Elmer 257 spectrophotometer, ^1H and ^{13}C -NMR spectra were recorded in CDCl_3 (δ ppm) on a Bruker AC 200 P spectrometer operating at 200 and 50 MHz, respectively. The EI-mass spectrum was obtained at 70 eV on a Nermag R 1010 C mass spectrometer. Silica gel GF₂₅₄ (Alufolien 60 F 254 Merck 5554) and silica gel 60 H (Merck 7736) were used for TLC and CC, respectively.

Plant material

Stem bark of *Ampelocera edentula* Kuhl. was collected by A. Fournet (A. F. 884) in September 1988 at Fatima de Chimane, in the Department of Beni, altitude 450 m, Bolivia. The plant material has been identified by Dr. C. Todzia of the Department of Botany, University of Texas, Austin, USA. A reference specimen is deposited in the National Herbarium of Bolivia (La Paz).

Extraction and isolation

Stem barks (3 kg) of *A. edentula* were acidified with 0.5 N HCl and extracted with chloroform. The crude extract (58 g) was chromatographed on silica gel H (1.5 kg) and eluted with a CHCl_3 -MeOH gradient. The active fraction (7.9 g) eluted with CHCl_3 -MeOH, 99/1, was further purified on silica gel H (240 g), followed by a column chromatography on Sephadex LH 20, using CHCl_3 , MeOH, 7/3, as eluent. The active compound (0.87 g) was identified by its physical and spectral data as 4-hydroxy-1-tetralone (1).

Spectral data for 4-hydroxy-1-tetralone

Yellow oil; $\text{C}_{10}\text{H}_{10}\text{O}_2$: 162; UV λ_{max} (EtOH) nm (log ϵ): 205 (4.35), 249 (4.04), 289 (3.20); IR (KBr) ν cm^{-1} : 3300, 2900, 1680, 770; EIMS, m/z (%): M^+ 162 (22), 147 (12), 134 (85), 115 (22), 105 (100), 77 (46); ^1H -NMR (200 MHz, CDCl_3): δ 2.07–2.84 (4H, H-2a, H-2b, H-3a and H-3b), 4.98 (1H, H-4), 7.40–8.05 (4H, H-5, H-6, H-7 and H-8); ^{13}C -NMR (50 MHz, CDCl_3): δ 197.9 (C-1), 145.5 (C-10), 134.0–126.9 (C-5, C-6, C-7 and C-8), 130.9 (C-9), 67.5 (C-4), 35.0 (C-2), 31.8 (C-3).

Biological assays

Parasites

Cultures of *Leishmania* ssp. and *Trypanosoma cruzi* were obtained from IBBA (Instituto Boliviano de Biología de Altura, La Paz) and identified by isoenzyme analysis. Five strains of *Leishmania* were used during these investigations: *L. braziliensis* (MHOM/BR/75/M 2903), *L. amazonensis* (IFLA/BR/67/PH8), *L. amazonensis* (MHOM/GF/84/CAY H-142), *L. donovani* (MHOM/IN/83/HS-70) and *L. donovani* (MHOM/Br/00/M 2682) and five strains of *Trypanosoma cruzi*: SC 43 CL2 (Bolivian strain), C8 CL1 (Brazilian strain), Tulahuén (isolated from *Triatoma infestans* in Brazil), TeCL2 (Brazilian strain) and 1979 CL1 (Bolivian strain).

Culture and maintenance of the promastigote forms of *Leishmania* ssp. and the spinstigote forms of *Trypanosoma cruzi*

Cultures of the extracellular forms of *Leishmania* ssp. and *Trypanosoma cruzi* were prepared and maintained as described previously (3, 4).

In vivo studies

Mice: Female and male BALB/c mice were supplied by the Charles Rivers Breeding Laboratory and were then bred in IBBA (Bolivia). Mice weighed between 18–20 g and were eight weeks old when experiments were initiated.

Parasites: The mouse footpad infection has been used as model for these experiments (5, 6).

Leishmania amazonensis MHOM/IFLA/BR/67/PH8 and *L. venezuelensis* MHOM/VE/74/PM-H3 were used. The source and history of these isolates have been previously described (7). All strains were maintained by passage every 6 to 8 weeks in hamsters. BALB/c mice ($n = 6$) were infected subcutaneously in the right rear footpad with 1×10^6 amastigotes obtained from donor hamsters. The parasites were delivered in 200 μl phosphate buffered saline (PBS).

The growth of lesions was determined weekly by measuring the diameter of both rear feet with a direct reading vernier caliper (Ref: Kroelin 10DI 00T6). The size of lesion, in millimeters (Index of Leishmaniasis), was calculated by subtracting measurements obtained from the uninfected foot from that of the infected foot. Measurements commenced one day prior to the inoculation of amastigotes and were continued for 8 or 9 weeks. For each experiment, the mean and standard error of the mean (S.E.M.) were calculated.

Drug treatment

N-Methylglucamine antimonate (Glucantime[®]) with a pentavalent antimony (Sb^v) content of 28 % by weight was purchased from the Rhône-Poulenc, France and was used as reference drug. Two experiments were conducted. Mice in the first experiment were treated by the subcutaneous route. Glucantime[®] was given at a dose of 56 mg Sb^v/kg daily and 4-hydroxy-1-tetralone at 25 mg/kg daily. This tetralone was dissolved in 40 μl of polysorbate (Tween 80, Prolabo) and Glucantime[®] in PBS. Untreated mice received PBS and Tween 80. Drug treatment commenced one day after the inoculation of amastigotes and was continued once daily for 14 days.

In a second set of experiments, mice were treated directly on the infected rear footpad with a single treatment 14 days after the inoculation of parasites. In these experiments, mice were treated with Glucantime[®] at 112 mg Sb^v/kg and with 4-hydroxy-1-tetralone at 50 mg/kg.

Results

In vitro effects on *Leishmania* ssp. and *Trypanosoma cruzi*

After 48 h incubation with 4-hydroxy-1-tetralone, the IC_{90} for five strains of promastigote forms of *Leishmania* was 10 $\mu\text{g}/\text{ml}$. For an easy comparison, results obtained in the presence of 4-hydroxy-1-tetralone, pentamidine, and meglumine antimonate are presented together in Table 1. 4-Hydroxy-1-tetralone was tested *in vitro* on five strains of epimastigote forms of *Trypanosoma cruzi*. The effects of this compound, nifurtimox, and benznidazole on the growth of *T. cruzi* are presented in Table 2. After 48 h, 4-hydroxy-1-tetralone showed an inhibitory activity on all strains, the IC_{90} was 10 $\mu\text{g}/\text{ml}$. Nifurtimox and benznidazole did not show any inhibitory activity against multiplication of epimastigote forms of *Trypanosoma cruzi* below 25 $\mu\text{g}/\text{ml}$.

Table 1 *In vitro* activity of 4-hydroxy-1-tetralone, Glucantime, and pentamidine against five strains of promastigote forms of *Leishmania* spp.

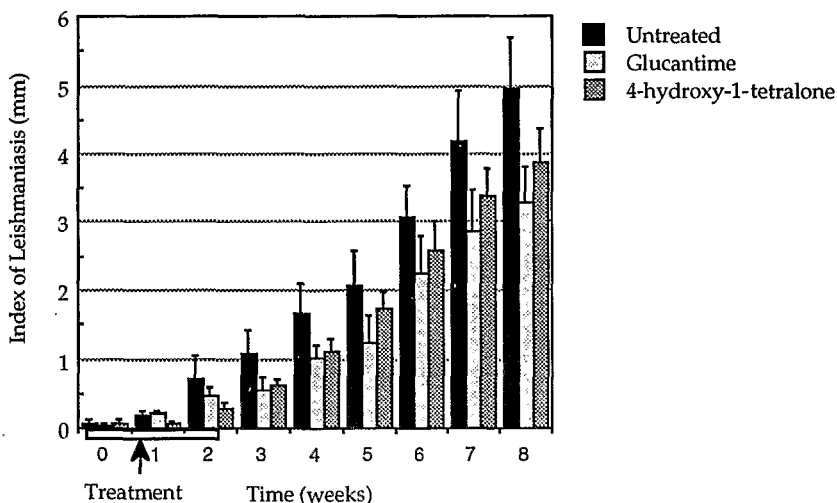
Compounds	IC ₉₀ (µg/ml)				
	<i>L. b.</i> ^a (2903)	<i>L. a.</i> ^b (PH 8)	<i>L. a.</i> ^b (H-142)	<i>L. d.</i> ^c (2682)	<i>L. d.</i> ^c (HS70)
4-Hydroxy-1-tetralone	10	10	10	10	10
Pentamidine	1	1	1	1	1
Glucantime	> 100	> 100	> 100	> 100	> 100

^a *Leishmania braziliensis*.^b *Leishmania amazonensis*.^c *Leishmania donovani*.**Table 2** *In vitro* trypanocidal activity of 4-hydroxy-1-tetralone, benznidazole, and nifurtimox against five strains of epimastigote forms of *Trypanosoma cruzi*.

Compounds	IC ₉₀ (µg/ml)				
	C8CL1	TeCL2	Tulahuen	1979 CL1	SC43 CL1
4-Hydroxy-1-tetralone	10	10	10	10	10
Benznidazole	100	25	50	25	50
Nifurtimox	25	25	25	50	50

Treatments for 14 days in mice infected with *L. amazonensis* (PH8)

The activity of 4-hydroxy-1-tetralone against experimental cutaneous leishmaniasis caused by *Leishmania amazonensis* in BALB/c mice is presented in Fig. 1. The mice treated with 4-hydroxy-1-tetralone and Glucantime developed similar lesion size after four weeks, 1.1 mm and 1 mm, respectively. We have observed an identical increase in the development of the lesion size in mice treated with 25 mg/kg of 4-hydroxy-1-tetralone daily or with 56 mg Sb^v/kg of Glucantime daily, respectively, 1.2 mm and 1 mm. Eight weeks after infection, mice treated with 4-hydroxy-1-tetralone had an average lesion size of 3.86 mm, this size was 3.28 mm in mice treated with the reference drug and 4.95 mm in the untreated mice. Preliminary toxicological evaluations of 4-hydroxy-1-tetralone administered in BALB/c mice indicated that this drug had a therapeutic index with an acute intraperitoneal 50 % lethal dose of 168 mg/kg. No apparent signs of drug toxicity, weight loss or hair loss, were observed in the experiments but an inflammatory effect was seen near the site of administration of drug.



Treatments against *L. venezuelensis*

The results obtained with this model are presented in Fig. 2. Four weeks after infection, mice treated with 4-hydroxy-1-tetralone or with the antimonial compound had an average lesion size of 1.28 mm and 0.95 mm, respectively, compared with 2.06 mm in untreated mice. During the last four weeks of the experiment, we observed that the sizes of lesions were similar in mice treated with 4-hydroxy-1-tetralone or reference drug, 3.77 mm and 3.3 mm, respectively. After eight weeks of experiment, the progression of the *Leishmania venezuelensis* infection was slower in mice treated with reference drug than in mice treated with 4-hydroxy-1-tetralone (respectively, 4.25 mm and 5.05 mm).

Single treatments on the footpad in mice infected with *Leishmania amazonensis* (PH8)

In this experiment, the treatment with 4-hydroxy-1-tetralone was administered at the site of parasitic infection, 14 days after infection with *Leishmania amazonensis* (PH8). The results obtained are shown in Fig. 3. We have observed a severe inflammatory effect in mice treated with 4-hydroxy-1-tetralone at 50 mg/kg. This inflammation was resorbed three weeks after the local treatment. Single treatment with 4-hydroxy-1-tetralone (at 50 mg/kg) reduced the severity of lesions in this model and appeared more effective than pentavalent antimony. Eight weeks after infection, mice treated with 4-hydroxy-1-tetralone had an average lesion size of 2.55 mm, compared to 2.85 mm in mice treated with Glucantime and 4.90 mm in untreated mice.

Fig. 1 Effect of 4-hydroxy-1-tetralone (25 mg/kg/d) and Glucantime (56 mg Sb^v/kg/d) on the development of *L. amazonensis* (PH8) in BALB/c mice. Treatments were given for 14 days period commencing 1 d after inoculation with *L. amazonensis*.

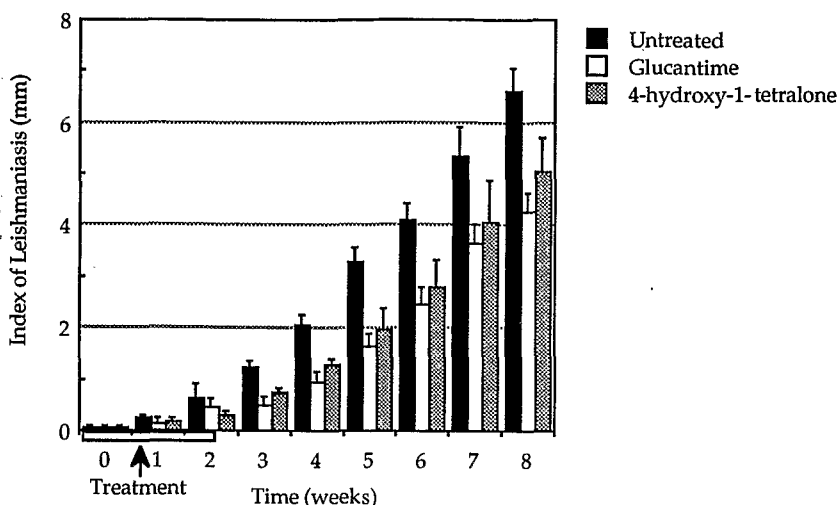


Fig. 2 Effect of 4-hydroxy-1-tetralone (25 mg/kg/d) and Glucantime (56 mg Sb^v/kg/d) on the development of *L. venezuelensis* (H-3) in BALB/c mice. Treatments were given for 14 days period commencing 1 d after inoculation with *L. venezuelensis*.

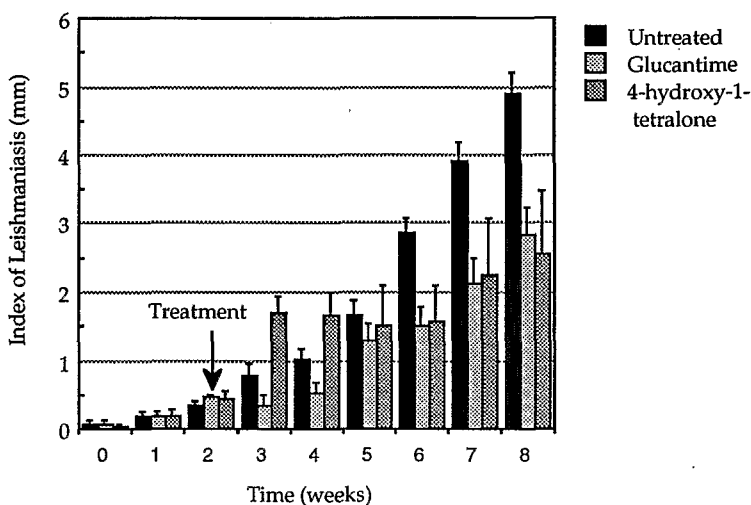


Fig. 3 Effect of 4-hydroxy-1-tetralone (50 mg/kg/d) and Glucantime (112 mg Sb^v/kg/d) on the development of *L. amazonensis* (PH8) in BALB/c mice. Drugs were given on the infected rear footpad with a single treatment 14 days after the inoculation of *L. amazonensis*.

Discussion

The bioassay guided fractionation of *Ampelocera edentula* led to one active compound identified as the known product, 4-hydroxy-1-tetralone or 4-hydroxy-3,4-dihydro-2*H*-naphthalenone. This product is considered as a metabolite from the degradation of naphthalene and 1-naphthol (8, 9) and as an intermediate in the biosynthesis of 1,4-naphthoquinones (8). It was previously encountered in fungi (10) and yeasts (11). It is also used as an intermediate in the synthesis of antibiotics (12). To our knowledge, this tetralone has been isolated here for the first time from higher plants.

The evaluation of the *in vivo* anti-leishmanial activity of 4-hydroxy-1-tetralone showed the efficacy of this compound. Results of the *in vivo* experiments indicated that all mice treated with 25 mg of 4-hydroxy-1-tetralone per kg daily for 14 days developed a similar lesion size when compared with mice treated with the antimony drug. We observed that 4-hydroxy-1-tetralone, administered at 50 mg/kg near the site of parasitic infection in *Leishmania amazonensis* infected

mice, is more effective than the antimony reference drug at 112 mg/Sb^v/kg. These results demonstrate that the traditional use of *Ampelocera edentula* in endemic areas of cutaneous leishmaniasis together with our clinical observations give excellent proof of the efficiency of 4-hydroxy-1-tetralone.

This class of compounds was previously described as antiprotozoal products (13–16). These compounds generate free oxygen radicals within parasites which lack protective mechanisms against such radicals (17), particularly catalase (18). They are described as cytotoxic, carcinogenic, and mutagenic for laboratory animals (19, 20). Some authors reported schistosomicidal activities of tetralones (21).

This study confirms that the poultices of stem bark of *Ampelocera edentula*, as used by Chimane Indians, are effective against cutaneous leishmaniasis. This work describes the first example of activity of a tetralone against *Leishmania* species, but the use of 4-hydroxy-1-tetralone as antileishmanial drug is critical because of its high cytotoxicity. Studies of derived 4-

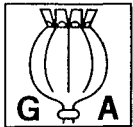
hydroxy-1-tetralone compounds are in progress in our laboratory for the treatment of cutaneous and visceral leishmaniasis.

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