Antimicrobial Activity of Isopteropodine

Rubén García[a,*], Cesia Cayunao[a], Ronny Bocic[a], Nadine Backhouse[a], Carla Delporte[a], Mercedes Zaldivar[b], and Silvia Erazo[a]

[a] Department of Pharmacological and Toxicological Chemistry, School of Chemical and Pharmaceutical Sciences, University of Chile, P. O. Box 233, Santiago 1, Chile. Fax: 56-2-22279 00. E-mail: rgarciam@uchile.cl

[b] Department of Biochemistry and Molecular Biology, School of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile

* Author for correspondence and reprint requests

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Bioassay-directed fractionation for the determination of antimicrobial activity of *Uncaria tomentosa*, has led to the isolation of isopteropodine (0.3%), a known *Uncaria* pentacyclic oxindol alkaloid that exhibited antibacterial activity against Gram positive bacteria.

Key words: Uncaria tomentosa, Isopteropodine, Antibacterial Activity

Introduction

Uncaria tomentosa (Willd. ex Roemer and Schultes DC.), commonly known as "cat's claw", is a large climbing shrub, belonging to the Rubiaceae family. Cat's claw is indigenous to the Amazon rainforest and other tropical areas of South and Central America, including Peru, Colombia, Ecuador, Guyana, Trinidad, Venezuela, Suriname, Costa Rica, Guatemala, and Panama (Keplinger *et al.*, 1999; Lock de Ugaz, 1995).

The bark of cat's claw is used in either folk medicine or in procuring phytotherapeutic drugs. This species contain several active compounds which were tested widely for possible medicinal value (rheumatism, tumors, anti-inflammatory effect and breast cancer) (Williams, 2001; Riva *et al.*, 2001; Aguilar *et al.*, 2002; Gattuso *et al.*, 2004).

Chemical studies on *Uncaria tomentosa* revealed the presence of diverse compounds as quinovic glycosides, polyoxygenated triterpenoids, catechins, sterols and alkaloids, with diverse pharmacological actions (Aquino *et al.*, 1989; Wagner *et al.*, 1985; Keplinger *et al.*, 1999; Kang *et al.*, 2002; Sandoval *et al.*, 2002; Mur *et al.*, 2002; Montoro *et al.*, 2004).

However, of all the extensive biological activities investigated in this plant, specially for pentacyclic oxindol alkaloids (Kang *et al.*, 2002; Lee *et al.*, 1999; Falkiewicz and Lukasiak, 2001) – only the quinovic glycosides were reported to have antimicrobial (antiviral) (Aquino *et al.*, 1989 ; Williams, 2001) activity – reputed in folk medicine for *Uncaria tomentosa.* These instances reinforced our interest for evaluating the antimicrobial activity of "cat's claw" bark against bacteria and fungi.

Materials and Methods

General experimental procedures

The solvent used for NMR studies was $CDCl_3$. The measurements of the NMR spectra were carried out on a Bruker AMX-300 [^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz)] spectrometer. Column chromatography (CC) was carried out using silica gel 60 G (Merck, 7734). TLC was performed on silica gel GF 254 plates (Merck, 5554); the spots were detected by UV light (254, 366 nm), Liebermann Burchard test and/or *p*-anisaldehyde and Dragendorf reagents.

GC-MS analyses were made on a Fisons MB 800 mass spectrometer (MS) coupled with a Hewlett-Packard mod. 5890 series II gas chromatograph (GC), equipped with a 25 m × 0.2 mm i.d. HP U-2 column, with 0.25 μm film thickness. The initial oven temperature was held at 40 °C for 6 min, it was then increased at 7 °/min up to 200 °C, at 110 KPa with helium as carrier gas.

HPLC was performed on a Shimadzu LC-10 AD, with a Shimadzu SPD-10 AV UV-VIS detector. Column: C_{18} (Phenomenex); solvent system: acetonitrile/buffer K_2HPO_4 (pH 7) 50:50 with a flow of 0.75 ml/min to 27 °C.

Plant material

U. tomentosa bark was kindly provided by Hochstetter Laboratory (collected from Peruvian

rainforest), and a voucher is kept at the botany faculty laboratory.

Extraction and isolation

Ground dried bark (720 g) was sequentially extracted at room temperature with *n*-hexane, dichloromethane and methanol, yielding after removal of the solvents *in vacuo*, 2.10 g (0.3%), 2.86 g (0.4%) and 82.5 g (11.5%), respectively.

Dichloromethane and methanol extracts were active in the preliminary bio-assay, showing the presence of a single bioactive alkaloid substance. The extracts were separately worked up dissolving the dried extracts in acetic acid (2 N), filtering, basifying with concentrated ammonia solution, and extracting with chloroform (5 × 25 ml). The chloroform extracts were joined and subjected to CC over silica gel, eluting an unpurified alkaloid with dichloromethane/ethyl acetate 9.5:0.5 to dichloromethane/ethyl acetate 8:2, in fractions 49–72, respectively. Preparative TLC on silica gel led to the isolation of the purified alkaloid compound. The purity of this substance was evaluated by gas chromatography-mass spectroscopy, showing a single peak at the retention time (Rt) of 21.5 min. Crystallization from MeOH gave colorless needles.

Isopteropodine: M.p. 207–209 °C. – $[\alpha]_D^{24}$ – 85 ° (c = 0.764, $CHCl_3$). – UV(MeOH): λ_{max} = 208, 243, 283 nm (sh). – MS: *m/z* (% rel. int.) = 368 (4.5) [M^{+*}], 223 (13), 208 (11), 180 (13), 146 (55), 130 (61), 117 (58), 103 (25), 77 (72), 69 (100), 55 (74); $C_{21}H_2N_2O_4$. – ^{13}C NMR (ppm): δ = 181.43, 167.64, 154.98, 140.23, 133.76, 127.67, 124.53, 122.52, 109.82, 109.70, 72.14, 71.25, 56.93, 54.14, 53.51, 51.02, 37.83, 34.84, 30.17, 30.42, 18.65. – ^1H NMR (ppm): δ = 8.56 (1H, brs), 7.42 (1H, s), 7.28 (1H, d, J = 7.5 Hz), 7.19 (1H, t, J = 7.6 Hz), 7.02 (1H, t, J = 7.5 Hz), 6.93 (1H, d, J = 7.6 Hz), 4.35 (1H, m), 3.60 (3H, s), 3.29 (2H, m), 2.46 (5H, m), 2.01(1H, m), 1.59 (2H, m), 1.41 (3H, d, J = 6.2 Hz), 0.87 (1H, appq, J = 12.8 Hz) (* brs: broad singlet; appq: apparent quartet).

Antimicrobial assays

The antimicrobial activity of the extracts was determined against *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (isolated from a patient), *Salmonella aviatum* (ATCC 2228), *Pseudomonas aeruginosa* (ATCC 14207), *Staphylococcus aureus* (ATCC 6538P), *Micrococcus flavus* (ATCC 10290) and *Bacillus subtilis* (ATCC 6633), *Candida albicans* and *Saccharomyces cerevisiae.*

Dilutions of 100 and 200 μg/ml of DMSO extract's solution were added to a fixed volume of Plate Count Agar (PCA) and Tryptic Soy Broth (TSB). They were then superficially inoculated with a single line of an overnight culture of the different microorganisms and incubated at 37 °C for 24 h for bacteria and 28 °C for fungi. Results were recorded as growth or growth inhibition at each extract concentration.

The active extracts were submitted to a bioautography agar overlay bioassay in order to determine the active compounds (Rahalison *et al.*, 1995).

The turbidimetric method (Balows *et al.*, 1991) was used for determining MIC values of the isolated substance against *S. aureus* and *B. subtilis.* It consisted in preparing serial dilutions of the compound (Table I) in TSB culture medium. Each tube (carrying the same volume) was inoculated with a fixed volume of the culture medium to obtain 2 ml of final volume. Each dilution of the sample in study was assayed in triplicate.

Results and Discussion

U. tomentosa active extracts (dichloromethane and methanol extracts) showed antimicrobial activity only against *M. flavus* and *B. subtilis* and was inactive against *C. albicans* and *S. cerevisiae.*

The methanol extract presented the major antibacterial activity at concentrations of 100 μg/ml and 200 μg/ml. A bioautographic agar overlay in TLC of the extracts and the isolated alkaloid was carried out on a silica gel 60 F_{254} plate developed with dichloromethane/ethyl acetate 1:1. The bioautograms were sprayed with an aqueous solution of thiazolyl blue (MTT). A positive antimicrobial reaction was observed as a clear inhibition zone against a purple background (Rahalison *et al.*, 1995).

The isolated alkaloid was identified unambiguously as isopteropodine (Uncarine E), by its mass fragmentation, ^1H NMR, ^{13}C NMR and literature data (Chan *et al.*, 1966; Shamma and Foley, 1967; Wagner *et al.*, 1985; Seki *et al.*, 1993).

The isopteropodine bark quantification (0.3%) was assayed by HPLC using the isolated alkaloid as internal reference (Rt: 12.1; Ganzera *et al.*, 2001).

Isopteropodine was inactive, in the same way as *Uncaria* extracts, against *C. albicans* and *S. cerevisiae*, and was active only against the Gram positive bacteria *S. aureus* and *B. subtilis*, with a MIC value

Table I. Isopteropodine MIC value determinations.

Bacteria	Isopteropodine concentration [μM]												
	353	380	408	435	462	489	516	543	571	598	625	652	679
S. aureus	+	+	–	–	–	–	–	–	–	–	–	–	–
B. subtilis	+	+	+	+	+	+	+	+	+	+	+	+	–

+, Bacterial growth.
–, No bacterial growth.

of 150 μg/ml (408 μM) and 250 μg/ml (679 μM), respectively (Table I) [ampicillin: MIC 5 μg/ml (14 μM) for *S. aureus* and 10 μg/ml (29 μM) for *B. subtilis*].

In conclusion, these results support scientifically the use of this species in popular medicine as antimicrobial, despite its weaker activity compared with the standard antibiotic.

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