Journal of Ethnopharmacology 157 (2014) 156–160

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

Neolignans from *Aristolochia elegans* as antagonists of the neurotropic effect of scorpion venom

Alejandro Zamilpa [a], Rodolfo Abarca-Vargas [a], Elsa Ventura-Zapata [b], Lidia Osuna-Torres [a], Miguel A. Zavala [c], Maribel Herrera-Ruiz [a], Enrique Jiménez-Ferrer [a], Manasés González-Cortazar [a,*]

[a] Southern Biomedical Research, Mexican Institute of Social Security, Argentina No. 1, Col. Centro, 62790 Xochitepec, Morelos, Mexico
[b] Development Center Biotic Products. Instituto Politécnico Nacional (IPN), 62761 Yautepec, Morelos, Mexico
[c] Departament of Biological Systems, UAM-Xochimilco, Calzada del Hueso 1100, Col. Villa Quietud, México, DF 04960 Mexico

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form
29 July 2014
Accepted 10 August 2014
Available online 30 September 2014

Keywords:
Aristolochia elegans
Aristolochiaceae
Centruroides limpidus limpidus
neolignans
scorpion venom

ABSTRACT

Ethnopharmacological relevance: The high frequency of poisoning by sting or bite from venomous animals has begun to be a serious public health problem in Mexico where scorpion sting is the most common. Because of this, there is the need to seek active substances in plant species with an antagonistic effect against neurotropic activity of scorpion venom. The aim of this work was to demonstrate which of the compounds contained in the *n*-hexane extract from *Aristolochia elegans* roots display activity against scorpion venom.

Material and methods: Antagonist activity displayed by extract, fractions and isolated compounds obtained from *Aristolochia elegans* was guided by the inhibition of smooth muscle contraction induced by scorpion venom (*Centruroides limpidus limpidus*) in a model of isolated guinea pig ileum. The neolignans obtained from this extract were isolated and analyzed by chromatographic methods including HPLC. The chemical characterization of these compounds was performed by the analysis of ^{1}H and ^{13}C NMR spectra.

Results: The bio-guided chromatographic fractionation allowed us to isolate 4 known neolignans: Eupomatenoid-7 (**1**), licarin A (**2**), licarin B (**3**), eupomatenoid-1 (**4**) and other new neolignan which was characterized as 2-(3′-hydroxy-4′-methoxyphenyl)-3-methyl-5-[(E)-α-propen-γ-al]-7-methoxy-benzo [b] furan (**5**). This compound was named as eleganal. Compounds **1** and **2** were purified from the most active fraction AeF3 (EC_{50} of 149.9 μg/mL, E_{max} of 65.66%). A doses–response analysis of eupomatenoid-7(**1**) and licarin A(**2**) allowed us to establish EC_{50} values (65.96 μg/mL and 51.96 μg/mL) respectively.

Conclusions: The antagonistic effect against *Centruroides limpidus limpidus* scorpion venom displayed by the *n*-hexane extract from *Aristolochia elegans* roots is due to the presence of neolignans **1–2** contained in the fraction AeF3. Chemical analysis of fraction AeF2 allowed the isolation of a new compound which was identified as 2-(3′-hydroxy-4′-methoxyphenyl)-3-methyl-5-[(E)-α-propen-γ-al]-7-methoxy-benzo[b] furan (**5**), denominated as eleganal.

1. Introduction

In some areas of the Mexican Pacific Coast and some central States in Mexico, poisoning scorpion sting is a serious public health problem (Bourée et al., 2006). The endemic species of scorpions in these areas are *Centruroides limpidus limpidus* and *Centruroides noxious* (Bourée et al., 2006). Severe cases of poisoning by scorpion sting present with symptoms related to muscle contraction of the different organs and tissues, especially of the respiratory and cardiovascular systems, which generate convulsions, pulmonary edema and cardiac arrest. These health problems maintain in force the need of new active substances with an antagonistic effect against neurotropic activity of scorpion venom (Possani, 2005). In Morelos State in the center of México, *Aristolochia elegans* Mast syn. *Aristolochia littoralis* between other *Aristolochia* species is known popularly as "guaco" (Martinez, 1969; Aguilar et al., 1994; Montes-Leyva and Rojas-Alba, 2011). This group of plants is widely used against the bite of poisonous animals, mainly against the scorpion and viper bite (Avilés and Suárez, 1994; Monroy-Ortiz and Castillo-España, 2000; Jiménez-

* Corresponding author. Tel./fax: +52 777 3 612155.
E-mail address: gmanases2000@gmail.com (M. González-Cortazar).

http://dx.doi.org/10.1016/j.jep.2014.08.045
0378-8741/

Ferrer et al., 2005). The effect of *Aristolochia elegans* against *Centruroides limpidus limpidus* scorpion venom was previously demonstrated. The non-polar extract of the roots from this plant displayed an inhibition 70% of the ileon contraction induced by scorpion venom (Jiménez-Ferrer et al., 2005). This effect was maintained when the *n*-hexane extract was obtained from the roots of a micropropagated plant (Mora et al., 2010). However, there are no reports of a chemical–pharmacological relaxant activity of this plant species, so the aim of this work was to perform a bio-guided chemical study directed on the *n*-hexane extract of wild root from *Aristolochia elegans* by measuring of this neurotropic effect induced with *Centruroides limpidus limpidus* venom.

2. Material and methods

2.1. Plant material

Adult wild specimens of *Aristolochia elegans* were collected in the district of Tepalcingo, Morelos, México. The plants were deposited at the Mexican Institute of Social Security (*Instituto Mexicano del Seguro Social* – IMSS) herbarium; they were identified by Abigail Aguilar Contreras and registered under number HPMIMS13595.

2.2. Experimental animals

Male Hartley strain guinea pigs were used. They were 3–5 months old and weighed 350–450 g; they were kept under controlled temperature conditions (25 ± 2 °C), with a 24 h cycle of 12 h light and 12 h darkness, and access to water and food (pellets from Harlan, Mexico). The guinea pigs were sacrificed by cervical dislocation following the guidelines of the Mexican Official Norm for the ethical use of laboratory animals (*Norma Oficial Mexicana* – NOM-062-ZOO-1999). The distal ileum was obtained by laparotomy and washed with Tyrode solution (mM concentrations of NaCl [136.9], glucose [11.1], $NaHCO_3$ [11.9], $CaCl_2$ [1.4], KCl [2.7], $MgCl_2$ [0.5], NaH_2PO_4 [0.4], with a 7.4 pH). It was kept with a gaseous blend of O_2 (95% v/v) and CO_2 (5% v/v) (Jiménez-Ferrer et al., 2005).

2.3. Preparation of extract

2 kg of wild roots was used, dried and after was extracted through maceration with *n*-hexane (12 L, Merck) for 24 h at room temperature for three times. The hexane extract from each material was concentrated in a rotatory evaporator (Heidolph Laborota 4000, Germany) under reduced pressure at 50–60 °C until completely dried, obtaining 22 g of extract.

2.3.1. Obtaining the compounds present in the n-hexane extract of Aristolochia elegans (**1**–**5**)

Hexane extracts (20 g) were adsorbed in silica gel and applied to a silica gel column (350 g, 70–230 mesh, Merck, Germany). A gradient of *n*-hexane/ethyl acetate was utilized to elute the column; obtaining 95 fractions 200 mL each were collected. The fractions were concentrated and regrouped according to their similarity in Thin-layer chromatography (TLC) in four groups; AeF1(1–18, 100:0, 7 g), AeF2(19–35; 80:20, 2.5 g), AeF3(36–51; 700:30, 3.5 g) and AeF4(51–95; 50:50, 5.5 g).

The AeF1 fraction (6 g) was separated using a chromatographic column with silica gel (180 g, 70–230 mesh, Merck, Germany) and eluted with a solvent mixture (hexane: ethyl acetate) with increasing polarity the 10%, obtaining 49 fractions of 100 mL each were collected. Fraction 10–17 were combined, obtaining a white solid amorphous (56 mg), which was elucidated by ^1H and ^{13}C NMR as licarin B (**3**). Fraction 18–25 showed a single spot; remain elucidated by ^1H and ^{13}C NMR as eupomatenoid-1(**4**). Spectroscopic data were compared with the literature data (Wenkert et al., 1976; Jiménez-Arellanes et al., 2012).

The AeF3 fraction (3 g) was separated using a chromatographic column (150×15 mm^2) with reverse phase silica (polygoprep® 60-50 C_{18}, Macherey-nagel, Germany, 90 g) and eluted with an isocratic system with a water/acetonitrile mixture (50:50), obtaining 17 fractions of 10 mL each were collected. Fractions 2–3 (AeF3–3) were combined and recrystallized, obtaining 22 mg the compound **1**, which was elucidated by ^1H and ^{13}C NMR as eupomatenoid-7 (**1**). Fraction 7 (AeF3–7) showed a single spot; remain elucidated by ^1H and ^{13}C NMR as licarin A (**2**). Spectroscopic data were compared with the literature data (Enriquez et al., 1984).

The AeF4 fraction (2.2 g) was separated using a chromatographic column with silica gel (80 g, 70–230 mesh, Merck, Germany) and eluted with a solvent mixture (hexane:ethyl acetate) with increasing polarity 10%, obtaining 31 fractions of 100 mL each were collected. Fractions 7–14 (AeF4–14) were combined and recrystallized, obtaining 55 mg compound **1**, which was isolated previously. Fraction 25 (AeF4–25) showed a single spot; remain elucidated by ^1H and ^{13}C NMR and spectroscopic data compared with the described (Barbosa-Filho et al., 1998), it was determined as 2-(3′-hydroxy-4′-methoxyphenyl)-3-methyl-5-[(E)-α-propen-γ-al]-7-methoxy-benzo[b]furan (**5**), this being a new neolignan previously unreported, which was named as eleganal.

The mixture of aristolochic acid I and II (Merk®, 55% and 45% p/p) was compared by TLC with hexane extract which was found to have no presence.

2.4. Phytochemicals analysis by HPLC

HPLC analysis was performed on a Waters 2695 separation module system equipped with a Waters 2996 photodiode array detector and Empower Chromatographic Manager version 1 software (Waters USA). Analysis was performed with a Merck supersper RP-18 (Merck, USA) column (5-μm, 4×120 mm^2). The mobile phase consisted of a gradient system H_2O (solvent A) and acetonitrile (solvent B). The system was started with solvent A (100%) during 1 min. The concentration of solvent B was gradually increased (3 min) to 30%. This mixture was maintained for 1 min; during the following min, solvent B was increased to 70%. This concentration was maintained for 4 min and finally, the solvent B concentration was increased to 100% (lineal change during 1 min) and maintained at this concentration for 3 min. The subsequent 3 min were employed to restore the initial conditions. The chromatographic method had a run time of 18 min. The sample injection volume was 10 μL with a 1 mL/min flow rate. The detection wavelength was scanned at 190–400 nm. The retention times (rt) to compounds **1**, **2**, **3**, **4** and **5** were 9.9, 10.7, 11.5, 13.6 and 8.9 min, respectively.

2.5. Spectroscopic analyses

All NMR spectra were recorded on Varian INOVA-400 at 400 MHz for ^1H NMR, ^1H–^1H COSY and 100 MHz for ^{13}C NMR and DEPT in $CDCl_3$ except for compound (**3**) which was performed at 200 MHz. Chemical shifts are reported in ppm relative to TMS.

2.6. Extraction of venom from Centruroides limpidus limpidus

Centruroides limpidus limpidus venom was obtained from a colony of scorpions grown and maintained at our laboratory in CIBIS-IMSS (Xochitepec, Morelos, México). The venom was