Revista Brasileira de Farmacognosia 25 (2015) 7–10

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

Chromenes from leaves of *Calea pinnatifida* and evaluation of their leishmanicidal activity

Tamires C. Lima[a], Alan Diego C. Santos[b], Danielle T.M. Costa[c], Rafaela J. Souza[a], Andersson Barison[b], Mário Steindel[c], Maique W. Biavatti[a,*]

[a] Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
[b] Departamento de Química, Universidade Federal do Paraná, Curitiba, PR, Brazil
[c] Departamento de Microbiologia e Parasitologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

ARTICLE INFO

Article history:
Received 23 October 2014
Accepted 26 January 2015
Available online 12 February 2015

Keywords:
Asteraceae
Calea pinnatifida
Chromenes
Leishmanicidal activity

ABSTRACT

Calea pinnatifida (R. Br.) Less., Asteraceae, is popularly known as “quebra-tudo”, “cipó-cruz” or “aruca”. This species is used in the folk medicine for the treatment of stomach pain, giardiasis and amoebiasis. The aim of this study was to isolate and identify chromenes from leaves of *C. pinnatifida* and evaluate their leishmanicidal activity. A fraction from leaves of *C. pinnatifida* was analyzed for their chemical constituents, resulting in the isolation and characterization of four known chromenes: 6-acetyl-7-hydroxy-2,2-dimethylchromene (**1**), 6-acetyl-7-methoxy-2,2-dimethylchromene (**2**), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**). Structure identification of isolated compounds involved analysis of spectral data of 1D and 2D-NMR. The isolated compounds are here reported for the first time in *C. pinnatifida*, and the chromenes **1** and **3** show a moderate leishmanicidal activity.

Introduction

Calea L. is a large genus of the Asteraceae family (tribe Heliantheae, subtribe Melampodiinae), containing approximately 125 species distributed essentially in tropical and subtropical zones of the Americas (Roque and Carvalho, 2011), with the greatest number of species being recorded in Brazil (Mondin and Bringel Jr., 2010). This genus has been reported in the literature to possess various biological properties, such as anti-inflammatory (Gomes and Gil, 2011), antiplasmodial (Kohler et al., 2002), antileishmanial (Wu et al., 2011), acaricidal (Ribeiro et al., 2011), antifungal (Flach et al., 2002), antidiabetic (Ramos et al., 1992), antimicrobial (Do Nascimento et al., 2004), antihypertensive (Guerrero et al., 2002), and cytotoxic activities (Nakagawa et al., 2005).

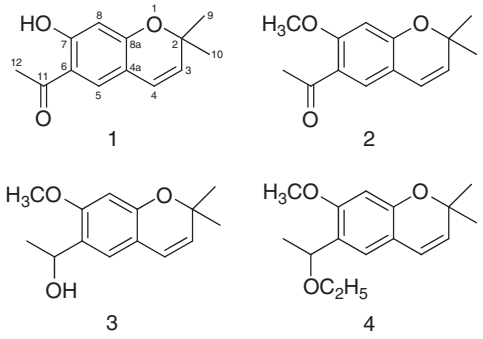
Calea pinnatifida (R. Br.) Less. is popularly known as “aruca”, “cipó-cruz” or “quebra-tudo” (Mors et al., 2000). This species is used in the folk medicine for treating digestive disorders, giardiasis and amoebiasis (Malhado Filho, 1947; Prusk and Urbatsch, 1988; Mors et al., 2000). Previous phytochemical investigations of the petroleum ether and ethyl acetate extracts from aerial parts of this plant and its essential oil revealed the presence of fatty esters, phenolic acids, sterols, monoterpenes, one polyacetylene, and one sesquiterpene lactone (Ferreira et al., 1980a,b; Kato et al., 1994).

Chromenes (benzopyrans) represent a class of secondary metabolites that have generated great attention because of their interesting biological and pharmacological properties (Ribeiro et al., 2011; Thomas and Zachariah, 2013). Several studies have demonstrated the insecticidal, antibacterial, fungicidal and cytotoxic activities of these substances (Bandara et al., 1992; Burkhardt et al., 1994; Iqbal et al., 2004; Chen et al., 2005). Furthermore, some compounds of this class of natural products have been described to have notable antiprotozoal effect (Alizadeh et al., 2008; Batista Jr. et al., 2008; Harel et al., 2011).

Herein, we report the isolation and the structure determination of four known chromenes, named as 6-acetyl-7-hydroxy-2,2-dimethylchromene (**1**), 6-acetyl-7-methoxy-2,2-dimethylchromene (**2**), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**). In addition, the isolated compounds were selected for leishmanicidal assays based on previously reported activity of related structurally compounds in other trypanosomatid protozoa (Harel et al., 2011).

* Corresponding author.
E-mail: maique.biavatti@ufsc.br (M.W. Biavatti).

http://dx.doi.org/10.1016/j.bjp.2015.01.004



Materials and methods

General experimental procedures

Melting point was determined using an MQAPF-301 melting point apparatus. Optical rotation was measured in the solvent $CHCl_3$ on a Jasco P-2000. The ^1H and ^{13}C NMR spectra was obtained using a high resolution Bruker AVANCE-400 and Ascend 600 spectrometers, frequency of 400 and 600 MHz for ^1H, and 100 and 150 MHz for ^{13}C, respectively. NMR spectroscopic data were acquired in $CDCl_3$, TMS was used as internal standard, chemical shifts (δ) were given in ppm, and coupling constants (J) in Hz. 2D NMR experiments (HSQC, HMBC) were also performed using Bruker AVANCE-400 and Ascend 600 spectrometers.

Plant material

The leaves from *Calea pinnatifida* (R. Br.) Less., Asteraceae, were collected in September 2012, at the "Costa da Lagoa", Florianópolis, Santa Catarina, Brazil. Plant identification was performed by Dr. John F. Pruski, New York Botanical Garden, and a voucher specimen (MO-2383318 number) is deposited in Missouri Botanical Garden Herbarium (MO), St. Louis, Missouri, USA.

Extraction and isolation

Fresh leaves from *C. pinnatifida* (800 g) were extracted by maceration for 15 days at room temperature (*ca.* 25 °C) with ethanol 92%. After evaporation of the solvent under reduced pressure, 12 g of the ethanol extract of *C. pinnatifida* were obtained. The ethanol extract was re-dissolved in H_2O and fractionated with solvents of increasing polarity. The partitioning of this extract was performed with *n*-hexane, dichloromethane and ethyl acetate, respectively, yielding *n*-hexane (4.5 g), dichloromethane (0.5 g) and ethyl acetate (1.5 g) fractions, as well as a residual aqueous fraction (5.5 g). Initially, an aliquot of the hexane fraction (2.0 g) of the extract was subjected to column chromatography with silica gel 60. Elution was carried out using a solvent gradient of *n*-hexane:acetone in increasing polarities (100:0, 98:2, 95:5, 90:10, 70:30, 50:50, 0:100, respectively), obtaining some sub-fractions rich in chromenes (subfraction 1: 35 mg, sub-fraction 2: 20 mg, subfraction 3: 15 mg). Subsequently, these sub-fractions were purified by preparative TLC (*n*-hexane:acetone 85: 15). Sub-fraction 1 afforded 8.0 mg of **1** and 4.0 mg of **4**, sub-fraction 2 yielded 18.0 mg of **2**, and sub-fraction 3 afforded 5.0 mg of **3**.

6-Acetyl-7-hydroxy-2,2-dimethylchromene (**1**)

Yellow needles; mp: 78–80 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.31 (br s, 1H, H-5), 6.33 (br s, 1 H, H-8), 6.28 (d, 1H, *J* 9.9 Hz, H-4), 5.58 (d, 1H, *J* 9.9 Hz, H-3), 2.54 (s, 3H, H-12), 1.44 (s, 3H, H-9), 1.44 (s, 3H, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 77.9 (C-2), 128.9 (C-3), 121.0 (C-4), 113.5 (C-4a), 128.5 (C-5), 113.8 (C-6), 165.2 (C-7), 104.5 (C-8), 160.4 (C-8a), 28.6 (C-9), 28.6 (C-10), 202.3 (C-11), 26.1 (C-12).

6-Acetyl-7-methoxy-2,2-dimethylchromene (**2**)

Yellow oil; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.54 (s, 1H, H-5), 6.38 (s, 1 H, H-8), 6.30 (d, 1H, *J* 9.8 Hz, H-4), 5.53 (d, 1H, *J* 9.8 Hz, H-3), 3.88 (s, 3H, H-13), 2.56 (s, 3H, H-12), 1.44 (s, 3H, H-9), 1.44 (s, 3H, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 77.7 (C-2), 128.4 (C-3), 121.4 (C-4), 114.0 (C-4a), 129.1 (C-5), 120.8 (C-6), 161.2 (C-7), 99.7 (C-8), 158.5 (C-8a), 28.4 (C-9), 28.4 (C-10), 197.6 (C-11), 31.9 (C-12), 55.6 (C-13).

6-(1-Hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**)

Green gum; $[\alpha]_D^{20}$ = +45.49 (c = 0.2133 g ml^{-1}; $CHCl_3$; *ca.* 20 °C); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.94 (d, 1H, *J* 0.3 Hz, H-5), 6.37 (s, 1 H, H-8), 6.27 (dd, 1H, *J* 9.7 0.3 Hz, H-4), 5.47 (d, 1H, *J* 9.7 Hz, H-3), 5.02 (q, 1H, *J* 6.5 Hz, H-11), 3.82 (s, 3H, H-13), 1.48 (d, 3H, *J* 6.5 Hz, H-12), 1.42 (s, 3H, H-9), 1.42 (s, 3H, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 76.5 (C-2), 127.9 (C-3), 121.9 (C-4), 113.7 (C-4a), 123.9 (C-5), 125.6 (C-6), 157.5 (C-7), 99.9 (C-8), 153.2 (C-8a), 28.2 (C-9), 28.2 (C-10), 65.8 (C-11), 22.9 (C-12), 55.6 (C-13).

6-(1-Ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**)

Green oil; ^1H NMR ($CDCl_3$, 600 MHz): δ 7.00 (s, 1H, H-5), 6.34 (s, 1 H, H-8), 6.29 (d, 1H, *J* 9.7 Hz, H-4), 5.45 (d, 1H, *J* 9.7 Hz, H-3), 4.74 (q, 1H, *J* 6.4 Hz, H-11), 3.77 (s, 3H, H-13), 3.39 (dq, 1H, *J* 9.4 7.0 Hz, H-1a′), 3.37 (dq, 1H, *J* 9.4 7.0, 1b′), 1.43 (s, 3H, H-9), 1.43 (s, 3H, H-10), 1.35 (d, 3H, *J* 6.4 Hz, H-12), 1.18 (dd, 3H, *J* 7.0 7.0 Hz, H-2′); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 76.3 (C-2), 127.5 (C-3), 122.2 (C-4), 113.9 (C-4a), 123.9 (C-5), 124.6 (C-6), 157.6 (C-7), 99.3 (C-8), 152.8 (C-8a), 28.2 (C-9), 28.2 (C-10), 70.9 (C-11), 22.8 (C-12), 55.5 (C-13), 63.8 (C-1′), 15.5 (C-2′).

Leishmanicidal screening

Human macrophage cell line THP-1 (ATCC TIB202) was grown in RPMI-1640 without phenol red (Sigma-Aldrich, CO. St. Louis, MO, USA) supplemented with 10% FBS (Life Technologies, USA), 12.5 mM HEPES, penicillin (100 U/ml), streptomycin (100 μg/ml) and Glutamax (2 mM), at 37 °C in a 5% CO_2 incubator. *L. amazonensis* MHOM/BR/77/LTB0016 promastigotes, expressing β-galactosidase, were grown at 26 °C in Schneider's insect medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 5% heat inactivated fetal bovine serum FBS and 2% of human urine.

For the leishmanicidal screening against intracellular *L. amazonensis* amastigotes, THP-1 cells (3.0×10^4 per well) were cultivated in 96 well plates in RPMI-1640 medium supplemented as described above and treated with 100 ng/ml of phorbol 12-myristate 13-acetate (PMA) for 72 h at 37 °C in a 5% CO_2, to allow THP-1 cells differentiation into non-dividing macrophages (Schwende et al., 1996).

Four days culture promastigotes (4.0×10^6 parasites/ml) were washed with phosphate buffered saline, pH 7.4 (PBS) an incubated in RPMI-1640 supplemented with 10% human AB+ serum heat-inactivated for 1 h at 34 °C to parasite opsonization. THP-1 cells were incubated with a parasite/cell ratio of 10:1 for 3 h at 34 °C and 5% CO_2. After this period non-adherent parasite were removed by one wash with PBS and infected cells were incubated with 180 μl of full supplemented RPMI-1640 medium for another 24 h to allow the transformation of promastigotes into intracellular amastigotes.

Compounds **1–4** were solubilized in dimethyl sulfoxide (DMSO) and serially diluted (50–0.8 μg ml^{-1}). Infected cell layer were