Steroids 106 (2016) 35–40

Contents lists available at ScienceDirect

Steroids

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

Spirostanol glucosides from the leaves of *Cestrum laevigatum* L.

Paulo Riceli Vasconcelos Ribeiro [a], Ana Jérsia Araújo [b], Letícia Veras Costa-Lotufo [c], Raimundo Braz-Filho [d,e], Hélio Vitoriano Nobre Junior [f], Cecília Rocha da Silva [f], João Batista de Andrade Neto [f], Edilberto Rocha Silveira [a], Mary Anne Sousa Lima [a,*]

[a] Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, CP 12.200, CEP 60.021-940 Fortaleza, CE, Brazil
[b] Departamento de Farmacologia e Fisiologia, Faculdade de Medicina, Universidade Federal do Ceará, CP 12.200, 60430-270 Fortaleza-CE, Brazil
[c] Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, CEP 05508-900 São Paulo, SP, Brazil
[d] Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013 602 Campos dos Goytacazes, RJ, Brazil
[e] Departamento de Química, Universidade Federal Rural do Rio de Janeiro, CP 74541, 23890-000 Seropédica, RJ, Brazil
[f] Departamento de Farmácia, Universidade Federal do Ceará, CP 12.200, 60430-170 Fortaleza, CE, Brazil

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 21 October 2015
Accepted 10 December 2015
Available online 17 December 2015

Keywords:
Solanaceae
Cestrum laevigatum
Spirostanol glycosides
Antifungal
Cytotoxic

ABSTRACT

Two new steroidal saponins, (25*R*)-spirost-5-ene-3β,26β-diol 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-[(1 → 2)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**1**) and (25*R*)-spirost-6-ene-3β,5β-diol 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-[(1 → 2)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**2**), along with the known diosgenin 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**3**), chonglouoside SL-5 (**4**) and Paris saponin Pb (**5**) were isolated from the leaves of *Cestrum laevigatum*. The structures of the compounds were determined using spectroscopic analyses including HRESI-MS, 1D and 2D NMR data, followed by comparison with data from the literature. Among them, two are particularly unique, compound **1** is the first $^{6}\Delta$-spirostanol saponin and compound **2** has an unusual C-26 hydroxyl in the $^{5}\Delta$-spirostanol skeleton. Antifungal testing showed a potent activity to formosanin C against *Candida albicans* and *Candida parapsilosis*. Evaluation of the cytotoxic activity indicated that compound **1** has a moderate activity against HL-60 and SF-295 cell lines, while compound **2** were active only against HL-60.

1. Introduction

Cestrum is the second largest genus of the Solanaceae family, with 150 species distributed in tropical and subtropical America, 50 of which occurring in Brazil [1–2]. As particular to some Solanaceae genera, several species have been reputed as poisonous plants and the search for active compounds revealed a prolific source of bioactive steroids saponins containing spirostanol or furostanol glycoside skeletons [2–7].

Cestrum laevigatum L. is an evergreen shrub native to South America and has been introduced to South Africa. It is widespread into natural grasslands, forests, riparian habitats, and coastal dunes, where is popularly known as "coerana", "lady of the night" and "corana". The dried leaves are used in traditional medicine as treatment for malaria and fever [8], and smoked by the Mapuche Indian of southern Chile as a substitute for cannabis [9]. Although commonly used for ornamental purposes, *C. laevigatum* is considered the most lethal plant to mammals, among the group of common invasive species that cause liver damage. For this reason, is one of toxic plants of greater importance in Brazilian livestock for its wide distribution and economic losses, and its growth is severely controlled or eradicated into pastureland [10,11].

In the course of our search for bioactive natural compounds we report the isolation and structural characterization of two new sapogenins (25*R*)-spirost-5-ene-3β,26β-diol 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-[(1→2)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**1**) and (25*R*)-spirost-6-ene-3β,5β-diol 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-[(1 → 2)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**2**) from the leaves of *C. laevigatum*, in addition to the known diosgenin O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**3**) [12], chonglouoside SL-5 (**4**) [13] and formosanin C (**5**) [14]. The screening for antimicrobial activities against *Candida parapsilosis* (ATCC® 22019™), *Candida albicans* (ATCC® 10231™), *Candida krusei* (ATCC® 14243™), *Pseudomonas aeruginosa* (ATCC® 9027™), *Staphylococcus aureus* (ATCC®6538™) and *Bacillus subtilis* (ATCC® 6633™), and evaluation of cytotoxicity using human

* Corresponding author.
E-mail address: mary@dqoi.ufc.br (M.A.S. Lima).

http://dx.doi.org/10.1016/j.steroids.2015.12.006
0039-128X/

promyelocytic leukemia (HL-60), ovarian carcinoma (OVCAR-8), colorectal adenocarcinoma (HCT-116) and glioma (SF-295) cell lines were performed (see Fig. 1).

2. Experimental

2.1. General experimental procedures

Melting points were obtained on a MetllerToledo-FP82HT and were uncorrected. IR spectra were recorded as KBr pallets on a Perkin-Elmer FT-IR Spectrum 1000 using KBr. The NMR spectra were performed on Bruker Avance DRX 300 or on Avance DRX500 MHz equipped with an inverse detection probe head and *z*-gradient accessory. The ^{1}H and ^{13}C chemical shifts are expressed in the δ scale and were referenced to TMS through the residual solvent. High-resolution mass spectra were recorded on a Waters Acquity UPLC system coupled with a Quadrupole/Time-of-Flight system (UPLC/Qtof MSE spectrometer) in the positive mode. The TOF conditions were as follow: source temperature 120 °C; desolvation temperature 350 °C; desolvation gas flow of 350 L/h; capillary voltage 2 kV; collision Energy Ramp 20 eV. The mode was acquired from 110 to 1200 Da. Optical rotations were obtained on a Perkin-Elmer Q-2000 polarimeter, at 589 nm and 25 °C. Column chromatography was performed over Sephadex LH-20 (Pharmacia) and SPE C18 cartridge (Phenomenex), while TLC was performed on precoated silica gel aluminum sheets (Merck). The compounds were visualized by UV detection and by spraying with vanillin/perchloric acid/EtOH solution, followed by heating.

2.2. Plant material

C. laevigatum L. was collected at the Guaramiranga Mountain, Pacoti, Ceará State, Northeast of Brazil. Voucher specimens (#38643) were deposited at the Herbário Prisco Bezerra (EAC) and identified by MSc. Edson de Paula Nunes, Departamento de Biologia, Universidade Federal do Ceará, Ceará, Brazil.

2.3. Extraction and isolation

Leaves of *C. laevigatum* (1.14 kg) were pulverized and extracted with EtOH (3 × 9.0 L) at room temperature. The solvent was removed under reduced pressure to yield a dark green residue (131.4 g).

Part of EtOH extract (91.0 g) was dissolved in a mixture of MeOH:H_2O (1:1 v/v) and submitted to liquid–liquid partition chromatography with hexane, $CHCl_3$, EtOAc and *n*-BuOH to give four fractions: hexane (0.11 g), CH_2Cl_2 (27.1 g), EtOAc (11.3 g) and *n*-BuOH (32.0 g).

An aliquot of the *n*-BuOH fraction (2.0 g) was rechromatographed on Sephadex LH-20 (10.0 g) (column 2.5 cm × 10 cm) to afford forty-five fractions (3.0 mL) that were pooled together into five resulting sub-fractions after TLC analysis. Sub-fraction F-3 (1.1 g) was further chromatographed on a SPE C18 (5.0 g) cartridge using MeOH/H_2O 1:1 (30.0 mL), MeOH/H_2O 7:3 (3.00 mL), MeOH/H_2O 8:2 (40.0 mL), MeOH/H_2O 9:1 (30.0 mL) and MeOH (50.0 mL), yielding five fractions. Sub-fraction F-5 (0.360 g) was submitted to semi-preparative RP-18 HPLC analysis, using MeOH/H_2O (87:13) as eluent, to afford **1** (10.6 mg).

An Aliquot of the EtOAc fraction (2.1 g) was rechromatographed on Sephadex LH-20 (10.0 g) (column 2.5 cm × 10 cm) to give forty sub-fractions (3.0 mL), which were combined into four resulting sub-fractions according to TLC analysis. Sub-fraction F-3 (0.70 g) which was then chromatographed on a SPE C18 cartridge by elution with MeOH/H_2O 1:1 (30.0 mL), MeOH/H_2O 7:3 (30.0 mL), MeOH/H_2O 8:2 (40.0 mL), MeOH/H_2O 9:1 (30.0 mL) and MeOH (40.0 mL), yielding five fractions. Fraction F-3 (0.26 g) was submitted to semi-preparative RP-18 HPLC chromatography, using an isocratic mixture MeOH/H_2O 8:1 to afford **2** (10.0 mg), **3** (8.0 mg), **4** (10.5 mg) and **5** (19.5 mg).

2.3.1. (25R)-spirost-5-ene-3β,26β-diol 3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-[(1 → 2)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**1**)

White amorphous powder, mp 240–242 °C; $[\alpha]_D^{20}$-172.8 (*c* 0.10, MeOH); Rf 0.22 (CH_2Cl_2/MeOH 25%); ^{1}H and ^{13}C NMR data: see

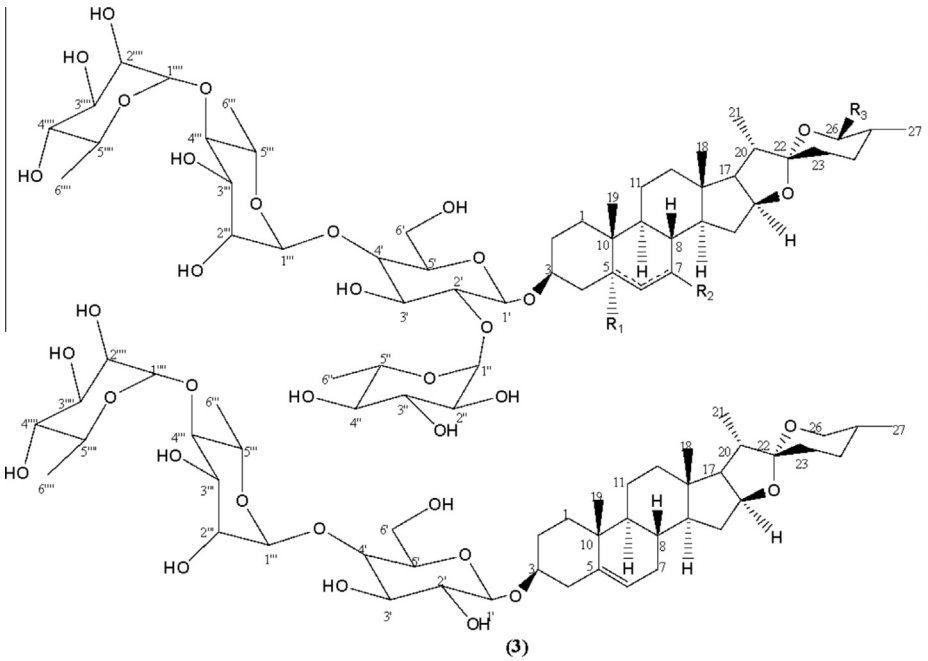

Fig. 1. Structures of compounds **1**–**5**.