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Original Article

Antileishmanial metabolites from *Lantana balansae*

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ABSTRACT

Eleven compounds, 12-oxo-phytodienoic acid (**1**), persicogenin (**2**), eriodictyol 3′,4′,7-trimethyl ether (**3**), phytol (**4**), spathulenol (**5**), 4-hydroxycinnamic acid (**6**), onopordin (**7**), 5,8,4′-trihydroxy-7,3′-dimethoxyflavone (**8**), quercetin (**9**), jaceosidin (**10**), and 8-hydroxyluteolin (**11**), were isolated from an ethanol extract of *Lantana balansae* Briq., Verbenaceae, that was found to possess antileishmanial activity. The structures of the compounds were determined by NMR spectroscopy and HR mass spectrometry, and **1**, **2**, **3**, **7**, **8** and **9** were investigated for antiprotozoal activity toward promastigotes of *Leishmania amazonensis* and *Leishmania braziliensis*. Compound **1** was shown to be the most potent, with the IC_{50} values 2.0 μM toward *L. amazonensis* and 0.68 μM toward *L. braziliensis*, although less potent than the positive control Amphotericin B. All compounds have been reported previously, but this is the first report of the isolation of a cyclopentenone fatty acid (**1**) and flavanones (**2** and **3**) from a *Lantana* species.

Introduction

The knowledge of the traditional uses of plants to treat different conditions has not only been helpful in the search for new biologically active compounds, but also contributed to preserve the information obtained directly from the people living in isolated rural communities. Based on such knowledge, extensive phytochemical studies of different *Lantana* species, particularly *L. camara*, have led to the identification of lantadenes (pentacyclic triterpenoids), flavonoids and phenylpropanoids as the characteristic secondary metabolites of this species. The biological and pharmacological evaluation of crude extracts, essential oils and isolated compounds have shown that they possess a broad range of biological activities, for example antiprotozoal (antiplasmodial, antimalarial, leishmanicidal), antiviral, antioxidant, antiproliferative and cytotoxic activities (Ghisalberti, 2000; Grace-Lynn et al., 2012; Sousa and Costa, 2012).

Lantana balansae Briq., Verbenaceae, is a perennial shrub with a pungent odor that grows in the mountain region of Cochabamba, Bolivia, where it is locally known as "k'ichita". An infusion of fresh leaves of *L. balansae* is used in the traditional medicine to treat digestive disorders and muscle spasms (personal communication with local people where the plant was collected). Previous studies of *L. balansae* have reported the antimicrobial activity of the methanol extract (Salvat et al., 2004) and the chemical composition of its essential oil (De Viana et al., 1973; Sena Filho et al., 2012). As part of our search for bioactive secondary metabolites from the native flora of Bolivia, an ethanol extract of *L. balansae* was assayed for leishmanicidal activity toward *Leishmania amazonensis* and *Leishmania braziliensis*. As the extract displayed significant activity toward both species of *Leishmania* it was selected for a more detailed study. Herein, we wish to report the secondary metabolites isolated from *L. balansae* as well as the leishmanicidal activities of six metabolites.

Materials and methods

General

1D and 2D NMR spectra were recorded at room temperature with a Bruker Avance II 400 or a Bruker Avance 500 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent signals (δ_H 7.16 and δ_C 128.0 for C_6D_6, δ_H 2.05 and δ_C 206.0 for acetone-d_6, and δ_H 2.49 and δ_C 39.5 for DMSO-d_6), while the coupling constants (J) are given in Hz. HR-ESI-MS experiments were performed with a Waters Q-TOF Micro system spectrometer, using H_3PO_4 for calibration and as internal standard. Vacuum liquid chromatography (VLC) separations were carried out on Merck silica gel 60G (Merck), while column chromatography (CC) was performed using silica gel 60 (230–400 mesh, Merck) and gel permeation on

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Sephadex LH-20 (GE-Healthcare). Analytical TLC plates were visualized with UV light at 254 nm and spraying with vanillin followed by heating. Preparative TLC (PTLC) was run on 20 cm × 20 cm glass-coated plates (1 mm thickness, Analtech).

Plant material

The aerial parts of *Lantana balansae* Briq., Verbenaceae, were collected near Independencia, Cochabamba, Bolivia at coordinates 17°11.17′ S 66°43.58′ W and an elevation of 2789 m. Voucher specimens, taxonomically identified by Lic. Modesto Zárate, are kept at "Herbario Forestal Martín Cárdenas", Cochabamba, under accession number MZ-3946.

Extraction and isolation

The air-dried and ground leaves and flowers of *L. balansae* (1308 g) were extracted twice at room temperature by maceration in 95% EtOH for 48 h. After filtration the combined solutions were concentrated under reduced pressure to yield 136.6 g of a dark residue. The crude organic extract was suspended in a mixture of H_2O:MeOH (9:1, v/v, 500 ml) and extracted four times with 500 ml hexane followed by the extraction with ethyl acetate (four times, 500 ml). After evaporation of the solvents, the two fractions (23.5 and 43.6 g, respectively) were fractionated. VLC chromatography (hexane:CH_2Cl_2 1:0 to 0:1) of the hexane extract yielded ten major fractions (A–J). Fraction E (3.0 g) was subjected to VLC (heptane:EtOAc 1:0 to 8:2) which gave nine subfractions (E1–E9). E4 (582.5 mg) was purified by CC on Sephadex LH-20 ($CHCl_3$:MeOH 1:1) to yield **4** (39.3 mg) and **5** (43.0 mg). F (1.2 g) was fractionated by Sephadex LH-20 CC ($CHCl_3$:MeOH 1:1) yielding six subfractions (F1–F6). Compound **10** (3.3 mg) was obtained pure from F5 (53.0 mg). G (485.0 mg) and H (789.0 mg) were fractionated by Sephadex LH-20 CC ($CHCl_3$:MeOH 1:1) to yield eleven (G1–G11) and five (H1–H5) subfractions, respectively. Compound **2** (9.0 mg) was obtained from G8 and **3** (7.7 mg) from G11. Sequential purification of H3 (510.0 mg) by Sephadex LH-20 CC ($CHCl_3$:MeOH 1:1) and VLC (PE:EtOAc 1:0 to 7:3) yielded **1** (26.1 mg). Purification of the EtOAC extract (8.0 g) by VLC (CH_2Cl_2:Me_2CO 1:0 to 0:1) yielded seven major fractions (A–G). C (1.4 g) was subjected to CC on Sephadex LH-20 (MeOH) to give fifteen subfractions (C1–C15), from which compounds **7** (6.4 mg), **11** (4.6 mg) and **9** (4.1 mg) were obtained pure from C10, C14 and C15, respectively. C8 and C11 were purified by PTLC (CH_2Cl_2:Me_2CO 8:2) to give **8** (3.3 mg) and **6** (5.2 mg).

*12-Oxo-phytodienoic acid (**1**)*

Colorless oil; ^{1}H (400 MHz, C_6D_6) δ_H 2.14 (H-2, 2H, t, 7.4 Hz), 1.50 (H-3, 2H, tt, 7, 7 Hz), 1.10 (H-4, 2H, m), 1.06 (H-5, 2H, m), 1.06 (H-6, 2H, m), 1.13 (H-7, 2H, m), 1.06 (H-8, 2H, m), 2.26 (H-9, 1H, m), 6.65 (H-10, 1H, dd, 5.8, 2.6 Hz), 6.00 (H-11, 1H, dd, 5.8, 2.2 Hz), 1.85 (H-13, 1H, ddd, 7.8, 4.5, 2.2 Hz), 2.50 (H-14a, H, m), 2.34 (H-14b, 1H, m), 5.33 (H-15, 1H, dtt, 11, 7, 1 Hz), 5.24 (H-16, 1H, dtt, 11, 7, 1 Hz), 1.97 (H-17, 2H, ddq, 7, 7, 1 Hz), 0.89 (H-18, 3H, t, 7.5 Hz); ^{13}C (100 MHz, C_6D_6) δ_C 179.9 (C-1), 34.1 (C-2), 24.9 (C-3), 29.2 (C-4), 29.7 (C-5), 27.5 (C-6), 29.3 (C-7), 34.5 (C-8), 46.9 (C-9), 166.1 (C-10), 133.1 (C-11), 209.8 (C-12), 51.5 (C-13), 28.5 (C-14), 125.9 (C-15), 133.8 (C-16), 20.9 (C-17), 14.4 (C-18); HR-ESI-MS *m/z* 293.2148 $[M+H]^+$ (caldc. for $C_{18}H_{29}O_3$ 293.2117); $[\alpha_D{}^{20}]$ +60.7 (*c* 0.89, $CDCl_3$).

*Persicogenin (**2**)*

Colorless oil; ^{1}H (400 MHz, C_6D_6) δ_H 4.70 (H-2, 1H, dd, 12.8, 3.1 Hz), 2.50 (H-3a, 1H, dd, 17.1, 12.8 Hz), 2.30 (H-3b, 1H, dd, 17.1, 3.1 Hz), 6.21 (H-6, 1H, d, 2.3 Hz), 6.08 (H-8, 1H, d, 2.3 Hz), 6.62 (H-2′, 1H, d, 2.1 Hz), 6.35 (H-5′, 1H, d, 8.4 Hz), 7.03 (H-6′, 1H, dd, 8.4, 2.1 Hz), 3.09 (OMe-7, 3H, s), 3.08 (OMe-4′, 3H, s), 12.84 (OH-5, 1H, brs), 5.47 (OH-3′, 1H, s); ^{13}C (100 MHz, C_6D_6) δ_C 79.0 (C-2), 43.4 (C-3), 196.2 (C-4), 165.2 (C-5), 95.3 (C-6), 168.3 (C-7), 94.5 (C-8), 163.3 (C-9), 103.8 (C-10), 132.5 (C-1′), 113.3 (C-2′), 146.5 (C-3′), 147.0 (C-4′), 110.1 (C-5′), 118.0 (C-6′), 55.1 (OMe-7), 55.3 (OMe-4′); HR-ESI-MS *m/z* 317.1046 $[M+H]^+$ (caldc. for $C_{17}H_{17}O_6$ 317.1025).

*Eriodictyol 3′,4′,7-trimethyl ether (**3**)*

Colorless oil; ^{1}H (400 MHz, C_6D_6) δ_H 4.76 (H-2, 1H, dd, 13.1, 2.9 Hz), 2.62 (H-3a, 1H, dd, 17.1, 13.1 Hz), 2.39 (H-3b, 1H, dd, 17.1, 2.9 Hz), 6.25 (H-6, 1H, d, 2.2 Hz), 6.16 (H-8, 1H, d, 2.2 Hz), 6.69 (H-2′, 1H, d, 2 Hz), 6.52 (H-5′, 1H, d, 8.8 Hz), 6.68 (H-6′, 1H, dd, 9, 2 Hz), 3.07 (OMe-7, 3H, s), 3.36 (OMe-4′, 3H, s), 3.40 (OMe-5′, 3H, s), 12.91 (OH-5, 1H, brs); ^{13}C (100 MHz, C_6D_6) δ_C 79.7 (C-2), 43.9 (C-3), 196.6 (C-4), 165.7 (C-5), 95.6 (C-6), 168.6 (C-7), 94.9 (C-8), 163.7 (C-9), 104.2 (C-10), 131.8 (C-1′), 111.0 (C-2′), 150.8 (C-3′), 150.6 (C-4′), 112.2 (C-5′), 119.4 (C-6′), 55.4 (OMe-7), 55.9 (OMe-4′), 55.9 (OMe-5′); HR-ESI-MS *m/z* 331.1207 $[M+H]^+$ (caldc. for $C_{18}H_{19}O_6$ 331.1182).

*Onopordin (**7**)*

Yellow powder; ^{1}H (500 MHz, Acetone-d_6) δ_H 6.58 (H-3, 1H, s), 6.60 (H-6, 1H, s), 7.50 (H-2′, 1H, d, 2.2 Hz), 6.99 (H-5′, 1H, d, 8.3 Hz), 7.47 (H-6′, 1H, dd, 8.3, 2.2 Hz), 3.87 (OMe-8, 3H, s), 12.25 (OH-5, 1H, brs); ^{13}C (125 MHz, acetone-d_6) δ_C 165.4 (C-2), 103.8 (C-3), 183.6 (C-4), 157.7 (C-5), 94.8 (C-6), 154.0 (C-7), 132.2 (C-8), 154.1 (C-9), 105.8 (C-10), 123.9 (C-1′), 114.2 (C-2′), 146.6 (C-3′), 150.3 (C-4′), 116.7 (C-5′), 120.2 (C-6′), 60.8 (OMe-8); HR-ESI-MS *m/z* 331.0685 $[M+H]^+$ (caldc. for $C_{16}H_{13}O_7$ 317.0661).

*5,8,4′-Trihydroxy-7,3′-dimethoxyflavone (**8**)*

Yellow powder; ^{1}H (500 MHz, acetone-d_6) δ_H 6.71 (H-3, 1H, s), 6.64 (H-6, 1H, s), 7.65 (H-2′, 1H, d, 2.0 Hz), 7.01 (H-5′, 1H, d, 8.3 Hz), 7.62 (H-6′, 1H, dd, 8.3, 2.0 Hz), 3.87 (OMe-7, 3H, s), 3.99 (OMe-3′, 3H, s), 13.25 (OH-5, 1H, brs); ^{13}C (125 MHz, acetone-d_6) δ_C 165.4 (C-2), 103.9 (C-3), 183.7 (C-4), 157.6 (C-5), 94.8 (C-6), 132.1 (C-7), 153.9 (C-8), 157.0 (C-9), 105.8 (C-10), 123.7 (C-1′), 110.5 (C-2′), 148.9 (C-3′), 151.5 (C-4′), 116.4 (C-5′), 121.4 (C-6′), 60.7 (OMe-7), 56.6 (OMe-3′); HR-ESI-MS *m/z* 331.0826 $[M+H]^+$ (caldc. for $C_{17}H_{15}O_7$ 331.0818).

*Quercetin (**9**)*

Yellow powder; ^{1}H (500 MHz, Acetone-d_6) δ_H 6.26 (H-6, 1H, d, 2.0 Hz), 6.53 (H-8, 1H, d, 2.0 Hz), 7.83 (H-2′, 1H, d, 2.2 Hz), 6.99 (H-5′, 1H, d, 8.5 Hz), 7.69 (H-6′, 1H, dd, 8.5, 2.2 Hz), 12.17 (OH-5, 1H, brs); ^{13}C (125 MHz, acetone-d_6) δ_C 164.9 (C-2), 136.7 (C-3), 176.6 (C-4), 162.3 (C-5), 99.1(C-6), 146.9 (C-7), 94.4 (C-8), 157.8 (C-9), 104.2 (C-10), 123.8 (C-1′), 115.8 (C-2′), 145.8 (C-3′), 148.3 (C-4′), 116.3 (C-5′), 121.5 (C-6′); HR-ESI-MS *m/z* 303.0546 $[M+H]^+$ (caldc. for $C_{15}H_{11}O_7$ 303.0505).

Leishmanicidal detection assay

The antileishmanial assay was performed using a colorimetric method-XXT (Salamanca et al., 2008). Briefly, the activity was measured *in vitro* on cultures of the *Leishmania* parasite in the promastigote forms, of complex *L. amazonensis* (clon 1: Lma, MHOM/BR/76/LTB-012) and complex *L. braziliensis* (strand M2904 C192 RJA) that were cultivated at 26 °C in Schneider medium (pH 6.8) supplemented with inactivated (by heating to 56 °C for 30 min) bovine calf serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites/ml, were seeded in the wells of 96-well plates. Solutions of compounds to be assessed at concentration range of 0.09–100 μg/ml were added. DMSO (1%) and amphotericin B (0.5 μg/ml) were used as negative and positive controls during the evaluations. All assays were performed in triplicate and the micro well plates were incubated for 72 h at 26 °C. After incubation, a solution of XTT (1 mg/ml) in PBS (pH 7.0 at 37 °C)