Published in final edited form as:

Phytochemistry. 2012 October ; 82: 128–135. doi:10.1016/j.phytochem.2012.06.012.

Sterols with antileishmanial activity isolated from the roots of *Pentalinon andrieuxii*

Li Pan^a, Claudio M. Lezama-Davila^b, Angelica P. Isaac-Marquez^c, Edward P. Calomeni^b, James R. Fuchs^a, Abhay R. Satoskar^b, and A. Douglas Kinghorn^{a,*}

^aDivision of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

^bDepartment of Pathology, Medical Center, The Ohio State University, Columbus, OH 43210, USA

^cCentro de Investigaciones Biomedicas, Universidad Autónoma de Campeche, Campeche, Campeche 24030, Mexico

Abstract

A new cholesterol derivative, pentalinosterol (cholest-4,20,24-trien-3-one, **1**), and a new polyoxygenated pregnane sterol glycoside, pentalinonside (**2**), together with 18 known compounds, including 14 sterols (**3–16**), three coumarins (**17–19**), and a triterpene (**20**), were isolated from a *n*-hexane partition of a methanol extract of the roots of the Mexican medicinal plant *Pentalinon andrieuxii*. Structure elucidation of compounds **1** and **2** was accomplished by spectroscopic data interpretation. All isolates were evaluated *in vitro* for their antileishmanial activity. Among these compounds, 6,7-dihydroneridienone (**15**) was found to be the most potent principle against promastigotes of *Leishmania mexicana* (*L. mexicana*). The cholesterol analogue, pentalinosterol (**1**), together with two known sterols, 24-methylcholest-4,24(28)-dien-3-one (**3**) and neridienone (**16**), also exhibited significant leishmanicidal activity in this same bioassay. Compounds **1**, **3**, **15**, **16**, cholest-4-en-3-one (**4**), and cholest-5,20,24-trien-3 β -ol (**7**), showed strong antileishmanial activity against amastigotes of *L. mexicana*, and **4** was found to be the most potent agent with an IC₅₀ value of 0.03 μ M. All the isolates were also evaluated for their cytotoxicity in non-infected bone marrow-derived macrophages, but none of these compounds was found active towards this cell line. The intracellular parasites treated with compounds **1**, **3**, **4**, **15**, and **16** were further studied by electron microscopy; morphological abnormalities and destruction of the amastigotes were observed, as a result of treatment with these compounds.

Keywords

Pentalinon andrieuxii (syn. *Urechites andrieuxii*); Apocynaceae; Sterols; Antileishmanial activity; *Leishmania mexicana*; Promastigotes; Amastigotes; Macrophages; Electron microscopy

1. Introduction

Pentalinon andrieuxii Mueller-Argoviensis (syn. *Urechites andrieuxii*, Apocynaceae), is a native plant in the Yucatan Peninsula of Mexico known as “bejuco guaco”, “cantibteac”, or “contrayerba”. This plant is used in Mayan folk medicine to treat cutaneous leishmaniasis lesions (Chiclero’s ulcer) in the states of Campeche and Quintana Roo, Mexico (Pulido

Salas and Serralta Peraza, 1993; Argüeta et al., 1994). Treatment of Chiclero's ulcer in Mayan traditional medicine uses the internal part of the root, which is fixed tightly to the skin lesions. This procedure is repeated each day after careful cleaning of lesions with an infusion of the roots and leaves, until visible healing is observed. In addition, this plant may be used to treat snakebites as well as to alleviate headaches and nervous disturbances (Pulido Salas and Serralta Peraza, 1993; Argüeta et al., 1994). Previous biological studies on extracts of this plant have led to reports of antiatherogenic, anti-inflammatory, antileishmanial, and antidepressant activities (Jiu, 1966; Lezama-Dávila and Isaac-Márquez, 1994; Lezama-Dávila et al., 2007a; Chan-Bacab et al., 2003). Among these activities, the antileishmanial effect of this plant is of particular interest. It has been reported that both the aqueous and organic extracts of *P. andrieuxii* exhibit *in vitro* antileishmanial activity, and the most active extract was determined as the hexane-soluble extract of the roots of this species. An extract of this plant part (10 µg/mL) was effective in killing 1 million *Leishmania mexicana* promastigotes cultured *in vitro*, and showed even more potent leishmanicidal activity than that of the control compound, meglumine antimoniate (GlucantimeTM) (Lezama-Dávila et al., 2007a). Thus far, the phytochemical investigation of *P. andrieuxii* is limited, with cardenolides, flavonoids, pregnane sterols, trinosesquiterpenoids and a triterpenoid, having been reported as secondary metabolites from extracts of the roots or leaves (De la Rosa et al., 1995; Yam-Puc et al., 2009, 2012; Domínguez-Carmona et al., 2010). There have been no active principles reported corresponding to the antileishmanial activity of this plant to date.

Development of alternative therapies to cure cutaneous leishmaniasis in the Yucatan peninsula and elsewhere is necessary, since access to the licensed drugs such as meglumine antimoniate in remote tropical areas may be either limited or entirely absent. As part of a collaborative effort leading to the discovery of new naturally occurring antileishmanial agents from plants, the roots of *P. andrieuxii* collected in a sub-tropical rainforest region of Mexico, were selected for activity-guided fractionation, following an initial screen for the lethal effect on the protozoa of *L. mexicana*. The present investigation on a hexane-soluble extract of *P. andrieuxii* led to the isolation and biological evaluation of 16 sterol derivatives (**1–16**), as well as three coumarins (**17–19**), and a triterpenoid (**20**) (Fig. 1). Among these compounds, **1** was determined to be a new cholestane derivative, and **2** a new sterol glycoside bearing a rare polyoxygenated 14,15-secopregane skeleton. The structures of these two new compounds were elucidated based on the analysis of their physical and spectroscopic data ($[\alpha]_D$, CD, ¹H NMR, ¹³C NMR, DEPT, 2D NMR, and HRESIMS). The known compounds isolated in the present study were identified as 24-methylcholesta-4,24(28)-dien-3-one (**3**) (Sheikh and Djerassi, 1974), cholest-4-en-3-one (**4**) (Kontiza et al., 2006), stigmast-4,22-dien-3-one (**5**) (Kontiza et al., 2006), stigmast-4-en-3-one (**6**) (Kontiza et al., 2006), cholest-5,20,24-trien-3 β -ol (**7**) (Cabrera et al., 1991), cholest-5,24-dien-3 β -ol (demosterol, **8**) (Yan et al., 2002), 24-methylcholest-5,24(28)-dien-3 β -ol (**9**) (Cabrera et al., 1991), cholesterol (**10**) (Seo et al., 1986), isofucosterol (**11**) (Anastasia et al., 1985), β -sitosterol (**12**) (McCarthy et al., 2005), 7-ketositosterol (**13**) (McCarthy et al., 2005), 7-ketostigmasterol (**14**) (Cui et al., 2009), 6,7-dihydroneridienone (**15**) (Schun and Cordell, 1987), neridienone (**16**) (Bai et al., 2007), serborosin (**17**) (de Melo Cazal et al., 2009), xanthoxyletin (**18**) (de Melo Cazal et al., 2009), xanthyletin (**19**) (de Melo Cazal et al., 2009), and oleanolic acid 3-acetate (**20**) (González et al., 1981), by comparison of their physical and spectroscopic data with published values. In this communication, the isolation and structure elucidation of new sterols (**1** and **2**) are described, as well as the *in vitro* leishmanicidal activities of all isolates against promastigotes and amastigotes of *L. mexicana*.

2. Results and discussion

2.1. Structure elucidation of new compounds

Compound **1** was obtained as a colorless gum. The molecular formula was assigned as $C_{27}H_{40}O$, based on the ion peak at m/z 381.3154 $[M+H]^+$ (Calc. for $C_{27}H_{41}O$ for $C_{27}H_{41}O$, 381.3157) in the HRESIMS. The typical IR absorptions supported the presence of methyl (2953 and 2873 cm^{-1}), methylene (2925 and 2852 cm^{-1}), ketone (1733 cm^{-1}), and double bond (1676 cm^{-1}) moieties. The UV maximum at 239 nm ($\log \epsilon$ 3.99) suggested the presence of an α,β -unsaturated ketone group. The 1H NMR spectroscopic data of **1** (Table 1) showed two characteristic broad singlets at δ_H 4.80 (1H) and 4.90 (1H), consistent with the presence of a vinylic group. A singlet at δ_H 5.73 (1H, H-4) and a broad triplet at 5.11 (1H, brt, $J = 7.0$ Hz, H-24) indicated the occurrence of two trisubstituted double bonds. In the high-field region, proton signals of two tertiary methyl groups at δ_H 0.62 (3H, s, H-18) and 1.19 (3H, s, H-19), together with resonances of two tertiary methyl groups linked to the double bond at δ_H 1.61 (3H, s, H-26) and 1.69 (3H, s, H-27), could be recognized. Besides these methyl group protons, the highly overlapped signals distributed in the high field region from 0.8 to 2.4 ppm suggested the presence of a group of alkyl methylene and methine resonances. Consistent with the 1H NMR data, the ^{13}C NMR spectroscopic data of **1**, which were sorted using DEPT and HSQC spectra, showed signals of a vinylic group at δ_C 149.5 (C-20) and 110.1 (C-21), two trisubstituted double bonds at δ_C 124.2 (C-4), 171.9 (C-5), 124.7 (C-24), and 132.0 (C-25), and four methyl carbons at δ_C 13.3 (C-18), 17.8 (C-19 and C-26), and 26.2 (C-27). Furthermore, the other carbon resonances in the ^{13}C NMR spectrum could be classified into 10 methylene carbons, four methine carbons, two quaternary carbons, and a ketone group. The NMR information combined with the molecular formula obtained from the HRESIMS, suggested that compound **1** is a cholestane steroid with an α,β -unsaturated ketone feature within the tetracyclic ring system and two double bonds in the side-chain. In the HMBC spectrum of **1**, correlations from H-4 to C-6 and C-10, and from H₃-19 to C-1, C-5, and C-9, confirmed the 3-keto-4-ene functionality of the A ring. The key HMBC correlations of the vinylic protons (H-21) with C-17 and C-22, H₃-26 and H₃-27 with C-24 and C-25, led to the placement of the two double bonds at C-20 and C-24 of the side-chain, respectively. Observed key NOE effects of H-8/H₃-19, H-8/H₃-18, H-14/H-9, H-14/H-17, and H-21/H-18 were used to establish the relative configuration of compound **1**. The absolute configuration of **1** was determined from the CD spectrum, and **1** showed a weak negative band around 317 nm ($\Delta \epsilon = -1.44$), corresponding to an α,β -unsaturated carbonyl $n-\pi^*$ excitation, and an intensive positive band around 238 nm ($\Delta \epsilon = +7.14$), representing a $\pi-\pi^*$ transition. These observed Cotton effects were very similar to those of the known compound, (8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*R*)-cholest-4-en-3-one (Gawronski, 1982). Thus, the structure of the new compound **1** was proposed as (8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-cholest-4,20,24-trien-3-one, and this was accorded the trivial name, pentalinosterol.

Compound **2** was obtained as a colorless resin. The HRESIMS of **2** afforded a sodiated molecular ion peak at m/z 513.2817, corresponding to an elemental formula of $C_{28}H_{42}O_7Na$ (Calc. m/z 513.2828). The IR spectrum exhibited typical absorptions of hydroxy (3477 cm^{-1}), alkyl methyl (2949 and 2889 cm^{-1}), and methylene (2933 and 2870 cm^{-1}) moieties. In the UV spectrum, no obvious absorption was observed within the 200–400 nm region. In the 1H NMR spectrum of **2**, a 2,6-deoxy sugar unit was evident from signals at δ_H 4.84 (1H, dd, $J = 9.6, 2.0$ Hz, H-1', the anomeric proton), 1.73 and 2.10 (each 1H, m, H-2), 3.43 (1H, ddd, $J = 12.0, 5.0, 2.0$ Hz, H-3), 3.91 (1H, brs, H-4), 3.60 (1H, q, $J = 6.5$ Hz, H-5), 1.55 (1H, d, $J = 6.5$ Hz, H-6), and 3.40 (3H, s, OCH₃-3). The chemical shifts and the coupling pattern of these proton signals were found to be comparable with those of diginose, and the large J value (9.6 Hz) of the anomeric proton indicated the β configuration of this 2,6-deoxyhexose

sugar unit (Zhao et al., 2007; Kuroda et al., 2010). The corresponding ^{13}C NMR spectroscopic data of this monosaccharide were observed at δ_{C} 98.8 (C-1, anomeric carbon), 33.3 (C-2), 79.3 (C-3), 67.1 (C-4), 71.5 (C-5), 17.8 (C-6), and 55.4 (OCH_3 -3). Besides the signals of the sugar unit, the ^1H NMR spectrum also displayed signals of seven oxygenated protons at δ_{H} 3.90 (1H, m, H-3), 4.02 (dd, J = 10.0, 4.4 Hz, H_α -15), 4.23 (1H, d, J = 10 Hz, H_β -15), 4.56 (1H, d, J = 4.5 Hz, H-16), 4.49 (1H, ddd, J = 7.7, 6.0, 1.5 Hz, H-20), 3.79 (dd, J = 12.4, 1.3 Hz, H_α -21), and 4.05 (dd, J = 12.0, 6.0 Hz, H_β -21), a signal for an olefinic proton at δ_{H} 5.52 (1H, t, J = 2.5 Hz, H-6), and the presence of two tertiary methyl groups at δ_{H} 0.97 (3H, s, H-19) and 1.08 (3H, s, H-18), as well as a number of highly overlapped resonances distributed in the high-field region from 0.8 to 2.4 ppm for alkyl methylenes and methines, all of which could be attributed to a highly oxygenated steroidal aglycone moiety. The 21 skeletal signals in the ^{13}C NMR spectrum were classified by the DEPT and HSQC spectra into two methyls, six methylene groups, two alkyl methines, three quaternary alkyl carbons, six oxygenated carbons (including two primary, three secondary and one tertiary), and a trisubstituted double bond. This suggested that compound **2** is a 3-oxy-4-en-14,15-secopregnane derivative (Plaza et al., 2003, 2005; Perrone et al., 2006, 2008). In the COSY spectrum, the oxygenated methine proton at δ_{H} 4.56 (1H, t, J = 4.5 Hz, H-16) showed correlations with an alkyl methine proton at δ_{H} 2.60 (1H, dd, J = 7.6, 4.4 Hz, H-17) and one geminal proton of an oxygenated methylene at δ_{H} 4.02 (1H, dd, J = 10.0, 4.4 Hz, H_α -15). Another oxygenated methine proton at δ_{H} 4.49 (1H, ddd, J = 7.6, 6.0, 1.5 Hz, H-20) also showed correlations with H-17, and two geminal protons of an oxygenated methylene at δ_{H} 4.05 (1H, dd, J = 12.4, 6.0 Hz, H_α -21) and 3.79 (1H, d, J = 12.4, 1.3 Hz, H_β -21), respectively. Thus, an important spin system including H-15, H₂-16, H-17, H-20 and H₂-21 in compound **2** was deduced as shown (Fig. 2). Furthermore, the key HMBC correlations from H-16, H-17 and H-21 to C-14, and from H-15 and H-16 to C-20, were used to construct a highly oxygenated unit. This unusual moiety in **2** involved the opening of the ring D of the pregnane skeleton between C-14 and C-15, and the connection through oxygen bridges of C-14 with C-16, C-15 with C-20, as well as C-21 with C-14 to generate a six-membered ring and two tetrahydrofuran rings with a ketal group on C-14.

The occurrence of 14,15-secopregnane derivatives with similar polyoxygenated features to **2** is rare in the plant kingdom, and other naturally occurring compounds of this class have been found from a limited number of plants such as species in the genera *Cynanchum* and *Solenostemma* of the family Asclepiadaceae, and the genus *Mandevilla* of the family Apocynaceae (Kennard et al., 1968; Lavault et al., 1999; Chen et al., 2008; Plaza et al., 2003, 2005; Perrone et al., 2006, 2008; Niero et al., 1999, 2002; Yunes et al., 1993). These compounds are characterized by the cleavage of the D ring between C-14 and C-15, a hexahydrofuro[3,4-*b*]furan ring on ring C, with C-20 connected to C-14 or C-18 through an oxygen atom, to generate ketal groups at C-14 and/or C-20. A structurally unusual feature of compound **2** is that it exhibits for the first time a C-21 methyl group in the pregnane skeleton incorporated into two tetrahydrofuran rings to form a keto group at C-14. The relative configuration of **2** was deduced from a NOESY NMR experiment. The key NOE effects between H-17 with H-16 suggested a *cis* junction of the two tetrahydrofuran rings of the highly oxygenated ring system, and the NOE effects between H-18 with H-20 and H_α -21 indicated the β -orientation of the C-21 oxygenated methylene group. The relative configurations of the remaining portion of the aglycone were consistent with those of known pregnane steroids, based on an analysis of the NOE spectrum. The β position of the diginosyl group on C-3 was deduced by the NOE correlations of H-3 with H_α -4 and H_α -2. The configuration of the β -diginose unit was confirmed by NOE correlations between H-1/ H_α -2, H-3, and H-5, H-4/H-5, OCH_3 -3/ H_β -2. The sugar unit obtained by acid hydrolysis of compound **1** exhibited a positive optical rotation value, which demonstrated that the absolute configuration of the sugar is in the D form. Furthermore, an energy-minimized model generated by Chem3D Ultro 10.0 based on a presumed configuration matched well with all

the observed NOESY correlations and served to explain the observed coupling patterns for H₂-15, H-16, H-17, H-20, and H₂-21 of the polyoxygenated ring system (Fig. 3). Thus, the structure of compound **2** (pentalinonside) was determined as 14,16-14,21-15,20-triepoxy-14,15-secopregnan-5-en-3-ol 3-*O*- β -D-diginopyranoside.

2.2. Antileishmanial activity evaluation

All the isolated compounds (**1–20**) from the roots of *P. andrieuxii* were evaluated for their antileishmanial activity on both the promastigote and amastigote stages of *L. mexicana* (Table 2). After 48 h of exposure against *L. mexicana* promastigotes, five sterols (**1**, **3**, **4**, **15**, and **16**) were found to show inhibitory effects and were more active than observed for the reference compound, pentostam. Of these compounds, 6,7-dihydroneridienone (**15**), a known C₂₁ sterol, was the most potent principle, with an IC₅₀ value of 9.2 μ M. The new cholesterol analogue, cholest-4,20,24-trien-3-one (pentalinonsterol, **1**), together with three known sterols, cholest-4-en-3-one (**4**), 24-methylcholest-4,24(28)-dien-3-one (**3**) and neridienone (**16**), also exhibited antileishmanial activity against promastigotes of *L. mexicana*.

All compounds with leishmanicidal activity against *L. mexicana* promastigotes, together with compound **7**, which was considered inactive for the extracellular stage, were observed to show significant activity against the amastigote stage (Table 2). Among these compounds, **1**, **3**, **15**, and **16**, gave IC₅₀ values of 3.3, 3.5, 1.4 and 3.5 μ M, respectively, and exhibited comparable potency to the positive control, Pentostam (IC₅₀ 2.7 μ M). Compound **7** was less active than Pentostam, with an IC₅₀ value of 14.5 μ M. Compound **4** showed the most potent leishmanicidal activity on this intracellular stage, with an IC₅₀ value of 0.03 μ M, nearly 100 times more potent than Pentostam (see Table 2).

It is notable that except compound **7**, all the other active sterols with leishmanicidal activity in the present study share a common 4-ene-3-oxo functionality in the steroidal ring system, while for the most of sterols (**8–14**) with a 3-ol-5-ene moiety, no obvious antileishmanial activity was observed. Variation of the side-chain on the five-membered D ring of these 4-ene-3-oxo sterols also influenced the resultant activity. Thus, compounds **5** and **6**, two stigmasterol derivatives with an extra ethyl group at C-24 of the side-chain when compared with the active cholesterol derivatives **1** and **4**, were both inactive in the bioassays used. The new compound, pentalinonsterol (**1**), with double bonds at C-20 and C-24, was found to be almost three times more active in the promastigote bioassay than cholest-4-en-3-one (**4**), for which the side-chain is saturated. None of the 3-ol-5-ene sterols was found active in the promastigote assay. Compound **7**, the only agent isolated with a 3-ol-5-ene functionality active in the amastigote assay, possesses the same side-chain on the D ring as that in compound **1**. For the two active C₂₁ sterols, 6,7-dihydroneridienone (**15**) and neridienone (**16**), both have an acetyl group on the D ring instead of a long alkyl chain that occurs in other active compounds. Compound **15** was found to be more active than **16** in both the promastigote and amastigote bioassay, with the latter possessing an extra double bond at C-5 in the B ring (Table 1).

All the isolates were also evaluated for their cytotoxicity in non-infected bone marrow-derived macrophages from C57BL/16 mice, the host cells. Since none were found active in this bioassay (IC₅₀ > 100 μ g/mL), the selectivity of the active antileishmanial compounds obtained in this investigation was established.

Electron microscopy (Em) of parasites treated for 2 h at a 100 μ M concentration of the individual bioactive molecules (compounds **1**, **3**, **4**, **15**, and **16**), demonstrated important morphological abnormalities and destruction as compared to sham control or reference drug-

treated parasites (100 μ M) (Fig. 4). Membrane alterations were observed clearly by treating the organisms with these active sterols. As a result, it is possible that the active sterols may replace cholesterol during the membrane biosynthesis of the parasites and induce membrane instability and disruption as seen clearly in the EM images.

3. Concluding remarks

Like fungi, the predominant endogenous sterols found in *Leishmania* species are ergosterol and its derivatives, and these sterols are constituents of cell membranes, which are essential for the normal structure and function of these parasites (de Souza and Rodrigues, 2009). Thus, the sterol biosynthesis pathway is considered as a promising target in the development of new therapeutic agents for the treatment of parasitic diseases (Werbovetz, 2002). In recent years, studies have shown that a number of sterol-derived compounds exhibit antileishmanial activities, and some of them such as azasterols are reported as inhibitors of certain enzymes involved in the sterol biosynthesis pathway of these parasites (Haughan et al., 1995; Magaraci et al., 2003; Bazin et al., 2006; Sartorelli et al., 2007). Based on these facts, the active sterols isolated from *P. andrieuxii* in the present study might exert their activity by acting as antagonists of endogenous sterols to interfere or inhibit sterol biosynthesis and lead to organism death.

The overall bioassay testing results observed in this investigation indicate that several sterol constituents should be at least in part responsible for the antileishmanial activity exhibited by the hexane-soluble extract of the roots of *P. andrieuxii*. Furthermore, some of these compounds represent promising antiprotozoal leads for further development.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 343 automatic polarimeter. UV spectra were measured with a Perkin-Elmer Lambda 10 UV/vis spectrometer. CD spectra were recorded on a JASCO J-810 spectrometer. IR spectra were run on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. NMR spectroscopic data were obtained on a Bruker Avance DRX-400 or 600 MHz spectrometer (with TMS as an internal standard). Accurate mass values were obtained on a Micromass LCT ESI spectrometer. Sodium iodide was used for mass calibration for a calibration range of m/z 100–2000. Column chromatography (CC) was performed with 65–250 or 230–400 mesh silica gel (Sorbent Technologies, Atlanta, GA). Analytical thin-layer chromatography (TLC) was conducted on precoated 250 μ m thickness silica gel plates (UV₂₅₄, glass backed, Sorbent Technologies), and preparative TLC was performed on precoated 20 cm $\times$ 20 cm, 500 μ m thickness silica gel plates (UV₂₅₄, glass backed, Sorbent Technologies). Analytical HPLC was conducted on a 150 mm $\times$ 4.6 mm i.d. Sunfire PrepC₁₈ column (Waters, Milford, MA), and semi-preparative HPLC was conducted on a 150 mm $\times$ 10 mm i.d. or a 150 mm $\times$ 19 mm i.d., 5 μ m Sunfire PrepC₁₈ column (Waters), along with a Waters system equipped with a 600 controller, a 717 Plus autosampler, and a 2487 dual wavelength absorbance detector. Electron micrographs were generated on JEOL JEM-1400 TEM (JEOL, Ltd., Tokyo, Japan), operating at 80 K equipped with a Veleta digital camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

4.2. Plant material

Roots of *P. andrieuxii* Muell.-Arg. were collected in the rainforest of Campeche, Mexico (municipalities of Campeche and Escarcega) in May and November 2009. The plant was identified in the Herbarium of the Autonomous University of Campeche (Universidad Autonoma de Campeche), Mexico, under voucher No. 6921 (Zamora-Crescencio &

Lezama-Dávila) and in the Herbarium of the Autonomous University of Yucatan (Universidad Autonoma de Yucatan), Mexico, under voucher No. 1 (Viscencio de la Rosa & Lezama-Dávila). The recent collection and positive identification of this plant was supervised by the Director of the Herbarium of UACAM, Mr. Pedro Zamora-Crescencio.

4.3. Extraction and isolation

Air-dried roots of *P. andrieuxii* (900 g) were milled and then extracted with methanol overnight at room temperature (3×4 L). The macerate was concentrated *in vacuo* (80 g) and partitioned to afford hexane-soluble (7.0 g) and CHCl_3 -soluble extracts (20 g). The hexane-soluble extract was found to be active against promastigotes of *L. mexicana* ($\text{IC}_{50} = 35 \mu\text{g/mL}$). Part of the hexane-soluble partition (6.5 g) was subjected to separation over a silica gel column using pure hexane initially, followed in turn by a gradient of increasing polarity using hexane–EtOAc and pure acetone to yield eight pooled fractions (F01–F08). Fractions F01, F02, F06, and F07 were found to be active against this assay using a concentration of $100 \mu\text{g/mL}$.

Fraction F01 (900 mg) was further purified by passage on a fine silica gel (3.0×45 cm) column using a gradient solvent of hexane–EtOAc, with nine pooled fractions obtained (F0101–F0109). Visualization with sulfuric acid detection showed fraction F0103 (120 mg) to be rich in compounds with a similar TLC behavior to terpenoids or steroids (Waksmundzka-Hajnos et al., 2008). This fraction was further purified repeatedly by HPLC on a semi-preparative RP18 column ($150 \text{ mm} \times 10 \text{ mm i.d.}$) using MeOH– H_2O (95:5; 5 mL/min) as the mobile phase, to yield compounds **1** (1.2 mg, $t_R = 11.2$ min), **3** (4.0 mg, $t_R = 16.7$ min), **4** (2.0 mg, $t_R = 19.2$ min), **5** (6.0 mg, $t_R = 22.3$ min), and **6** (8.0 mg, $t_R = 25.2$ min).

Fraction F02 (980 mg) was subjected to separation over a silica gel column and eluted with a hexane–EtOAc gradient solvent system. Of the subfractions obtained, F0203 was detected by TLC as a sterol-rich fraction, which was further purified on a semi-preparative RP18 column ($150 \text{ mm} \times 10 \text{ mm i.d.}$) by HPLC using MeOH– H_2O (90:10; 5 mL/min) as the solvent system, to yield compounds **20** (4.0 mg, $t_R = 7.2$ min), **7** (4.0 mg, $t_R = 12.0$ min), **8** (6.0 mg, $t_R = 15.7$ min), **9** (6.0 mg, $t_R = 17.8$ min), **10** (2.0 mg, $t_R = 20.2$ min), **11** (4.0 mg, $t_R = 21.7$ min), and **12** (24.0 mg, $t_R = 27.4$ min). Compound **17** (2.0 mg) was purified from subfraction F0202 by repeated chromatography on silica gel columns, using hexane–acetone for elution.

Fractions F06 and F07 were combined (380 mg) and applied to a silica gel column and eluted with a CH_2Cl_2 –acetone gradient solvent system (30:1 to pure acetone) to yield 14 subfractions. Compound **2** (5.0 mg) was purified from subfraction F06F12 by repeated chromatography using silica gel columns with a CH_2Cl_2 –acetone gradient solvent system (15:1 to 4:1). Subfraction F06F03 was subjected to semi-preparative RP18 ($150 \text{ mm} \times 10 \text{ mm i.d.}$) HPLC using CH_3CN – H_2O (35:65; 5 mL/min) as mobile phase, to give compounds **15** (1.0 mg, $t_R = 13.6$ min), and **16** (0.8 mg, $t_R = 15.8$ min).

In order to find certain common flavonoids or coumarins as potential marker compounds to provide a basis for extract standardization, fraction F04, which was found to be rich in compounds showing fluorescence under UV light at 365 nm on TLC plates, was also investigated. Compounds **14** (1.5 mg, $t_R = 17.2$ min) and **13** (4.0 mg, $t_R = 19.5$ min) were purified from a less polar subfraction of F04 by preparative HPLC on a semi-preparative RP18 column ($150 \text{ mm} \times 10 \text{ mm i.d.}$), using MeOH– H_2O (92:8, 5 mL/min) as eluting solvent. Compounds **18** (3.0 mg, $t_R = 6.9$ min) and **19** (2.0 mg, $t_R = 8.1$ min) were purified from a more polar subfraction of F04 by preparative HPLC on a semi-preparative RP18 column ($150 \text{ mm} \times 10 \text{ mm i.d.}$), using MeOH– H_2O (70:30, 5 mL/min) as eluting solvent.

4.4. Pentalinonsterol (1)

Colorless gum; $[\alpha]_D^{20} +56.0$ (*c* 0.1, CH₂Cl₂); UV (MeOH) $\lambda_{\max}$ (log *e*) 239 (3.99), 316 (0.90) nm; CD (*c* 1.31×10^{-5} M, CH₂Cl₂/MeOH) $\lambda_{\max}$ ($\Delta\epsilon$) 238 (+7.14), 317 (−1.44) nm; IR (film) $\nu_{\max}$ 2952, 2925, 2873, 2852, 1733, 1676, 1456, 1376, 1230, 1170 cm^{−1}; for ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (100 MHz, CDCl₃) data, see Table 1; HRESIMS *m/z* 381.3154 [M+H]⁺ (Calc. for C₂₇H₄₁O, 381.3157).

4.5. Pentalinonside (2)

Colorless gum; $[\alpha]_D^{20} -30.0$ (*c* 0.1, MeOH); UV (MeOH) end absorption; IR (film) $\nu_{\max}$ 3477, 2949, 2933, 2889, 2870, 1640, 1460, 1381, 1258, 1168, 1098, 1067 1025, and 977 cm^{−1}; for ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (100 MHz, CDCl₃) data, see Table 1; HRESIMS *m/z* 513.2817 [M+Na]⁺ (Calc. for C₂₈H₄₂O₇Na, 513.2828).

4.6. Acid hydrolysis of compound 2

Compound **2** (2.0 mg) in MeOH (0.8 mL) was added to 0.05 M H₂SO₄ (0.8 mL), and the solution was then heated at 70 °C for 3 h. The reaction mixture was neutralized by passage through a Dowex® 1 × 2 column (2.0 × 2.0 cm), and was then applied to an ODS column (1.5 × 2.0 cm), with MeOH–H₂O (40:60 to 70:30) as eluent, to give diginose (0.2 mg). This 2,6-deoxyhexose sugar unit was assigned in the D-form based on its optical rotation value.

D-diginose: $[\alpha]_D^{20} +21.0$ (*c* 0.02, 24 h after dissolution in H₂O) [lit. $[\alpha]_D^{20} +16.4$ (*c* 0.05, H₂O)] (Kuroda et al., 2010).

4.7. Parasite strain

L. mexicana (MNYC/BZ/62/M379) was maintained by serial passage of amastigotes inoculated subcutaneously into the shaven rumps of 129SvE mice and was obtained by *in vitro* culture of amastigotes in RPMI-1640 supplemented with 10% fetal calf serum (FCS, Sigma, St. Louis, MO) and incubated at 28 °C. All work including experimental animals was approved by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University.

4.8. Promastigote assay

One million log phase promastigotes were seeded in 1 mL of complete RPMI-1640 medium, and parasite numbers, and their mobility and morphology were measured from 24 and 48 h. This was performed by flow cytometry measurements using experimental and control groups of parasites stained with propidium iodide and with *Quillaja saponaria* saponin as positive control, as reported in the literature (Delgado et al., 2001). The test compounds isolated from *P. andrieuxii* were compared with the antimonial drug, Pentostam.

4.9. Amastigote assay

Bone marrow-derived macrophages (BMDM) (0.5×10^6) were adhered on top of glass rounded coverslips deposited at the bottom of an individual plate well (24-well tissue culture plate, Corning, Inc.). Next, macrophages were infected overnight with 2.5×10^6 stationary phase promastigotes of the *L. mexicana* (ratio 5:1). After this time, cells were extensively washed with Hank's balanced salt solution (HBSS) to eliminate non-phagocytosed parasites, and, after stimulation with test compounds for 48 h cells, were stained with Giemsa stain (Sigma). In each experiment, infection rates were recorded by counting the number of parasites in 100 macrophages on each slide in triplicate in a blinded fashion (Lezama-Dávila et al., 2007b).

4.10. Cytotoxicity assay in non-infected macrophages

Bone marrow-derived macrophages (0.5×10^6) from C57BL/6 mice were seeded into 24-well tissue culture plates (Corning, Inc.) and co-cultured (48 h) with a 100 μ M concentration of each test compound, including non-treated sham controls. Cells were stained with trypan blue for viability determination (Ferreira et al., 2011). Viability of treated cells was similar to sham controls (more than 80%) in experiments ran in triplicate.

4.11. Electron microscopy

Parasites were co-cultured, as previously described by treating a 100 μ M concentration of each pure compound for 2 h and suspending them in glutaraldehyde. Samples were further processed in an electron microscopy unit as follows: Incubation steps were carried out on a Lab-Line orbital shaker operating at 700 rpm (Barnstead/Thermolyne, Melrose Park, IL). After the initial fixation in 3% buffered glutaraldehyde, cell pellets were washed twice with sodium cacodylate buffer (pH 7.4, 10 min each) and spun down after each wash at 1500 rpm for 5 min. Cell samples were then post-fixed in 1% osmium tetroxide in sym-collidine buffer (pH 7.6) for 1 h at room temperature. Following two washes with sym-collidine buffer (10 min each) the cell pellet was stained with a saturated aqueous uranyl acetate solution (pH 3.3) for 1 h. Cell pellets were dehydrated in a graded EtOH series up to absolute (10 min each). Acetone was used as the transitional solvent, with two changes for 10 min each. The cell suspensions were infiltrated overnight with a 1:1 mixture of acetone and Spurr's epoxy resin (Electron Microscopy Sciences, Fort Washington, PA). Finally, cell pellets were placed into BEEM embedding capsules containing 100% Spurr's resin. Polymerization of epoxy blocks was carried out at 70 °C overnight. Polymerized blocks were sectioned with a Leica Ultrat UCT ultramicrotome (Leica Microsystem GmbH, Wein, Austria). Methylene Blue-Basic Fuchsin stained semi-thin (750 nm) sections were evaluated and two representative areas were thin-sectioned for ultrastructural examination. Ultrathin (80 nm) sections were collected on 200 mesh copper grids (Electron Microscopy Sciences) and post-stained with lead citrate (3 min).

Acknowledgments

This study was supported by NIH Grants AI 076309, AT 004160, AI 090803 (all to A.R.S.), RC4 AI 092624 (to A.R.S. and A.D.K.), and CONACYT (Fondo sectorial salud 140091) grants to APIM and CMLD. Mr. P. Zamora-Crescencio is acknowledged for the identification of the plant material used in this study. We thank Mr. John Fowble, College of Pharmacy, and Dr. Chun-hua Yuan, Campus Chemical Instrumentation Center (CCIC), The Ohio State University, for facilitating the acquisition of NMR spectra. We are also grateful to Mr. M.E. Apsega and Ms. J.C. Hach of CCIC, The Ohio State University, for the mass spectrometric data.

References

- Anastasia M, Allevi P, Ciuffreda P, Fiecchi A, Gariboldi P, Scala A. Conversion of nor-ketones into prochiral terminal methylene groups: synthesis of (24*E*) and (24*Z*)-[28-²H]ergosta-5,24(28)-dien-3 β -ols. *J Chem Soc Perkin Trans.* 1985; 1:595–599.
- Argüeta, A.; Cano, L.; Rodarte, M. *Atlas de las Plantas de la Medicina Tradicional Mexicana*. Vol. 2. Instituto Nacional Indigenista; Mexico, DF: 1994.
- Bai L, Wang L, Zhao M, Toki A, Hasegawa T, Ogura H, Kataoka T, Hirose K, Sakai J, Bai J, Ando M. Bioactive pregnanes from *Nerium oleander*. *J Nat Prod.* 2007; 70:14–18. [PubMed: 17253842]
- Bazin MA, Loiseau PM, Bories C, Letourneux Y, Rault S, El Kihel L. Synthesis of oxysterols and nitrogenous sterols with antileishmanial and trypanocidal activities. *Eur J Med Chem.* 2006; 41:1109–1116. [PubMed: 16949702]
- Cabrera G, Palermo JA, Seldes AM, Gros EG, Oberti JC. Steroidal derivatives from the roots of *Mandevilla pentlandiana*. *Phytochemistry.* 1991; 30:1239–1243.

- Chan-Bacab MJ, Balanza E, Deharo E, Muñoz V, Durán-García R, Peña-Rodríguez LM. Variation of leishmanicidal activity in four populations of *Urechites andrieuxii*. *J Ethnopharmacol*. 2003; 86:243–247. [PubMed: 12738094]
- Chen H, Xu N, Zhou Y, Qiao L, Cao J, Yao Y, Hua H, Pei Y. Steroidal glycosides from the roots of *Cynanchum amplexicaule* Sieb. et Zucc *Steroids*. 2008; 73:629–636.
- Cui JG, Fan L, Huang LL, Liu HL, Zhou AM. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure/activity studies. *Steroids*. 2009; 74:62–72. [PubMed: 18838084]
- Delgado G, Puentes F, Moreno A, Patarroyo ME. Flow cytometry, a useful tool for detecting the lethal effect of pentamidine on *Leishmania viannia* complex promastigote forms. *Pharmacol Res*. 2001; 44:281–286. [PubMed: 11592861]
- De la Rosa, GV.; Tamay-Segovia, P.; Isaac-Márquez, AP.; Lezama-Dávila, CM. Memorias de la III Reunión de Investigación Química, en el Sureste de México. Universidad Autónoma de Yucatán; Mérida, Yucatán, México: 1995 Mar 8–10. p. 93
- de Melo Casal C, de Cássia Domingues V, Batalhão JR, Corrêa Bueno O, Rodrigues Filho E, da Silva MFGF, Cezar Vieira P, Fernandes JB. Isolation of xanthyletin, an inhibitor of ants' symbiotic fungus, by high-speed counter-current chromatography. *J Chromatogr A*. 2009; 1216:4307–4312. [PubMed: 19296958]
- de Souza, W.; Rodrigues, JCF. Sterol biosynthesis pathway as target for anti-trypanosomatid drugs; Interdiscip Perspect Infect Dis. 2009. p. Article ID 642502 <http://dx.doi.org/10.1155/2009/642502>
- Domínguez-Carmona DB, Escalante-Erosa F, García-Sosa K, Ruiz-Pinell G, Gutierrez-Yapu D, Chan-Bacab MJ, Giménez-Turba A, Peña-Rodríguez LM. Antiprotozoal activity of betulinic acid derivatives. *Phytochemistry*. 2010; 17:379–382. [PubMed: 19748254]
- Ferreira C, Soares DC, Barreto-Junior CB, Nascimento MT, Freire-de-Lima L, Delorenzi JC, Lima ME, Atella GC, Folly E, Carvalho TM, Saraiva EM, Pinto-da-Silva LH. Leishmanicidal effects of piperine, its derivatives, and analogues on *Leishmania amazonensis*. *Phytochemistry*. 2011; 72:2155–2164. [PubMed: 21885074]
- Gawronski JK. Circular dichroism and stereochemistry of chiral conjugated cyclohexenones. *Tetrahedron*. 1982; 38:3–26.
- González AG, Fraga BM, González P, Hernandez MG, Ravelo AG. ¹³C spectra of olean-18-ene derivatives. *Phytochemistry*. 1981; 20:1919–1921.
- Haughan PA, Chance ML, Goad LJ. Effects of an azasterol inhibitor of sterol 24-transmethylation on sterol biosynthesis and growth of *Leishmania donovani* promastigotes. *Biochem J*. 1995; 308:31–38. [PubMed: 7755579]
- Jiu J. A survey of some medicinal plants of Mexico for selected biological activities. *Lloydia*. 1966; 29:250–259.
- Kennard O, Fawcett JK, Watson DG, Kerr KA, Stöckel K, Stöcklin W, Reichstein T. Hirundigenin and anhydrohirundigenin, two natural 15-oxa steroids of plant origin. Chemical and X-ray investigation. *Tetrahedron Lett*. 1968; 35:3799–3804.
- Kontiza I, Abatis D, Malakate K, Vagias C, Roussis V. 3-Keto steroids from the marine organisms *Dendrophyllia cornigera* and *Cymodocea nodosa*. *Steroids*. 2006; 71:177–181. [PubMed: 16280145]
- Kuroda M, Kubo S, Uchida S, Sakagami H, Mimaki Y. Amurensiosides A-K, 11 new pregnane glycosides from the roots of *Adonis amurensis*. *Steroids*. 2010; 75:83–94. [PubMed: 19883671]
- Lavault M, Richomme P, Bruneton J. Acetophenones and new pregnane glycosides from the roots of *Vincetoxicum hirundinaria*. *Fitoterapia*. 1999; 70:216–220.
- Lezama-Dávila CM, Isaac-Márquez AP. Las plantas medicinales y su utilidad en el tratamiento de las leishmaniosis en México Estudio preliminar con *Urechites andrieuxii*. *Muell-Arg Divulg Bioméd*. 1994; 2:13–16.
- Lezama-Dávila CM, Isaac-Márquez AP, Zamora-Crescencio P, Úc-Encalada MR, Justiniano-Apolinar SY, Angel-Robles R, Satoskar A, Hernández-Rivero L. Leishmanicidal activity of *Pentalinon andrieuxii*. *Fitoterapia*. 2007a; 78:255–257. [PubMed: 17355903]
- Lezama-Dávila CM, Isaac-Márquez AP, Barbi J, Oghumu S, Satoskar AR. 17β-Estradiol increases *Leishmania mexicana* killing in macrophages from DBA/2 mice by enhancing production of nitric

- oxide but not pro-inflammatory cytokines. *Am J Trop Med Hyg.* 2007b; 76:1125–1127. [PubMed: 17556622]
- Magaraci F, Jimenez CJ, Rodrigues C, Rodrigues JC, Braga MV, Yardley V, de Luca-Fradley K, Croft SL, de Souza W, Ruiz-Perez LM, Urbina J, Gonzalez J. Azasterols as inhibitors of sterol 24-methyltransferase in *Leishmania* species and *Trypanosoma cruzi*. *J Med Chem.* 2003; 46:4714–4727. [PubMed: 14561091]
- McCarthy FO, Chopra J, Ford A, Hogan SA, Kerry JP, O'Brien NM, Ryan E, Maguire AR. Synthesis, isolation and characterisation of beta-sitosterol and beta-sitosterol oxide derivatives. *Org Biomol Chem.* 2005; 3:3059–3065. [PubMed: 16186940]
- Niero R, Calixto JB, de Silva Dias, Filho J, Yunes RA, Sant'Ana AEG. Acetylillustrol. A new compound from *Mandevilla illustris*. *Nat Prod Lett.* 1999; 13:63–70.
- Niero R, Alves RV, Cechinel Filho V, Calixto JB, Hawkes JE, Sant'Ana AEG, Yunes RA. A new anti-oedematogenic nor-pregnane derivative isolated from *Mandevilla illustris*. *Planta Med.* 2002; 68:850–853. [PubMed: 12357405]
- Perrone A, Plaza A, Ercolino SF, Hamed AI, Parente L, Pizza C, Piacente S. 14,15-Secopregnane derivatives from the leaves of *Solenostemma argel*. *J Nat Prod.* 2006; 69:50–54. [PubMed: 16441067]
- Perrone A, Plaza A, Hamed AI, Pizza C, Piacente S. *Solenostemma argel*: a rich source of very unusual pregnane and 14,15-secopregnane glycosides with antiproliferative activity. *Curr Org Chem.* 2008; 12:1648–1660.
- Plaza A, Bifulco G, Hamed AI, Pizza C, Piacente S. Argelosides A and B, two novel 14,15-secopregnane glycosides from *Solenostemma argel*. *Tetrahedron Lett.* 2003; 44:8553–8558.
- Plaza A, Perrone A, Balestrieri C, Balestrieri ML, Bifulco G, Carbone V, Hamed AI, Pizza C, Piacente S. New antiproliferative 14,15-secopregnane glycosides from *Solenostemma argel*. *Tetrahedron.* 2005; 61:7470–7480.
- Pulido Salas, MT.; Serralta Peraza, L. Lista Anotada de las Plantas Medicinales de Uso Actual en el Estado de Quintana Roo, México. Centro de Investigaciones de Quintana Roo; Chetumal, Quintana Roo, Mexico: 1993.
- Sartorelli P, Andrade SP, Melhem MSC, Prado FO, Tempone AG. Isolation of antileishmanial sterol from the fruits of *Cassia fistula* using bioguided fractionation. *Phytother Res.* 2007; 21:644–647. [PubMed: 17397117]
- Seo S, Yoshimura Y, Satoh T, Uomori A, Takeda K. Synthesis of (25*S*)-[26-²H₁] cholesterol and ¹H N.M.R. signal assignments of the *pro-R* and *pro-S* methyl groups at C-25. *J Chem Soc Perkin Trans.* 1986; 1:411–414.
- Schun Y, Cordell GA. Cytotoxic steroids of *Gelsemium sempervirens*. *J Nat Prod.* 1987; 50:195–198. [PubMed: 3655795]
- Sheikh YM, Djerassi C. Steroids from sponges. *Tetrahedron.* 1974; 30:4095–4103.
- Waksmundzka-Hajnos, M.; Sherma, J.; Kowalska, T., editors. Thin Layer Chromatography in Phytochemistry. CRC Press/Taylor and Francis Group; Boca Raton, FL: 2008. p. 576-597.
- Werbovets KA. Promising therapeutic targets for antileishmanial drugs. *Expert Opin Ther Targets.* 2002; 6:407–422. [PubMed: 12223057]
- Yunes RA, Brum RL, Calixto JB, Rimpler H. Illustrol, a seco-norpregnane derivative from *Mandevilla illustris*. *Phytochemistry.* 1993; 34:787–790.
- Yam-Puc A, Escalante-Erosa F, Pech-López M, Chan-Bacab MJ, Arunachalampillai A, Wendt OF, Sterner O, Peña-Rodríguez LM. Trinorsesquiterpenoids from the root extract of *Pentalinon andrieuxii*. *J Nat Prod.* 2009; 72:745–748. [PubMed: 19388707]
- Yam-Puc A, Chee-González L, Escalante-Erosa F, Arunachalampillai A, Wendt OF, Sterner O, Godoy-Hernández G, Peña-Rodríguez LM. Steroids from the root extract of *Pentalinon andrieuxii*. *Phytochem Lett.* 2012; 5:45–48.
- Yan SJ, Su JY, Zeng LM, Liu LJ, Wang YH. Chemical constituents of alga *Halimeda incrassata* (II). *Redai Haiyang Xuebao.* 2002; 21:92–95.
- Zhao M, Bai L, Wang L, Toki A, Hasegawa T, Kikuchi M, Abe M, Sakai JI, Hasegawa R, Bai Y, Mitsui T, Ogura H, Kataoka T, Oka S, Tsushima H, Kiuchi M, Hirose K, Tomida A, Tsuruo T,

Ando M. Bioactive cardenolides from the stems and twigs of *Nerium oleander*. J Nat Prod. 2007; 70:1098–1103. [PubMed: 17595134]

Fig. 1.
Structures of compounds (1–20) isolated from *P. andrieuxii*.

Fig. 2.
Selected ^1H - ^1H COSY (—) and HMBC (---) correlations observed for compounds **1** and **2**.

Fig. 3. Preferred conformation and selected NOESY ($\leftrightarrow$) correlations observed for compound **2**.

Fig. 4. Electron microscopy images of *L. mexicana*. Morphological abnormalities and destruction observed in parasites treated with pentalinonsterol (**1**).

Table 1

¹H and ¹³C NMR Chemical Shifts of Compounds **1** and **2**^a

position	1		2	
	¹ H (mult. J, Hz) ^b	¹³ C, mult. ^c	¹ H (mult. J, Hz) ^b	¹³ C, mult. ^c
1	1.73 ^d	36.1, CH ₂	1.05 α, ^d	37.5, CH ₂
	2.02 ^d		1.75 β, ^d	
2	2.40 ^d	34.4, CH ₂	2.13 α, ^d	30.4, CH ₂
			1.68 β, ^d	77.3, CH
3		200.1, qC	3.90, m	39.4, CH ₂
4	5.73, s	124.2, CH	2.59 α, ^d	
			2.40 β, ^d	
5		171.9, qC		139.7, qC
6	2.30 ^d	33.4, CH ₂	5.52 t (2.5)	122.6, CH
	2.40 ^d			
7	1.02 ^d	32.4, CH ₂	1.67 ^d	25.5, CH ₂
	1.84 ^d		2.50 ^d	
8	1.58 ^d	36.4, CH	1.85 ^d	36.2, CH
9	1.00 ^d	54.4, CH	1.42 ^d	46.1, CH ₂
10		39.0, qC		37.1, qC
11	1.43 ^d	21.5, CH ₂	1.43 ^d	20.6, CH ₂
	1.59 ^d			
12	1.25 α, ^d	38.9, CH ₂	1.38 α, ^d	34.9, CH ₂
	1.88 β, ^d		2.40 β, ^d	
13		43.5, qC		41.4, qC
14	1.15 ^d	56.2, CH		108.2, qC
15	1.22 ^d	24.5, CH ₂	4.02 a, dd (10.0, 4.4)	74.3, CH ₂
	1.73 ^d		4.23 b, d (10.0)	
16	1.76 ^d	26.2, CH ₂	4.56 t (4.5)	80.9, CH
	1.80 ^d			
17	2.10 ^d	56.3, CH	2.60, dd (7.6, 4.4)	54.6, CH
18	0.62, s	13.3, CH ₃	1.08, s	17.3, CH ₃
19	1.19, s	17.8, CH ₃	0.97, s	19.6, CH ₃
20		149.5, qC	4.49, ddd (7.6, 6.0, 1.5)	76.3, CH
21	4.80, brs	110.1, CH ₂	4.05 ^a , dd (12.4, 6.0)	66.2, CH ₂
	4.90, brs		3.79 b, dd (12.4, 1.3)	
22	2.06 ^d	36.4, CH ₂		
23	2.10 ^d	27.5, CH ₂		

position	1		2	
	¹ H (mult. J, Hz) ^b	C, mult. ^c	¹ H (mult. J, Hz) ^b	C, mult. ^c
24	5.11, brt (7.0)	124.7, CH		
25		132.0, qC		
26	1.61, s	17.8, CH ₃		
27	1.69, s	26.2, CH ₃		
1			4.84, dd (9.6, 2.0)	98.8, CH
2			1.73, m	33.3, CH ₂
			2.10, m	
3			3.43, ddd (12.0, 5.0, 2.0)	79.3, CH
4			3.91, brs	67.1, CH
5			3.60, q (6.5)	71.5, CH
6			1.55, d (6.4)	17.8, CH ₃
OCH ₃ -3			3.40, s	55.4, CH ₃

^a¹H NMR spectrum measured at 400 MHz, ¹³C NMR measured at 100 MHz; NMR data of **1** were obtained in CDCl₃ and the NMR data of **2** were obtained in pyridine-d₅. Assignments are based on HSQC and HMBC NMR spectra.

^bJ values (Hz) are given in parentheses. Some geminal protons are indicated with the α- or β-orientation based on a NOESY experiment.

^cMultiplicity obtained from the DEPT spectra.

^dFor overlapped signals, only chemical shift values are given.

Table 2*In vitro* antileishmanial activities of compounds isolated from the roots of *Pentalinon andrieuxii*.^a

Compound	<i>L. mexicana</i>	
	Promastigotes ^b	Amastigotes ^c
Cholestra-4,20,24-trien-3-one (1)	30.0	3.3
24-Methylcholesta-4,24(28)-dien-3-one (3)	24.0	3.5
Cholest-4-en-3-one (4)	81.0	0.03
Cholest-5,20,24-trien-3 β -ol (7)	>262	14.5
6,7-Dihydroneridienone (15)	9.2	1.4
Neridienone (16)	26.2	3.5
Pentostam ^d	346.1	2.7

^aCompounds with IC₅₀ > 100 μ g/mL are considered to be inactive.^bResults are expressed as IC₅₀ values (μ M), calculated by linear regression analysis from the *K_C* values at the concentrations used (1, 10, 50 and 100 μ g/mL) at 48 h culture.^cIC₅₀ for *L. mexicana* in bone marrow-derived macrophages from C57BL/6 mice (48 h experiment ran in triplicate).^dUsed as a positive control substance.