

8,9-Seco-Eremophilanolides from *Roldana ehrenbergiana*[†]

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Dedicated to Professor Pedro Joseph Nathan

Abstract. Two new *seco*-eremophilanolides, roldehrenbergins C and D, were isolated from *Roldana ehrenbergiana*. Structural elucidation was achieved by spectroscopic methods and confirmed by X-ray crystallographic analysis.

Key Words Index: *Roldana ehrenbergiana*, Asteraceae, Senecioneae, Hierba del Perro, *seco*-eremophilanolides.

Resumen. Dos *seco*-eremofilanolidas, roldehrenberginas C y D, fueron aisladas de *Roldana ehrenbergiana*. La elucidación estructural se llevó a cabo por métodos espectroscópicos y confirmada por análisis cristalográfico de rayos-X.

Palabras Clave: *Roldana ehrenbergiana*, Asteraceae, Senecioneae, Hierba del Perro, *seco*-eremofilanolidas.

Introduction

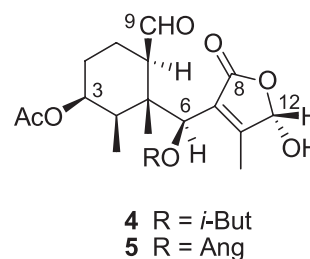
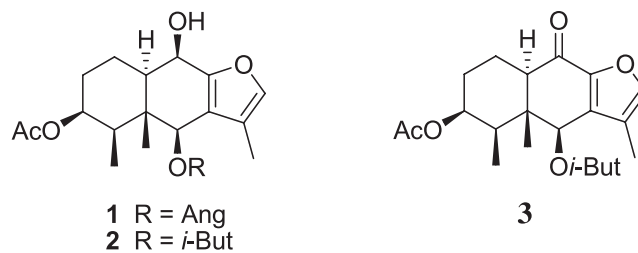
Most of the 48 species that constitute the genus *Roldana* (Asteraceae, Senecioneae) were segregated from the genus *Senecio* [1]. Chemical studies of six *Roldana* species [2-8] show plastoquinone, oplopane and eremophilane derivatives as their main secondary metabolites. In these studies no pyrrolizidine alkaloids were detected, in spite of taxonomic relationship between *Roldana* and *Senecio*. The fact that the aerial parts of *Roldana ehrenbergiana* (Klatt.) H. Robinson & Brettell (Itzcuimpatli or Hierba del Perro) have been used to kill rabid dogs in San José Tequiluca, Puebla, Mexico, induced us to carry out a chemical research of its leaves and roots [8]. In the mentioned study, the MeOH extract of roots was fractionated in A, B, and C fractions. Purification of A and C afforded β -sitosterol, stigmasterol, β -sitosteryl glucopyranoside, and **1**. The B fraction yielded roldehrenbergins A and B (**2** and **3**), and a B₁ residue. The toxicity of compounds **1-3** was tested against leukemia (K562), prostate (PC-3), colon (HCT-15), and breast (MCF-7) human cancer cells, but only **1** showed moderate activity against PC-3 line. In continuation of the above work, we undertook the search for chemical constituents of flowers of *R. ehrenbergiana* and of the B₁ residue from roots [8].

Results and Discussion

The purification of the MeOH extract of flowers afforded the known compounds: β -sitosterol, stigmasterol and 3-*O*-calenduladiol myristate and isopalmitate. The B₁ residue of the MeOH extract of roots gave a mixture of two labile compounds,

which presented in TLC a single spot. Even that it was not possible to resolve the mixture, the structures of its components were established as **4** and **5**.

The molecular formulae of roldehrenbergin C (**4**, C₂₁H₃₀O₈) and roldehrenbergin D (**5**, C₂₂H₃₀O₈) were determined by HR-FABMS. The IR spectrum of the mixture showed absorption bands due to hydroxyl (3588 and 3500 cm⁻¹), α,β -unsaturated γ -lactone (1771 and 1680 cm⁻¹), and to carbonyl groups of saturated and unsaturated esters and of aldehyde (1731 and 1717 cm⁻¹). The ¹H and ¹³C NMR spectra exhibited the characteristic signals of the acetoxy, isobutyryloxy, and angeloyloxy groups. In the same spectra, the presence of signals due to vinylic, tertiary, and secondary methyls, suggested the structural relationship of **4** and **5** with an eremophilane. The position of the acyloxy groups was determined



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by the HMBC spectrum, which showed cross peaks of the acetoxy carbonyls (δ 170.5 and δ 170.6) with H-3 (**4** and **5**, δ 4.93), and of both isobutyryloxy (**4**, δ 176.3) and angeloyloxy carbonyls (**5**, δ 166.6) with H-6 (**4**, δ 5.65; **5**, δ 5.79). The correlations of the vinyl carbons C-7 (δ 125.6 and δ 126.0) and C-11 (δ 162.4 and δ 163.2) and of the γ -lactone carbonyl (δ 169.7 and δ 169.9) with H-6 indicated the linkage between C-6 and the α -carbon of the γ -lactone. The aldehyde function was deduced from the chemical shifts of H-9 (**4**, δ 10.16; **5**, δ 10.12) and C-9 (**4**, δ 205.4; **5**, δ 205.7) in the ^1H and ^{13}C NMR spectra, respectively, and its position at C-10 was supported by the observed correlations in the HMBC spectrum. Thus, H-6 correlated with C-10 (δ 52.7 and δ 52.9) and H-10 correlated with the aldehyde carbonyl. The downfield shifts of the C-12 (δ 97.6 and δ 98.0) and H-12 (**4** and **5**, δ 5.84) signals suggested the presence of a hemiacetalic alcohol at C-12.

The stereochemistry of the A-ring was deduced from the NOE effects of H-9 with CH_3 -14, of H-10 with H-4, and of the acetoxy group with CH_3 -14 and CH_3 -15, which were observed in the NOESY spectrum of the mixture, nevertheless, the stereochemistry at C-12 remained unknown. On the other hand, during the NMR spectra acquisition time, almost all the signals of **4** and **5** were duplicated. The above, together with the presence of a hemiacetalic alcohol at C-12 in both compounds, induced us to propose the existence of a chemical equilibrium of **4** and **5** with their respective epimers at C-12, in the CDCl_3 solution. Fortunately, the mixture of **4** and **5** gave appropriate crystals for an X-ray crystallographic analysis, which allowed to establish the relative stereochemistry of roldehrenbergin C (**4**) and roldehrenbergin D (**5**) as shown in Figures 1 and 2.

Although *seco*-eremophilanes have been isolated from different genera, such as *Senecio* [9, 10] *Euryops* [11], and *Petasites* [12], these compounds are relatively uncommon, and this is the second report of *seco*-eremophilanes isolated from genus *Roldana* [7]. On the other hand and considering the toxicity attributed to the title plant, the mixture of compounds **4** and **5** was evaluated against leukemia (K562), prostate (PC-3), colon (HCT-15), and breast (MCF-7) human cancer cells [13]. The results were similar to those reported in the previous paper [8], roldehrenbergin C (**4**) and roldehrenbergin D (**5**) were inactive against the tested cell lines. Taking into account the above results and in order to prove the cytotoxic activity attributed to *R. ehrenbergiana*, we consider that additional tests of the extracts and isolated compounds are necessary.

Experimental

General Experimental Procedures. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV. FAB-HR-MS was obtained on a JEOL JMS-SX102A mass spectrometer (matrix: nitrobenzyl alcohol). ^1H and ^{13}C NMR data were obtained on a Varian Unity Plus 500 instrument. Chemical shifts are given in δ values (ppm) from TMS.

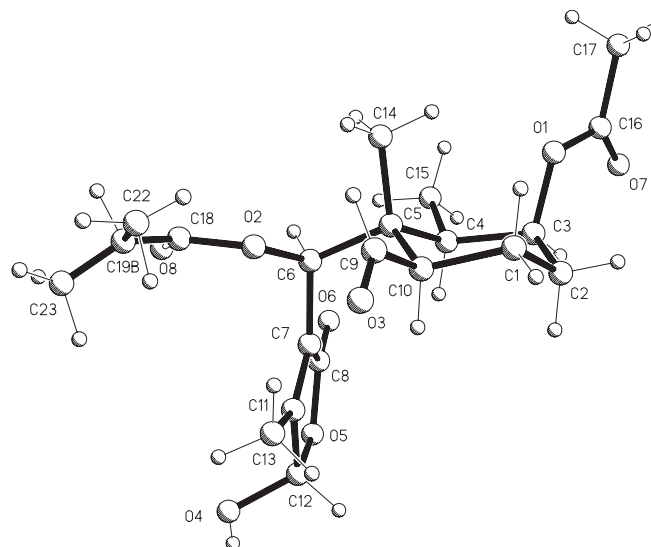


Fig 1. ORTEP projection of **4** (crystallographic numbering).

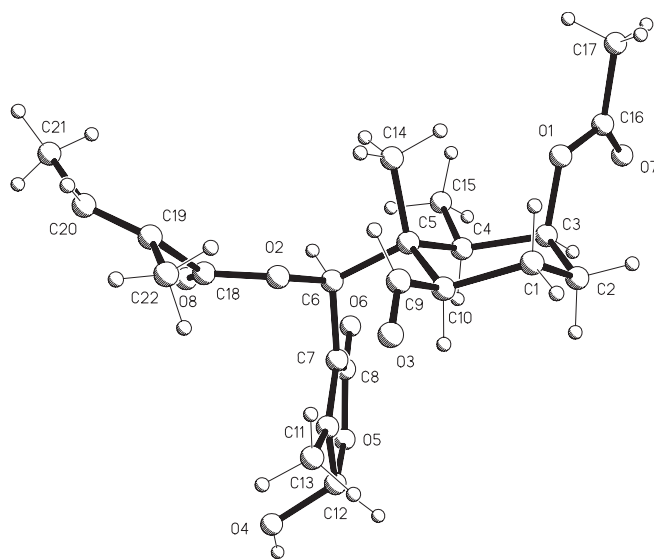


Fig 2. ORTEP projection of **5** (crystallographic numbering).

Vacuum column chromatographies (VCCs) were carried out on Si gel 60 G (Merck).

Plant Materials. *Roldana ehrenbergiana* (Klatt.) H. Robinson & Brettell was collected in San José Tejiulca, Puebla State, Mexico, in July 2000. A voucher specimen (HUAP 10800) was deposited at the Herbario de la Benemérita Universidad Autónoma de Puebla, México.

Extraction and Isolation. Dried and ground flowers (783 g) were extracted with MeOH to afford 75 g of MeOH extract, which gave a negative Dragendorff test. The extract was purified by three consecutive VCC (hexane- Me_2CO polarity gradi-

ent systems) to give a mixture of 3-*O*-calenduladiol myristate and isopalmitate (250 mg) and a mixture of β -sitosterol and stigmaterol (561 mg). Dried and ground roots were extracted with MeOH and the extract was purified by successive VCC as described on the preceding paper [8]. The purification of the eluates obtained with hexane-EtOAc 9:11 (fraction B) yielded compounds **1-3** and a residue (B₁, 859.6 mg). The latter was submitted to consecutive VCC (hexane-Me₂CO 4:1) to give a 2:3 mixture (122.5 mg) of roldehbergins C and D (**4** and **5**).

Roldehbergin C (4) and Roldehbergin D (5)

Mixture. White crystals: mp 180-8°C (hexane-EtOAc); IR (CHCl₃) ν_{\max} 3588, 3500, 1771, 1731, 1645, 1603 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz, assignments by COSY, HMQC and HMBC) δ 10.16 (1H, d, *J* = 3.0 Hz, H-9, **4**), 10.12 (1H, d, *J* = 3.0 Hz, H-9, **5**), 6.14 (1H, qq, *J* = 7.5, 1.5 Hz, H-3', Ang, **5**), 5.84 (2H, s, H-12, **4** and **5**), 5.79 (1H, s, H-6, **5**), 5.65 (1H, s, H-6, **4**), 4.93 (2H, ddd, *J* = 8.5, 6.5, 3.5 Hz, H-3, **4** and **5**), 2.67 (1H, dd, *J* = 11.5, 3.0 Hz, H-10, **4**), 2.59 (1H, dd, *J* = 11.5, 3.0 Hz, H-10, **5**), 2.56 (1H, hept, *J* = 7.0 Hz, H-2', *i*-But, **4**), 2.18 (3H, s, H-13, **4**), 2.17 (3H, s, H-13, **5**), 2.08 (6H, s, AcO, **4** and **5**), 1.96 (3H, dq, *J* = 7.5, 1.5, H-4', Ang, **5**), 1.91 (2H, m, H-2b, **4** and **5**), 1.89 (3H, dq, *J* = 7.5, 1.5, H-5', Ang, **5**), 1.85 (2H, m, H-1a, **4** and **5**), 1.61 (2H, m, H-4, **4** and **5**), 1.54 (2H, m, H-1b, **4** and **5**), 1.47 (2H, m, H-2b, **4** and **5**), 1.28 (3H, s, H-14, **5**), 1.25 (3H, s, H-14, **4**), 1.15 (3H, d, *J* = 7.0 Hz, H-3', *i*-But, **4**), 1.13 (3H, d, *J* = 7.0 Hz, H-4', *i*-But, **4**), 1.06 (3H, d, *J* = 7.0 Hz, H-15, **5**), 1.03 (3H, d, *J* = 7.0 Hz, H-15, **4**); ¹³C NMR (CDCl₃, 125 MHz, assignments by DEPT, HMQC and HMBC) δ 205.7 (C-9, **5**), 205.4 (C-9, **4**), 176.3 (C-1', *i*-But, **4**), 170.6 and 170.5 (CH₃CO), 169.9 and 169.7 (C-8), 166.6 (C-1', Ang, **5**), 163.2 and 162.4 (C-11), 141.4 (C-3', Ang, **5**), 126.5 (C-2', Ang, **5**), 126.0 and 125.6 (C-7), 98.0 and 97.6 (C-12), 73.9 (C-6, **4**), 73.4 (C-6, **5**), 73.1 (C-3), 52.9 and 52.7 (C-10), 45.0 and 44.9 (C-5), 38.2 and 38.0 (C-4), 34.0 (C-2', *i*-But, **4**), 28.5 and 28.2 (C-2), 20.5 (CH₃CO; C-5', Ang, **5**), 18.8 (C-3' and C-4', *i*-But, **4**), 18.6 (C-1), 15.8 (C-4', Ang, **5**), 14.5 and 14.4 (C-14), 13.3 and 13.2 (C-13), 12.9 and 12.8 (C-15); EIMS *m/z* (rel. int.): 423 [M₅+H]⁺ (1), 411 [M₄+H]⁺ (1), 405 [M₅-H₂O] (1), 323 [M+H-RCO₂H]⁺ (4), 263 [323-RCO₂H-HOAc]⁺ (11), 262 [M-RCO₂H-HOAc]⁺ (12), 234 (9), 126 (54), 109 (45), 83 (100), 71 (35), 55 (27), 43 (49); HRFABMS *m/z*: 411.2024 (**4**: calcd. for C₂₁H₃₁O₈, 411.2019), 423.2020 (**5**: calcd. for C₂₂H₃₁O₈, 23.2019).

Crystal data of Roldehbergin C (4) and Roldehbergin D (5) as mixture.

C₂₁H₃₀O₈, C₂₂H₃₀O₈, MW = 410, MW = 422; crystal of dimensions 0.50 × 0.18 × 0.086 mm, monoclinic, *P*2₁; *a* = 11.070 (1) Å, *b* = 7.124 (1) Å, *c* = 13.838 (1) Å, β = 96.462 (3)°, *V* = 1084.4(2) Å³, *Z* = 2, *D*_{calcd} =

1.295 Mg/m³, Mo K α (λ = 0.71073 Å), *F*(000) = 453. On a Bruker Smart Apex CCD diffractometer at 293 (2)°K a total of 8923 reflections were collected in the range 1.85° = θ = 25.00°, of which 3818 were unique reflections with *I* > 2 σ (*I*), and were used for refinement. The final *R* and *R*_w were 0.0544 and 0.0648, respectively. The structures were solved by the direct methods using the program SHELXTL-97. Hydrogen atoms were included at calculated positions and were not refined. Crystallographic data for the structures reported in this paper have been deposited in the Cambridge Crystallographic Data Center (CCDC 242954). Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Rd., Cambridge CB2 1EZ, UK (fax: +44-(1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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