Furanoeremophilanes from *Roldana ehrenbergiana**

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Two new furanoeremophilanes, roldehrenbergin A and roldehrenbergin B, and several known compounds were isolated from *Roldana ehrenbergiana*. Structural determination of the new compounds was achieved by spectroscopic analyses and chemical evidence.

Key words: *Roldana ehrenbergiana*, Asteraceae, Senecioneae, Hierba del Perro, Furanoeremophilanes

Introduction

Several plants of the tribe Senecioneae, family Asteraceae, have shown toxic effects in livestock, attributed to the presence of pyrrolizidine alkaloids [1]. Nevertheless, no one of the species of the genus *Roldana* studied so far [2 – 7], has shown presence of pyrrolizidine alkaloids, in spite of its close relationship with *Senecio* [8], which is rich in such metabolites. The genus *Roldana* contains, as characteristic secondary metabolites, oplopane, eremophilane and plastoquinone derivatives. An interesting case is *Roldana ehrenbergiana* (Klatt.) H. Robinson & Brettell, which was used in prehispanic times to treat leprosy and to kill dogs, rabbits and another animals [9]. Due to the above, we became interested in the chemical composition of this plant, which was collected in San José Tejiluca, Puebla, Mexico, where is locally known as Itzcuimpatli or Hierba del Perro, and considered as a livestock poison and used to kill rabid dogs. The study of the leaves and roots afforded two new furanoeremophilanes, roldehrenbergins A (**1**) and B (**2**), and the known compounds β-sitosterol, stigmasterol, β-sitosterol glucoside, the flavonoid quercitrin [10], and L-δ-hydroxynor-valine [11]. Pyrrolizidine alkaloids were neither isolated nor even detected.

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Results and Discussion

Roldehrenbergin A (**1**) presented a molecular formula $C_{22}H_{30}O_6$ determined by HRMS. The IR spectrum exhibited absorption bands at 3613, 1727, 1645, 1603, and 1562 cm^{-1} which can be attributed to hydroxyl, ester, and furan groups. The presence of acetoxy and angeloyloxy groups was evidenced from the characteristic signals observed in ^1H and ^{13}C NMR spectra (Table 1). The ^1H NMR spectrum showed signals for three methyl groups which were assigned to CH_3-13, CH_3-14, and CH_3-15 of a furanoeremophilane skeleton. The position of the angeloyloxy group at C-6, was deduced from the HMBC spectrum, which exhibited a correlation of the H-6 signal ($\delta = 6.01$) with that of the carbonyl ester group ($\delta = 167.7$). The acetoxy and alcohol groups should be attached to C-3 and C-9 respectively, due to the chemical shifts of H-3 ($\delta = 4.92$) and H-9 ($\delta = 4.56$). In addition, the ^1H-^1H COSY spectrum showed correlation of the H-3 signal with those of H-2a and H-2b, and that of H-9 with H-6 and H-10 signals. The relative stereochemistry of **1** was deduced by means of NOESY spectroscopy (Fig. 1). This spectrum showed cross-peaks between H-10 and the protons 6 and 9, between H-9 and H-1b, as well as between H-1a and CH_3-14, suggesting a *trans* junction of the A/B rings and a β-orientation of the C-6 acyloxy group. According to the coupling constant of H-3 (br d, J = 3.0 Hz), and the observed

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** and **2** ($CDCl_3$)[a].

[a] Coupling constants (J) in Hz are given in parentheses.

No.	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1α		1.50 dddd (14.0, 4.5, 3.0, 1.5)		1.49 dddd (14.0, 4.5, 3.0, 1.5)
1β	20.8	2.17 dtd (14.0, 14.0, 3.5)	21.0	2.17 dtd (14.0, 14.0, 4.0)
2α		1.64 dddd (14.0, 14.0, 4.5, 3.0)		1.64 dddd (14.0, 14.0, 4.4, 3.0)
2β	30.5	2.11 br dtd (14.0, 4.0, 3.0)	30.5	2.00 br dtd (14.0, 4.0, 3.0)
3	75.9	4.92 dt (5.5, 2.5)	75.9	4.92 dt (5.5, 2.5)
4	44.7	1.78 m	44.8	1.75 m
5	42.2		42.3	
6	76.2	6.01 d (1.0)	76.5	5.93 d (1.0)
7	150.9		151.1	
8	119.5		119.5	
9	65.3	4.56 br s	65.3	4.53 d (4.0)
10	46.6	1.76 br dt (14.0, 3.0)	46.8	1.73 m
11	118.9		118.8	
12	140.1	7.36 q (1.2)	140.2	7.13 q (1.0)
13	8.9	1.85 s	9.2	1.81 d (1.0)
14	11.6	1.13 s	11.5	1.26 s
15	13.7	0.96 d (7.2)	14.0	0.91 d (6.5)
1’	167.7		176.7	
2’	127.7		34.7	2.62 hept (7.0)
3’	140.3	6.20 qq (7.5, 1.5)	18.8	1.23 d (7.0)
4’	15.9	2.07 dq (7.5, 1.5)	18.6	1.24 d (7.0)
5’	20.5	1.94 dq (1.5, 1.5)		
1”	170.6		170.6	2.08 s
2”	21.3	2.07 s	21.3	

NOESY cross peak between CH_3-14 and the acetoxy methyl group, a β-orientation for the acetate was assumed. Therefore, the structure of roldehrenbergin A is that drawn as **1**.

Roldehrenbergin B (**2**) presented a molecular formula of $C_{21}H_{30}O_6$, which was determined by HRMS. The IR, ^{1}H and ^{13}C NMR spectra (Table 1) were very similar to those of **1**, since the only difference was an isobutyryloxy group attached to C-6 instead of an angeloyloxy. Thus, both compounds (**1** and **2**) have the same relative stereochemistry. In order to corroborate the C-9 position of the alcohol in **1** and **2**, a mixture of both compounds was treated with Jones reagent, affording 9-keto derivatives **3**, also isolated as a natural product in this study, and **4**. Compound **3** was identified as sendarwin I, previously isolated from *Senecio darwinii* [12]. Even though furanoeremophilane **4** was previously isolated from *Senecio grandifolius* Less [13] its spectral data were not described; therefore they are included in Table 2.

The alkaline treatment of **3** produced **5**, which exhibited an ^{1}H NMR spectrum in $CDCl_3$ (Table 2), with broad signals. The broad multiplet at $\delta = 2.50 - 2.70$ assigned to H-10 suggested an inversion on the configuration at C-10 leading to a *cis*-junction of the A/B rings [14]. A similar complexity was observed in the ^{1}H NMR spectrum in DMSO-d_6 at room temperature; however, when the experiment was carried out at 70 °C (Table 2), the H-10 signal was transformed into a multiplet similar to a dd ($\delta = 2.26$). Thus, a con-

Table 2. ^{13}C NMR (75 MHz) Data of **4** and ^{1}H NMR (300 MHz) Data of **4**–**6** ($CDCl_3$)[a].

[a] Coupling constants (J) in Hz are given in parentheses; [b] in DMSO-d_6.

Position	**4**		**5**	**5** + Δ(70°)[b]	**6**
	^{13}C	^{1}H	^{1}H	^{1}H	^{1}H
1α	15.7	2.10 m		1.48 m	1.6–2.0 m
1β		1.72 br dtd (14.0, 12.3, 3.6)		1.48 m	1.6–2.0 m
2α	29.5	1.53 br tt (14.1, 3.3)		1.28 m	1.6–2.0 m
2β		2.10 m		1.28 m	1.6–2.0 m
3	75.4	4.94 dd (5.4, 3.3)	4.2 br	3.72 br	5.09 br
4	44.1	2.00 m		1.39 m	1.6–2.0 m
5	49.5				
6	75.9	6.43 s	6.61 br	6.27 br	6.4 br
7	146.8				
8	121.0				
9	185.7				
10	55.0	2.47 dd (11.4, 3.2)	2.50–2.70 m	2.26 m	2.6–2.8 m
11	134.7				
12	145.1	7.13 q (1.5)	7.04 br s	7.59 br s	7.41 q (1.2)
13	8.5	1.79 d (1.5)	1.96 br s	1.70 d (0.3)	1.99 br s
14	10.0	1.27 s	1.24 s	0.86 s	1.22 m
15	14.2	0.91 d (7.0)	0.96 d (6.9)	0.67 d (7.2)	0.93 d (6.9)
1’	167.2				
2’	127.0		2.63 m	2.49 hept (6.9)	2.6-2.8 m
3’	141.8	6.30 qq (7.2, 1.5)	1.22 d (7.0)	0.99 d (7.5)	1.22 m
4’	16.0	2.10 m	1.22 d (7.0)	0.95 d (7.2)	1.22 m
5’	20.3	1.97 m			
1”	170.4				
2”	21.2	2.07 s			2.06 s

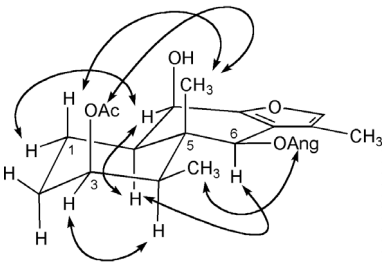
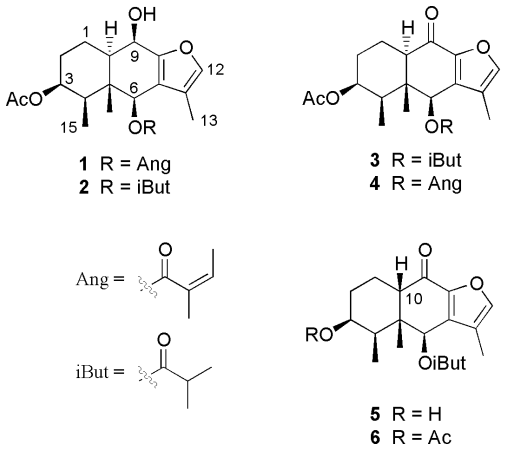


Fig. 1. Selected NOESY correlations of **1**.



formational equilibrium between stereoisomers with a steroidal and non-steroidal conformation of ring A [14] was proposed. A different junction of the A/B rings between **3** and **5**, was corroborated when the latter was acetylated producing **6**. The ^{1}H NMR spectrum of the acetyl derivative (**6**) presented the H-10 signal with a multiplicity and chemical shift similar to that of **5** (Table 2) but different from that reported for sendarwin I (**3**, $\delta = 2.41$, dd, $J = 9$, 3 Hz) [12].

On the other hand, considering that the toxic properties of *Senecio bonariensis* [15] and *Tetradymia glabrata* [16] were attributed to their content of eremophilane derivatives, we evaluated the toxicity of

the mixture of compounds **1** and **2**, and compound **3** against leukemia (K562), central nervous system (U251), prostate (PC-3), colon (HCT-15), and breast (MCF-7) human cancer cells, following the protocols established by the National Cancer Institute, Bethesda, Maryland [17]. Unfortunately, the mixture showed a very low activity, nevertheless eremophilane **3** exhibited a moderate activity against PC-3 cell line, with IC_{50} values of 16.6 μM. The reference compound, doxorubicin, presented IC_{50} values of 0.32 μM.

Experimental Section

General experimental procedures

Optical rotations were determined on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. EI MS data were determined on a JEOL JMS-AX505HA mass spectrometer. FAB^+ and HR-FAB^+ MS were 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 or Varian Unity 300 instruments. Chemical shifts are given in δ values (ppm) from TMS. Vacuum column chromatographies (VCCs) were carried out on Kieselgel G (Merck, Darmstadt, Germany). Preparative TLC on Si gel GF_{254} (Merck), 20 × 20, layer thickness 2 mm. Semi-preparative HPLC was performed on a Water deltaPrep 4000 instrument using a UV detector, 214 nm, column μPorasil (150 × 3.9 mm i.d., 10 μm particle size) with a flow of 1 ml/min.

Plant material

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

Extraction and isolation

Dried leaves (333 g) and roots (767 g) were separately ground and extracted with MeOH to yield two extracts: E_1 (136 g) and E_2 (61 g), respectively. All extracts gave a negative Dragendorff test. E_1 extract was partitioned with hexane and MeOH. Hexane residue (22.9 g) was purified by VCC (hexane-Me_2CO gradient system) to yield β-sitosterol glucopyranoside (24.6 mg). MeOH residue afforded the amino acid L-δ-hydroxynor-valine (16.46 g) [11]. Mother liquors (92.64 g) were purified by two consecutive VCCs (CH_2Cl_2-MeOH gradient systems) to yield quercitrin (141.7 mg) [10]. E_2 extract was analyzed by VCC (hexane-EtOAc gradient system). Fractions eluted with hexane-EtOAc (19:1) (6.41 g) were submitted to a VCC (hexane-EtOAc 19:1) to give a mixture of β-sitosterol and stigmasterol (83.6 mg) and **3** (3.36 g) [12]. Fractions eluted with hexane-EtOAc (9:11) (13.38 g) were analyzed by VCC (hexane-Me_2CO 19:1) to yield **3** (1.10 g), and a (3:2) mixture of roldehrenbergins A (**1**) and B (**2**) (3.22 g). Fractions obtained with EtOAc gave β-sitosterol glucopyranoside (158.4 mg). The mixture of **1** and **2** (20 mg) was purified by semi-preparative HPLC (hexane-isopropyl alcohol 99:1) afforded 9.2 mg of **1** and 4.2 mg of **2**.

*Roldehrenbergin A (**1**)*

White crystals from hexane-EtOAc, m.p. 130 – 135 °C. – $[\alpha]_D^{28} + 25°$ (c = 0.2, MeOH). – UV (MeOH): λ_{max} ($\log \varepsilon_{max}$) = 220 (4.4), 272 (4.2) nm. – IR ($CHCl_3$): ν = 3613, 1727, 1645, 1603, 1562 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 390 (2) [M^+], 290 (25) [M^+-RCO_2H], 230 (5) [M^+-RCO_2H-HOAc], 83 (100), 55 (18), 43 (10). – HRMS (FAB^+): *m/z* = 391.2120 [M^++H], calcd. for $C_{22}H_{31}O_6$ (391.2121).

*Roldehrenbergin B (**2**)*

Unstable substance; colorless gum. – $[\alpha]_D^{28} + 21.7°$ (c = 0.17, MeOH). – IR ($CHCl_3$): ν = 3612, 1725, 1645, 1562 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 378 (1) [M^+], 361 (1) [M^+-OH], 290 (55) [M^+-RCO_2H], 230 (11) [M^+-RCO_2H-HOAc], 140 (100), 71 (28), 43 (33); HRMS (FAB^+): *m/z* = 379.2137 [M^++H], calcd. for $C_{21}H_{31}O_6$ (379.2121).

*Oxidation of the mixture of **1** and **2***

A mixture of **1** and **2** (100 mg) in Me_2CO (4 ml) at 2 °C was treated with Jones reagent. After 10 min, MeOH (1 ml) was added and the solvent eliminated by air stream. The residue was analyzed by preparative TLC (hexane-Me_2CO 4:1) afforded 103.5 mg of a mixture of **3** and **4**, which was further purified (20 mg) by semi-preparative HPLC (hexane-isopropyl alcohol 99:1) to yield 12.7 mg of **3** and 1.8 mg of **4**. Compound **4**: colorless gum. – $[\alpha]_D^{28} - 33°$ (c = 0.32, $CHCl_3$). – IR ($CHCl_3$): ν = 1731, 1683, 1603, 1533 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Table 2. – MS (EI, 70 eV): *m/z* (%) = 388 (1) [M^+], 328 (4) [M^+-HOAc], 288 (46) [M^+-RCO_2H], 246 (95), 228 (40), 83 (27), 43 (100).

*Alkaline treatment of **3***

Compound **3** (50 mg), K_2CO_3 (100 mg) and MeOH (3 ml) were stirred 5 h at r.t. The reaction mixture was purified by preparative TLC (hexane-Me_2CO 7:3) affording **5** as colorless gum (28.2 mg). – $[\alpha]_D^{28} - 38°$ (c = 0.3, $CHCl_3$). –

IR ($CHCl_3$): ν = 3614, 1730, 1674, 1603, 1532 cm^{-1}. – ^1H NMR spectral data: see Table 2. – MS (EI, 70 eV): *m/z* (%) = 334 (17) [M^+], 316 (8) [M^+-H_2O], 264 (76) [M^+-C_4H_6O], 246 (100) [M^+-RCO_2H], 71 (72), 43 (48).

Acetylation of **5**

Compound **5** (16.6 mg) was treated with pyridine (0.2 ml) and acetic anhydride (0.2 ml). After 1 h at r.t., the reaction mixture was worked up in the usual manner to yield **6** as colorless gum (12.7 mg). – $[\alpha]_D^{28} - 59°$ (c = 0.23, $CHCl_3$). – IR ($CHCl_3$): ν = 1737, 1680, 1532 cm^{-1}. – ^1H NMR spectral data: see Table 2. – MS (FAB^+): *m/z* (%) = 289 (4) [M^+-$C_4H_7O_2$], 229 (6) [289^+-HOAc], 71 (89), 55 (81), 43 (98), 41 (100).

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