Eremophilanolides from *Roldana lobata*

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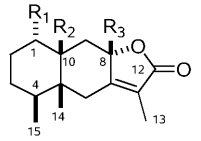
Three new eremophilanolides (**1** – **3**) and several known compounds were isolated from *Roldana lobata*. The structures of the new compounds were elucidated by spectroscopic methods. The cytotoxicity of the isolated compounds was tested against selected cancer cell lines.

Key words: Roldana lobata, Senecioneae, Eremophilanolides

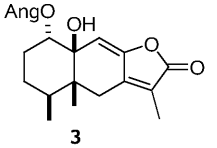
Introduction

The genus *Roldana* (Asteraceae, Senecioneae, Tussilagininae), spread from southern Arizona and New Mexico to Panamá, groups 48 species segregated from the genus *Senecio* [1, 2]. Some are used in Mexican folk medicine for a variety of ailments [3].

Previous investigations have shown that sesquiterpenes of the eremophilane or oplopane type, flavonoids, and plastoquinones are the main secondary metabolites reported in the eight members of this genus studied chemically so far [4 – 12]. This paper describes the first chemical study of *Roldana lobata* La Llave & Lex which resulted in the isolation and structure determination of three new eremophilanolides, 1α-senecioyloxy-10β-hydroxyeremophil-7(11)-en-8α,12-olide (**1**), 1α-angeloyloxy-10β-hydroxy-8β-methoxyeremophil-7(11)-en-8α,12-olide (**2**), and 1α-angeloyloxy-10β-hydroxyeremophila-8,7(11)-dien-8,12-olide (**3**), in addition to the known eremophilanolides **4** – **6** [6, 7], and the flavonoids hyperin [13] and rutin [14]. Structures of the new compounds were determined by spectroscopic methods. The cytotoxicity of compounds **1** – **6** was tested against selected cancer cell lines.



1 R_1 = OSen, R_2 = OH, R_3 = H
2 R_1 = OAng, R_2 = OH, R_3 = OCH_3
4 R_1 = OAng, R_2 = OH, R_3 = H
5 R_1 = OAng, R_2 = H, R_3 = OH
6 R_1 = OAng, R_2 = OH, R_3 = OH



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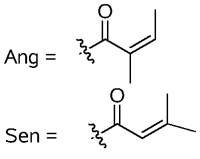

Results and Discussion

Compound **1** showed a molecular formula $C_{20}H_{28}O_5$by HRMS, and exhibited in the IR spectrum bands of hydroxyl, unsaturated γ-lactone and ester groups at 3519, 1742 and 1709 cm^{-1}, respectively. In the ^1H NMR spectrum (Table 1) the signal at δ = 5.73 (hept, J = 1.5 Hz) was attributed to the vinylic proton of the senecioyloxy group. COSY and HMBC experiments permitted to assign the signal at δ = 5.05 (dd, J = 11.5, 5.0 Hz) to H-1 and that at δ = 4.95 (br dd, J = 11.0, 8.0 Hz) to H-8. At higher field, in addition to the signals of the vinylic methyls of the senecioyloxy group (δ = 2.18, d, J = 1.5 Hz and 1.94, d, J = 1.5 Hz), appeared those of the methyl groups C-13 (δ = 1.82, t, J = 1.5 Hz), C-14 (δ = 1.07, s) and C-15 (δ = 0.85, d, J = 6.5 Hz), characteristic of an eremophilanolide skeleton. On the basis of the HMBC experiment, a hydroxyl group was localized at C-10 (δ = 76.9). The NOESY experiment showed cross peaks of CH_3-14 with H-1 and H-6β, of H-8 with H-9β and of H-9α with H-4, suggesting the α orientation of both, the ester group and the γ-lactone function, since, on biogenetic grounds, the CH_3-14

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1** – **3** ($CDCl_3$)[a].

No.	**1**		**2**[b]		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	5.05 dd (11.5, 5.0)	74.9 d	5.18 dd (11.7, 5.4)	73.5 d	5.17 dd (11.0, 4.5)	75.2 d
2a	1.85 m	27.6 t	1.94 m	27.6 t	1.97 m	28.6 t
2b	1.45 m		1.37 m		1.49 m	
3	1.57 m	28.4 t	1.53 m	28.2 t	1.48 m	28.2 t
4	1.50 m	32.8 d	1.43 m	32.9 d	1.64 m	33.3 d
5		46.1 s		47.8 s		44.8 s
6α	2.61 d (14.0)	32.2 t	2.55 d (13.8)	31.1 t	2.57 d (17.0)	29.7 t
6β	2.53 dd (14.0, 1.5)		2.35 dq (13.8, 1.5)		2.73 dq (17.0, 2.0)	
7		160.8 s		155.6 s		146.6 s
8	4.95 br dd (11.0, 8.0)	78.3 d		105.2 s		151.4 s
9β	2.54 dd (13.0, 8.0)	35.4 t	2.58 d (14.8)	37.8 t	5.82 s	105.3 d
9α	1.71 dd (13.0, 11.0)		2.17 d (14.8)			
10		76.9 s		75.2 s		75.9 s
11		120.9 s		126.0 s		122.7 s
12		175.0 s		170.9 s		170.8 s
13	1.82 t (1.5)	8.3 q	1.91 d (1.5)	8.5 q	1.91 d (2.0)	8.4 q
14	1.07 s	14.8 q	1.10 s	14.9 q	1.13 s	14.5 q
15	0.85 d (6.5)	15.7 q	0.87 d (6.0)	15.8 q	0.82 d (6.5)	15.7q
1′		167.7 s		167.4 s		168.2 s
2′	5.73 hept (1.5)	115.5 d		128.1 s		127.5 s
3′		151.1 s	6.06 qq (7.2, 1.5)	137.7 d	6.15 qq (7.0, 1.5)	139.4 d
4′	2.18 d (1.5)	20.5 q	1.98 dq (7.2, 1.5)	15.8 q	2.02 dq (7.0, 1.5)	15.9 q
5′	1.94 d (1.5)	27.5 q	1.91 quint (1.5)	20.7 q	1.94 quint (1.5)	20.6 q
OCH_3			3.51 s	50.1 q		

[a] Coupling constants (J) in Hz are in parentheses; [b] ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz).

is β-oriented [15]. The homoallylic couplings of H-8 and H-6β with CH_3-13, observed in the COSY experiment, are also in agreement with the β orientation of H-8 [16]. Finally, the *cis*-decaline configuration of **1** is in accordance with the chemical shift of the tertiary methyl group which resonates at lower field than the secondary methyl group [17].

Compound **2** also exhibited spectroscopic data consistent with an eremophilanolide skeleton. The molecular formula $C_{21}H_{30}O_6$(HRMS) showed one CH_2O unit more than in compound **1**. In the ^{1}H NMR spectrum (Table 1), the H-8 signal was absent, and those of a methoxy (δ = 3.51) and of an angeloyloxy group (δ = 6.06, qq, J = 7.2, 1.5 Hz; 1.98, dq, J = 7.2; 1.5 Hz; 1.91, quint, J = 1.5 Hz) were observed. COSY and HMBC NMR experiments allowed to place the methoxy group at C-8 (δ = 105.2, s) and the angeloyloxy group at C-1 (δ = 73.5, d). NOESY correlations between H-1 and CH_3-14 and between the methoxyl group and H-6β, which also correlates with CH_3-14, permitted to propose for compound **2** the same stereochemistry as observed in **1**.

Eremophilanolide **3** presented a molecular formula $C_{20}H_{26}O_5$ by HRMS with an additional degree of unsaturation as compared to compound **1**. In the ^{1}H NMR spectrum, the signal at δ = 6.15 (qq, J = 7.0, 1.5 Hz) was assigned to the vinylic proton of an angeloyloxy group attached to C-1, and that at δ = 5.82 to H-9, by 2D NMR experiments. The ester function should be α-equatorial, as in compounds **1** and **2**, since H-1 (δ = 5.17, dd, J = 11.0, 4.5 Hz) showed an axial-axial coupling and correlated with CH_3-14 in the NOESY experiment. Finally, the hydroxyl group was placed β-orientated at C-10 (δ = 75.9, s), as in compounds **1** and **2**.

The structures of the known compounds 1α-angeloyloxy-10β-hydroxyeremophil-7(11)-en-8α,12-olide (**4**) and 1α-angeloyloxy-10βH,8β-hydroxyeremophil-7(11)-en-8,12-olide (**5**) were determined by comparison of their physical constants and spectroscopic features with those reported in the literature. 1α-Angeloyloxy-10β,8β-dihydroxyeremophil-7(11)-en-8,12-olide (**6**), hyperin, rutin, a sitosterol-stigmasterol mixture, sucrose and β-sitosterol glucoside were identified by comparison with authentic samples and by analysis of their spectroscopic data and physical constants.

Compounds **1** – **6** were tested against central nervous system (U-251), prostate (PC-3), leukemia (K562), colon (HCT-15), breast (MCF-7), and lung

(SKLU-1) human cancer cells following the protocols established by the National Cancer Institute [18]. Unfortunately, none of these compounds were active.

Experimental Section

General experimental procedures

Melting points were determined on a Fisher-Jones melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. 1D and 2D NMR spectra were obtained on Eclipse JEOL 300 MHz, Bruker Avance 300 MHz or a Varian-Unity Inova 500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. EIMS data were determined on a Bruker Daltonics Analysis 3.2 mass spectrometer. HRFABMS were performed at 10.000 resolution using electric field scans and polyethylene glycol ions (Fluka 200 and 300) as reference material. Column chromatography was carried out under vacuum on silica gel G 60 (Merck, Darmstadt, Germany). TLC was performed on silica gel 60 and preparative TLC on silica gel GF_{254} (Merck), layer thickness 2.0 mm.

Plant material

Roldana lobata La Llave & Lex. was collected in Ozumba, Mexico State, Mexico, in October 2004. A voucher specimen (MEXU 394121) has been deposited at the Herbario del Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and isolation

Dried and ground roots (880 g) were extracted successively with hexane and methanol at r. t. The hexane extract (20 g) was separated by column chromatography eluted with a hexane-acetone gradient mixture system. Fractions eluted with hexane-acetone 19 : 1 and 9 : 1 (4.3 g) after column chromatography eluted with a hexane-acetone gradient system afforded compound **4** (860 mg) [6], fraction A and fraction B. Mother liquors of compound **4** (920 mg) were submitted to a new column chromatography eluted with hexane-acetone (19 : 1) to obtain **1** (10.5 mg) and **4** (127 mg). Compound **5** (40 mg) [6] was isolated from fraction A (350 mg) after column chromatography eluted with CH_2Cl_2-acetone 99 : 1, followed by preparative TLC eluted with hexane-acetone 19 : 1. Fraction B (405 mg) was worked up by successive column chromatographies in hexane-acetone 9 : 1 and CH_2Cl_2-acetone 99 : 1 to afford compound **6** [7] (68 mg) as colorless prisms from EtOAc-*i*Pr_2O, m. p. 158 – 160 °C, m. p. lit. 94 – 95 °C (a sample kindly supplied by the authors was a hydrate which lost water at 94 – 95 °C and melted at 156 – 157 °C). Fractions obtained from the first column with hexane-acetone 8 : 2 (4 g) were purified by column chromatography eluted with hexane-acetone 9 : 1 to obtain **6** (760 mg).

The methanolic extract (71 g) was separated by column chromatography eluted with EtOAc-MeOH mixtures of increasing polarity. Fractions eluted with EtOAc (11.5 g) were fractionated by column chromatography eluted with hexane-EtOAc mixtures of increasing polarity. Fractions eluted with hexane-EtOAc 93 : 7 afforded 250 mg of **4**. Mother liquors of **4** were purified in a new column chromatography eluted with hexane-acetone 19 : 1 to afford 200 mg of **4** and fraction C. Fraction C (350 mg) was fractionated through a column chromatography eluted with CH_2Cl_2-hexane 8 : 2 and fractions 1 – 3 (80 mg) and 4 – 9 (120 mg) were further purified. Fractions 1 – 3 were submitted to preparative TLC eluted with hexane-acetone 9 : 1 to obtain compound **2** (25 mg). Fractions 4 – 9 were purified by preparative TLC eluted with C_6H_6-acetone 19 : 1 to produce compounds **2** (15 mg) and **3** (13 mg).

Dried and ground leaves (1 kg) were extracted with hexane and methanol, successively at r. t. The hexane extract (7.3 g), after purification by column chromatography eluted with hexane-acetone of increasing gradient of polarity afforded a sitosterol-stigmasterol mixture (430 mg). The methanolic extract (80 g) was fractionated by column chromatography eluting with EtOAc-MeOH mixtures of increasing polarity. Fractions eluted with EtOAc-MeOH 19 : 1 afforded sitosterol β-glucoside (50 mg). Fraction eluted with EtOAc-MeOH 9 : 1, after a new chromatography eluted with EtOAc-MeOH 19 : 1, produced hyperin (525 mg) [13]. Fractions eluted with EtOAc-MeOH 8 : 2 were purified on a sephadex column eluted with MeOH-H_2O 8 : 2 to obtain hyperin (300 mg), rutin (13 mg) [14], and sucrose (880 mg).

*1α-Senecioyloxy-10β-hydroxyeremophil-7(11)-en-8α,12-olide (**1**)*

Colorless needles (EtOAc-*i*Pr_2O), m. p. 198 – 200 °C. – $[\alpha]_D^{25}$ = −36.3° (*c* = 0.19, $CHCl_3$). – UV (MeOH): λ_{max} (lg ε_{max}) = 220 nm (4.2). – IR ($CHCl_3$): ν = 3519, 1742, 1709 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 348 (2) $[M]^+$, 330 (10), 230 (30), 83 (100). – HRMS (FAB^+): *m/z* = 349.2017 (calcd. 349.2015 for $C_{20}H_{29}O_5$, $[M+H]^+$).

*1α-Angeloyloxy-10β-hydroxy-8β-methoxyeremophil-7(11)-en-8,12-olide (**2**)*

Colorless needles (EtOAc-*i*Pr_2O), m. p. 98 – 100 °C. – $[\alpha]_D^{25}$ = +38.3° (*c* = 0.23, MeOH). – UV (MeOH): λ_{max} (lg ε_{max}) = 218 nm (4.3). – IR ($CHCl_3$): ν = 3522, 1770, 1713 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 378 (2) $[M]^+$, 346 (10), 228 (30), 278 (30), 83 (100). – HRMS (FAB^+): *m/z* = 379.2128 (calcd. 379.2121 for $C_{21}H_{31}O_6$, $[M+H]^+$).

*1α-Angeloyloxy-10β-hydroxyeremophila-8,7(11)-dien-8,12-olide (**3**)*

Colorless oil. – $[\alpha]_D^{25} = +42.9°$ (c = 0.27, MeOH). – UV (MeOH): $\lambda_{max}(\lg \varepsilon_{max})$ = 220 nm (4.1), 272 nm (4.1). – IR ($CHCl_3$): ν = 3486, 1776, 1715, 1677 cm^{-1}. – ^1H NMR and ^{13}C NMR spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 346 (15) $[M]^+$, 263 (100), 245 (70), 83 (95). – HRMS (FAB^+): *m/z* = 347.1857 (calcd. 347.1858 for $C_{18}H_{19}O_7$, $[M+H]^+$).

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