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Eremophilanes and Pyrrolizidine Alkaloids of Senecioneae Species

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Abstract. The chemical study of two species of the tribe Senecioneae afforded two eremophilanes and two pyrrolizidine alkaloids from *Senecio subauriculatus* and four modified eremophilanes from *Roldana oaxacana*. The chemistry of these species is in accord with that reported for species of *Senecio* and *Roldana* studied so far, and therefore, with the already described for the tribe Senecioneae.
Key words: Senecioneae, *Senecio*, *Roldana*, eremophilanes, pyrrolizidine alkaloids.

Resumen. El estudio químico de dos especies de la tribu Senecioneae permitió obtener dos eremofilanos y dos alcaloides pirrolizidínicos de *Senecio subauriculatus* y cuatro eremofilanos modificados de *Roldana oaxacana*. Los metabolitos obtenidos de estas especies concuerdan con el tipo de metabolitos aislados de especies de *Senecio* y *Roldana* estudiadas hasta ahora, y en consecuencia, concuerdan con lo descrito para la tribu Senecioneae.
Palabras clave: Senecioneae, *Senecio*, *Roldana*, eremofilanos, alcaloides pirrolizidídicos.

Introduction

Mexico is one of the countries with the highest endemism in its flora and the Asteraceae family occupies a prominent place since 65.9% of its 3021 species are endemic [1]. Oaxaca is the richest state with 133 endemic species, and 116 whose endemism is shared with the states of Guerrero, Veracruz, and Puebla [2]. Two examples are *Senecio subauriculatus* and *Roldana oaxacana*, the first one is endemic to Oaxaca state and the second one to the states of Oaxaca, Veracruz, and Puebla [2]. Although both species belong to different genera, they are placed in the same tribe, Senecioneae. A large number of the species of the genus *Senecio*, which is cosmopolitan and complex, have been subjected to diverse taxonomic interpretations and divided on sections or groups and even some species have been segregated and added to Tussilaginoid or Senecionoid genera [2-4]. Thus, the genus *Roldana* is constituted by species separate from *Senecio* [5]. Additionally, both genera contain eremophilane derivatives as characteristic metabolites, in concordance with their taxonomic relationship. Other typical metabolites of the genus *Senecio* are the pyrrolizidine alkaloids (PAs), compounds not isolated from species of *Roldana*, so far. Continuing with our chemical research of Mexican species of the Senecioneae tribe [6] and in order to contribute to the chemotaxonomy of the tribe, we carried out the chemical study of *Senecio subauriculatus* and *Roldana oaxacana* which were collected in Oaxaca state.

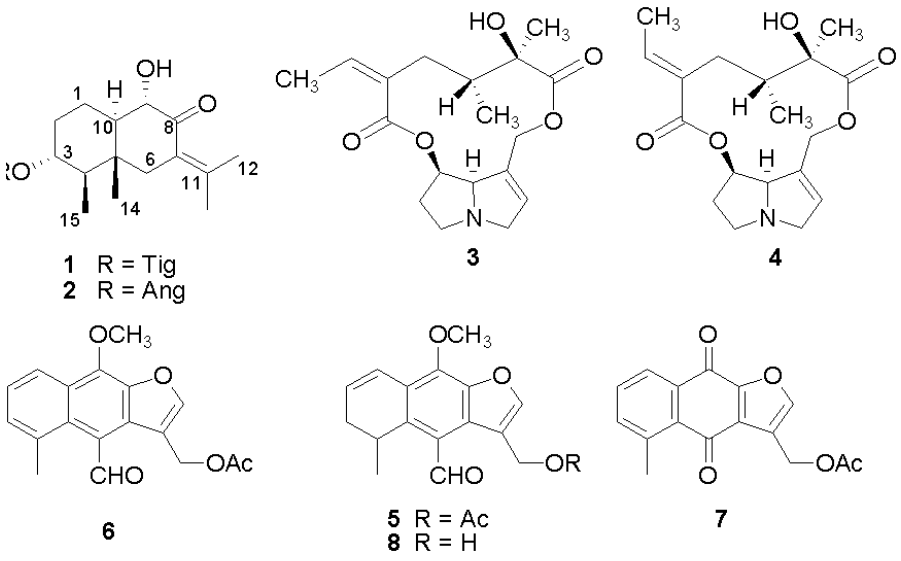
Results and Discussion

The roots and aerial parts of *Senecio subauriculatus* were extracted of independent manner with hexane and methanol, successively. The purification of the hexane extract of roots afforded the eremophilane **1** [7] and two mixtures, one constituted by the eremophilanes **1** and **2** [7b, 8], and the second one by the sterols *β*-sitosterol and stigmasterol. Of the hexane extract of the aerial parts only a mixture of *β*-sitosterol and stigmasterol was obtained. Since the methanol extracts of roots and aerial parts gave positive Dragendorff test, they were submitted to reductive treatments (Zn/H^+) [9] to produce the respective alkaloidal extracts. The analysis of the alkaloidal extract of the roots led the isolation of the PA senecionine (**3**) [10] and the analysis of that of the aerial parts afforded senecionine (**3**) and integerrimine (**4**) [10a, 11].

The methanol extract of roots of *Roldana oaxacana* gave the modified eremophilanes 13-acetoxy-14-oxocacalohastin (**5**) [12], maturin acetate (**6**) [13], maturone acetate (**7**) [14], and 13-hydroxy-14-oxocacalohastin (**8**) [12]. Additionally, *β*-sitosterol and stigmasterol as a mixture and *β*-sitosteryl *β*-D-glucopyranoside were obtained. The purification of the methanol extract of the aerial parts afforded 13-acetoxy-14-oxocacalohastin (**5**), maturin acetate (**6**), the sterols above mentioned, and sucrose.

The structural elucidation of all isolated metabolites was carried out by the analysis of their physical and spectroscopic data and, with exception of compounds **1** and **2**, they were compared with authentic samples. The ^{13}C NMR data of compound **1** are described in this paper because, to our knowledge, they have not been reported yet.

In conclusion, the isolation of eremophilanes and pyrrolizidine alkaloids from *Senecio subauriculatus* and of modified eremophilanes from *Roldana oaxacana* is in agreement with the chemistry of the genera *Senecio* and *Roldana* reported so far. On the other hand, the toxicity of some *Senecio* species has been attributed mainly to the pyrrolizidine alkaloids [15]. In fact, the acute toxic activity of senecionine (**3**) and integerrimine (**4**) was already evaluated [15b,16], therefore, *S. subauriculatus* should be considered a plant with high toxic potential.



General Experimental Procedures

Melting points were determined on a Fisher-Johns melting points apparatus and are uncorrected. NMR spectra were obtained on a Varian-Unity 300 MHZ, a Bruker Avance 300 MHz, a Varian Inova 500 MHz, or a Bruker Avance III 400 MHz spectrometer with TMS as internal standard and as solvent $CDCl_3$ or CD_3OD. Vacuum column chromatography (VCC) was carried out with silica gel G 60 (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was performed with silica gel 60 (230-400 mesh, Macherey-Nagel). Preparative TLC was carried out on precoated Sil G-100 UV_{254} plates (Macherey-Nagel) and on Sil RP-18W/UV254 plates of 1.0 mm thickness (Macherey-Nagel).

Plant Material

Senecio subauriculatus Greenm. and *Roldana oaxacana* (Hemsl.) H. Rob. & Brettell were collected in state of Oaxaca, Mexico, the first one in Miahuatlán and the second one in Ixtlán de Juárez, January 2009, and they were authenticated by Professor J. L. Villaseñor (Instituto de Biología, UNAM). Voucher specimens (MEXU 1256449 and MEXU 1256442, respectively) were deposited at the Herbario Nacional, Instituto de Biología, UNAM.

Extraction and Isolation

Dried and ground roots (108 g) of *Senecio subauriculatus* were extracted successively with hexane and MeOH. Evaporation of the hexane yielded 250 mg of extract. The MeOH extract which gave a positive Dragendorff test, was evaporated to a tenth of its volume and submitted to a reductive procedure (5.5 g of Zn, acidification to pH 4 with 5% aqueous H_2SO_4, and stirred overnight at room temp. [9]) to produce 0.4 g of alkaloidal extract. The aerial parts (411 g) were extracted of similar manner to afford 2.3 g of hexane extract and the MeOH extract after a reductive procedure produced 1.6 g of alkaloidal extract. The hexane extract of roots was purified by VCC eluted with mixtures of hexane-EtOAc (100:0 → 90:10). Fractions obtained with hexane-EtOAc 98:2 were combined (97.7 mg) and submitted to a preparative RP-TLC (eluent MeOH-H_2O 70:30 × 2). Two fractions were obtained, that of lower Rf yielded 2.0 mg of a mixture of *β*-sitosterol and stigmasterol as white crystals (EtOH), mp 135-136 °C. The fraction of higher Rf (38.3 mg) was purified by preparative TLC (eluent C_6H_6-Me_2CO 98:2 × 2) to afford fractions A and B. Fraction A (11 mg) was purified by VCC (eluent C_6H_6-Me_2CO 99:1) to give 4.5 mg of compound **1** as yellow pale oil [7]. Purification of fraction B (17.6 mg; CC, eluent C_6H_6-Me_2CO 99:1) produced 11.9 mg of a yellow pale oil constituted of an 1:1.3 mixture of compounds **1** and **2** [7b, 8]. Hexane extract of the aerial parts (2.3 g) was worked up as the roots extract to yield 19.7 mg of a mixture of *β*-sitosterol and stigmasterol. The alkaloidal extract of roots (0.4 g) presented solids which were crystallized (hexane-EtOAc) to give 21.2 mg of senecionine (**3**) [10] as white solids, mp 222-225 °C. The mother liquors were purified by FCC (eluent CH_2Cl_2-MeOH 95:5) to afford 30.7 mg of **3**. The alkaloidal extract of the aerial parts (1.69 g) was purified by a VCC (eluent CH_2Cl_2-MeOH 85:15) to produce 35 fractions. Fractions 13-19 were combined (377.0 mg) and submitted to a reductive procedure (120 mg of Zn, acidification to pH 4 with 5% aqueous H_2SO_4, and stirred overnight at room temp.) because, their ^1H NMR analysis showed the presence of pyrrolizidine alkaloids as N-oxides. The reaction mixture was crystallized from MeOH to give 20.3 mg of **3**. The crystallization (EtOAc) of the mother liquors produced 33.8 mg of integerrimine (**4**) [10a, 11] as white solids, mp 172-175 °C. The last mother liquors were crystallized (MeOH) to yield 16.0 mg of **3**.

Compound **1**: ^{13}C NMR ($CDCl_3$, 125 MHz, assignments based on the DEPT, HSQC, and HMBC spectra): δ 204.4 (C, C-8), 167.8 (C, C-1'), 143.3 (C, C-11), 136.9 (CH, C-3'), 128.9

(C, C-2'), 128.8 (C, C-7), 76.3 (CH, C-9), 73.3 (CH, C-3), 52.1 (CH, C-10), 46.9 (CH, C-4), 42.9 (CH_2, C-6), 38.5 (C, C-5), 31.5 (CH_2, C-2), 23.4 (CH_2, C-1), 22.6 (CH_3, C-12), 21.5 (CH_3, C-13), 14.3 (CH_3, C-4'), 12.4 (CH_3, C-14), 12.1 (CH_3, C-5'), 10.5 (CH_3, C-15).

Dried and ground roots (112 g) and aerial parts (255 g) of *Roldana oaxacana* were exhaustively extracted with MeOH at room temp. The extract of roots (13 g) was fractioned by VCC eluted with a hexane-Me_2CO-MeOH gradient system to afford fractions A-E. A was eluted with hexane-Me_2CO 19:1, B with hexane-Me_2CO 9:1, C with hexane-Me_2CO 4:1, D with hexane-Me_2CO 1:1, and E grouped the Me_2CO-MeOH 9:1 eluates. Purification of fraction A (850 mg) by FCC (eluent hexane-Me_2CO 9:1) gave 47.8 mg of 13-acetoxy-14-oxocacalohastin (**5**) [12] as pale yellow oil, a mixture of *β*-sitosterol and stigmasterol (18.0 mg) and fractions A1 and A2. Fraction A1 (174 mg) produced 8.0 mg of compound **5** and 22.0 mg of maturin acetate (**6**) [13] as yellow needles from hexane-EtOAc, mp 82-84 °C, by means of a preparative TLC (hexane-Me_2CO 9:1 × 3). Fraction A2 (74.6 mg) was purified by preparative RP-TLC (MeOH-H_2O 7:3 × 3) to obtain compound **6** (7.0 mg) and maturone acetate (**7**) [14] as yellow needles from hexane-EtOAc, mp 147-150 °C, 5.0 mg. Fraction B (730 mg) was purified by VCC using hexane-Me_2CO gradient system as eluent to obtain compound **5** (53.0 mg). Fraction C (520 mg) was purified by FCC (hexane-Me_2CO 4:1) to produce 15.0 mg of 13-hydroxy-14-oxocacalohastin (**8**) [12] as pale yellow oil. Fraction D produced 250 mg of *β*-sitosteryl glucopyranoside and from fraction E, 150 mg of sucrose were isolated. The aerial parts extract (23 g) was worked out in a similar way as the extract of the roots to produce compound **5** (85.0 mg), compound **6** (17.0 mg), a mixture *β*-sitosterol and stigmasterol (18.0 mg), and *β*-sitosteryl glucopyranoside (300.0 mg).

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