

Sesquiterpenes and Other Natural Products from *Roldana reticulata*

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Resumen. De *Roldana reticulata* se aislaron dos 9-oxo-furanoeremofilanos (**1**, **2**), además de senecrassidiol, tirosol, icarisida D₂ y rutina; no se detectaron alcaloides pirrolizidínicos, coincidiendo con el patrón químico del género reportado hasta ahora. La actividad anti-inflamatoria de los extractos y productos aislados fue evaluada en el modelo de edema de inflamación aguda inducido con TPA.

Palabras clave: *Roldana reticulata*, Asteraceae, Senecioneae, Tussilaginiaceae, furanoeremofilanos, actividad anti-inflamatoria.

Abstract. The chemical study of *Roldana reticulata* led to the isolation of two 9-oxo-furanoeremophilanes (**1**, **2**), together with senecrassidiol, tyrosol, icariside D₂, and rutin; no pyrrolizidine alkaloids were detected, in agreement with the chemistry of the genus reported so far. The anti-inflammatory activity of extracts and isolated products was tested using the TPA model of induced acute inflammation.

Key words: *Roldana reticulata*, Asteraceae, Senecioneae, Tussilaginiaceae, furanoeremophilanes, anti-inflammatory activity.

Introduction

Roldana reticulata is one of the 48 species of the genus *Roldana* (Asteraceae, Senecioneae, Tussilaginiaceae) which groups species segregated mainly from the genus *Senecio*, spread from southern Arizona and New Mexico to Panama [1, 2]. Some species of *Roldana* are known to have medicinal properties; *R. sessilifolia* is used as part of the “cachani” complex [3], in Mexican traditional medicine to treat female sterility, hemorrhoids and rheumatic pains, and *R. ehrenbergiana* (Itzcuimpatli or hierba de perro) has been used to treat leprosy [4]. Products with anti-oxidant, anti-inflammatory and insecticidal properties have been isolated from *R. barba-johannis* [5], while bioactive metabolites against *Trichophyton mentagrophytes* have been isolated from *R. lineolata* [6]. Previous phytochemical reports have shown that eremophilane derivatives are the main secondary metabolites isolated from eight of the ten species of this genus studied so far: *R. angulifolia* [7], *R. aschenborniana* (also studied as *Senecio aschenbornianus*) [8, 9], *R. ehrenbergiana* [10], *R. heterogama* [11], *R. lineolata* [6], *R. lobata* [12], *R. sessilifolia* [13], and *R. barba-johannis* (also studied as *Senecio barba-johannis*) [5]. The phytochemical studies of *R. mexicana* (studied as *Senecio mexicanus*) and *R. sundbergii* (studied as *Senecio sundbergii*) report the isolation of opiplane sesquiterpenes [14], caryophyllene, and open chain sesquiterpenes [9], respectively. This paper describes the first chemical study of *Roldana reticulata*.

Results and Discussion

The chemical study of roots and aerial parts of *Roldana reticulata* afforded two 9-oxofuranoeremophilanes (**1** and **2**) [9,15], tyrosol (**3**) [16], icarioside D₂ (**4**) [17], senecrassidiol (**5**) [18], rutin (**6**) [19], sucrose, a mixture of β -sitosterol-stigmasterol, and β -sitosteryl glucopyranoside. Structures of the

isolated products were determined by comparing their physical constants and spectroscopic data with those reported in the literature. The ¹³C NMR data of 6 β -(2-methyl-2-vinylacetoxy)-furanoeremophil-9-one (**1**) are included because they are not yet available in the literature. Assignments were achieved by means of 1D and 2D NMR. The characteristic signals of the carbonyl groups, a conjugate ketone at δ 186.7 and an ester function at δ 173.9 were evident. The HMBC correlations of H-10 and H-1 with the first one allowed to position the ketone at C-9 and the interaction of H-6 with the second one determined the position of the ester group at C-6. Additionally, the presence of four aromatic and two vinyl carbon atoms accounted for the furan ring and the vinyl acetoxy substituent.

Due to the medicinal use of some *Roldana* species [3, 5], the anti-inflammatory activity of extracts and isolated products of *R. reticulata* were evaluated using the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) model of induced acute inflammation [20]. All extracts, with exception of the methanol extract of the roots which was not active, were moderately active showing values between 35.41 to 64.75 % of inhibition (Table 1), being more active those of the aerial parts. None of the isolated metabolites showed relevant activity compared with indomethacin, the reference compound.

The results of the phytochemical study of *R. reticulata*, showing the presence of furanoeremophilanes and phenolic products and the fact that pyrrolizidine alkaloids were not detected, nor isolated, coincide with the chemistry of the genus and of the tribe Senecioneae and subtribe Tussilaginiaceae [21].

General Experimental Procedures

Melting points were determined using a Fisher Jones melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. EIMS data were

Table 1. Effect of extracts and isolated products from *R. reticulata* on TPA-induced mouse edema

Sample	Edema (mg)	Inhibition (%)
Hexane extract of the roots	9.53 ± 0.42**	37.59**
Acetone extract of the roots ^a	9.87 ± 1.33**	35.41**
Methanol extract of the roots ^b	17.20 ± 3.62**	-15.18
Hexane extract of the aerial parts ^a	7.23 ± 0.39**	52.65**
Acetone extract of the aerial parts ^c	4.70 ± 0.64**	64.75**
Methanol extract of the aerial parts ^a	6.07 ± 0.41**	60.28**
Metabolite 1 ^d	7.50 ± 2.28**	48.06**
Metabolite 2 ^d	7.38 ± 1.16**	48.93**
Tyrosol (3) ^e	14.50 ± 0.45**	19.29**
Icariside D ₂ (4) ^a	13.47 ± 0.08**	11.84**
Senecrassidiol (5) ^d	9.97 ± 0.98*	30.98*
Rutin (6) ^e	12.08 ± 0.96**	32.79**
Indomethacin ^{f,g}	2.06 ± 0.30**	87.61**
Indomethacin ^f	1.99 ± 0.69*	83.73*

Dose: 1 mg/ear for extracts, 1 μmol/ear for pure compounds. Each value represents the mean of three animals ± standard error. Control:

^amethanol-acetone 1:1, 15.28 ± 0.29; ^bmethanol 14.93 ± 0.52 ^cacetone-CH₂Cl₂ 1:1, 13.33 ± 0.66, ^dacetone-CH₂Cl₂ 1:1 14.44 ± 0.61, ^emethanol 17.97 ± 0.48, ^facetone, 13.53 ± 104, ^g1 mg/ear. Results were analysed by the *t* Student test. **p* ≤ 0.05. ***p* ≤ 0.01.

determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV. ¹H NMR and ¹³C NMR data were obtained on a Varian Unity 300 or on a Varian Unity Plus 500 instrument. Chemical shifts were referred to TMS. Preparative HPLC was carried out in a Waters Delta Prep 4000 instrument using a Luna 5u C18(2) 100A, AXIA packed column of 50 × 21.2 mm at 20 ml/min, Detector UV Waters 486 at 280 nm. Vacuum column chromatography (VCC) was performed using Silica gel 60 G (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was run using Silica gel 60 (230-400 Macherey-Nagel). TLC was carried out on Silica gel 60 GF₂₅₄ and preparative TLC on Silica gel GF₂₅₄ (Macherey-Nagel), layer thickness 2.0 mm.

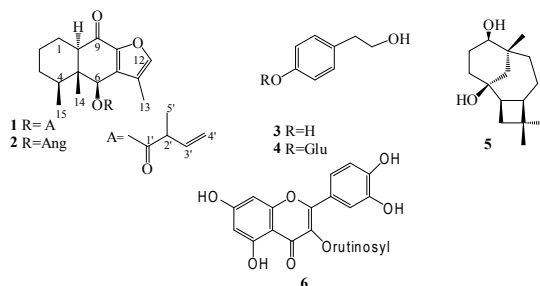
Plant Material

Roldana reticulata (DC.) H. Rob. & Bretell was collected at Llano de la Cantimplora, Tlalpan, México D. F., México, in December 2003. A voucher specimen was deposited at the Herbarium del Instituto de Biología, UNAM, México (MEXU 1156423).

Extraction and Isolation

Dried and ground roots (383 g) and aerial parts (569 g) of *R. reticulata* were separately and successively extracted with hexane, acetone and methanol. Solvents were removed at reduced pressure to obtain the corresponding extracts which gave negative tests with the alkaloids Dragendorff reagent. The hexane extract of the roots (12 g) was subjected to VCC eluting with hexane-EtOAc mixtures of increasing polarity. Fractions eluted

with hexane-EtOAc 19:1 (2.5 g) were further purified by a VCC (hexane-EtOAc 49:1) and FCC (hexane-EtOAc 19:1) to produce a mixture (680 mg) which was purified by HPLC (MeOH-H₂O 70:30) to yield **1** (8 mg, colorless gum, [α]₂₅ -40.5°, *c* 0.25, CHCl₃) [**9**] and **2** (5.6 mg, colorless needles, mp 103-105 °C, [α]₂₅ -90.2°, *c* 0.2, CHCl₃, lit.: [α]₂₅ -90.3°, *c* 0.4, Me₂CO) [**15**]. The acetone extract of the roots (5.6 g) was purified by VCC eluting with hexane-acetone mixtures of increasing polarity to obtain 25 mg of icariside D₂ (**4**) (9.5 mg, amorphous powder, [α]₂₅ -59.5°, *c* 0.1, MeOH, lit.: [α]₂₅ -61.9°, *c* 0.11, MeOH) [**17**] from the hexane-acetone 3:2 eluates. The methanol extract of the roots (24 g) was fractionated by VCC eluting with EtOAc-MeOH mixtures of increasing polarity to produce sucrose (350 mg) and β-sitosterol glucopyranoside (180 mg, amorphous powder). Successive VCC purifications of the hexane extract of the aerial parts (12.7 g) using hexane-EtOAc mixtures of increasing polarity as eluent produced senecrassidiol (**5**) (9 mg, colorless needles, 93-95 °C, [α]₂₅ -10.1°, *c* 0.1, CHCl₃, lit.: [α]₂₄ -10.9°, *c* 0.23, CHCl₃) [**18**] and a mixture of β-sitosterol and stigmasterol (23 mg, colorless plates,



mp 130-132 °C). Similarly, the VCC purification (hexane-acetone mixtures of increasing polarity) of the acetone extract of the aerial parts (35 g) resulted in fractions: A (eluted with hexane-acetone 9:1), B (eluted with hexane-acetone 4:1 and 7:3), and C (eluted with hexane-acetone 3:2 and 1:1). Purification of fraction A (5.6 g) by FCC (hexane-acetone 9:1) produced mixture of β -sitosterol-stigmasterol (36 mg). Fraction B (8.5 g) was purified by FCC (hexane-acetone 7:3) to yield 8 mg of tyrosol (**3**) as colorless needles (mp 89-91 °C) [16] and 85 mg of icariside D₂ (**4**). Fraction C produced an amorphous solid (1.3 g) which, by recrystallization (EtOAc-MeOH), afforded 458 mg of icariside D₂ (**4**). The methanol extract of the aerial parts (24 g) was purified by VCC (EtOAc-MeOH mixtures of increasing polarity); while fractions eluted with EtOAc afforded 165 mg of **4**, the EtOAc-MeOH 9:1, 4:1 and 7:3 eluates afforded a mixture (11 g), a portion of which (2 g) was passed through a Sephadex LH 20 column (MeOH-H₂O 4:1) to obtain 1.2 g of sucrose and 65 mg of rutin (**6**) (yellow powder, $[\alpha]_{25} -3.0^\circ$, c 0.25, MeOH, lit.: $[\alpha]_{25} -8.8^\circ$, c 0.14, MeOH) [19].

6 β -(2-Methyl-2-vinylacetoxy)-furanoremerophil-9-one (**1**): ¹³C NMR (CDCl₃, 125 MHz): δ 186.7 (C, C-9), 173.9 (C, C-1'), 146.9 (C, C-8), 144.9 (CH, C-12), 136.2 (CH, C-3'), 134.3 (C, C-7), 120.8 (C, C-11), 116.7 (CH₂, C-4'), 75.9 (CH, C-6), 55.2 (CH, C-10), 49.9 (C, C-5), 44.4 (CH, C-2'), 42.5 (CH, C-4), 32.2 (CH₂, C-1), 24.9 (CH₂, C-2), 20.7 (CH₂, C-3), 18.6 (CH₃, C-15), 16.2 (CH₃, C-5'), 8.8 (CH₃, C-13), 7.2 (CH₃, C-14).

Evaluation of the anti-inflammatory activity

Animals. Male NIH mice weighing 25-30 g were maintained under standard laboratory conditions in the animal house (temperature 27 ± 1 °C) with a 12/12 h light-dark cycle, according with the Mexican official norm MON-062-Z00-1999. They were fed laboratory diet and water *ad libitum*.

TPA-induced edema model

The TPA-induced ear edema assay in mice was performed as previously reported [20]. A solution of TPA (2.5 μ g) in ethanol (10 μ l) was applied topically to both faces (5 μ l each face) of the right ear of the mice. Solutions of the test substances in their respective solvents were applied 10 minutes after (10 μ l, to each face). The left ear received ethanol (10 μ l) first and ten minutes after 20 μ l of the respective solvent. Four hours later, the mice were killed by cervical dislocation. A 7 mm diameter plug was removed from each ear and the swelling was assessed as the difference in weight between the left and the right ear. Control animals received the correspondent solvent in each case. Edema inhibition (EI %) was calculated by the equation: EI = 100 - (B x 100/A), where A is the edema induced by TPA alone and B is the edema induced by TPA plus sample. Indomethacin was used as the reference compound (Table 1).

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