



Sesquiterpene lactone variability in *Parthenium hysterophorus* L.

J.R. de la Fuente^a, M.L. Uriburu^a, G. Burton^b, V.E. Sosa^{c,*}

^aConsejo de Investigación, Universidad Nacional de Salta, 4400 Salta, Argentina

^bFacultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

^cFacultad de Ciencias Químicas, Universidad Nacional de Córdoba, Instituto Multidisciplinario de Biología Vegetal IMBIV (CONICET-UNC), 5016 Córdoba, Argentina

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Abstract

A population of *Parthenium hysterophorus* collected in Salta Argentina afforded two ambrosanolides, 2 β -hydroxycoronopilin and 1 α ,2 β ,4 β -trihydroxypseudoguaian-6 β ,12-olide, as well as five known others. Plants of the fructification from those transplanted from the Puna Argentina at 1200 m over the sea level produced hymenin. © 2000 Elsevier Science Ltd. All rights reserved.

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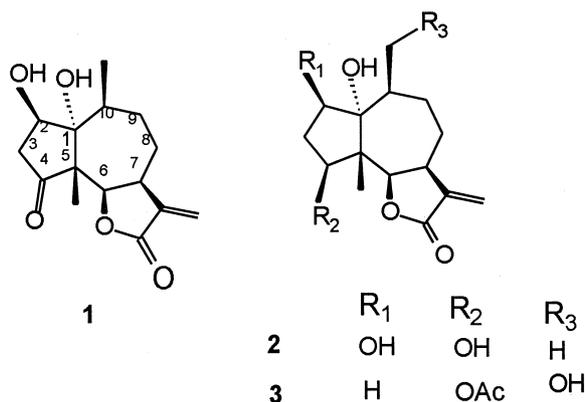
1. Introduction

In previous phytochemical and taxonomic investigations carried out with five populations of *Parthenium hysterophorus*/*Parthenium glomeratum* growing in different geographical regions of Salta Province, Argentina, the isolation of ambrosanolides was reported (de la Fuente et al., 1997) these being considered characteristic of delimited zones of the world (Picman and Towers, 1982). Taking into account the morphological, anatomical and chromosomal examinations, it was suggested that *P. hysterophorus* and *P. glomeratum* are synonyms, with the latter being a clinal variation (Piazzano et al., 1998).

We now report the isolation and structural determination of two new ambrosanolides from a Tonco population. We also report the chemical results from plants of the first generation of those transplanted from the 3400 m above sea level to the subtropical habitat at 1200 m altitude (de la Fuente et al., 1997). These findings indicate that *P. glomeratum*, considered as an independent species, should be considered as a synonym and thus subordinate to *P. hysterophorus*.

2. Results and discussion

The aerial parts of *P. hysterophorus* collected in Tonco, at 3000 m above sea level gave two new ambrosanolides 2 β -hydroxycoronopilin (**1**) and 1 α ,2 β ,4 β -trihydroxypseudoguaian-6 β ,12-olide (**2**) together with 4 α -*O*-acetylpsuedoguaian-6 β ,12-olide (de la Fuente et al., 1997), 1 α -hydroxy-4 β -*O*-acetylpsuedoguaian-6 β ,12-olide (de la Fuente et al., 1997); coronopilin (Herz et al., 1961); parthenin (Herz and Watanabe, 1959; Rodríguez et al., 1971); hysterin (Romo de Vivar et al., 1966), tetranurin E (**3**) (Yoshioka et al., 1970) and *p*-methoxybenzoic acid. Table 1 shows a comparison of the compounds isolated from this population with those studied previously.



* Corresponding author. Tel.: +54-351-4334170; fax: +54-351-4333030.

E-mail address: vesosa@dqo.fcq.unc.edu.ar (V.E. Sosa).

Table 1
Distribution of sesquiterpene lactones in populations of *P. hysterophorus*^a

Compound	A	I	II	III	IV	V	B	B ₁
1 α -2 α , 4 β -Trihydroxypseudoguaian-6 β , 12-olide (2)	×							
1 α -Hydroxy-4-oxo-14- <i>O</i> -(2-methylbutyryl)-pseudoguaian-6 β ,12-olide and 1 α -hydroxy-4-oxo-14- <i>O</i> -isovaleoylpseudoguaian-6 β ,12-olide			×					
1 α -Hydroxy-4 β - <i>O</i> -acetylpseudoguaian-6 β , 12-olide	×					×		×
2 β -Hydroxycoronopilin (1)	×							×
4 α - <i>O</i> -Acetylpseudoguaian-6 β , 12-olide	×					×		×
Confertdiolide		×						
Coronopilin	×	×	×	×	×	×	×	×
Chiapin B					×			
Hymenin		×						×
Hysterin	×	×						
Parthenin	×			×	×		×	
Tetraneurin A			×	×				
Tetraneurin E (3)	×							

^a A, Tonco population; I–V, populations studied previously (de la Fuente et al., 1997); B, transplanted taxon; B₁, fructification taxon from B.

Compound **1**, C₁₅H₂₀O₅, was isolated as a solid of melting point 185–187°C. Its positive ion FABMS showed an [M+1]⁺ ion at *m/z* 281 and the EIMS exhibited a peak at *m/z* 262 due to the loss of one molecule of water. The presence of a γ -lactone ring with an exocyclic double bond was evident from the IR bands at 1750 and 1658 cm⁻¹, the two signals in the ¹H

NMR spectrum (Table 2) at δ 5.65 (*d*, *J*=2 Hz) and δ 6.08 (*d*, *J*=2.64 Hz) and the ¹³C- resonances at δ 170.4 (C) and 120.8 (CH₂). The deshielding effect observed on C-5 and C-10 methyl group signals at δ 1.40 (*s*) and δ 1.50 (*d*, *J*=7.6 Hz), respectively, may be attributed to the four bond *syn-axial* interaction with the β -hydroxyl group at C-2 (Balza and Towers, 1988). The H-2 resonance at δ 4.28 (*dd*, *J*=6.1, 0.8 Hz) is part of an AMX system with H-3 α and 3 β , the correlations appearing in the H,H-COSY experiment. Furthermore in the long-range H,H-COSY spectrum, the interaction between the C-5 methyl group and H-3 confirmed the presence of a hydroxyl group at C-2. The stereochemistry at this position was deduced from a comparison of coupling constants (*J*_{2,3 α} 6.1 Hz, *J*_{2,3 β} 0.8 Hz), with those calculated from model structures. The presence of a carbonyl group at C-4 was confirmed by both the ¹³C NMR resonance at δ 219.1 and the chemical shifts of H-3 α (δ 2.88) and H-3 β (δ 2.25) in the ¹H NMR spectrum.

Three signals of carbon atoms attached to oxygen [δ _C 80.7 (CH), δ _C 79.3 (CH) and δ _C 84.7 (C)] were assigned to C-2, C-6 and C-1.

The NOESY spectrum confirmed the above arrangement and the configurations proposed for (**1**) as 2 β -hydroxycoronopilin. Strong NOESY correlation peaks were observed for the pairs H-6/H-7; H-14/H-15; H-15/H-3 β (δ 2,25) as well as between H-3 α (δ 2,88) and H-2 α (δ 4.28).

Compound **2** was obtained as an amorphous solid and showed signals at 1745 and 1655 cm⁻¹ in the IR spectrum due to the presence of an α,β -unsaturated γ -lactone ring. In the ¹H NMR spectrum carried out in DMSO-*d*₆ (Table 2), the H-2 and H-4 signals at δ 3.71 and δ 4.22 showed coupling to two non-equivalent protons on C-3. The H,H-COSY experiment revealed the sequences of H-3 α/β to H-2 and H-4. As in ambrosanolide **1** the C-14 methyl group signal showed a deshielding effect by a *syn-axial* interaction with the

Table 2
¹H NMR spectral data for compounds **1** and **2**^a

H	1		2
	Me ₂ CO- <i>d</i> ₆	DMSO- <i>d</i> ₆	
H-2	4.28 <i>dd</i> (6.1, 0.8)	3.71 <i>ddd</i> (7.7, 4.8, 3.7)	3.98 <i>dd</i> (7.9, 3.5)
H-3 α	2.88 <i>dd</i> (18.8, 6.1)	2.52 <i>m</i>	2.66 <i>ddd</i> (14.0, 8.3, 7.9)
H-3 β	2.25 <i>dd</i> (18.8, 0.8)	1.35 <i>m</i>	1.45 <i>ddd</i> (14.0, 8.4, 3.5)
H-4		4.22 <i>td</i> (7.0, 5.3)	4.40 <i>dd</i> (8.4, 8.3)
H-6	4.71 <i>d</i> (7.8)	4.89 <i>d</i> (8.9)	5.11 <i>d</i> (9.2)
H-7	3.36 <i>m</i>	3.33 <i>m</i>	3.40 <i>m</i>
H-8	1.70 <i>m</i>	1.80 <i>m</i>	1.90 <i>m</i>
H-9	2.15 <i>m</i>	2.25 <i>m</i>	2.22 <i>m</i>
H-9'	1.37 <i>m</i>	1.40 <i>m</i>	1.35 <i>m</i>
H-10	2.38 <i>m</i>	2.10 <i>m</i>	2.04 <i>m</i>
H-13a	5.65 <i>d</i> (2.0)	5.58 <i>d</i> (3.2)	5.56 <i>d</i> (3.8)
H-13b	6.08 <i>d</i> (2.5)	5.98 <i>d</i> (3.2)	6.04 <i>d</i> (3.8)
H-14	1.50 <i>d</i> (7.8)	1.32 <i>d</i> (7.3)	1.45 <i>d</i> (7.3)
H-15	1.40 <i>s</i>	0.94 <i>s</i>	1.08 <i>s</i>
2-OH		4.82 <i>d</i> (4.8)	
1-OH		4.00 <i>s</i>	
4-OH		3.87 <i>d</i> (5.3)	

^a Multiplicity (*J* in Hz).

β -hydroxyl group at C-2. Deuterium oxide exchange in the ^1H NMR spectrum in $\text{DMSO}-d_6$ of **2** confirmed the presence of three hydroxyl groups (Table 2). Signals at $\delta 84.5$ (C-1), 80.4 (C-2) and 86.2 (C-4) in the ^{13}C NMR spectrum (Table 3) supported the proposed substitution pattern. Selective heteronuclear decoupling at $\delta 3.71$ (H-2) collapsed the doublet at 80.4 (C-2) into a singlet confirming this assignment.

The stereochemistry at C-2 and C-4 was deduced from the coupling constants calculated from the model structures as well as from ^1H and ^{13}C simulated spectra. The theoretical coupling constants were as follows (Altona equation, Haasnoot et al., 1980): $J_{3\alpha,4}$ (8.5 Hz), $J_{3\beta,4}$ (8.0 Hz), $J_{2,3\alpha}$ (9.7 Hz) and $J_{2,3\beta}$ (4.4 Hz), showed good correspondence with the experimental data (Table 2, $\text{Me}_2\text{CO}-d_6$).

Selective heteronuclear decoupling at $\delta 3.33$ (H-7) collapsed the doublet at $\delta 42.9$ (Table 3, $\text{DMSO}-d_6$) into a singlet. Therefore, the doublet at $\delta 40.4$ was assigned to C-10.

The above data led us to propose the structure for **2** as: $1\alpha,2\beta,4\beta$ -trihydroxypseudoguaian-6 β ,12-olide.

The spectroscopic data of the known compounds were in agreement with published data (de la Fuente et al., 1997). Tetraneurin E was identified from comparison of its spectral data (^1H NMR, IR, MS) with literature values (Yoshioka et al., 1970); the previously unreported ^{13}C NMR spectral assignments is listed in Table 3.

We also studied the chemical composition of the fructification plants of *P. hysterophorus* from a transplanted specimen from the Argentine Puna to lower altitude. The fructification plants yielded *p*-methoxy-

benzoic acid, 2β -hydroxycoronopilin (**1**), 4α -*O*-acetyl-pseudoguaian-6 β ,12-olide, 1α -hydroxy-4 β -*O*-acetyl-pseudoguaian-6 β ,12-olide, coronopilin, hysterin and hymenin.

From our point of view, the most significant result from this study is the isolation of hymenin from the specimens grown from seeds of a transplanted member from an altitude of 3400 m down to 1220 m above sea level. Hymenin has been considered as an ambrosanoid typical from members growing at lower altitude (Balza and Towers, 1988). These results suggest an adaptive response to various physical and biotic factors in the environment over *P. hysterophorus*, as previously proposed by Rodriguez (1977) and Cabrera (1978) and confirm the synonym between *P. glomeratum* and this species.

3. Experimental

3.1. General

Mps uncorr. Mass spectra were obtained by EI at 70 eV, IR spectra were measured as dispersions in KBr. ^1H and ^{13}C NMR experiments were performed on a Bruker AC 200 with TMS as internal standard. CC was performed on silica gel (70–230 mesh).

3.2. Plant material

P. hysterophorus was collected in March 1997 and identified by Ing. L. Novara in road 33 entrance to Tonco, Cachi Department of Salta Argentina. A voucher specimen, MCNS No. 10891, is deposited at Facultad de Ciencias Naturales, Universidad Nacional de Salta.

3.3. Extraction and isolation

The air-dried aerial parts (500 g) of *P. hysterophorus* were ground and extracted with CHCl_3 (de la Fuente et al., 1997) to yield, following solvent evaporation in vacuo, a gummy residue (20 g). A portion of the crude extract (6 g) was separated by CC, using mixture (100 ml) of C_6H_6 , EtOAc, Me_2CO and MeOH of increasing polarity. Frs were collected and the course of the column followed by thin layer chromatography on silica gel, IR and ^1H NMR spectra. The purification of frs was carried out by 'dry column'; method (Hostettmann et al., 1986) on Sephadex LH 20 and by recrystallization. The following compounds were obtained: *p*-methoxybenzoic acid, 8.4 mg; 2β -hydroxycoronopilin (**1**), 150 mg; 4α -*O*-acetyl-pseudoguaian-6 β ,12-olide, 48 mg; 1α -hydroxy-4 β -*O*-acetyl-pseudoguaian-6 β ,12-olide, 60 mg; coronopilin, 48 mg; parthenin, 52 mg; hysterin, 110 mg; tetraneurin E, 35 mg and $1\alpha,2,4\beta$ -trihydroxy-pseudoguaian-6 β ,12-olide (**2**), 15 mg.

Table 3
 ^{13}C NMR spectral data for compounds 1–3

C	1	2		3
	($\text{Me}_2\text{CO}-d_6$)	($\text{DMSO}-d_6$)	($\text{Me}_2\text{CO}-d_6$)	CD_3OD
1	84.7	84.5	86.0	85.5
2	80.7	80.4	82.5	34.8
3	45.6 ^a	42.1	42.6	23.1
4	219.1	86.2	87.2	86.3
5	58.7	53.2	55.0	55.3
6	79.3	78.1	79.9	82.7
7	45.5 ^a	42.9	43.8	44.2
8	29.0 ^b	24.7 ^a	25.1 ^a	27.4 ^a
9	32.9 ^b	29.6 ^a	30.4 ^a	25.7 ^a
10	42.1	40.4	— ^c	52.6
11	143.3	141.9	143.0	142.7
12	170.4	— ^c	170.0	172.7
13	120.8	119.0	119.20	120.5
14	17.2	16.5	17.00	61.6
15	15.7	9.8	10.3	11.3

^a Assignments may be interchanged in each vertical column.

^b Assignments may be interchanged in each vertical column.

^c — Not observed.

3.4. 2 β -Hydroxycoronopilin (1)

Colourless needles from EtOAc, melting point 185–187°C, IR_{v_{max}} cm⁻¹: 3500, 1750, 1733, 1655, 1275, 1380, 1340, 1172, 971, 956. FABMS *m/z* [M + 1]⁺: 281. EIMS *m/z* (rel. int.): 262 [M-H₂O] (1.4), 244 (1.9), 233 (2.2), 216 (2.2), 204 (4.7), 191 (12.8), 55 (52.5), 44 (58.9), 43 (100). ¹H and ¹³C NMR data are listed in Tables 2 and 3.

3.5. 1 α ,2 β ,4 β -Trihydroxy-pseudoguaian-6 β ,12-olide (2)

Amorphous solid from Me₂CO, IR_{v_{max}} cm⁻¹: 3500, 1745, 1655, 1416, 1390, 1302, 1103, 1061, 974. EIMS *m/z* (rel. int.): M⁺ (not observed) 264 [M-H₂O] (1.8) 246 [M-2H₂O] (4.7), 235 (8.9), 220 (9.6), 203 (4.0), 191 (11.3), 175 (7.3), 165 (20.7), 151 (13.0), 135 (13.2), 123 (18.6), 107 (19.7), 85 (32.4), 69 (23.6), 55 (69.3), 43 (100). ¹H and ¹³C NMR data are listed in Tables 2 and 3.

3.6. Tetraneurin E (3)

Colourless needles from Me₂CO, melting point 196–198°C. EIMS *m/z* (rel. int.): M⁺ (not observed), 246 (M-H₂O), 3.7), 233 (2.8), 216 (4.1), 207 (3.0), 189 (3.8) and 161 (6.5). The ¹³C NMR data are listed in Table 3.

3.7. Fructification plants from the transplanted specimens

The fructification plants cultivated at 1220 m above sea level from transplanted specimens of *P. hysterophorus* previously studied, that grow at 3400 m above sea level were collected in April 1997. A voucher specimen (MCNS No. 1088) is deposited at Facultad de Ciencias Naturales, Universidad Nacional de Salta. Dried and powdered aerial parts (30 g) were extracted with CHCl₃ and processed as described above. The

crude extract (400 mg) yielded 1 α -hydroxy-4 β -O-acetyl-pseudoguaian-6 β ,12-olide; 2 β -hydroxycoronopilin (1); 4 α -O-acetyl-pseudoguaian-6 β ,12-olide; coronopilin and hymenin (Toribio and Geissman, 1968).

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