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CHEMOTAXONOMY OF *PARTHENIUM*: *P. HYSTEROPHORUS*–*P. GLOMERATUM*

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Key Word Index—*Parthenium hysterophorus*; *Parthenium glomeratum*; Asteraceae–Heliantheae; 'altamisa'; sesquiterpene lactones; pseudoguaianolides; ambrosanolides; chemotaxonomy.

Abstract—From aerial parts of five plants lots of *Parthenium hysterophorus–P. glomeratum* collected along 72 km in Salta Province, Argentina, seven known and four new pseudoguaianolides have been found. *p*-Methoxybenzoic acid was also isolated from plants growing 2500 m above sea level. The taxonomic situation of both species is discussed. © 1997 Elsevier Science Ltd. All rights reserved

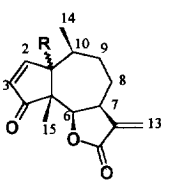
INTRODUCTION

Two species of genus *Parthenium*, tribe Heliantheae (Asteraceae) have been described for Argentina: *Parthenium hysterophorus* L. and *P. glomeratum* Rollins [1].

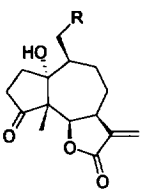
P. hysterophorus, commonly known as 'altamisa' is a weed, probably originally from America, growing in central and northern Argentina, whereas *P. glomeratum* is found only in the north of the country and was described by Rollins as a new species [2]. The difference between both species is the relatively smaller plant size and the densely agglomerated capitulum (flowering head) of *P. glomeratum*. According to Cabrera [1], *P. glomeratum* would be an extreme form of *P. hysterophorus* 'one of them is probably only an alpine form' [1]. There is much information about chemical studies carried out on *P. hysterophorus* from different regions of the world [3, 4] and on a sample of *P. glomeratum* from Pampa Blanca, Argentina [3]. Preliminary investigations on the sesquiterpene lactone chemistry in populations of *P. hysterophorus* from different Argentine geographical regions reported hymenin (**1**), coronopilin (**3**), dihydroisoparthenin and hysterin (**8**) [3, 4], while in samples from other American countries and from other continents, parthenin (**2**) and tetraneurin A (**4**) have been identified [4, 5]. *P. glomeratum* yielded hysterin (**8**) and damsin [3]. According to Rodríguez [3] the sesquiterpene lactone composition in *Parthenium* is characteristic of delimited zones or regions.

The present paper deals with taxonomic and phytochemical studies of five populations (Lots I–V) (Table 1) of *P. hysterophorus* and/or *P. glomeratum* growing in different geographical regions of Salta Province, Argentina; from Salta City at 1220 m above sea level along 72 km up to 3400 m to the southwest of the province. Additionally, transplanted plants from 3400 to 1220 m were studied.

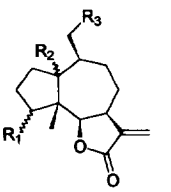
In the morphological study we analysed the influ-



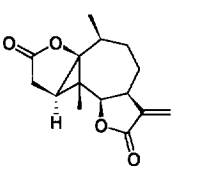
R	
1	α OH
2	β OH



R	
3	H
4	OAc
5	
6	
7	



	R_1	R_2	R_3
8	α OAc	α H	OH
9	α OAc	α H	H
10	β OAc	α OH	H



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ence of the altitude above sea level over the variation of the plant attributes and the different habitat to which they belong and the individual aspects of one population transplanted to an opposite and contrasting habitat. The phytochemical studies were carried out on the aerial parts after the morphological evaluation.

RESULTS AND DISCUSSION

The exhaustive morphological analysis of the collected taxa (Plant Lots I–V, see Experimental) led us to the following conclusions: the applied statistic techniques based on the morphology of the vegetative and reproductive organs do not solve the taxonomic problem between *P. hysterophorus* and *P. glomeratum* that could justify the synonymy or the difference between both species.

The morphological characters such as plant height, stems and leaves do not provide by themselves conclusive data to establish whether the taxa belong to *P. hysterophorus* or to *P. glomeratum*. Transplanted plants from 3400 m, after 1 year adopted shape and size similar to those of *P. hysterophorus* growing at 1220 m. However, the colour and density of flowerheads were intermediate when comparing the transplanted specimen attributes with those of its congeners from the highest and lowest altitude. As is evident, the registered differences of the taxa are largely dependent upon the ecophysiological factors.

Seven known sesquiterpene lactones were isolated from the studied plant lots together with the new ambrosanolides **5**, **6** (as a mixture), **9** and **10**. Our chemical studies showed (Table 1) that coronopilin (**3**) [6] was common to all the plant lots while hymenin (**1**) [7] was the major sesquiterpene lactone in Lot I where it was accompanied by its photolytic product confertdiolide (**11**) [8] and hysterin (**8**) [9]. Lot II also yielded tetraneurin A (**4**) [10] as the main constituent and a mixture of the new pseudoguaianolides 1α-hydroxy-4-oxo-14-*O*-(2-methyl-butyroyl)-pseudoguaian-6β,12-olide (**5**) and 1α-hydroxy-4-oxo-14-*O*-isovaleroyl-pseudoguaian-6β,12-olide (**6**) whose structures will be discussed below. Parthenin (**2**) [11, 12], tetraneurin A (**4**) [10] and *p*-methoxybenzoic acid were identified in Lot III while Lot IV produced parthenin (**2**) [11, 12], chiapin B (**7**), previously found in *P. fruticosum* [13] and *p*-methoxybenzoic acid. This acid together with hysterin (**8**) [9] and the new ambrosanolids 4α-*O*-acetyl-pseudoguaian-6β-olide (**9**) and 1α-hydroxy-4β-*O*-acetyl-pseudoguaian-6β,12-olide (**10**) were isolated from Lot V. Finally, the transplanted members from 3400 to 1220 m yielded coronopilin (**3**) and parthenin (**2**) [11, 12]. Known compounds were identified by comparison of their spectral properties with those reported in the literature.

Ambrosanolides **5** and **6** were obtained as a 4:1 mixture (NMR criteria). EI-mass spectrometry of the mixture gave the molecular ion at *m/z* 364 while the IR spectrum showed an absorption band at 3530 cm^{-1}, characteristic of a tertiary hydroxyl group, and a broad signal at 1739 cm^{-1} assigned to the carbonyl groups of the γ-lactone moiety and the ester function of the side chain. The ^1H NMR spectrum (see Experimental) was very close to that of tetraneurin A (**4**) [10], the only differences were the signals due to the acyl moieties at C-14. The ^{13}C NMR spectrum (Table 3) was in total agreement with that of tetraneurin A (**4**).

The EI mass spectrum of ambrosanolide **9** gave the molecular ion at *m/z* 292 while the IR spectrum showed a broad absorption band at 1749 cm^{-1} assigned to the carbonyl groups of the conjugated γ-lactone and the acetyl moiety. The ^1H NMR spectrum (Table 2) was assigned by analogy with hysterin (**8**) [9] while the ^{13}C NMR spectrum (Table 3) confirmed the proposed structure.

The positive ion FAB mass spectrum of ambrosanolide (**10**) showed the $[M+1]^+$ ion at *m/z* 309 suggesting an extra oxygen with respect to **9**. The ^1H NMR spectrum (Table 2) was similar to those of hysterin (**8**) and ambrosanolide (**9**). However, there were noticeable deshielding effects on both the H-4 and H-6 signals. This effect may be explained because of the opposite stereochemistry at C-4 of ambrosanolide (**10**) that would imply the absence of the anisotropic effect on H-6 caused by the β-orientation of the acetyl group as it has been demonstrated for tetaneurin E and related compounds [14]. The singlet at δ 85.9 in the ^{13}C NMR (Table 3) confirmed the presence of the tertiary hydroxyl group at C-1.

Full proof of the skeleton of ambrosanolide (**10**) was achieved by a combination of homonuclear H-H COSY, NOESY and ^1H-^{13}C HETCOR experiments. The NOESY spectrum of abrosanolide (**10**) (Table 4) showed a nOe between H-4 and H-6 indicating that

Table 1. Distribution of sesquiterpene lactones in Lots I–V of *Parthenium hysterophorus–P. glomeratum*

Lot	**1**	**2**	**3**	**4**	**5–6***	**7**	**8**	**9**	**10**	**11**
I	92.2		1.4				4.1			2.3
II			41.3	57.5	1.2					
III		48.8	36.6	14.6						
IV		50.0	41.5			8.5				
V			5.5				33.0	8.8	52.7	

Numbers indicate percentage with respect to the total sesquiterpene lactone content of each plant lot.
* In 4:1 mixture.

Table 2. ^{1}H NMR spectra of compounds **9** and **10**

H	9	10
2α		1.37 *ddd*
2β		2.47 *ddd*
3α		2.41 *dddd*
3β		1.53 *dddd*
4	5.10 *t*	5.68 *dd*
6	4.45 *d* (10)	5.17 *d* (10)
7	3.31 *m*	3.32 *ddd*
8α		2.04 *dddd*
8β		1.92 *dddd*
9α		1.60 *m*
9β		1.60 *m*
10		1.98 *dd*
13a	6.16 *d* (3.6)	6.17 *d* (3.4)
13b	5.44 *d* (3.2)	5.47 *d* (3.2)
14	1.03 *d** (7.2)	1.16 *d** (7.2)
15	0.90 *s**	0.95 *s**
OAc	2.08 *s**	2.08 *s**

At 200.13 MHz, in $CDCl_3$, δ in ppm relative to TMS as internal standard.

* Intensity three protons.

Numbers in parentheses indicate coupling constants in Hz.

Table 3. ^{13}C NMR spectra of compounds of **4–6**, **9** and **10**

C	4	5–6*		9	10†
1	83.4 *s*	83.8 *s*		33.3 *d*	85.9 *s*
2	33.8 *t*	33.1 *t*		23.0 *t*	35.0 *t*
3	32.3 *t*	31.8 *t*		24.0 *t*	26.5 *t*
4	217.5 *s*	216.8 *s*		88.8 *d*	80.6 *d*
5	58.4 *s*	58.3 *s*		50.7 *s*	54.3 *s*
6	79.2 *d*	78.9 *d*		80.8 *d*	83.7 *d*
7	44.6 *d*	44.7 *d*		47.4 *d*	43.0 *d*
8	25.2 *t*	28.1 *t*		26.9 *t*	23.9 *t*
9	28.0 *t*	25.3 *t*		32.5 *t*	27.6 *t*
10	44.6 *d*	48.4 *d*		43.4 *d*	42.9 *d*
11	140.8 *s*	140.6 *s*		140.4 *d*	140.9 *d*
12	170.4 *s*	170.1 *s*		170.9 *s*	168.0 *s*
13	122.1 *t*	122.0 *t*		118.9 *t*	119.3 *t*
14	63.2 *t*	62.8 *t*		14.1 *q*	17.0 *q*
15	14.0 *q*	14.2 *q*		10.1 *q*	11.3 *q*
1′	171.0 *s*	176.6 *s*		174.2 *s*	170.9 *s*
2′	20.9 *q*	41.0 *d*	43.3 *t*	21.4 *q*	21.0 *q*
3′		26.7 *t*	25.3 *t*		
4′		11.6 *q*	22.4 *q*		
5′		16.6 *q*	22.4 *q*		

At 50.03 MHz, in $CDCl_3$, δ in ppm, TMS as internal standard. Multiplicities by DEPT.

* Taken from **4**:1 mixture.

† Assignments from HETCOR spectrum.

the acetyl moiety is in a β-orientation. Other features in this spectrum showed the spatial vicinity of H-14/H-2β, H-15/H-2β, H-15/H-3β, H-15/H-8β and H-13β/H-8α. Finally, the HETCOR experiment allowed the unambiguous assignments reported in Tables 2 and 3.

Our chemical results are in good agreement with the proposal of Rodríguez that 'the distribution of secondary products in *Parthenium* is probably a result of adaptive responses to various physical and biotic factors in the environment' [3].

On the other hand, evaluation of both the taxonomic and photochemical studies suggests that *P. glomeratum*, described as an autonomous and independent species, could be an alpine form of *P. hysterophorus* as suggested by Cabrera [1].

Table 4. Relevant nOe correlations for compound **10***

δ	NOESY
5.68 (H-4)	2.41 (H-3α) 5.17 (H-6)
5.17 (H-6)	5.68 (H-4) 3.32 (H-7)
3.32 (H-7)	5.17 (H-6)
0.95 (H-15)	{1.16 (H-14)} 1.53 (H-3β) 1.92 (H-8β) 2.47 (H-2β)
1.16 (H-14)	{0.95 (H-15)} 1.98 (H-10) 2.47 (H-2β)
6.17 (H-13a)	5.47 (H-13b)
5.47 (H-13b)	2.04 (H-8α)

EXPERIMENTAL

General. Mps are uncorr. CC were performed on silica gel 60 (70–230 mesh); TLC was carried out on precoated Silica gel 60 F_{254} plates (Merck). Detection was achieved by viewing under UV light and spraying with H_2SO_4 soln as reagent followed by heating.

Plant material. The plant lots were collected in December 1990 and identified by Ing. Lázaro Novara. Voucher specimens are deposited at the Museo of the Facultad de Ciencias Naturales, Universidad Nacional de Salta. The plant material was collected along road no. 33 (Salta Province) as follows: Lot I, Salta City at 1220 m (NOVARA 10076); Lot II, at 1620 m (NOVARA 10062); Lot III, at 2500 m (NOVARA 10065); Lot IV, from 3000 to 3150 m (NOVARA 10068); Lot V, from 3300 to 3400 m (NOVARA 10069). One of these specimens was transplanted and cultivated at 1220 m (MCNS 928).

General extraction procedure. Air dried aboveground parts were exhaustively extracted with $CHCl_3$. The residue obtained after evapn of the solvent was dissolved in hot EtOH and a soln of 4% $Pb(AcO)_2$ was added. After standing overnight the ppt. was filtered and the organic solvent evapd and the aq. soln extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent evaporated under red. press. to yield a gummy residue.

Isolation and purification. The residues obtained as described above were fractionated by CC, eluted with benzene and the polarity increased with EtOAc and Me_2CO. Further purification of the fractions were achieved by dry column chromatography and/or on Sephadex LH-20.

Lot I: 825 g were extracted and purified as described above to yield 250 mg of confertdiolide (**11**) [8], 150

mg of coronopilin (**3**) [6, 15], 10.2 g of hymenin (**1**) [7] and 458 mg of hysterin (**8**) [9, 16].

Lot II: extraction and purification of 245 g produced 24 mg of a 4:1 mixt. of **5** and **6**, 790 mg of coronopilin (**3**) [6, 15] and 1.1 g of tetraneurin A (**4**) [10].

Lot III: 200 g were processed as described above to yield 6 mg of *p*-methoxybenzoic acid, 181 mg of coronopilin (**3**) [6, 15], 241 mg of parthenin (**2**) [11, 12, 15] and 72 mg of tetraneurin A (**4**) [10].

Lot IV: extraction and purification of 460 g gave 17 mg of *p*-methoxybenzoic acid, 22 mg of chiapin B (**7**) [13], 108 mg of coronopilin (**3**) [6, 15] and 130 mg of parthenin (**2**) [11, 12, 15].

Lot V: 590 g were extracted and purified as described above to give 23 mg of *p*-methoxybenzoic acid, 101 mg of **9**, 602 mg of **10**, 63 mg of coronopilin (**3**) [6, 15] and 377 mg of hysterin (**8**) [9, 16].

Extraction and purification of 3 g of the cultivate specimen yielded parthenin (**2**) [11, 12, 15] and coronopilin (**3**) [6, 15].

1α-*Hydroxy*-4-*oxo*-14-O-(2-*methylbutyroyl*)-*pseudoguaian*-6β,12-*olide* (**5**) *and* 1α-*hydroxy*-4-*oxo*-14-*O*-*isovaleroyl pseudoguaian*-6β,12-*olide* (**6**). A 4:1 mixt. (NMR) was isolated as a gum IR ν_{max} cm^{-1}: 3530 (OH), 1739 (C=O), 1658 (conj. C=C). EIMS (70 eV) *m/z* (rel. int.): 364 $[M]^+$ (2), 294 (2), 276 (4), 189 (30), 161 (15), 57 (100). ^1H NMR (Cl_3CD, J = Hz) δ: 6.28 (*d*, 2.8, H-13a); 5.59 (*d*, 2.3, H-13b); 4.88 (*d*, 8.6, H-6); 4.41 (*dd*, 9.6, 1.9, H-14a); 4.12 (*t*, 9.6, H-14b); 3.37 (*m*, H-7); 1.15 (*d*, 7.2, H-5′); 1.06 (*s*, H-15); 0.92 (*t*, 7.2, H-4′, H-5′); 0.90 (*t*, 7.2, H-4′); ^{13}C NMR spectra in Table 3.

4α-*Acetoxypseudoguaian*-6β,12-*olide* (**9**). Amorphous solid that could not be induced to crystallize. IR ν_{max} cm^{-1}: 1749 (conj. γ-lactone), 1735 (ester). CIMS: *m/z* (rel. int.): 291 $[M+1]^+$, 250 $[M+1-COCH_3]$, 234 $[M+1-OCOCH_3]$, 43 (100). ^1H NMR spectrum in Table 2, ^{13}C NMR spectrum is described in Table 3.

1α-*Hydroxy*-4β-*acetoxypseudoguaian*-6β,12-*olide* (**10**). Needles from Me_2CO, mp 135–137°. IR ν_{max} cm^{-1}: 3508 (OH), 1741 *s* (λ-lactone, ester). FAB-MS *m/z* (rel. int.) 326 (10) $[M+H_2O]^+$, 209 (8) $[M+1]^+$, 291 (7), 267 (12), 248 (15), 232 (100). ^1H and ^{13}C NMR spectra in Tables 2 and 3, respectively.

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