

Bioactive Flavones and Terpenes from *Baccharis calliprinos* and *B. rhetinodes* (Asteraceae)

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SUMMARY. The chemical study of *Baccharis calliprinos* Griseb. yielded 2 α ,3 α -dihydroxycaticic acid (I) together with the flavonoids luteolin-7,3'-dimethylether (II), homoeriodictyol (III) and eriodictyol-3',4'-dimethylether (IV). On the other hand, from the aerial parts of *B. rhetinodes* Meyen & Walp bacchotricuneatin A (V), oleanolic acid (VI) and the flavone (II) were isolated. The three isolated flavonoids were subjected to the antiinflammatory test using the carrageenan-induced mouse paw edema test.

RESUMEN. "Flavonas y Terpenos Bioactivos a partir de *Baccharis calliprinos* y *B. rhetinodes* (Asteraceae)". El estudio fitoquímico de *Baccharis calliprinos* Griseb. permitió el aislamiento del ácido 2 α ,3 α -dihidroxicatívico (I) y de los flavonoides luteolina-7,3'-dimetiléter (II); homoeriodictyol (III) y eriodictyol-3',4'-dimetiléter (IV). Por otra parte, de las partes aéreas de *B. rhetinodes* Meyen & Walp fueron aislados bacchotricuneatina A (V), ácido oleanólico (VI) y la flavona (II). Los tres flavonoides informados, fueron sometidos al bioensayo de actividad antiinflamatoria utilizando el método del edema inducido por carragenina en pata de ratón.

INTRODUCTION

Growing interest in the study of the pharmacological potential of plant natural products has led to the search of several kinds of compounds such as sesquiterpene lactones¹, diterpenes², and flavonoids³⁻⁴ as antiinflammatory agents.

It has been reported that flavonoids appear to be capable of selectively reacting with free radicals or systems related to the induction of inflammatory processes inhibiting leukotriene synthesis and histamine release, as well as acting as superoxide scavengers^{5,6}. In this way, the flavonoids have been considered as the active principles of many plants extracts with antiinflammatory properties.

In the past few years, nearly 100 species from the large American genus *Baccharis* (Asteraceae, Astereae) have been chemically investigated. The most widespread compounds reported are clerodane⁷ and labdane⁸ diterpenoids as well as triterpenoids of the oleanane series. In addition, kaurene terpenoids, cinnamic

acid esters, coumarin derivatives and flavonoids with different oxidation pattern are common secondary metabolites. Considerable attention has been devoted to the isolation of the clerodane-type furan-diterpenoids due to its antifeedant activity toward insect larvae^{9,10}. On the other hand, the aqueous extracts of some species such as *B. articulata* (Lam.) Pers., *B. crispa* Spreng. and *B. trimeria* (Less) DC., have been reported as antiinflammatory using the carrageenan mouse paw edema test¹¹. In the latter case, it has been proposed that rutin (quercetin 3-O- α -D-rhamnosyl-(1-6), β -D-glucoside) and a saponin mixture are the active principles³.

As a continuation of our investigations on the chemical constituents of species of this genus growing in the Cuyo region¹² (Argentina), we report herein the isolation and identification of the labdane and clerodane diterpenoids, together with one oleanane triterpene and several flavonoids from *Baccharis calliprinos* Griseb. and *B. rhetinodes* Meyen & Walp.

KEY WORDS: Antiinflammatory activity, *Baccharis calliprinos*, *B. rhetinodes*, Diterpenes, Flavonoids.

PALABRAS CLAVE: Actividad antiinflamatoria, *Baccharis calliprinos*, *B. rhetinodes*, Diterpenos, Flavonoides.

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Extracts of *B. calliprinos* are used in the form of decoctions due to their topical antiinflammatory effects for the treatment of skin ulcers and colics¹³. In view of these properties, the isolated flavones were subjected to the antiinflammatory activity test on the carrageenan-induced mouse paw edema.

MATERIAL & METHODS

General

The ¹H NMR spectra were recorded in CDCl₃ at 200.13 MHz, the ¹³C NMR were obtained at 50.23 MHz. COSY and XH-CORR experiments were resolved using standard software (Bruker AC-200). EIMS were collected at 70 eV (Finnigan-Mat GCQ-Plus). CC were performed on Silica gel G 70-230 mesh and Kieselgel 60 H; TLC were carried out on Silica gel 60 F₂₅₄ 0.2 mm thick plates using C₆H₆-dioxane-AcOH, 30:5:1 as solvent. Gel permeation chromatography was run using Sephadex LH-20 and MeOH as solvent.

Plant material

Aerial parts of *B. calliprinos* were collected at 920 m in El Salto, Potrerillos, Provincia de Mendoza, Argentina, and a voucher specimen was deposited at the Herbarium of the UNSL-Del Vitto N° 8809. *B. rhetinodes* was collected at

1500 m in Valle de Las Leñas, Mendoza, voucher UNSL-Del Vitto N° 1367.

Extraction and isolation

The dried material (300 g of each specimen) was extracted twice (one week each time) with Me₂CO at room temperature and purified using solvent partition and column chromatography, as previously described⁸. Identification of the isolated compounds was performed by one and two-dimensional ¹H and ¹³C NMR spectroscopy and EIMS, physical constants, as well as by comparison with authentic samples.

Biological assay

Groups of five male albino mice received *i.p.* 0.5 ml (75 mg/kg) of test substance of phenylbutazone suspended in normal saline. The control group received only the vehicle¹⁴. After one hour, 0.05 ml of 3% suspension of carrageenan in saline was injected into the subplantar area of the right hind paw. Paw volumes were measured with a plethysmometer 1.0, 3.0, 5.0 and 7.0 hours after injection. The volume of edema was expressed as the difference between the carrageenan-injected and the contralateral paw. The percent inhibition of edema was calculated for each group with respect to its vehicle-treated control group (see Table 1).

Flavone ^a	Carrageenan-Edema Inhibition (%) ^b			
	1 h	3h	5h	7h
luteolin-7,3'-dimethylether (II)	18	32 *	40 **	13
homoeriodictyol (III)	0	11	24	38 *
eriodictyol-3',4'-dimethylether (IV)	4	5	24	23
phenylbutazone (control)	19	44	40 **	37 *

Table 1. Antiinflammatory activity for the isolated flavonoids. ^a 75 mg/kg, intraperitoneally; ^b percentages of edema reduction are expressed by the mean with S.E.M.; Dunnet's t-test for unpaired data was used for statistical evaluation (n = 6 animals); * p<0.05; ** p<0.01.

RESULTS AND DISCUSSION

B. calliprinos Griseb. is a nanophanerophyte (nearly 2 m high) growing in regions of the Los Andes Mountain from Tucumán to Mendoza. *B. rhetinodes* Meyen & Walp. is a resinous nanophanerophyte 0.2-0.5 m high growing in an area ranging from Mendoza to Chubut near the Chile-Argentina border^{15,16}.

From aerial parts of *B. rhetinodes*, collected at 700 m, in Junín de Los Andes (Argentina), has been reported the isolation of baccharis oxide and bacchotricuneatin A, together with a furane-

diterpene acid possessing a clerodane skeleton¹⁷.

From the polar fraction of *B. calliprinos* a white solid was recovered, after several column chromatographic runs. Its ¹H NMR resembled that of compounds with a labdane skeleton with a broad singlet at δ 5.37 allylically coupled with a three proton broad singlet at δ 1.66. One secondary methyl group at 0.97 ppm (J= 7.0 Hz) and three methyl singlets at δ 0.79, 0.90 and 0.98 were consistent with the aforementioned labdane structure. The acidic nature of the compound under study was confirmed from the ¹³C

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