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

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SUMMARY. The chemical study of *Baccharis calliprinos* Griseb. yielded $2\alpha,3\alpha$ -dihydroxycativic 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 2α,3α-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 lactones ¹, diterpenes ², and flavonoids ³⁻⁴ 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 clerodane ⁷ and labdane ⁸ 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. trimera* (Less) DC., have been reported as antiinflammatory using the carrageenan mouse paw edema test 11 . 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 principles 3 .

As a continuation of our investigations on the chemical constituents of species of this genus growing in the Cuyo region ¹² (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 colics ¹³. 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 Nº 8809. *B. rhetinodes* was collected at

1500 m in Valle de Las Leñas, Mendoza, voucher UNSL-Del Vitto Nº 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 described ⁸. 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'-dimethyleher (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 1 H NMR ressembled that of compounds with a labdane skeleton with a broad singlet at δ 5.37 allilically 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 13 C

NMR spectrum (C-15, δ 175.2). Proton resonances at δ 3.97 (1H, d, J=11.0 Hz) and 3.41 (br s), were in agreement with a *vec*-diol system. The comparison of all the spectral data (¹H NMR, ¹³C NMR, EIMS) as well as the physical constants (m.p. and optical rotation) allowed us to identify the compound under study as 2α , 3α -dihydroxycativic acid (I), a diterpene previously isolated from *B. petiolata* DC. collected in the same phytogeographical area ⁸.

By repeated column chromatography (silica gel) and gel permeation (Sephadex LH-20, MeOH), the flavonoids luteolin-7,3′-dimethylether (II); homoeriodictyol (III) and eriodictyol-3′,4′-dimethylether (IV) were obtained and their structures were determined by spectroscopical means (UV, ¹H NMR).

The aerial parts of B. rhetinodes afforded, after several purifications, a crystalline white solid. Its ¹H NMR showed the typical pattern of the furan-clerodane type diterpenes with signals at δ 6.4 (br s, H-14) and the two proton multiplet centered at δ 7.45, clearly coupled from the ¹H-¹H-COSY spectrum. A double-doublet at δ 6.78 (J=7.0 and 2.5 Hz) was consistent with the presence of an olefinic proton on a β -carbon of an α,β-unsaturated carbonyl group. Two one-proton signals at δ 3.95 (dd, J=9.0 and 1.5 Hz) and 4.30 (d, J=9.0 Hz) were in agreement with an AB as part of a lactone moiety. The ¹H NMR also displayed a dd (J=11.0 and 8.0 Hz) centered at δ 5.40 typical of a C-20-C-12 olide system. Only one methyl group as singlet was observed at δ 0.9. The comparison of all spectral data as well as the physical constants, allowed us to establish the structure as bacchotricuneatin A (V), a diterpene previously isolated from B. tricuneata 18. This diterpene showed potent antifeedant activity using *Tenebrio molitor* L. (Coleoptera:Tenebrionidae) larvae as insect model ^{9,10}. As minor components, the anti-inflammatory triterpene oleanolic acid (**VI**) ¹⁹, and the flavone luteolin-7,3´-dimethylether (**II**), were isolated and their structures determined by spectroscopical methods as well as comparison with authentic samples.

Flavonoids have been considered as the active principles of many antiinflammatory plants. Taking into account the properties of the aqueous extracts of B. calliprinos, the isolated flavones were subjected to an antiinflammatory biotest. In Table 1 the results from the first to the seventh hour post-injection are shown. It is possible to observe that the most active compound was (II), with an inhibition percentage similar to phenylbutazone in the first five hours. Interestingly, the compound (III), with the same B ring oxidation pattern, but with C-2-C-3 single bond, reached the higher activity seven hours after injection. On the other hand, compound (IV) was inactive. It is noteworthy that the hydroxyl radical scavenging activity of flavonoids is closely related to the number and position of the hydroxyl groups on the B-ring 20; additionally, in the present case the C-2-C-3 unsaturation seems to play an important role in the bioactivity. It would be desirable to extend this investigation to other related flavones and flavanones before drawing more general conclusions.

Acknowledgement. Financial support of CONICET (PIP 5030) and UNSL (Project 7301). Thanks are also due to Dra. L. Pelzer for the antiinflammatory test and Ing. L.A. Del Vitto for the plant material identification.

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