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Il Farmaco 55 (2000) 502–505

IL FARMACO

Short Communication

Phytochemical study and anti-inflammatory properties of *Lampaya hieronymi* Schum. ex Moldenke

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Received 30 March 255; accepted 12 June 2000

Abstract

The chemical study of aerial parts of *Lampaya hieronymi* Schum. ex Moldenke yielded oleanolic acid, *epi*-oleanolic acid, *epi*-maslinic acid, 4′7-dimethoxyapigenin, *p*-hydroxyacetophenone, and *p*-hydroxyacetophenone-β-glucoside. In searching for natural products as potential anti-inflammatory agents, all the compounds, except 4′7-dimethoxyapigenin were evaluated in vivo for their ability to inhibit acute inflammation. Our studies demonstrated that *p*-hydroxyacetophenone and the triterpenes produced protective effects in carrageenan induced paw edema in mouse, at 1–3 h and 3–5 h, respectively, after the injection of carrageenan. These results indicated that their effects might correlate with the release of histamine, serotonin, kinin and prostaglandins.

Keywords: *Lampaya hieronymi*; Anti-inflammatory activity; Carrageenan-induced mouse paw edema

1. Introduction

The plant genus *Lampaya* (family Verbenaceae, subfamily Verbenoideae, tribe Lantanae) is endemic of South America, and is represented by three species distributed in the 'Puna' and high mountains of Bolivia, Northwestern Argentina and Northern Chile, in sandy or salty soils [1]. They are used in popular medicine as antispasmodic and antirheumatic plants [2]; additionally, they are dye plants.

The shrub formation of *Lampaya* is typical of the dummy lands in the 'puneña' phytogeographical province [3]. *Lampaya hieronymi* Schum. ex Moldenke is a low branched shrub with viscous stems. It is known by the vernacular names 'Lampaya' or 'Lampayo'. To our knowledge no chemical investigation of this species has been reported.

In our effort to find new compounds showing biological activity we have investigated the anti-inflammatory properties of the plant in the model of paw edema in mouse. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow [4]. Paw edema has been characterized by an early phase caused by the release of histamine, 5-hydroxytryptamine and bradykinin, followed by a late phase mainly sustained by prostaglandin release [5]. This paper describes the isolation, characterization and biological activity on an in vivo model of components from *L. hieronymi* Schum. ex. Moldenke.

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0014-827X/00/$ - see front matter
PII: S0014-827X(00)00067-7

2. Experimental

2.1. Plant material

The plant was collected in February 1993 in the surroundings of 'Valle del Río Chaschuil', Ruta Nacional 60, Catamarca Province, Argentina. It was identified by Ing. L.A. Del Vitto. A voucher specimen was deposited at the Herbarium UNSL, MERL N° 8950.

The dried material (1.5 kg) was defatted with *n*-hexane and then extracted with chloroform to give, after evaporation, 90 g of extract. This extract was purified by 'flash' chromatography [6], eluted with *n*-hexane–EtOAc mixtures having increasing polarity.

Fractions eluted with *n*-hexane–EtOAc (90:10) were purified by repeated column chromatography on Silicagel 60 G and yielded *p*-hydroxyacetophenone (**4**) (118 mg) and 4′7-dimethoxyapigenin (**5**) (80 mg) [7].

Two white solids were obtained by the elution of fractions with *n*-hexane–EtOAc (80:20) in several chromatographic procedures. The ^{1}H and ^{13}C NMR spectra showed the typical pattern of Δ^{12}-oleananes [8] and allowed us to establish their structures as oleanolic acid (**1**) (2.5 g) and *epi*-oleanolic acid (**2**) (230 mg).

Fractions eluted with *n*-hexane–EtOAC (70:30), after several column chromatographic runs, yielded *epi*-maslinic acid (**3**) (130 mg.) and *p*-hydroxyacetophenone-β-glucoside (**6**) (160 mg). The ^{1}H, ^{13}C NMR and mass spectra of **3** resembled those of triterpene Δ^{12}-oleananes containing hydroxyl groups at C-2 and C-3. The stereochemistry at the C-2 and C-3 carbons was determined by ^{13}C NMR chemical shift values (66.4 and 78.9 ppm, respectively) [9]. On the other hand, the relationship between both hydroxyl groups 2α, 3α, was confirmed by acetonide formation.

***Lampaya hieronymi*- (Isolated compounds)**

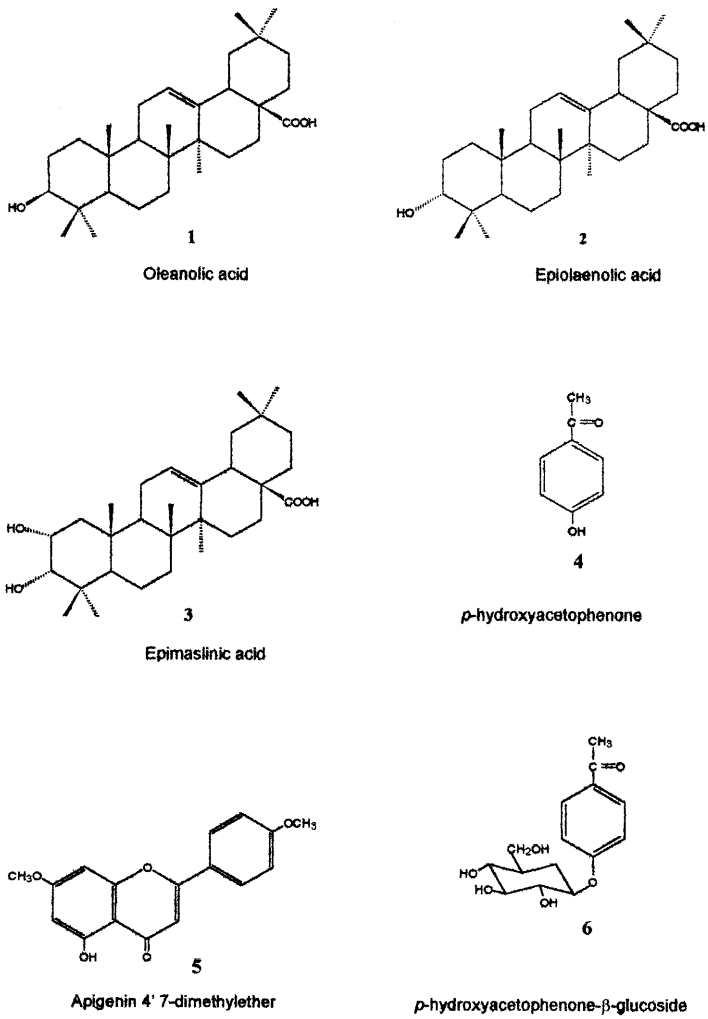

Fig. 1. *L. hieronymi* (isolated compounds).

Table 1
Effects of intraperitoneal administration of compounds from *L. hieronymi* on the carrageenan-induced mouce hind paw edema [a]

Product	Number of animals	Inhibition (%) of carrageenan edema (acute test)			
		1 h	3 h	5 h	7 h
Oleanolic acid	6	10	15	36*	24
epi-Oleanolic acid	6	22	15	35*	24
epi-Maslinic acid	6	27	33*	35*	40*
p-Hydroxyacetophenone	6	35*	40*	30	28
p-Hydroxyacetophenone-β-glucoside	6	22	22	20	30
Phenylbutazone	6	35*	42*	40*	37

[a] Each value represents the mean value obtained from six mice.
* $P < 0.005$ from respective control group (ANOVA and Dunnet's test).

All the isolated compounds have a carboxylic group at C-28 (Fig. 1). This moiety is required to increase the activity in carrageenan induced edema [10].

2.2. Animals

Forty-two mice, weighing 25–30 g, of both sexes, were used in this study. The animals were kept at a constant temperature of 22 ± 1°C and humidity of 55 ± 5%. They were fed laboratory diet ad libitum and allowed free access to drinking water.

2.3. Anti-inflammatory evaluation: carrageenan paw edema in the mouse

Anti-inflammatory activity on carrageenan-induced paw edema was determined by the method of Sugishita et al. [11] and compared with results for phenylbutazone (Sigma Chemical Co.). The animals were divided into three groups (control, standard and test), each consisting of six mice. Edema was induced by subcutaneous injection of 0.05 ml of carrageenan type IV (3.5% w/v) (Sigma Chemical Co.) in 0.9% NaCl solution into the subplantar region of the left hind paw. The volume of each paw up to the tibiatarsal articulation was measured by plethysmometry (Ugo Basile) before the injection of carrageenan and 1, 3, 5 and 7 h later.

The vehicle (tween 80:water 5:95 v/v), phenylbutazone (80 mg/kg) and the compounds (80 mg/kg) were administered intraperitoneally 1 h before the carrageenan injection. The results were obtained by measuring the volume differences between the right and the left paws. The percentage of inhibition of the edema was taken by comparing the data of the control group which received only tween 80 solution.

The experimental design was in accordance with the U.S. National Institute of Health (U.S.) Guidelines for the use of experimental animals.

2.4. Statistical analysis

The results reported are the means $\pm$ SEM of N observations, where N represents the number of animals studied. The data obtained from mouse paw edema measurement were analyzed using two way analysis of variance (ANOVA) and Dunnet's test [12] was applied for multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

3. Results

The results of the inhibition caused by intraperitoneal administration of the test agents are shown in Table 1. As can be seen, oleanolic acid and *epi*-oleanolic acid show similar inhibition at 5 h; *epi*-maslinic acid produces an inhibitory effect more prolonged at 3 and 5 h; *p*-hydroxyacetophenone shows significant inhibition at 1 and 3 h but for its glucoside no significant activity was detected.

4. Discussion

The present results indicate that agents isolated from *L. hieronymi* exhibit anti-inflammatory effects. The carrageenan paw edema test produced an acute inflammation that results from the sequential action of several mediators. According to Di Rosa [13] and Vineger et al. [14,15] histamine and serotonin were mainly released during first 1.5 h after carrageenan injection.

Kinin was released until 2.5 h and at the last step inflammation was continued until 5 h by prostaglandins. From the results obtained in this work, the intraperitoneal administration of *p*-hydroxyacetophenone gave stronger inhibition at 1–3 h after injection of carrageenan comparable to phenylbutazone, indicating that their effects might correlate with the release of histamine, serotonin and kinin. However, the triterpenes produced inhibition of edema at 5 h, and

epi-maslinic acid at 3–5 h. These data suggest that the effect may principally relate to inhibition of prostaglandin release. In conclusion, in the present study we have demonstrated that the triterpenes and *p*-hydroxyacetophenone exert a beneficial effect on acute inflammation. The relative contributions to possible mechanisms of action need to be determined in further studies.

Acknowledgements

The authors wish to thank Ing. Luis A. Del Vitto for his collaboration in the collection of plant specimens and for the botanical identification, and tec Mario Soloa for his technical assistance. Financial support of CONICET (PIP 5030) and UNSL (Project 7301) is acknowledged.

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