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BIOACTIVITY OF THE ESSENTIAL OIL OF *BUPLEURUM FRUTICOSUM*

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ABSTRACT.—The essential oil of *Bupleurum fruticosum* was investigated qualitatively and quantitatively together with the anti-inflammatory activity of the whole essential oil and its major components. In addition, antispasmodic activity was determined in rat uterus preparations using acetylcholine and oxytocin as agonists. The anti-inflammatory activity shown by the essential oil can be attributed in part to the two major components, α-pinene and β-pinene, although the presence of thymol and carvacrol, minor components capable of potentiating the action of these hydrocarbons, was also confirmed.

Several species of the genus *Bupleurum* are widespread in the region surrounding Granada, and some members are commonly used in folk remedies as anti-inflammatory and antimicrobial agents (1). Previous studies in our laboratory have shown the essential oil of another species, *Bupleurum gibraltaricum* Lam., to possess considerable anti-inflammatory activity attributable to Δ-3-carene, its major component (2,3). In the present investigation a qualitative and quantitative analysis of the essential oil of *Bupleurum fruticosum* L. (Umbelliferae) was carried out, and the degree of anti-inflammatory activity (or lack thereof) was determined.

Pharmacodynamic studies were completed with assays aimed at measuring antispasmodic activity in rat uterus preparations, as such action has been previously shown by the essential oils of other species of the genus *Bupleurum* (2).

EXPERIMENTAL

PLANT MATERIAL.—The fruiting apexes of *B. fruticosum* were collected from slopes of the Sierra Cogollos mountains (province of Granada, Spain) in September 1987, at an altitude of 960 m. The plant material was dried at room temperature (27–29°) and ground preparatory to the extraction of the essential oil. A total of 1.2 kg of dried material yielded 1.85% (v/w) essential oil, which was steamed out with a Clevenger device (4). The essential oil was frozen at −5° with anhydrous Na_2SO_4 until use.

In all pharmacodynamic assays a 9:1 emulsion of the essential oil with Tween-80 (5) was administered orally and ip (total volume administered = 1.0 ml). The pure components (also in a 9:1 emulsion with Tween-80) were administered ip.

QUALITATIVE AND QUANTITATIVE ANALYSIS.—Qualitative data were determined by gc and gc-ms. The gc analyses were performed using a Perkin-Elmer gas chromatograph (model 3810 B) equipped with a flame ionization detector and a Perkin-Elmer (model GP-100) computing integrator. A semi-capillary column (12 m × 0.53 mm i.d.) was coated with Carbowax 20 M. The temperature program adopted was: 60° (4 min), 140° (1 min), 180° (3 min) by increments of 10°/min. The detector and injector point heaters were set at 300° and 250°, respectively. The carrier gas was H_2 at a working flow rate of 10 ml/min.

Gc-ms analyses were done on a Hewlett-Packard mass spectrometer combined with a Hewlett-Packard gas chromatograph (model 5992 A) with a Carbowax 20 M capillary column, 25 m × 0.25 mm i.d. Split injection was used throughout the analyses at a ratio of 80:1. The temperature program was 50° (2 min), 150° (3 min), 190° (5 min), in increments of 8°/min. The detecter and injector point heaters were set at 250°. The carrier gas was He at a flow rate of 2 ml/min.

Components were identified by adding quantities of pure component to the essential oil and comparing their mass spectra with those of authenticated samples and/or data reported in the literature.

Gc quantitative analysis was performed by the relative proportions method under the conditions specified above. An internal standard was likewise used in the quantification of α- and β-pinene.

ANTI-INFLAMMATORY ACTIVITY ASSAYS.—Anti-inflammatory activity of the essential oil was tested against carrageenan-induced edema in the hindpaw of the rat: i.e., in acute exudative inflammation (6).

Female Wistar rats weighing an average of 200 g were divided into groups of eight animals each. An aqueous suspension of carrageenan (1%, 0.1 ml) was injected into the subplantar region of the left hindpaw. The volume of the injected paw was recorded phlethysmographically before administering car-

rageenan and 1, 3, and 5 h thereafter. The drugs or vehicle were administered 1 h before carrageenan (orally and ip).

Anti-inflammatory activity of the essential oil was also investigated against a foreign body granuloma produced by implanting a pellet of cotton in a model of subchronic proliferative inflammation (7). Female Wistar rats (200 g) were divided into groups of eight animals each, and granuloma was induced by implanting a small piece of sterile cotton into the dorsal area. During the next 8 days a target dose (200 mg/kg) of the essential oil emulsified with Tween-80 (9:1) was administered ip, and on the ninth day the granulomas were excised, dried at 50° for 24 h, and weighed.

ISOLATED RAT UTERI.—Virgin Wistar rats weighing 200 g were killed and the uteri removed. Each uterine horn was mounted in a 30-ml organ bath and bathed in Garcia Jalon solution (8). Contractions were quantified with a Stathman isometric transducer and recorded with a Beckman R-411 Dynograph recorder set at a tension of 0.5 g. Oxytocin and acetylcholine were used as the agonist (EC_{50} was calculated by the accumulative method). The essential oil, previously emulsified in Tween-80, was added to the bath at 60-min intervals and left in contact with the preparation for 2 min. After adding the various doses of essential oil and washing several times, the contractile activity of the uterine preparations against the agonist was similar to that recorded at the beginning of the experience, thus suggesting that the antispasmodic action of the essential oil cannot be attributed to a possible toxic effect.

Student's *t*-test was used to compare the means in the different pharmacodynamic assays.

RESULTS AND DISCUSSION

Data obtained in the qualitative and quantitative determination of the essential oil sample are reported in Table 1. The major fraction was represented by the monoterpenic hydrocarbons, at about 80% by far the most abundant compounds, of which α- and β-pinene were found to be the major components (40.93% and 46.47%). Conspicuous by its absence was Δ-3-carene, the major component in the essential oil of *B. gibraltaricum* (2).

The essential oil of *B. fruticosum* showed a potent anti-inflammatory activity when administered both orally and ip against carrageenan-induced plantar edema (Figures 1 and 2). When activity was compared following oral and ip administration, the former

TABLE 1. Composition of the Essential Oil of *Bupleurum fruticosum*.

Component	Relative concentration (%)	$[M]^+$	*m/z* (100%)
Hydrocarbons			
α-Pinene	41.21	136	93
β-Pinene	35.89	136	93
Myrcene[a]	3.10	136	93
α-Phellandrene[a]	3.10		
Limonene[b]	4.10		
γ-Terpinene	2.51		
p-Cymene	2.91	148	119
Caryophyllene		189	93
Epoxides			
1,8-Cineole[b]	4.10		
Alcohols			
Linalool	T[c]		
Terpinen-4-ol	T		
Borneol	T		
Acetates			
Linalyl acetate	T		
Aromatic alcohols			
Estragol	T		
Thymol	T	186	135
Carvacrol	1.47		

[a,b]Compounds not resolved for individual quantitation.
[c]T = trace (below 1%).

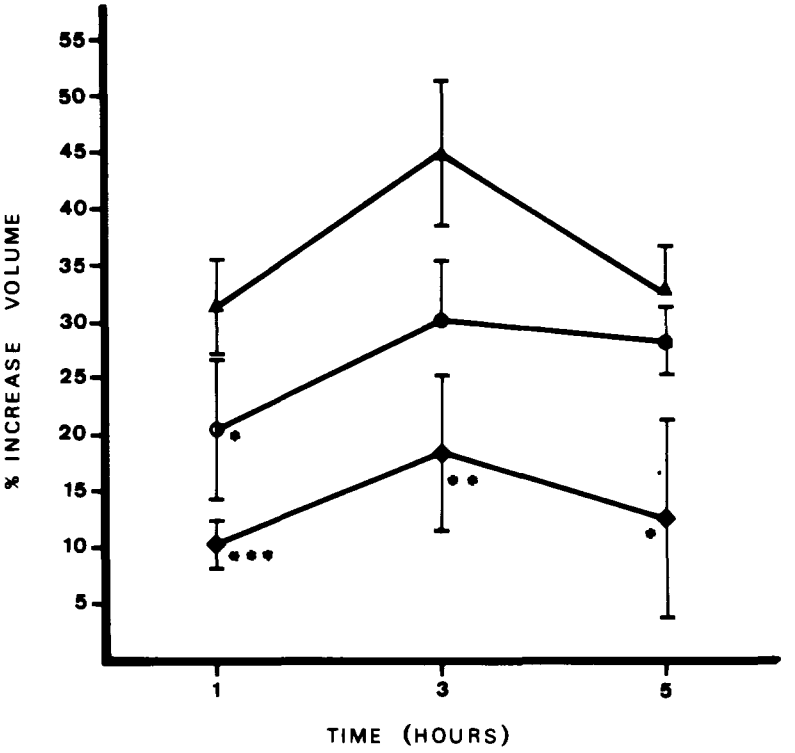

FIGURE 1. Percent increase in hindpaw volume with 400 mg/kg (○), 800 mg/kg (◆) and without (▲) oral pretreatment with *Bupleurum fruticosum* essential oil. Each point indicates the mean of the % increase in volume ± SEM ($n = 8$). Asterisks refer to significance relative to the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

route was found to require much higher doses than the latter in order to produce the anti-inflammatory effect.

Essential oils are known to provoke irritation; hence the ip route of aministration would be expected to modify anti-inflammatory activity against carrageenan-induced plantar edema. This fact, which might initially be looked upon as an obstacle to the use of this route, can be considered an advantage in view of the fact that the basic goal of the present study was to determine the active component(s) of the essential oil. The considerably enhanced anti-inflammatory activity observed following ip administration allows us to follow the course of each component more accurately, a factor which in turn makes it a relatively simple matter to identify reliably the component responsible for the pharmacological action recorded. This reason alone led us to choose the ip route in the present study.

The major components of the essential oil, α- and β-pinene, exhibited a clear anti-inflammatory activity at doses equivalent to those of whole essential oil (Table 2). The pharmacological action of these hydrocarbons was nevertheless weaker than that of whole essential oil, both when administered alone and in artificial mixture in which the relative proportion of each hydrocarbon is equivalent to that found in the essential oil. These observations imply that while α- and β-pinene are indeed responsible for a large part of the activity shown by the essential oil, there must also exist a minor component capable of potentiating the anti-inflammatory action of the major components. In

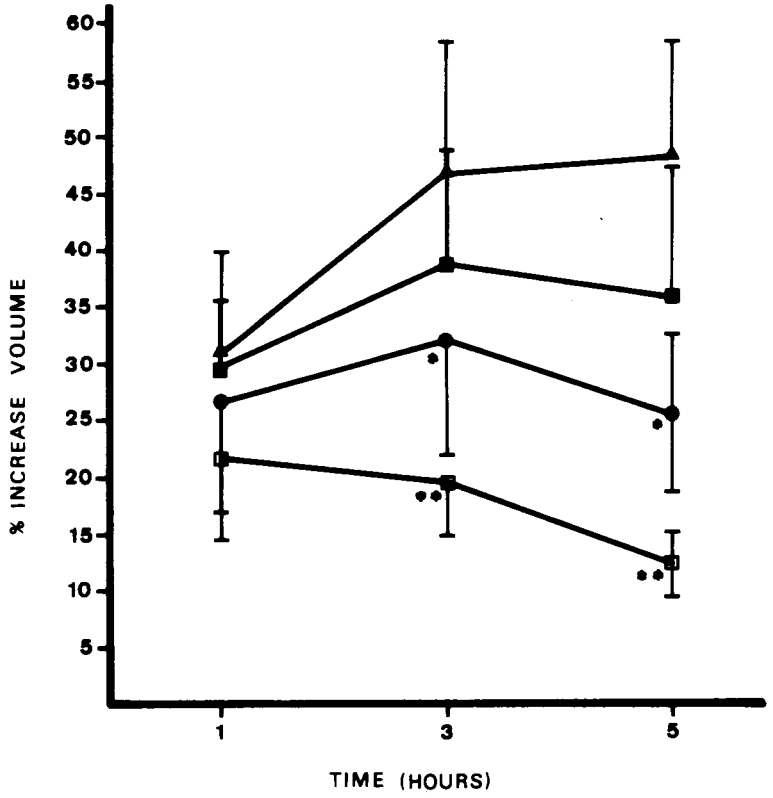

FIGURE 2. Percent increase in hindpaw volume with 50 mg/kg (■), 100 mg/kg (●), 200 mg/kg (□) and without (▲) ip pretreatment with *Bupleurum fruticosum* essential oil. Each point indicates the mean of the % increase in volume ± SEM ($n = 8$). Asterisks refer to significance relative to the control group: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

earlier studies we tested all the components making up more than 3% of the whole essential oil (2): myrcene, α-phellandrene, limonene, *p*-cymene, and 1,8-cineole. None of the compounds was found to produce anti-inflammatory activity at doses equivalent to their proportional content in whole essential oil. This led us to suspect the presence of an extremely minor component(s) capable of potentiating the anti-inflammatory action of α- and β-pinene. Hence, 1.0 mg/kg thymol plus 3.0 mg/kg carvacrol were added to the previously assayed artificial mixture of the above-mentioned hydrocarbons (α- and β-pinene) in order to reproduce their naturally occurring proportions. This new mixture confirmed that the anti-inflammatory activity of *B. fruticosum* essential oil is due not entirely to α- and β-pinene but also to the aromatic alcohols thymol and carvacrol, which although present in extremely low levels nonetheless potentiate the pharmacodynamic action under study.

Upon comparing the present findings with the anti-inflammatory activity of the essential oil of *B. gibraltaricum* (2), the latter species was noted to be considerably more active. This is not surprising in view of the lack in *B. fruticosum* essential oil of Δ-3-carene, the major component of the essential oil of *B. gibraltaricum*. The anti-inflammatory activity of this compound, as a previous study has shown (2), is considerably more potent than that of either α- or β-pinene. At doses of 33.0 and 66.0 mg/kg ip, for example, anti-inflammatory activity 3 h after carrageenan administration was calculated as 50.3% and 77.0%, respectively.

TABLE 2. % Anti-inflammatory Activity of the Essential oil of *Bupleurum fruticosum* and Major Components Against Plantar Edema Induced by Carrageenin.[a]

Sample	(mg/kg)	Time after carrageenan (h)		
		1	3	5
Essential oil	200	29.4 ± 23.1	58.2 ± 10.0	74.5 ± 5.7
	100	13.7 ± 31.0	31.3 ± 12.2	47.3 ± 14.3
	50	3.6 ± 19.0	17.1 ± 21.1	26.2 ± 23.3
α-Pinene	80	19.0 ± 7.4	26.2 ± 9.1	13.2 ± 3.9
	40	20.4 ± 10.5	21.0 ± 10.8	14.0 ± 8.2
	20	5.6 ± 8.4	8.9 ± 7.3	−1.6 ± 5.3
β-Pinene	80	8.7 ± 2.5	32.2 ± 6.2	31.9 ± 5.7
	40	27.5 ± 4.8	19.2 ± 5.8	14.9 ± 7.3
	20	20.5 ± 7.8	15.1 ± 4.7	16.7 ± 5.4
α-Pinene + ...	80 + 80	13.2 ± 7.5	34.1 ± 5.7	39.6 ± 1.6
β-Pinene	40 + 40	8.8 ± 11.0	28.1 ± 7.0	41.7 ± 5.4
	20 + 20	−4.1 ± 20.3	−5.9 ± 13.6	0.8 ± 13.0
α-Pinene + ...				
β-Pinene +	80 + 80 +	25.5 ± 10.3	62.1 ± 16.7	70.5 ± 15.4
Thymol +	1 + 3			
Carvacrol				

[a]Values are given as mean (A.A.I.) + SEM.

$\%A.A.I. = \frac{I_b - I_x}{I_b} \times 100$, where I_b = % inflammation volume to the control group and I_x = % inflammation volume to the treated group.

The essential oil also showed anti-inflammatory activity against inflammation provoked by implanting a piece of cotton wool. This activity, nevertheless, was less intense than that observed in the previous assay.

Although *B. fruticosum* essential oil showed a clearly antispasmodic activity against acetylcholine- and oxytocin-induced contractions in rat uteri (Table 3), this behavior varied depending on the agonist tested. The former was antagonized in a noncompetitive way, as E_{max} was modified but not EC_{50}, whereas antagonism against oxytocin was both competitive and noncompetitive, as indicated by the change in both indices. Noncompetitive antagonism can probably be attributed to α- and β-pinene (9,10). A more likely explanation for the bimodal activity against oxytocin (i.e., changes in both EC_{50}

TABLE 3. Changes in EC_{50} (μM) and E_{max} of Acetylcholine and Oxytocin in Response to Increasing Doses of *Bupleurum fruticosum* Essential Oil (n = 6).[a]

Agonist	Bath concentration of essential oil (μg/ml)	EC_{50} ± SEM	E_{max} ± SEM
Acetylcholine	—	0.090 ± 0.030	100
	40	0.046 ± 0.020	57.6 ± 6.3**
(1×10^{-9}–	80	0.045 ± 0.004	42.9 ± 7.6***
0.5×10^{-6} M)	160	0.060 ± 0.030	20.7 ± 4.4***
Oxytocin	—	0.070 ± 0.004	100
	40	0.130 ± 0.020*	67.6 ± 7.4**
(1×10^{-8}–	80	0.140 ± 0.040*	13.4 ± 8.2***
1×10^{-6} M)			

[a]Values are given as mean ± SEM. Asterisks refer to significance relative to the control group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

and E_{max}), however, is the presence in the essential oil of additional pharmacologically active compounds capable of competitively antagonizing this agonist.

Upon comparing the antispasmodic activity against oxytocin shown by the essential oils of *B. fruticosum* and *B. gibraltaricum*, we find that the latter was able to modify the EC_{50} of the agonist at much lower doses, a finding attributable to the presence of Δ-3-carene as the major component. This hydrocarbon, which is lacking in *B. fruticosum* essential oil, was markedly effective against oxytocin-induced contractions: Doses of 1.1 μg/ml and 2.2 μg/ml raised oxytocin EC_{50} by 3.1 and 6.4 times more than control values (2).

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