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Antiinflammatory Principle of *Bupleurum longiradiatum* Roots

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A methanol extract of the roots of *Bupleurum longiradiatum* (Umbelliferae) showed antiinflammatory activity in rats. Hexane, chloroform, butanol, and aqueous fractions from the methanol extract were tested with the result that only the hexane fraction exhibited positive activity. Fractionation of the hexane extract resulted in isolation of arborinone as an active principle.

Key words: *Bupleurum longiradiatum*, Umbelliferae, Triterpenoid, Arborinone, Antiinflammatory

INTRODUCTION

The roots of *Bupleurum longiradiatum* have occasionally been substituted for Bupleuri Radix and used as a remedy to treat gout and inflammatory illness (Perry, 1980). Preliminary pharmacological screening showed a marked antiinflammatory activity for its methanol extract. For this reason, it was decided to investigate which component(s) was/were responsible for the antiinflammatory activity. We report here the isolation of arborinone as an active principle from the roots.

EXPERIMENTAL METHODS

Plant material

The dried roots of *B. longiradiatum* were purchased from a crude drug market in Seoul and identified botanically by Prof. H. J. Chi of NPRI. A voucher specimen is deposited in the NPRI herbarium.

Extraction and fractionation

The powdered root (10 kg) was refluxed with MeOH at 95℃, four times. The pooled MeOH solution was concentrated *in vacuo* to afford 1,038 g of concentrated extract. An aliquot (100 g) of the MeOH extract was partitioned with *n*-hexane and 10% aqueous MeOH. The hexane phase was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a hexane fraction (19 g). The aqueous phase was partitioned with $CHCl_3$ and BuOH, successively, to yield $CHCl_3$ (29.5 g) and BuOH (25 g) fractions, respectively. The aqueous layer was concentrated *in vacuo*, frozen, and lyophilized to afford an aqueous fraction (30 g).

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Isolation of arborinone

The hexane soluble fraction was subjected to SiO_2 column chromatograpy using hexane-EtOAc (gradient) and the eluates were collected in 250 m*l* portions and combined to give 6 main subfractions. Subfractions 1 and 2 were combined and chromatographed over SiO_2 ($CHCl_3$-MeOH=100:0.5) to obtain pure arborinone as rhombic (300 mg), mp. 214-216°, LB test: brown; Zimmermann test: yellow brown. IR (KBr): 1708 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 0.77 (3H, s), 0.81 (6H, s), 0.83 (3H, d, J=5.5), 0.90 (3H, d, J=5.5), 1.07 (6H, s), 1.22 (3H, s), 2.5 (2H, m), 5.25 (1H, m); ^{13}C NMR ($CDCl_3$): δ 14.0 (C-28), 15.3 (C-27), 16.9 (C-26), 20.2 (C-19), 21.7 (C-25), 22.0 (C-24), 22.1 (C-30), 22.7 (C-7), 22.9 (C-29), 25.7 (C-23), 26.4 (C-6), 28.2 (C-20), 29.7 (C-15), 30.7 (C-22), 34.8 (C-2), 36.0 (C-16), 36.2 (C-12), 36.7 (C-1), 36.8 (C-13), 38.3 (C-14), 39.4 (C-10), 41.2 (C-8), 42.9 (C-17), 47.6 (C-4), 52.1 (C-18), 53.4 (C-5), 59.6 (C-21), 115.7 (C-11), 147.5 (C-9), 216.3 (C-3). EIMS *m/z* (rel. int.): 424 (M^+, 31), 409 (43), 271 (23), 259 (12.6), 257 (100), 245 (21.8), 205 (4.6).

Animals

Male Sprague-Dawley rats (CD strain) weighing 150-180 g were used. The animals were housed in cages at least one week before the experiments. The laboratory chows (Samyang Yuji Co.) and tap water were given *ad libitum*. The room temperature was main-

Table I. Effect of MeOH extract and fractions carrageenin-induced edema in rats

Treatment	Dose[a] (mg/kg, i.p.)	No. of animals	Percent increased (mean± S.E.) 1	2	3	5[b]
Control	–	5	36.2± 11.64	61.6± 16.30	65.4± 16.25	47.5± 18.06
MeOH	200	5	14.5± 3.30*	14.2± 1.58*	15.4± 2.14*	29.0± 2.48
Hexane	50	5	14.0± 1.53	14.7± 1.41*	18.2± 2.40*	21.5± 3.06*
$CHCl_3$	50	5	26.0± 10.65	53.8± 12.79	66.4± 12.92	48.5± 11.92
BuOH	50	5	43.3± 8.76	88.8± 12.94	97.5± 12.33	85.9± 10.67
H_2O	50	5	13.5± 3.10	34.4± 9.67	39.1± 7.11	55.4± 8.09
Hydrocortisone acetate	40	5	10.3± 3.70*	15.8± 6.79*	16.8± 3.80*	16.0± 11.33

[a]Samples were given 60 min before carrageenin injection (s.c.)
[b]Hours after carrageenin injection
*Significantly different from the Control group ($p<0.01$)

Table II. Effect of subfractions from hexane soluble fraction on carrageenin-induced edema in rats

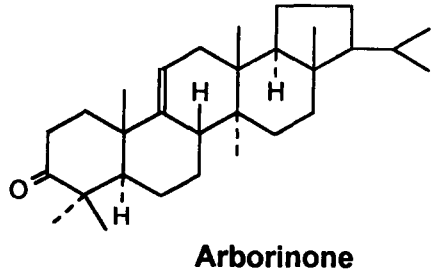
Subfraction No.	Dose[a] (mg/kg, i.p.)	No. of animals	Percent increased (mean± S.E.) 1	2	3	5[b]
C	–	5	15.4± 2.61	46.8± 3.84	59.7± 2.50	54.9± 3.03
1	6.1	5	7.3± 2.36	13.7± 3.89***	22.4± 5.71***	37.4± 6.63
2	15.5	5	9.1± 1.30	28.4± 3.52*	34.4± 3.43**	38.0± 4.50*
3	4.9	5	9.5± 4.78	19.9± 12.36	31.3± 17.34	43.2± 14.47
4	28.7	5	18.1± 1.13	43.0± 3.54	47.9± 8.95	50.4± 9.90
5	40.8	5	16.5± 5.54	44.3± 13.27	51.1± 17.08	62.0± 21.61
6	12.7	5	17.9± 2.64	39.9± 4.90	55.9± 12.57	56.2± 4.94
H	40.0	5	17.4± 1.80	36.7± 1.51*	42.1± 3.10**	50.7± 6.57

[a]Samples were given 60 min before carrageenin injection (s.c.)
[b]Hours after carrageenin injection
Significantly different from the Control group ($p<0.05$, **$p<0.01$, **$p<0.001$)
C: Control, H: Hydrocortisone acetate

Table III. Antiinflammatory activity of arborinone against carrageenin-induced edema in rats

Compound	Dose[a] (mg/kg, i.p.)	No. of animals	Percent increased[b] (mean± S.E.)	% Inhibition	p
Control	–	7	58.3± 3.50	–	–
Arborinone	10	7	33.7± 4.41	42.20	<0.05
Hydrocortisone Acetate	40	7	23.6± 2.10	59.75	<0.001

[a]Samples were given 60 min before carrageenin injection (s.c.)
[b]Three hours after carregeenin injection



Arborinone

tained at 20-23℃.

Biological evaluation for antiinflammatory activity

The samples were tested for antiinflammatory activity by carrageenin-induced rat paw edema test according to Winter *et al.* (1962). The paw edema was induced in the left paw by subplantar injection of 0.1 m*l* of 1% carrageenin suspension in physiological saline solution and the volume of the injected paw was measured before and every 1 hr after carrageenin injection with modified plethysmometer of Singh and Ghosh (1968). The samples were suspended in 0.5% CMC soluton and administered intraperitoneally 1 hr before injection of carrageenin. Hydrocortisone acetate was used as a positive control drug.

RESULTS AND DISCUSSION

As shown in Table I, the MeOH extract at a dose of 200 mg/kg i.p. exhibited a significant inhibition of carrageenin-induced pedal edema in rats.

In order to pursue the compound(s) responsible for the activity, the MeOH extract was fractionated as described in the experimental methods and the fractions were tested. The result indicated that the hexane fraction had positive activity at a dose of 50 mg/kg i.p., whereas the $CHCl_3$, BuOH, and H_2O fractions did not show any activity at the same dosage (Table I).

Bioassay guided silica gel column chromatography of the hexane extract showed that subfractions 1 and 2 were found to be active (Table II). Further column chromatograpy of combined subfractions of 1 and 2 gave a compound which was homogeneous on TLC and it was identified as arborinone by comparison of its physical and spectral data with those described in the literature (Nishimoto *et al.*, 1968; Akihisa *et al.*, 1992).

Arborinone exhibited a remarkable reduction in edema formation at a dose of 10 mg/kg i.p. (Table III). Therefore, it can be concluded that arborinone be responsible for the antiinflammatory activity of this drug.

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