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Pharmacological Activities of the Constituents of Atractylodes Rhizomes

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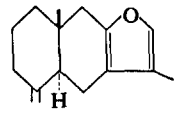
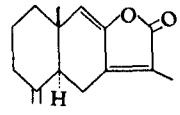
Abstract□The anti-microbial and anti-inflammatory activities of the constituents from Atractylodes rhizomes were evaluated. Atractylone showed anti-microbial activity. Atractylone and atractylenolide I possessed considerable anti-inflammatory activity utilizing rat cotton pellet granuloma bioassay.

Keywords□atractylone, atractylenolide I, atractylenolide III, anti-microbial and anti-inflammatory activity.

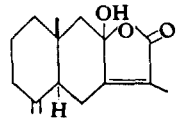
The entire part of Atractylodes rhizomes has been used in fevers, catarrh, chronic dysentery, rheumatism, apolexy[1]. The known constituents of Atractylodes rhizomes are atractylone, atractylenolide I, atractylenolide III and recently identified (2'-E/Z)-2-(3', 7'-Dimethyl-octa-2', 6'-dienyl)-6-methyl-2,5-cyclohexadien-1,4-dione[2-4]. Among these compounds, atractylenolide I and III were previously reported to show anti-inflammatory activity in chichen egg granuloma method[5]. But, there is no report mentioning anti-microbial activity of these constituents. Recently, in our laboratory, the above three constituents of Atractylodes rhizomes have been purified. In this investigation, the anti-microbial and anti-inflammatory activities of these compounds were evaluated.



Atractylone



Atractylenolide I



Atractylenolide III

Fig. 1. Constituents of Atractylodes rhizomes.

EXPERIMENTAL METHODS

The constituents of Atractylodes rhizomes were purified as previously published[4]. The dried plant (2 kg) was extracted with petroleum ether/ether (1:1) and concentrated *in vacuo* (65 g). This extract (20 g) was chromatographed over 1 kg of silica gel 60 (4.9 × 92 cm) and eluted with n-hexane to give 68 mg of atractylone. Subfraction 2 (200 mg, colorless crystal, Ve = 3,600-5,100 m*l*) was eluted with n-hexane/AcOEt (95:5) to give 120 mg of atractylenolide I. Subfraction 3 (90 mg, yellow crystal, Ve = 17,000-17,900 m*l*) was eluted with n-hexane/AcOEt (80:20) and gave 60 mg of atractylenolide III. These three compounds were analyzed with m.p., IR, UV, ^{1}H-NMR and revealed to be identical as previously published (Fig. 1)[4,6].

For these three compounds, the anti-microbial activity was tested with conventional filter paper method. In brief, *Bacillus subtilus* BD170 and *Escherichia coli* HB101 were spread in the nutrient agar media. The filter papers (Whatman No. 2) previously cut with puncher were layered on the solidified media. Antibiotics or test compounds dissolved

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in 5 μl of methanol were carefully added to each filter disk. Following incubation at 37 °C for 36 hrs, diameters of the growth inhibition zone were measured. The anti-inflammatory activity was evaluated by rat cotton pellet granuloma test as previously published[7]. Each cotton pellet (Richimond dental Co., 35 ± 1 mg) was impregnated with 0.2 m*l* of test compound solution (acetone). The pellets were dried at room temp. overnight. SD rats (♂, 100-150 g) were lightly anethesized and cut along the middle line. Each pellet was inserted under right axilla. The surgery sites were closed with autoclip (Clay Adams). The rats were maintained at the animal room of College of Pharmacy, with Purina lab chow and given water *ad libitum* (12 hr/12 hr, light/dark cycle). After 7 days, the rats were sacrificed with cervical dislocation and pellets were obtained. The pellets were dried at 55 °C for 2 days. The dried pellets were weighed and the granuloma weights were calculated after substracting the original pellet weight.

RESULTS AND DISCUSSION

The results of the anti-microbial activity of the each constituents were shown in Table I. Among the test compounds, only atractylone showed the anti-microbial activity against both *B. subtilus* and *E. coli*. Minimum detectable activity was seen at the dose of 100 μg/disk. The other two compounds did not show any activity up to the dose of 400 μg/disk. To the authors' knowledge, this is the first report showing that atractylone possesses the anti-microbial activity.

Table I. The anti-microbial activity of the constituents

Compounds	Dose/disc (μg)	Inhibition zone (mm)[a]	
		Bacillus subtilus	*Escherichia coli*
Penicillin G	3.5	11	—[b]
	7.0	19	—
	10.0	24	—
Chlorampenicol	3.0	—	11
	6.0	—	16
	13.0	—	22
	27.0	—	29
Atractylone	100	8	7
	200	11	10
	600	20	18
Atractylenolide I	400	NA[c]	NA
Atractylenolide III	400	NA	NA

[a]The diameter of the filter paper disc was 6 mm and the inhibition zone shown here was the total inhibition diameter (filter paper + inhibition zone). [b]not tested. [c]NA indicates that no activity was shown.

Table II. The anti-inflammatory activity of the constituents

Compounds[a]	Dose/pellet (mg)	Granuloma weight (mg)	% Inhibition of granuloma formation
Control	0.0	47.4 ± 2.3[b]	—
Prednisolone	2.5	17.5 ± 1.8	60.0
	1.0	25.6 ± 3.2	46.1
Atractylone	5.0	31.3 ± 4.2	34.0
Atractylenolide I[c]	1.0	31.1 ± 1.6	34.4
Atractylenolide III	5.0	36.6 ± 3.2	22.0
	3.0	39.7 ± 6.1	16.3

[a]Five rats (SD, ♂, 100-150 g) were used in each group. Compounds were impregnated at one side. [b]Data represents mean ± S.D. [c]Due to the limited amount of pure atractylenolide I, two rats were used and the data showed mean ± range.

To evaluate the anti-inflammatory activity, rat cotton pellet granuloma bioassay was employed. The results were shown in Table II. All of these compounds showed anti-inflammatory activity more or less. Among them, atractylenolide I showed the highest activity, which was well correlated with the previous findings of Endo *et al.*[5]. Considering the nature of plant constituents, the anti-inflammatory activity shown by this compound seemed to be relatively high. It is suggested that the chemical modification of atractylenolide I might lead to the novel anti-inflammatory compound of clinical interest.

ACKNOWLEDGEMENT

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