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Antiinflammatory Principles of *Atractylodes* Rhizomes[1)]

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The crude drug "jutsu" prepared from *Atractylodes* rhizomes has been used for antiinflammatory purposes in Oriental medicine. In fact, a preparation from *A. japonica* was found to show significant inhibition of the increased vascular permeability induced by acetic acid. Fractionation of the extract, monitoring by bioassay, resulted in the isolation of two active principles, (+)-eudesma-4(14),7(11)-dien-8-one (VI) and atractylenolide I (VII). The structurally related principles atractylenolide II and III (VIII and IX) also had the tendency to show antiinflammatory activity.

Keywords——*Atractylodes japonica*; Compositae; sesquiterpenoids; eudesmadienone; atractylenolides; antiinflammatory activity

The rhizomes of *Atractylodes* spp. (Compositae) are used for the preparation of the important crude drug "jutsu" in Oriental medicine, and are prescribed for diuretic and analgesic purposes, as well as for stomach disorders, mainly to improve water metabolism. The crude drug is empirically classified into two groups depending on the original plant.[3)] Thus the preparations originating from *A. japonica* Koidzumi and *A. ovata* De Candolle are called "byaku-jutsu", while those originating from *A. lancea* De Candolle and its varieties are called "sō-jutsu". The two groups of the crude drug are said to have different therapeutic effects: the former having antisudorific activity and the latter diaphoretic activity, although this remains to be conclusively demonstrated. When the two groups of crude drug are evaluated in the light of their sesquiterpenoid constituents, "byaku-jutsu" is characterized by an intense reaction in the vanillin-hydrochloric acid test owing to its high content of the furan-containing atractylon (I).[4,5)] On the other hand "sō-jutsu" is characterized by weak or no reaction in the vanillin-hydrochloric acid test due to the low content or absence of furano-analogs. Instead, "sō-jutsu" contains the alcohols β-eudesmol (II) and hinesol (III).[6)] The two groups can also be differentiated by the presence of the polyacetylenes diacetylatractylodiol (IV) in *A. japonica*,[7)] and atractylodin (V) in *A. lancea* and its varieties.[8)]

Quite recently, "sō-jutsu" was shown, mainly by behavioral pharmacological studies, to have central nervous system depressant action; the active principles were primarily the sesquiterpenoid alcohols β-eudesmol (II) and hinesol (III).[9)] However, very little information has been obtained on the interrelation of the constituents of the two groups and their pharmacological effects.

1) Part LV in the series on sesquiterpenoids. This paper is also part XIII on the validity of the Oriental medicines.
2) Location: *a) Aoba-yama, Sendai*; *b) Tsukasa-machi 40, Gifu.*
3) S. Takahashi and S. Murayama, *Shoyakugaku Zasshi*, **15**, 239 (1961); S. Takahashi and K. Namba, *ibid.*, **15**, 246 (1961); S. Takahashi, *ibid.*, **19**, 49 (1965).
4) S. Takagi and G. Hongo, *Yakugaku Zasshi*, **44**, 539 (1924).
5) H. Hikino, Y. Hikino, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **10**, 641 (1962).
6) I. Yosioka, S. Takahashi, H. Hikino, and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **7**, 319 (1959).
7) I. Yosioka, T. Tani, M. Hirose, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 1943 (1974).
8) I. Yosioka, S. Takahashi, H. Hikino, and Y. Sasaki, *Yakugaku Zasshi*, **80**, 1564 (1960); I. Yosioka, H. Hikino, and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **8**, 949, 952, 957 (1960).
9) J. Yamahara, T. Sawada, T. Tani, T. Nishino, I. Kitagawa, and H. Fujimura, *Yakugaku Zasshi*, **97**, 873 (1977).

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During the course of our investigation of the chemical constituents and pharmacological effects of Oriental medicines, we have found that prescriptions used for antiinflammatory purposes frequently contain the crude drug "jutsu" as a component. In order to confirm the supposed effect, a preparation from *A. japonica* was subjected to pharmacological bioassay for antiinflammatory activity using the Whittle method.[10] It was found that when a 50% aqueous ethanol extract was administered orally to mice at a dose equivalent to 10 g of crude drug/kg, a 42% inhibition of the increase of vascular permeability induced by acetic acid was observed. Since the antiinflammatory activity of the preparation of *Atractylodes* rhizomes was thus confirmed, characterization of the active principles was then undertaken.

Preliminary work indicated that the active principles could be effectively extracted with methanol (42% inhibition at a dose equivalent to 10 g of crude drug/kg), and that the activity could subsequently be concentrated in the ethyl acetate-soluble neutral fraction of the extract (41% inhibition at a dose equivalent to 20 g of crude drug/kg). Repeated chromatography of this fraction over silica gel yielded an oily substance (VI) and a crystalline substance (VII) as the active principles. The isolation procedures for these active principles are summarized in Chart 1.

The oily substance was found by mass spectroscopy to be a sesquiterpenoid with the composition $C_{15}H_{22}O$. The spectral data indicated it to be (+)-eudesma-4(14),7(11)-dien-

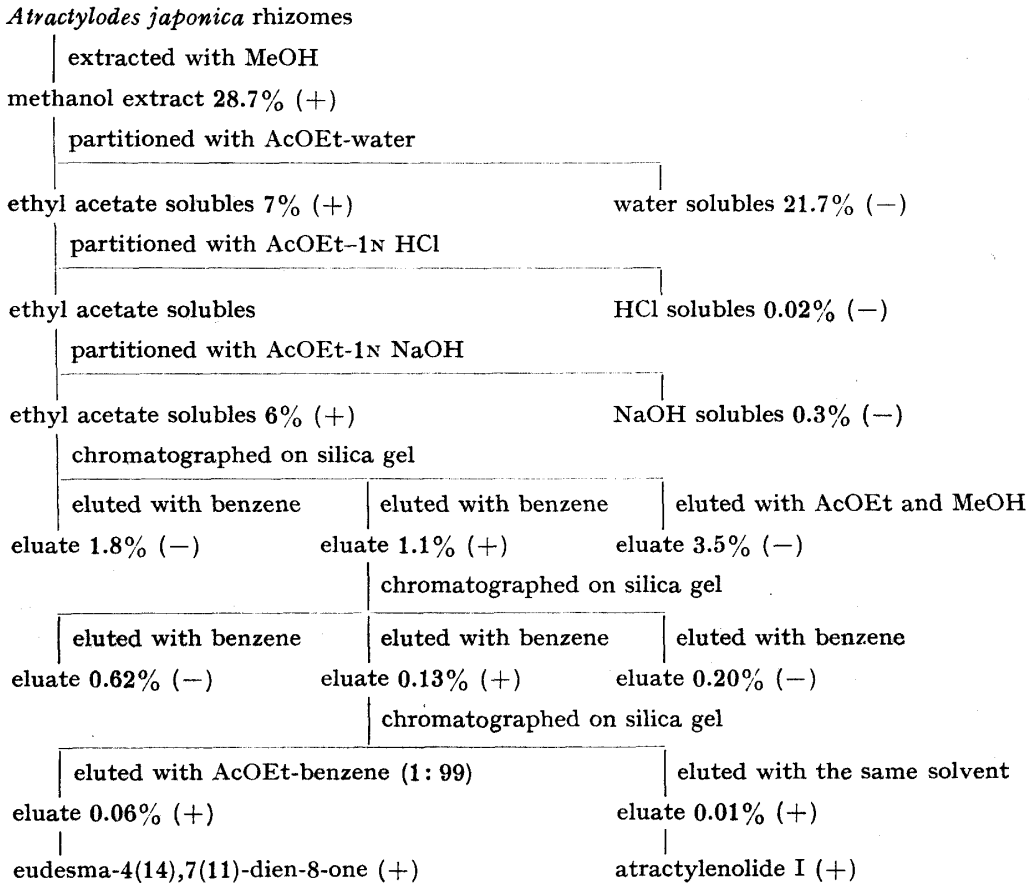

Chart 1. Flow Diagram of the Isolation of Eudesmadienone and Atractylenolide I from *Atractylodes japonica* Rhizomes

Yields (%) were calculated on the basis of the plant material, and scores in parentheses represent the relative antiinflammatory activities of the fractions

10) B.A. Whittle, *Br. J. Pharmac. Chemother.*, **22**, 246 (1964).

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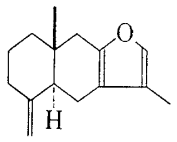
8-one (VI), previously obtained from a preparation of *A. japonica*,[11] and this conclusion was substantiated by direct comparison. We have recently clarified the absolute configurations of (+)- and (−)-eudesma-4(14),7(11)-dien-8-one,[12] which have hitherto been confused.[13,14]

The crystalline substance was shown to be a sesquiterpenoid having the composition $C_{15}H_{18}O_2$. It exhibited an ultraviolet (UV) absorption maximum at 274 nm and an infrared (IR) band at 1758 cm^{-1}, suggesting the presence of an α,β-butenolide having extended conjugation which involves an enol lactone moiety. The IR and ^1H nuclear magnetic resonance (NMR) spectra displayed resonances attributable to a tertiary methyl group (δ 0.92 ppm), a vinylic methyl group (δ 1.85 ppm). an exo-methylene group (ν_{max} 1640, 886 cm^{-1}, δ 4.59, 4.86 ppm) and an isolated olefinic hydrogen (δ 5.45 ppm). These spectral properties were in accord with the structural features of the dehydration product (VII) of atractylon autoxidation product B (IX),[5] and its identity was confirmed by direct comparison. The name atractylenolide I has been given to this new natural product. As will be described later, atractylon autoxidation products A and B (VIII and IX)[5] have now been obtained from natural sources, and are also designated as atractylenolide II and III, respectively.



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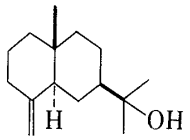

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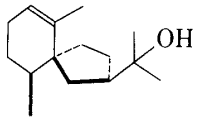

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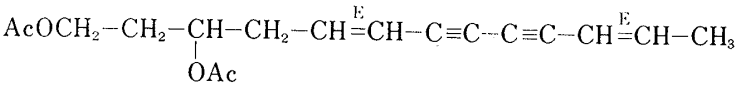

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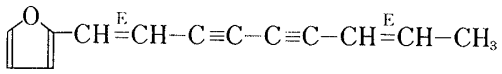

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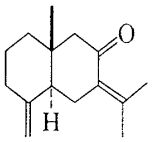

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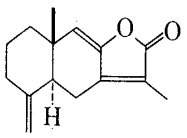

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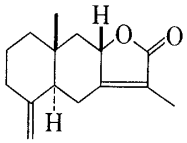
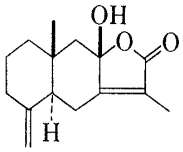


VIII



IX

Chart 2

The antiinflammatory activities of atractylenolide I and its analogs have been examined in various experimental models. The inhibitory effect on increased vascular permeability induced by acetic acid in mice using the Whittle method,[10] and the inhibitory effect on granulation tissue formation in chick embryos using the D'Arcy method[15] are shown in Tables I and II, respectively. It can be seen that each of the substances tested has an inhibitory effect in both test systems, compound VII being the most effective. The study was further extended to examine the inhibitory effect on the swelling of rat paw induced by carrageenin.[16] As shown in Fig. 1, none of the substances (VII—IX) has an appreciable

11) Y. Nishikawa, I. Yasuda, Y. Watanabe, and T. Seto, *Shoyakugaku Zasshi*, **30**, 132 (1976).
12) K. Endo and H. Hikino, *Bull. Chem. Soc. Japan*, **52**, 2439 (1979).
13) J. Endo and M. Nagasawa, *Yakugaku Zasshi*, **94**, 1574 (1974).
14) F. Bohlmann and A. Suwita, *Phytochemistry*, **17**, 567 (1978).
15) P.F. D'Arcy and E.M. Haward, *Br. J. Pharmac. Chemother.*, **29**, 378 (1967); H. Otsuka, M. Tsukui, T. Matsuoka, M. Goto, H. Fujimura, Y. Hiramatsu, and T. Sawada, *Yakugaku Zasshi*, **94**, 796 (1974).
16) C.A. Winter, E.A. Rinsley, and G.W. Nuss, *Proc. Soc. Exper. Biol.*, N.Y., **111**, 544 (1962); S. Garattini, "Non-Steroidal Antiinflammatory Drugs," Excerpta Medica Foundation, Amsterdam, 1965, p. 256.

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effect on oral administration at doses as high as 400 mg/kg. As has been mentioned above, the crude drug "sō-jutsu" and its components β-eudesmol (II) and hinesol (III) were shown to exhibit central nervous system depressant action.[9] The antiinflammatory principles of "byaku-jutsu" were also subjected to examination for analgesic action.[17] It was found that the compounds (VII—IX) failed to exhibit analgesic activity at an oral dose of 200 mg/kg (Table III).

Table I. Effects of Eudesmadienone and Atractylenolides on the Increased Vascular Permeability in Mice induced by Acetic Acid using the Whittle Method

Substance	Dose (mg/kg)	No. of animals	Inhibition (%)
Control	—	10	—a)
Eudesma-4(14),7(11) dien-8-one	30	10	−4.6±10.7
	100	10	9.6±11.5
	300	10	31.6±11.9
Atractylenolide I	30	5	−1.0±16.0
	100	10	18.3± 8.1
	300	10	33.7± 3.0*
Atractylenolide II	100	5	34.6± 4.9
	300	5	26.4±10.3
Atractylenolide III	100	5	11.7±18.3
	300	10	19.5± 1.1
Aminopyrine	100	10	47.2± 8.5**

a) Dye permeated/animal, 74.1±8.6 μg.
Significantly different from the control, $p < 0.05$* or $p < 0.01$**.

Table II. Effects of Atractylenolides on Granulation Tissue Formation using the Fertile Egg Method

Substance	Dose (μg/disc)	Granulation tissue		Survival ratio
		Dry Wt. (mg)	Inhibition (%)	
Control	—	6.2	—	18/20
Atractylenolide I	25	5.3	15.4	15/20
	50	4.2	31.4	14/20
	100	3.1	49.7	11/20
Atractylenolide II	25	5.1	17.7	13/20
	50	4.3	30.2	13/20
	100	4·1	33.4	13/20
Atractylenolide III	25	5.1	17.6	12/20
	50	4.7	23.5	15/20
	100	4.5	27.8	16/20
Berberine chloride	12.5	2.4	61.0	13/20
	25	1.9	69.0	15/20

Table III. Effects of Atractylenolides on Acetic Acid-induced Stretching in Mice

Substance	Inhibition of AcOH stretching ED_{50} (95% C.L.), mg/kg, *p.o.*
Atractylenolide I	> 200
Atractylenolide II	> 200
Atractylenolide III	> 200
Aminopyrine	78.0 (55.7—109.2)

17) R. Koster, M. Anderson, and E.J. de Beer, *Fed. Proc.*, **18**, 412 (1959).

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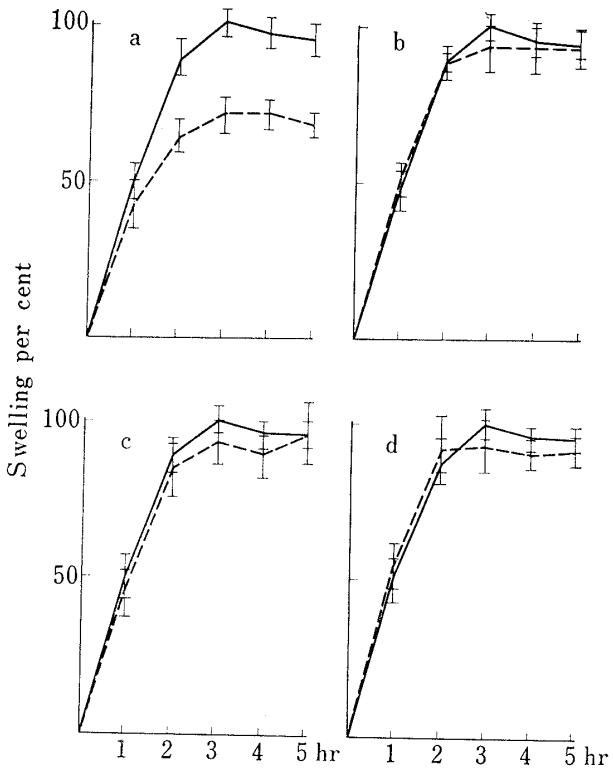

Fig. 1. Effects of Atractylenolides on the Swelling of Rat Hind Paw Induced by Carrageenin

——: control. ----: treated.
Vertical bars represent standard errors.
a : phenylbutazone 100 mg/kg, *p.o.*
b : atractylenolide I 400 mg/kg, *p.o.*
c : atractylenolide II 400 mg/kg, *p.o.*
d : atractylenolide III 400 mg/kg, *p.o.*

Summarizing data so far obtained on the pharmacological actions of the crude drug, it can be concluded that "byaku-jutsu" shows antiinflammatory activity, while "sō-jutsu" has a central nervous system depressant action. It is not at present known whether the differences in the pharmacological effects described above are related to the differences in the alleged therapeutic effects of "byaku-jutsu" and "sō-jutsu".

Experimental

All mp's are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz unless otherwise stated. Chemical shifts (δ) are expressed in ppm downfield from internal tetramethylsilane. Abbreviations: s=singlet, br=broad.

Assays for Antiinflammatory Activity——The effect of the crude drug "jutsu" and its principles on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle.[10] Evaluation of the antiinflammatory activity of the atractylenolides in the carrageenin-edema test followed the method reported by Garattini.[16] For assaying the antiinflammatory activity employing the chorioallantoic membrane of the chick embryo, the procedure described by D'Arcy and Howard[15] was used. Test samples were suspended in 5% gum arabic solution.

Assays for Analgesic Activity——The analgesic effects of the atractylenolides on the acetic acid-induced stretching in mice was determined according to Koster *et al.*[17]

Isolation of (+)-Eudesma-4(14),7(11)-dien-8-one and Atractylenolide I from *Atractylodes japonica* Rhizomes——A commercial crude drug preparation, consisting of the dried rhizomes of *Atractylodes japonica* (435 g), was extracted 6 times with refluxing MeOH (1 l) for 5 hr (each extraction). The MeOH solutions were combined and concentrated to dryness to afford an extract (117.6 g) which was partitioned between AcOEt and water. The organic layer was washed successively with dilute HCl and NaOH solutions. After concentration, the neutral fraction was fractionated by successive chromatographies on silica gel as shown in Chart 1 to afford the substances VI and VII.

These substances may be obtained more conveniently and effectively by the following procedure. A MeOH extract (104 g) obtained from a preparation (450 g) of *A. japonica* was diluted with water and extracted with light petroleum, yielding a petroleum-soluble portion (27 g) and a water-soluble portion (77 g). The former was applied to a column of silica gel (300 g) and eluted with benzene to give an active fraction (2.7 g) which was again chromatographed on silica gel (100 g). Elution with AcOEt–*n*-hexane (1: 49) afforded crude VI (1.0 g), and purification by repeated silica gel chromatography gave eudesma-4(14),7(11)-dien-8-one (VI) as a colorless oil.[12] Continuous elution with the same solvent, AcOEt–*n*-hexane, yielded crude VII (0.7 g) which was crystallized from *n*-hexane to afford atractylenolide I (VII) as colorless needles: mp 109—110.5°. *Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.31; H, 7.63. $[\alpha]_D^{20}$ +222° (c=0.010, MeOH). MS *m/e*: 230 [M$^+$]. UV λ_{max}^{hexane} nm (log ε): 274 (4.16); IR ν_{max}^{KBr} cm^{-1}: 1758 (C=O), 1640 (C=C), 1005 (C–O–C), 886 (C=CH$_2$); ^{1}H NMR (CCl_4) δ: 0.92 (3H s, CH$_3$), 1.85 (3H s, CH$_3$), 4.59 and 4.86 (1H each br s, C=CH$_2$), 5.45 (1H s, C=CH). The identity of this compound was confirmed by comparison of mp (mixed mp), as well as IR and NMR spectra with those of an authentic sample.

Acknowledgement We are indebted to Dr. M. Goto, Takeda Chemical Industries, Ltd., for performing some of the bioassays.

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