

Spasmolytic Principle of *Asarum sieboldii*

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Abstract—Fractionation of methanolic extract of *Asarum sieboldii* monitoring by bioassay resulted in the isolation of methyleugenol as a spasmolytic principle. Its pD'_2 value was 4.3.

Keywords—*Asarum sieboldii* · Aristolochiaceae · methyleugenol · spasmolytic compound

Asarum sieboldii (Aristolochiaceae) is a perennial herbaceous plant growing in woods in Korea and some other Asian countries. In oriental medicine the plant has been used for control of cough, pain and fever. Several phytochemical¹⁻¹²⁾ and pharmacological^{13,14)} studies on *Asarum* spp. have been previously reported.

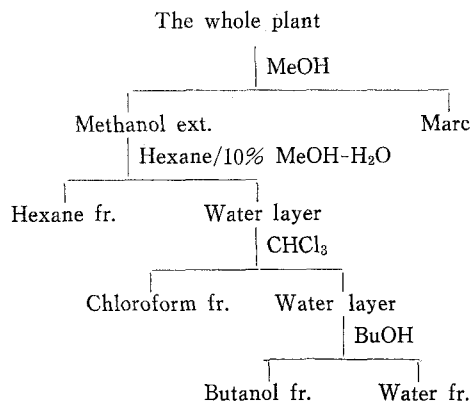
In a course of biological screening of medicinal plants it was found that the methanolic extract of the whole plant showed anti-cholinergic and anti-oxytocic activities in ileum and uterus of rats, respectively¹⁵⁾. Gondo¹³⁾ has reported that the essential oil from this plant caused relaxation of rabbit small intestine which was not affected by addition of muscarine, pilocarpine and barium chloride solution. These findings suggested the presence of the compounds with papaverine-like action in the plant. This paper deals with the establishment of a spasmolytic principle of the plant.

Materials and Methods

Plant material—The dried plant was purchased from local herb store and identified taxonomically.

Extraction and fractionation—One kilogram of crushed whole plant was extracted

three times with 95% boiling methanol for 6 hrs. and filtered off. All the filtrates were concentrated *in vacuo* under 45°. The methanolic extract obtained was fractionated according to the procedure illustrated in Scheme 1.



Scheme 1. Fractionation of the whole plant of *A. sieboldii*.

Drugs—Acetylcholine chloride (ACh) and methyleugenol were obtained from Sigma Chemical Co. and papaverine from Tokyo Kasei Kogyo Co.

Sample preparation for bioassay—Methanolic extract and all the fractions and drugs except chloroform fraction, were suspended in 1% sodium carboxymethylcellulose solution. The chloroform fraction was coprecipitated with

polyvinylpyrrolidone (PVP) 10,000 according to the method of Simonelli *et al.*¹⁶⁾ One part of the extract and four parts of PVP were dissolved in methanol, respectively. These solutions were mixed together and the solvent was removed by rotary evaporator. The fluffy product obtained was dissolved in 1% sodium carboxymethylcellulose solution for bioassay.

Test of spasmolytic activity—Male rats weighing 150-250g of body weight were fasted for 24 hrs and the water was given *ad libitum*. For bioassay the rats were stunned by a blow on the head and decapitated. The stomach was dissected and the fundus strip was prepared according to the procedure described by Vane¹⁷⁾. The strip was suspended in Locke-Ringer solution bubbled with a mixture of 95% O₂ and 5% CO₂. Each strip was mounted with 0.5g of initial tension. The responses were recorded isometrically on a Harvard Biograph (Model 2120). The ability of each test material to inhibit the contractile response to Ach was determined by obtaining cumulative dose response curves to Ach, first in the absence and then in the presence of several increasing concentrations of the agent in question. The ED₅₀ for each of the curves was determined, and the apparent affinities were calculated as pD'₂ values by the method of van Rossum¹⁸⁾.

GC-MS—Mass spectra were obtained with a

Hewlett Packard Model 5985B GC/MS spectrometer. Operating conditions; glass capillary column (12m × 0.2mm), OV 101, temp. programme, 10°/min from 60 to 280°, ionization potential, 70eV.

Results and Discussion

In order to isolate the active components, systematic fractionation was carried out, and the methanolic extract and fractions were bioassayed. The spasmolytic activity of each test sample is summarized in Table I.

The affinity of Ach expressed as pD₂ value was 6.3 in the stomach fundus preparation used in this experiments. The minimum dose of Ach which showed the response was 1 × 10⁻⁸ g/ml and the maximum contraction was observed at a dose of 1 × 10⁻³g/ml. It is noted that the dose response curve of Ach in the fundus preparation comprises wide range of the doses as compared with that in preparations of ileum and uterus.

The hexane fraction afforded the greatest effect and the activity was a half of that of papaverine weight for weight.

GC-MS data (Table II) of the hexane fraction indicate the presence of eucarvone, dimethoxytoluene, safrole and methyleugenol which were already isolated from this plant⁸⁾.

Each compound was identified by comparison of their retention times and mass spectra with

Table I. Spasmolytic activity of the extract and fractions of *A. sieboldii*

Agonist	Antagonist	No. of assays	pD ₂ value (M±S.E.)	pD' ₂ value (M±S.E.)	Relative potency
Acetylcholine chloride		32	6.3±0.28	—	—
	Papaverine	10	—	4.6±0.38	1
	MeOH Ext.	10	—	2.9±0.45	0.02
	Hexane Fr.	10	—	4.3±0.53	0.5
	CHCl ₃ Fr.	10	—	3.3±0.53	0.05
	BuOH Fr.	6	—	1.6±0.42	0.001
	H ₂ O Fr.	2	—	No inhibition	—

Table II. GC-MS of the hexane fraction

No.	Rt (min)	Area(%)	M ⁺ (m/z)	Fragment ions(m/z)					Assignment
1	8.30	5	150	135	122	107	91	77	eucarvone
2	9.93	5.5	152	137	123	109	91	77	dimethoxytoluene
3	10.38	5.5	162	135	131	104	91	77	safrole
4	12.77	84.0	178	163	151	147	135	115	methyleugenol
				103	91	77			

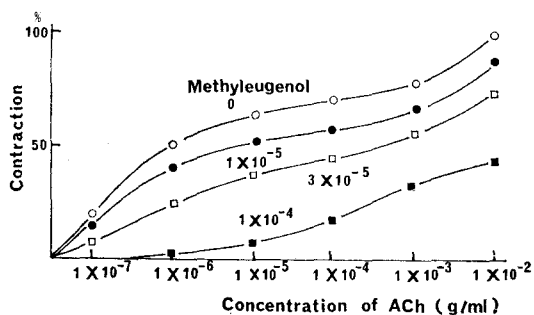


Fig. 1. Cumulative dose response curves of acetylcholine in the presence of methyleugenol.

those of authentic samples and/or with data reported in literatures^{6,19}). Comparison of each peak area shows methyleugenol which is a major component. Therefore, spasmolytic activity test was contacted with pure methyleugenol (Fig. 1) to give pD'_2 value of 4.3 ± 0.2 , suggesting that methyleugenol is a principal spasmolytic constituent of the plant.

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