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Allelopathic Substances in Egoma, *Perilla frutescens* var. japonica

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Synopsis

A plant growth inhibitor was isolated from the egoma plant, and its allelopathic activity on various plant species was investigated. The essential oils obtained from egoma leaves and stems strongly inhibited the growth of lettuce and large crabgrass seedlings. The essential oil from the roots had only weak inhibition. The inhibitor isolated from egoma leaf oil was identified as a monoterpene, the perilla ketone.

Perilla ketone isolated from egoma leaves or the leaf oil also inhibited the growth of lettuce and large crabgrass. The ketone completely inhibited the radicle elongation of lettuce and the large crabgrass at concentrations of from 50 to 100 ppm, but it did not inhibit the germination of lettuce seeds. The egoma leaf oil and perilla ketone from leaves also inhibited the growth of microorganisms. These results suggested that the best source of the inhibitor is egoma leaves and that the inhibition of the growth of other plant by egoma arises from the perilla ketone.

Introduction

Fields continuously cultivated with egoma, Perilla frutescens var. japonica, are often observed to produce fewer weeds than nomar[1,2]. This phenomenon suggests that egoma is allelopathic to competing plant species. Harada[3] identified perilla ketone as a substance that inhibits plant growth. Okada et al.[4] investigated plant growth inhibitory substances in organic solvent extracts from egoma roots, and identified several fatty acids. However, to prove the presence of allelopathic effects, it is necessary to demonstrate the release or secretion of the inhibitory substances from the donor plant.

Here, a plant growth inhibitor in egoma was isolated and identified. The allelopathic activity on certain plant species under various conditions was studied.

Materials and Methods

1. Isolation and identification of essential oils

Egoma plants were cultivated from April to September 1986 on lant previously used for the same purpose in Kyoto Prefecture. Twenty kilograms egoma plants were separated into roots, stems, and leaves, and the essential oils were obtained from each kind of tissue by steam distillation for 20 hr followed by hexane extraction of the distillate. The components of the

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essential oils were fractionated by silica gel column chromatography with hexane, and the inhibitory substance in each fraction was detected by a lettuce bioassay test. After purification by thin-layer chromatography (plate, Merc Kieselgel 60, 2 mm, with hexane as developer), the component was identified by gas chromatography (GC), gas chromatography-massspectroscopy (GC-MS), ^{1}H-NMR, ^{13}C-NMR, IR, and UV spectroscopy. GC was done with a Yanagimoto apparatus (G-2800) on a column (0.2×200 cm) of 3% OV-17 Gaschrom Q, 60-80 mesh (100-180°C, 4°C/min). GC-MS was performed with a Shimadzu LKB-9000B combined gas chromatography-mass spectrometer. The component was chromatographed on a glass column (3×300 cm) packed with 3% OV-17 on Chromosorb W at 100-180°C. The molecular separator and ion source were kept at 240°C. The electron energy was 20 eV, the accelerating voltage, 3500 V, and the ionizing current, 60 uA. ^{1}H-NMR and ^{13}C-NMR were recorded with a JEOL model JNM-FX60Q Fourier transform in $CDCl_3$ with Me_4Si as the internal standard. IR spectra were recorded on a Shimadzu IR-27C spectrophotometer and UV spectra on a Shimadzu UV-240 apparatus.

2. Inhibitory activity of the essential oils

Lettuce, *Lactuce sativa* L. c.v. new york, and large crabgrass, *Degitaria adsendens* Henr., were used for the bioassays. The inhibition of seedling growth was tested at 23°C for 72 hr by transplantation of 20 germinated onto filter paper moistened with an essential oil. The lengths of the hypocotyl, leaf sheath, and radicle were measured.

Goseous components released from the essential oils were bioassayed as described here. A 3 cm petri dish containing 5 ml of an aqueous suspension of a known amount of the essential oil was placed at the center of a 9 cm petri dish in which a moistened piece of filter paper was placed.

Fifty lettuce seeds were placed evenly over the wet paper. The seeds were covered with a lid and incubated at 23°C in the dark for 5 days.

3. Detection of the volatile compound released from egoma leaves

A 500 ml flask was filled with 100 g of egoma leaves, stoppered, and kept at 20°C for GC analysis. One kilogram of soil collected from the egoma field was extracted with hexane, the extract was concentrated to a small volume and then studied by GC.

4. Antimicrobial activities of the essential oils

The following microorganisms were used for bioassays, *Helminthosporium velutinum*, *Corynebacterium aquaticum*, *Fusarium solani*, *Aspergillus niger*, *Aspergillus oryzae*, *Escherichia coli*, and *Bacillus subtilis*. The surface of an agar culture medium in a 9 cm petri dish was inoculated. An 8 mm paper disk on which a known amount of an essential oils had been placed was put at the center of each culture, which was incubated at 27-30°C for 2 days.

Results and Discussion

1. Composition of the essential oils

Egoma has a characteristic aroma, so we adopted the common steam distillation method for preparation of the essential oils. The oil content was 0.124% in the leaves, 0.046% in the stems, and 0.003% in the roots on the basis of fresh weight. The gas chromatograms of the essential oils from leaves and stems were simple, showing compound A as a main component, but the gas chromatogram from the roots was quite different, showing compound A as a minor component (Fig. 1).

The gas chromatograms of the gas released from the leaves and of the extract from soil in which egoma had been cultivated were similar to those of essential oils from egoma leaves and stems. Compound A was the main compound (Fig. 2). For its identification, compound A was isolated from the oils of leaves and stems by column chromatography and was studied by instrumental analysis. From the results shown in Table 1 and Fig. 3, we identified compound A

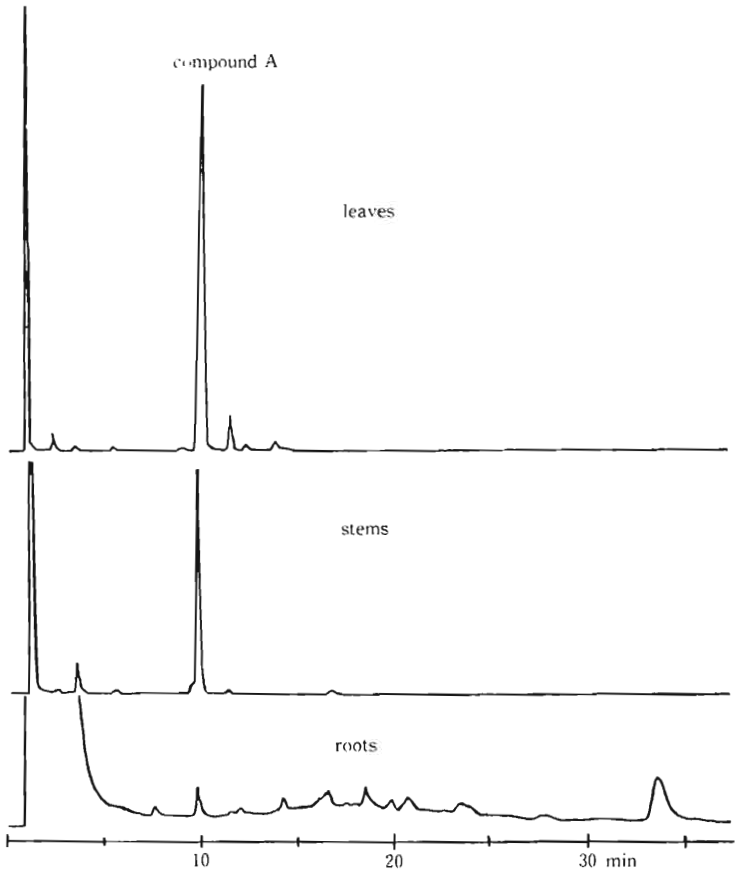

Fig. 1. GC of the essential oils from egoma plant.

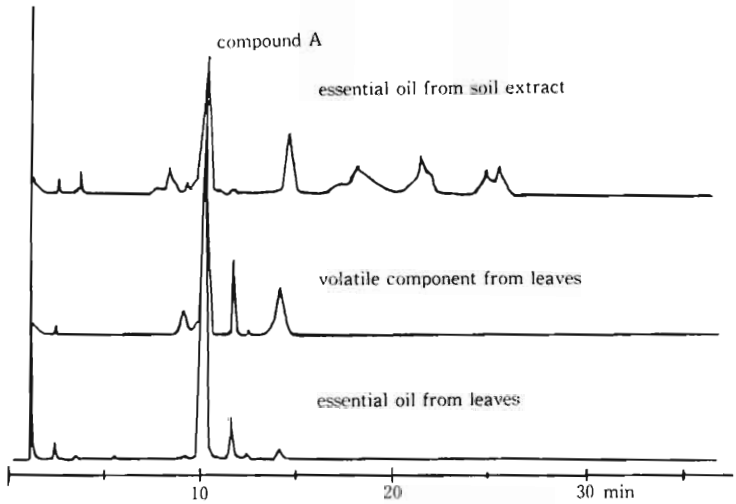

Fig. 2. GC of the essential oils extracted from leaves or from soil in which egoma had been cultivated and the volatile component from egoma leaves.

Table 1. Spectral data for compound A.

IR $\nu_{max}^{CCl_4}$ cm^{-1}	2960, 1685(C=O), 1562, 1510(C=C) 1159(-O-), 872(-O-)
Mass m/z	166(M^+), 151, 123, 110, 95
UV λ_{max}^{MeOH}	209, 244(α, β-unsaturated C=O)
^{1}H-NMR($CDCl_3$, δ)	0.9-1.00(6H, d, $CH<^{CH_3}_{CH_3}$) 1.6(3H, m, $-CH_2-CH_2-CH$) 2.8(2H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-$) 6.8(1H, S, furan) 7.5(1H, S, furan) 8.1(1H, S, furan)

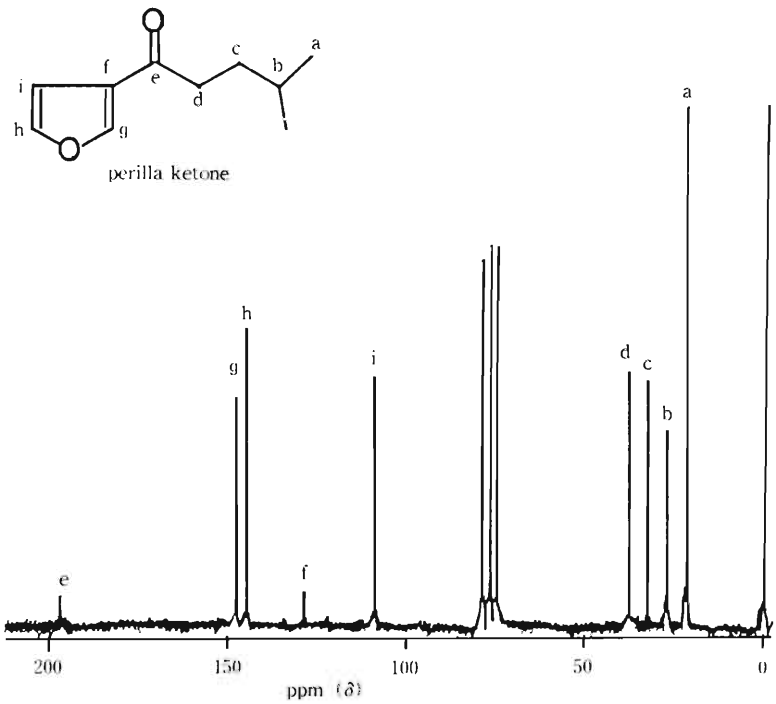

Fig. 3. ^{13}C-NMR spectrum of compound A

as perilla ketone[5], 1-(3-furanyl)-4-methyl-1-pentanone. GC-MS showed that the molecular ion (M^+) has the M/Z ratio of 166.

2. Biological activity of the essential oils

Table 2 shows the inhibitory effects of the oils on lettuce seedlings. The essential oils from leaves and stems strongly inhibited the elongation of radicles at 100 ppm and completely inhibited elongation at 500 ppm. The essential oil from roots. had little inhibitory effect at 500 ppm. None of these essential oils inhibited the germination of lettuce seeds. Table 3 shows the inhibition by the essential oils of the growth of large crabgrass. The development of radicles was completely inhibited by the oils from leaves and stems at 100 ppm and strongly inhibited development at 50 ppm. The oil from the roots caused weak inhibition at 100 ppm as in the lettuce bioassay.

Table 2. Effects of egoma essential oils on the growth of lettuce seedlings.

oil	conc.(ppm)	hypocotyl length(%)	radicle length(%)	fresh weight(%)
I	500	—	—	—
	100	62.5*	48.5*	50.1*
	50	76.4*	50.7*	68.0*
II	500	—	—	—
	100	77.4*	52.0*	59.8*
	50	81.1*	58.5*	66.4*
III	500	92.0	89.0	84.6*
	100	102.2	100.5	97.3
	50	101.9	105.8	100.0
IV	500	—	—	—
	100	51.4*	50.7*	47.2*
	50	69.1*	53.4*	64.9*

*Experimental values are significantly different from the control at the 5% level

—, Brown withering during incubation

Control (zero concentration) = 100%

I. Extract from leaves; II. Extract from stems; III. Extract from roots; IV. Isolated compound A, the perilla ketone

Lengths of control: hypocotyl, 11.0 mm; radicle, 20.5 mm.

Fresh weight, 24.2 mg, n=20 seedlings.

Table 3. Effects of egoma essential oils on the growth of large crabgrass seedlings

oil	conc.(ppm)	plant length(%)	radicle length(%)	fresh weight(%)
I	500	—	—	—
	100	18.7*	—	20.6*
	50	41.0*	15.4*	46.9*
II	500	—	—	—
	100	28.9*	12.5*	29.4*
	50	55.0*	30.1*	46.2*
III	500	78.3*	64.6*	67.0*
	100	91.5	88.7	92.8
	50	101.3	90.2	96.5
IV	500	—	—	—
	100	9.4*	—	15.4*
	50	37.8*	27.5*	29.6*

*Experimental values are significantly different from control at the 5% level.

—, Brown withering during incubation

Control (zero concentration) = 100%

I. Extract from leaves; II. Extract from stems; III. Extract from roots; IV. Isolated compound A, the perilla ketone

Lengths of control: Plant length, 10.4 mm; radicle, 13.8 mm Fresh weight, 26.1 mg, n=20 seedlings

An experiment was next done, and the results used to make deductions about the allelopathic effects of egoma. The inhibitory activity of the volatile component released from the essential oils is shown in Table 4. Elongation of both hypocotyles and radicles of lettuce was inhibited by the bolatile component released from an 50 ppm aqueous suspension of an essential oil. Large

crabgrass was more susceptible to inhibition than lettuce. Similar results were obtained by fumigation of the test plants with perilla ketone. The results suggested that the main source of the inhibitor was the leaves of the egoma plant and that the inhibition of the growth of other plants by egoma was caused by perilla ketone.

Table 4. Effects of volatile component from egoma leaf oil and of perilla ketone on the growth of lettuce and large crabgrass seedlings

	oil	conc.(ppm)	hypocotyl length(%)	radicle length(%)	fresh weight(%)
A	I	500	35.6*	21.6*	43.3*
		100	42.3*	74.3*	68.0*
		50	48.8*	62.0*	71.3*
	II	500	37.4*	29.3*	41.3*
		100	43.5*	86.9*	96.0
		50	44.7*	75.8*	86.7*
B	I	500	11.8*	9.0*	23.5*
		100	14.0*	8.5*	37.4*
		50	59.5*	20.1*	56.2*
	II	500	15.2*	14.4*	27.8*
		100	15.5*	22.1*	39.7*
		50	46.4*	25.0*	41.1*

A. Lettuce ; B. Large crabgrass
I. egoma leaf oil ; II. perilla ketone isolated from leaf oil
*Experimental values are singificantly different from control at the 5% level.
Control (zero concentration) = 100%
Lengths of control : hypocotyl, 16.4 mm ; radicle, 23.0 mm
Fresh weight, 22.8 mg, n = 20 seedlings

The egoma leaf oil and perilla ketone also strongly inhibited the growth of microorganisms (Table 5), although *Aspergillus niger* and *Aspergillus oryzae* were somewhat resistant.

In general, compounds with an unsaturated carbonyl group readily undergo the Michael reaction with nucleophilic groups such as SH groups. This reaction seems to inhibit plant growth[5,6]. Accordingly, the inhibitory effect of perilla ketone may be due to the unsaturated ketone in its structure. The compound seems to act on other plants after vaporizing from the leaves.

Table 5. Inhibition by leaf oil and perilla ketone of the growth of microorganisms.

microorganism	essential oil	perilla ketone
Cladosporium cucumerinum	+	++
Helminthosporium velutinum	+	++
Corynebacterium aquaticum	+	++
Fusarium solani	+	+
Aspergillus niger	−	±
Aspergillus oryzae	−	±
Escherichia coli	++	++
Bacillus subtilis	++	++

++ ; Strong activity, + ; Moderate activity, − ; Low activity

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エゴマの他感作用について

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摘　　要

エゴマの他感作用に関与する物質の調査を実施した．エゴマ植物体の茎葉部から得た精油に強い生長抑制作用が示された．しかし根茎部の精油には強い抑制活性は認められなかった．そこで茎葉部からの精油より活性成分モノテルペンケトン，ペリラケトンを単離同定した．

本成分はレタス及びメヒシバの幼苗の生長を50 ppmから100 ppmで著しく阻害した．その阻害は茎葉部よりも根部の伸長に対して著しかった．しかし本成分はレタス種子の発芽には全く阻害作用を示さなかった．更にペリラケトンは数種微生物にも強い抑制作用を示した．

ペリラケトンはエゴマの葉部よりガス状で揮散し，アレロパシー活性を示すと共にその一部は土壌表面にも吸着されることが確認された．従ってエゴマの他感作用は主として葉部組織から揮散したペリラケトンがガス状又は土壌を介して近傍の植物及び土壌微生物に影響を及ぼすものと推察される．