

Insecticidal activities of asarones identified in *Acorus gramineus* rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutoidea)

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Abstract

The insecticidal activities of compounds derived from the rhizomes of *Acorus gramineus* against four agricultural insect pests were examined using direct contact application method. The biologically active constituents of *A. gramineus* rhizomes were characterized as the phenylpropenes, *cis*- and *trans*-asarones by spectroscopic analyses. Potencies varied according to insect species, compound, and dose. In a test with female adults of *Nilaparvata lugens*, *cis*-asarone caused 100, 83, and 40% mortality at 1,000, 500, and 250 ppm, respectively, whereas 67% mortality was achieved at 1,000 ppm of *trans*-asarone. Against 3rd instar larvae of *Plutella xylostella*, *cis*-asarone gave 83 and 50% mortality at 1,000 and 500 ppm, respectively, whereas *trans*-asarone at 1,000 ppm showed 30% mortality. Against female adults of *Myzus persicae* and 3rd instar larvae of *Spodoptera litura*, *cis*- and *trans*-asarones both were almost ineffective at 2,000 ppm. The *A. gramineus* rhizome-derived materials merit further study as potential insect-control agents or as lead compounds against *N. lugens* and *P. xylostella*.

Key words: Natural insecticide, *Nilaparvata lugens*, *Plutella xylostella*, *Acorus gramineus*, asarone

INTRODUCTION

Over several decades, insect pests have been controlled mainly by synthetic insecticides. Although effective, their repeated use has disrupted natural biological control systems and led to resurgence of insect species, sometimes resulting in the development of resistance, undesirable effects on non-target organisms, and fostered environmental and human health concerns (Brown, 1978; Georgiou and Saito, 1983; Hayes and Laws, 1991). These problems have highlighted the need for the development of new strategies for selective insect pest control.

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Much effort has been focused on plant-derived materials as potential sources of commercial insect-control agents or as lead compounds (Arnason et al., 1989a; Miyakado et al., 1989; Isman, 1995; Hedin et al., 1997). Jacobson (1989) has

pointed out that the most promising botanical insect-control agents are in the families Annonaceae, Asteraceae, Canellaceae, Labiatae, Meliaceae, and Rutaceae. Methanol extract of the rhizomes from *Acorus gramineus* Solander, belonging to the family Araceae, has insecticidal activity against female adults of *Nilaparvata lugens* (Stål) and larvae of *Plutella xylostella* (L.) (Kim et al., 2001).

In the laboratory study described herein, we have examined the methanol extract of the rhizomes from *A. gramineus* for insecticidal constituents active against four economically important agricultural insect pests [*N. lugens*, *Myzus persicae* (Sulzer), *P. xylostella*, and *Spodoptera litura* (F.)] using direct contact application method.

MATERIALS AND METHODS

Chemicals. *cis*- and *trans*-Asarones were purchased from Aldrich (Milwaukee, WI, USA). Triton X-100 was supplied by Coseal (Seoul, Korea). All other chemicals were of reagent grade.

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Insects. The susceptible strains of *N. lugens*, *M. persicae*, and *P. xylostella* have been maintained in the laboratory without exposure to any insecticide on *Oryza sativa* L. seedlings (7–10 days after germination), *Nicotiana tabacum* L., and *Raphanus sativus* L. seedlings (5–6 days after germination), respectively, in acrylic cages at $25 \pm 1^\circ\text{C}$, 40–60% RH, and a photoregime of 16 : 8 (L : D) h. The laboratory-reared strain of *S. litura* was reared on an artificial diet (Im et al., 1988) in plastic containers (28×20×9 cm).

Isolation and identification. Dried rhizomes (5 kg) of *A. gramineus* were purchased from Boeun medicinal herb shop, Kyungdong market, Seoul. It was finely powdered, extracted twice with methanol (20 l) at room temperature and filtered. The combined filtrate was concentrated under vacuum at 45°C to yield about 15% (based on the weight of the dried rhizome). The extract (40 g) was sequentially partitioned into hexane (10.0 g), chloroform (14.8 g), ethyl acetate (2.4 g), and water (12.8 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 45°C , and the water portion was freeze-dried.

Isolation procedures for constituents of *A. gramineus* rhizomes active against test insects were performed as previously described (Park, 2000). For isolation, 2,500 ppm of each *A. gramineus* rhizome-derived fraction in acetone was applied as described below. The hexane portion (10 g) was chromatographed on a silica gel column (Merck 230–400 mesh, 500 g, 6×80 cm), and successively eluted with a stepwise gradient of chloroform/methanol (100/0, 95/5, 90/10, 75/25, 50/50, and 0/100, v/v). Column fractions were analyzed by TLC (silica gel G), and fractions with a similar TLC pattern were pooled. The bioactive fraction (7.3 g) was successively rechromatographed on a silica gel column, using hexane/ethyl acetate (150 : 1). For further separation of the constituents from the active fraction (2.3 g), preparative high-performance liquid chromatography (HPLC) (Spectra System P2000, Thermo Separation Products) was used. The column was a 39 i.d.×300 mm μ Bondapak C_{18} (Waters) with methanol/water (3 : 7) at a flow rate of 3 ml/min and detection at 254 nm. Finally, two active principles **1** (89 mg) and **2** (23 mg) were isolated.

The structures of the active isolates were deter-

mined by spectroscopic analyses. ^1H - and ^{13}C -NMR spectra were recorded in deuteriochloroform with a BRUKER AM-500 spectrometer at 400 and 100 MHz, respectively. UV spectra were obtained in methanol with a JASCO V-550 spectrometer and EI-MS spectra on a JEOL GSX 400 spectrometer.

Bioassay. Spray method was used for *N. lugens*. Ten female adults (3 to 5 days old) were transferred into a test tube (3×15 cm) containing five rice plant seedlings (7–10 days after germination) wrapped with cotton and water (10 ml). Each *A. gramineus* rhizome-derived fraction and isolate (dissolved in 4 ml acetone) was suspended in distilled water with Triton X-100 (0.1 ml/l). Controls received acetone-Triton X-100 solution. Test material solutions were applied at a rate of 0.1 ml per test tube by a glass spray unit connected to a forced air supply (Pacific Chemical, Seoul).

The toxicity of *A. gramineus* rhizome-derived fractions and isolates to the aphid and two lepidopteran larvae was examined by leaf dipping assay. Cabbage (*Brassica oleracea* L., 25 days old) leaves for 3rd instar larvae of *P. xylostella* and *S. litura*, and tobacco leaves for *M. persicae* female adults from each plant species grown in a glasshouse were collected, and discs (5.5 cm in diameter) were punctured from each leaf. Leaf discs were dipped in each test solution (20 ml) described above for 30 s. Controls received acetone-Triton X-100 solution. After drying in a fume hood for 30 min, 10 individuals each of *P. xylostella*, *S. litura*, and *M. persicae* were separately placed onto the treated and the control leaf discs in Petri dishes (6×1.5 cm).

Treated and control insects were held at the same conditions used for colony maintenance. Mortalities were determined 48 h after treatment. Test insects were considered dead if appendages did not move when prodded with a camel's hair brush. All treatments were replicated nine times.

Statistical analysis. The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffé's test at $p=0.05$ (SAS Institute, 1990). Means (\pm SE) of untransformed data are presented.

RESULTS

Identification

When fractions obtained from the methanol extract of *A. gramineus* rhizomes were bioassayed according to direct contact application, significant differences were observed in toxicity to the insect species (Table 1). At a concentration of 2,500 ppm, the hexane fraction showed potent insecticidal activity against *N. lugens* females and *P. xylostella* larvae. However, moderate and weak activity against *M. persicae* females and *S. litura* larvae was observed. Purification of the biologically active constituents from the hexane fraction was done by silica gel chromatography and HPLC.

Bioassay-guided fractionation of *A. gramineus* rhizome extract afforded two active constituents identified by spectroscopic analyses, including MS and NMR, and by direct comparison with authentic compounds. The active constituents were characterized as the phenylpropenes, *cis*-asarone (1) and

trans-asarone (2) (Fig. 1).

Insecticidal activity

The toxicity of *A. gramineus* rhizome-derived *cis*- and *trans*-asarones against *N. lugens* females was examined by spray application method (Table 2). Potencies varied according to compound and dose. *cis*-Asarone (1) caused 100 and 83% mortality at 1,000 and 500 ppm, respectively, whereas

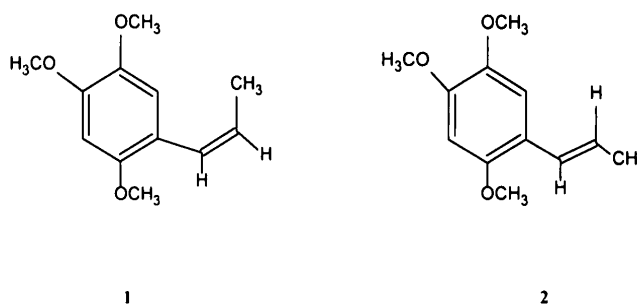


Fig. 1. Structures of the phenylpropenes, (*cis*-)asarone (1) and (*trans*-)asarone (2), insecticidal constituents of *Acorus gramineus* rhizome.

Table 1. Toxicity of *A. gramineus* rhizome-derived materials to four insect pests, evaluated by direct contact application

Material ^a	Mortality, % (mean ± SE) ^b			
	<i>N. lugens</i>	<i>M. persicae</i>	<i>P. xylostella</i>	<i>S. litura</i>
Hexane	100 ± 0.0 a	50 ± 3.7 a	100 ± 0.0 a	20 ± 2.9 a
Chloroform	57 ± 1.7 b	13 ± 3.3 b	93 ± 1.7 b	0 ± 0.0 c
Ethyl acetate	10 ± 2.9 c	0 ± 0.0 c	27 ± 1.7 c	10 ± 1.7 b
Water	0 ± 0.0 d	0 ± 0.0 c	0 ± 0.0 d	0 ± 0.0 c

^a Exposed to 2,500 ppm.

^b Means within a column followed by the same letter are not significantly different at $p=0.05$ (Scheffé's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (\pm SE) of untransformed data are reported.

Table 2. Toxicity of *A. gramineus* rhizome-derived asarones to four insect pests, evaluated by direct contact application

Compound	Conc. (ppm)	Mortality, % (mean ± SE) ^a			
		<i>N. lugens</i>	<i>M. persicae</i>	<i>P. xylostella</i>	<i>S. litura</i>
<i>cis</i> -Asarone	2,000	100 ± 0.0 a	53 ± 1.7 a	100 ± 0.0 a	13 ± 1.7 ab
	1,000	100 ± 0.0 a	20 ± 3.3 b	83 ± 1.7 b	10 ± 2.4 ab
	500	83 ± 1.7 b	7 ± 1.7 c	50 ± 2.9 c	11 ± 3.5 ab
	250	40 ± 2.9 d	0 ± 0.0 d	33 ± 1.7 d	3 ± 1.7 b
<i>trans</i> -Asarone	2,000	100 ± 0.0 a	0 ± 0.0 d	100 ± 0.0 a	20 ± 4.1 a
	1,000	67 ± 2.9 c	3 ± 1.7 cd	30 ± 3.7 d	13 ± 1.7 ab
	500	40 ± 2.4 d	0 ± 0.0 d	7 ± 1.7 e	13 ± 1.7 ab
	250	13 ± 1.7 e	0 ± 0.0 d	0 ± 0.0 f	7 ± 1.7 ab

^a Means within a column followed by the same letter are not significantly different at $p=0.05$ (Scheffé's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (\pm SE) of untransformed data are reported.

40% mortality was achieved at 250 ppm. *trans*-Asarone (**2**) was much less active than *cis*-asarone. There was no mortality in the untreated controls.

The insecticidal activity of the test compounds against *M. persicae* females was determined by leaf dipping application method (Table 2). *cis*- and *trans*-Asarones exhibited weak or no insecticidal activity at 1,000 ppm.

Toxic effects of the test compounds on 3rd instar larvae of *P. xylostella* and *S. litura* were assessed by leaf dipping application method (Table 2). Significant differences were observed in toxicity to the lepidopteran larvae. Against *P. xylostella* larvae, *cis*-asarone showed 83 and 50% mortality at 1,000 and 500 ppm, respectively, whereas *trans*-asarone at 1,000 ppm gave 30% mortality. *cis*- and *trans*-Asarones showed no insecticidal activity against *S. litura* larvae at 2,000 ppm. No mortality was observed in the untreated controls.

DISCUSSION

In East Asia, *A. gramineus* rhizomes has long been considered to have medicinal properties attributable to various constituents such as *cis*-asarone, *trans*-asarone, isoasarone, caryophyllene, *cis*-methyl isoeugenol, and safrol (Tang and Eisenbrand, 1992). It has been reported that extracts of *A. gramineus* rhizomes possess insecticidal activity against adults of *Sitophilus oryzae* (L.), *Callosobruchus chinensis* (L.), and *Lasioderma serricorne* (F.) (Park, 2000) and *N. lugens* females and *P. xylostella* larvae (Kim et al., 2001). Bioefficacy of *Acorus* species against insects has been well described (Koul, 1995). In this present study, *A. gramineus* rhizome-derived materials exhibited insecticidal activity against *N. lugens* females and *P. xylostella* larvae, whereas weak activity against *M. persicae* females and *S. litura* larvae was observed.

It has been well recognized that plant extracts and phytochemicals can be developed into products suitable for integrated pest management because many of them are selective to pests, have no or little harmful effects on non-target organisms and the environment, act in many ways like neem extracts on various types of pest complex, and may be applied to the plant in the same way as conventional insecticides (Arnason et al., 1989a; Schmutterer, 1992; Hedin et al., 1997). Derivatives of *Azadirachta indica* A. Juss, belonging to the family

Meliaceae, are found to have a variety of biological activities including insecticidal activity against nearly 200 species of insects without any adverse effects on most non-target organisms (Saxena, 1989; Lowery and Isman, 1995). Additionally, certain plant-derived compounds were found to be highly effective against insecticide-resistant insect pests (Arnason et al., 1989b; Schmutterer, 1992; Ahn et al., 1997). Much concern has been focused on the distribution, nature, and practical use of plant-derived chemical substances having insecticidal activity. In the present study, the insecticidal constituents of *A. gramineus* rhizomes were identified as the phenylpropenes, *cis*- and *trans*-asarones with species selective activity. *cis*- and *trans*-Asarones were effective against 3rd instar larvae of *P. xylostella* but not *S. litura*. The differential susceptibility of asarones to these two lepidopteran larvae might be attributable to differences in biological factors (e.g. body weight), environmental factors (e.g. temperature), and physiological or biochemical characteristics such as penetration and detoxifying enzyme activity (e.g. mixed-function oxidases, hydrolases, and glutathione *S*-transferases) (Hollingworth, 1976; Eda, 1985). *cis*-Asarone exhibits antifeeding and growth-inhibiting effects on larvae of *Peridroma saucia* (Hübner) (Koul et al., 1990), antigonadal activity against some insect species (Saxena et al., 1977; Matolcsy et al., 1981; Schmidt and Brochers, 1981), repellent effects on many insects (Koul et al., 1990), and attractant effects for *Ceratitis capitata* (Wiedemann), *Dacus cucurbita* (Coquillett), and *D. dorsalis* (Hendel) (Jacobson et al., 1976). In contrast, *trans*-asarone has antifeeding and growth-inhibiting effects on larvae of *P. saucia* (Koul et al., 1990), oviposition-stimulating effects on the carrot rustfly (Stadler and Buser, 1984), and feeding-deterrent activity against some stored foods Coleoptera (Poplawski et al., 2000).

Structure-activity relationships in insects have been well studied. Park (2000) reported that the insecticidal activity of *cis*-asarone was more pronounced against adults of *S. oryzae*, *C. chinensis*, and *L. serricorne* than that of *trans*-asarone. It has been well established that changes in the phenyl substituents influence the chemosterilant activity of asarones against *Dysdercus koenigii* (Walk) significantly (Saxena et al., 1977). In this study, *cis*-asarone was a more potent insecticidal agent

against *N. lugens* females and *P. xylostella* larvae than *trans*-asarone. These results indicate that the toxicity of asarones might be due to the *cis* configuration rather than to the position of the double bond.

Results of this and earlier studies indicate that *A. gramineus* rhizome-derived materials against *N. lugens* and *P. xylostella* can be developed as insect-control agents or lead compounds. However, *cis*-asarone is known to possess *in vivo* hepatocarcinogenic effects (Wiseman et al., 1987), *in vitro* mutagenic activity against *Salmonella typhimurium* TA 100 (Gogglemann and Schimmer, 1983), and effects of induction of structural chromosome aberrations in human lymphocytes *in vitro* (Abel, 1987), whereas *trans*-asarone has sister-chromatid exchange induction effects *in vivo* and *in vitro* (Morales-Ramirez et al., 1992) and *in vivo* hepatocarcinogenic effects (Wiseman et al., 1987). Direct use of asarones as insecticides would not be allowed due to the above mentioned chronic toxicity, although they have low acute toxicity to mammals (Budavari et al., 1989). For practical use of these compounds as novel insecticides, further studies are essential to evaluate the hazards of natural and synthetic asarone analogs to workers during application of the compounds or consumers from residual materials on crops, their exact mode-of-action, and their effects on non-target organisms and the environment.

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