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MOSQUITO LARVICIDAL ACTIVITY OF PIPERNONALINE, A PIPERIDINE ALKALOID DERIVED FROM LONG PEPPER, *PIPER LONGUM*

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ABSTRACT. A methanol extract of *Piper longum* fruit was found to be active against mosquito larvae of *Culex pipiens pallens* at 10 μg/ml after 24 h. A piperidine alkaloid, pipernonaline, was found to be responsible for this activity, with the 24-h median lethal dose (LD_{50}) value for this compound being 0.21 mg/liter. The LD_{50} value of pipernonaline was not much higher than those for the 3 organophosphorous insecticides malathion, chlorpyrifos-methyl, and pirimiphos-methyl, used for comparative purpose in this study. Structural elucidation of pipernonaline was by means of mass spectrometry (^1H and ^{13}C nuclear magnetic resonance imaging).

KEY WORDS *Piper longum*, pipernonaline, mosquito larvicidal activity, *Culex pipiens*

INTRODUCTION

Mosquito-borne diseases such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis contribute significantly to disease burden, death, poverty, and social debility in the world. Among these diseases, malaria continues to be a major public health problem in most countries of the tropical world. For example, the World Health Organization (WHO) reported that 2200 million people were exposed to malarial infections in some 90 countries or areas and that malaria was also the cause of an estimated 1.4–2.6 million deaths worldwide every year, with more than 90% of these deaths occurring in Africa alone (WHO 1992). However, control of malarial and other mosquito-borne diseases is becoming increasingly difficult. The effectiveness of vector control has declined because of reduced effectiveness of insecticides caused by emergence of resistance in mosquitoes against the currently used insecticides (Chandre et al. 1998, Penilla et al. 1998). It is also important to recognize that only a limited number of pesticides are available for use in public health. Therefore, an effort to find alternatives for the currently used insecticides in relation to mosquito control may be needed.

The use of botanical derivatives in the control of mosquito larvae may offer a more environmentally safe method of insect control than the use of synthetic chemicals. In this paper, I report isolation procedures and structural determinations of a piperidine alkaloid active against mosquito larvae of *Culex pipiens pallens* Coq. from the methanolic extract of the dried fruits of the long pepper, *Piper longum* L.

MATERIALS AND METHODS

Extraction and isolation.

Dried fruits (2.5 kg) of *P. longum*, obtained from a traditional market in Seoul, were crushed and extracted twice with methanol (10 liters) at room temperature and filtered (Toyo filter paper no. 2, Tokyo, Japan). The combined filtrate was concentrated in vacuo at 35°C to yield about 9.8% (on the basis of the weight of the dried fruit). The extract (20 g) was sequentially partitioned into hexane (3.8 g), chloroform (4.3 g), ethyl acetate (1.4 g), and water-soluble (10.5 g) portions for subsequent bioassay with 3rd instars of *Cx. p. pallens*. The organic solvent portions were concentrated to dryness by rotatory evaporation at 35°C, and the water portion was freeze-dried. The active hexane portion was chromatographed on a silica gel column (Merck 70-230 mesh, 300 g, 4.5 inner diameter × 60 cm; Merck, Whitehouse Station, NJ) and successively eluted with hexane-ethyl acetate, ethyl acetate, and ethyl acetate-methanol. The active fractions eluted with hexane-ethyl acetate (3:1) were chromatographed on a silica gel column and eluted with hexane-ethyl acetate (4:1). Column fractions were collected and analyzed by thin-layer chromatography (TLC; hexane-ethyl acetate, 3:1). Fractions with a similar TLC pattern were combined. For further separation of the mosquito larvicidal substances, a Waters Delta Prep 4000 high-performance liquid chromatography (Waters Co., Milford, MA) was used. The column was 29 inner diameter × 300 mm Bondapak C18 (Waters) using methanol:water (3:7) at a flow rate of 7 ml/min and detection at 260 nm. Compound 1 (28 mg) was isolated.

Structural determination of the active isolate was based on spectral analysis. ^1H (400 MHz) and ^{13}C nuclear magnetic resonance (NMR; 100 MHz) spectra were measured in dimethylsulfoxide-d_6 at room temperature on a Bruker AMX-400 (Bruker Instrument Inc., Fremont, CA) with tetramethylsilane as an internal standard. Mass spectra were done on a JEOL JMS-DX30 spectrometer (JEOL USA Inc., Fremont, CA).

Bioassay

Five hundred female mosquitoes, *Cx. p. pallens* (F-256 larvae were acquired from Korean Food and

Table 1. Regression parameters for mortality response of 3rd-stage *Culex pipiens pallens* Cog. larvae exposed to pipernonaline and 3 organophosphorous insecticides: chlorpyrifos-methyl, pirimiphos-methyl, and malathion.[1]

Compound	LD_{50}		LD_{95}		Slope
Pipernonaline	0.21	(0.17–0.28)	0.52	(0.48–0.57)	2.1
Chlorpyrifos-methyl	0.00037	(0.00022–0.00058)	0.0011	(0.00078–0.0015)	4.6
Malathion	0.016	(0.009–0.023)	0.078	(0.056–0.096)	2.5
Pirimiphos-methyl	0.11	(0.09–0.14)	0.17	(0.15–0.18)	7.8

[1] Doses expressed in mg/liter; LD_{50}, median lethal dose; LD_{95}, 95% lethal dose.

Drug Administration, Seoul, Korea), were used in this study. All bioassays were done in an environmentally controlled room maintained at 30 ± 1°C and 80 ± 5% relative humidity. Larval testing was done on 5 acetone serial dilutions of pipernonaline, chlorpyrifos-methyl, malathion, and pirimiphos-methyl. One hundred milliliters of each test solution was placed into a Pyrex dish (100 × 50 mm; Fisher Scientific, Pittsburgh, PA) along with thirty 3rd instars. Each dilution, along with an untreated control group (acetone carrier and water), was replicated 5 times. Mortality recorded at 24 h after treatment, median lethal dose (LD_{50}), and 95% lethal dose values were calculated by probit analysis (Finney 1971). Control mortality was accounted for by Abbott's formula (Abbott 1925).

RESULTS AND DISCUSSION

During the initial experiments, we observed that methanolic extract of *P. longum* fruits possessed mosquito larvicidal activity against *Cx. p. pallens,* and also only 1 mg/liter of hexane fraction had a strong larvicidal activity of 100% mortality. Moderate activity and little activity were produced from other organic solvent fractions. No activity was observed in the water fraction. One active isolate from the hexane fraction showed potent larvicidal activity, shown in Table 1, and it was characterized by the spectral analyses as pipernonaline (Fig. 1).

Pipernonaline

Pale yellow needles. UV λ_{max} EtOH: 364.2 nm. Found $[M]^+$ *m/z* 341.2336. EI-MS *m/z* (rel. int.): 341 (45), 273 (18), 228 (37), 206 (61), 193 (20), 166 (100), 153 (30), 131 (75), 103 (75), 84 (45). ^{1}H NMR (δ): 1.28–1.74 (10H, br m), 2.00–2.36 (4H, br m), 3.50 (4H, br m), 5.90 (1H, d, J = 15.0 Hz, C_2-H), 5.92 (2H, s, -OCH_2O-), 6.01–6.38 (2H, m), 6.73 (2H, br s, $C_{5',6'}$-H), 6.80 (1H, dt, J = 15.0, 4.0 Hz, C_3-H), 6.88 (1H, s, $C_{2'}$-H). ^{13}C NMR: Table 2.

The fruits of some *Piperaceae* plants have been known for their use as food flavoring agents and also for containing insecticidal properties (Miyakado et al. 1979, Su and Horvat 1981, Tyagi et al. 1993). Among these components, unsaturated amides constitute a major group of secondary metabolites. Dry black pepper, *Piper nigrum* L., which is available from the supermarket, has been reported to be toxic to houseflies (*Musca domestica* L.), rice weevils (*Sitophilus oryzae* L.), and cowpea weevils (*Callosobruchus maculatus* Fabr.; Su 1977, Scott and McKibben 1978, Su and Horvat 1981). Piperine is easily isolated from the fruit of the black pepper plant, although it is apparently inactive as a contact toxicant (Su and Horvat 1981). However, several insecticidal amides, such as pipericide, (*E,E*)-*N*-(2-methylpropyl)-2,4-decadienamide, (*E,E,E*)-13-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,12-tridecatrienamide, and (*E,E,E*)-11-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,10-undecatrienamide, have been isolated from the pepper plant (Miyakado et al. 1979, Su and Horvat 1981).

Pipernonaline has been isolated from *P. longum* (Tabuneng et al. 1983), but its biological activity has not been determined. Herein, I report that this compound has a potent larvicidal activity against 3rd-stage larvae of *Cx. p. pallens,* with 24-h LD_{50} values of just 0.21 mg/liter (Table 1). This LD_{50} value of pipernonaline was 27-fold, 110-fold, and 245-fold stronger than those of obacunone, nomilin, and limonin, respectively (Jayaprankasha et al. 1997). However, this LD_{50} value of pipernonaline

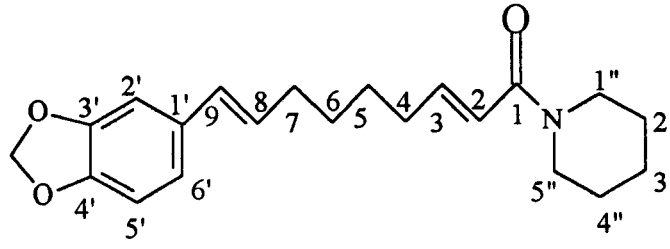

Fig. 1. Structure of pipernonaline isolated from *Piper longum* L.

Table 2. ^{13}C Nuclear magnetic resonance ($CDCl_3$) data of pipernonaline.

	Pipernonaline ^{13}C
1	165.2
2	120.5
3	145.2
4	32.2
5	27.9
6	28.9
7	32.6
8	128.6
9	129.5
1′	132.2
2′	105.2
3′	146.5
4′	147.9
5′	108.0
6′	120.1
–OCH_2O–	100.8
1″	42.9
2″	26.5
3″	24.5
4″	25.6
5″	46.6

was not stronger than those of the 3 organophosphorous insecticides malathion, chlorpyrifos-methyl, and pirimiphos-methyl (Table 1). Recently, 2 reports showed that extract of *Tagetes minuta* L. had strong biocidal effects on both the larvae and adults of *Aedes aegypti* L. and *Anopheles stephensi* L. (Perich et al. 1994). The insecticidal components isolated from the plant extract were 4 thiophenes, 5-(but-3-ene-1-ynyl)-2,2′-bithiophene, 5-(but-3-ene-1-ynyl)-5′-methyl-2,2′-bithiophene, 2,2′,5′,5″-terthiophene, and 5-methyl-2,2′,5′,2″-terthiophene (Perich et al. 1995). These compounds may be considered as alternatives to the currently used insecticides. With these 4 thiophenes, pipernonaline may be a potential candidate for a mosquito larvicidal agent.

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