# ACTINIDINE, A DEFENSIVE SECRETION OF STICK INSECT, *MEGACRANIA ALPHEUS* WESTWOOD (ORTHOPTERA: PHASMATIDAE)

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(Accepted for publication April 21, 1986)

## ABSTRACT

The largest of the stick insects, *Megacrania alpheus* Westwood, has paired thoracic glands which produce five volatile, protective chemicals. In response to irritation, five special odor compounds are ejected of which actinidine is identified as the major constituent by IR, NMR, and GC-Mass spectrometry and elemental analysis. Different juvenile stages contain varying amounts of actinidine. Few nymphs deprived of actinidine survive to maturity because of high incidence of mortality from natural predators.

Key Words: Stick insects, *Megacrania alpheus* Westwood, walking sticks, Phasmatidae, thoracic glands, actinidine, defensive allomones as pheromones, screw-pine, *Pandanus tectorius*, predators.

J. Entomol. Sci. 21(2): 97-101 (April 1986)

## INTRODUCTION

Two classes of defensive compounds, irridodials and nepetalactone, from two species of Phasmida, viz. *Graeffea crouani* (Mainwald et al. 1962) and *Anisomorpha buprestoides* (Smith et al. 1979), have been detected with mass spectrometer combined with gas-liquid chromatographic techniques. In Taiwan, the walking stick insect, *Megacrania alpheus* Westwood, causes considerable damage to screw pine, *Pandanus tectorius* Sol (Fig. 1a) along the southern coast. The insect is the largest species of stick insect in Taiwan and is also found in many other tropical countries such as the Philippines and Solomon Islands. Its morphological features were first described by Shiraki (1935) who considered it a new species and named it *Megacrania tsudai* Shiraki. More recently it was redescribed by Wang and Chu (1982) who offered the present name. When disturbed by other animals, secretions of the gland discharge as a defensive allomone (Fig. 1b, 1c and 1d) in a manner similar to a related species, *A. buprestoides* (Eisner 1965). Because the food plant of *M. alpheus* differs from species studied earlier (Eisner 1965), and because our preliminary study showed the presence of two new defensive pheromone components (Chow and Lin 1983), we studied the chemical composition of the secretions again and report here the true allomone constituents or defensive pheromones.

## MATERIALS AND METHODS

Adults of *Megacrania alpheus* were collected at the O-luan-bi, southern coast area of Taiwan. The adults were reared on the leaves of screw pine, *Pandanus*

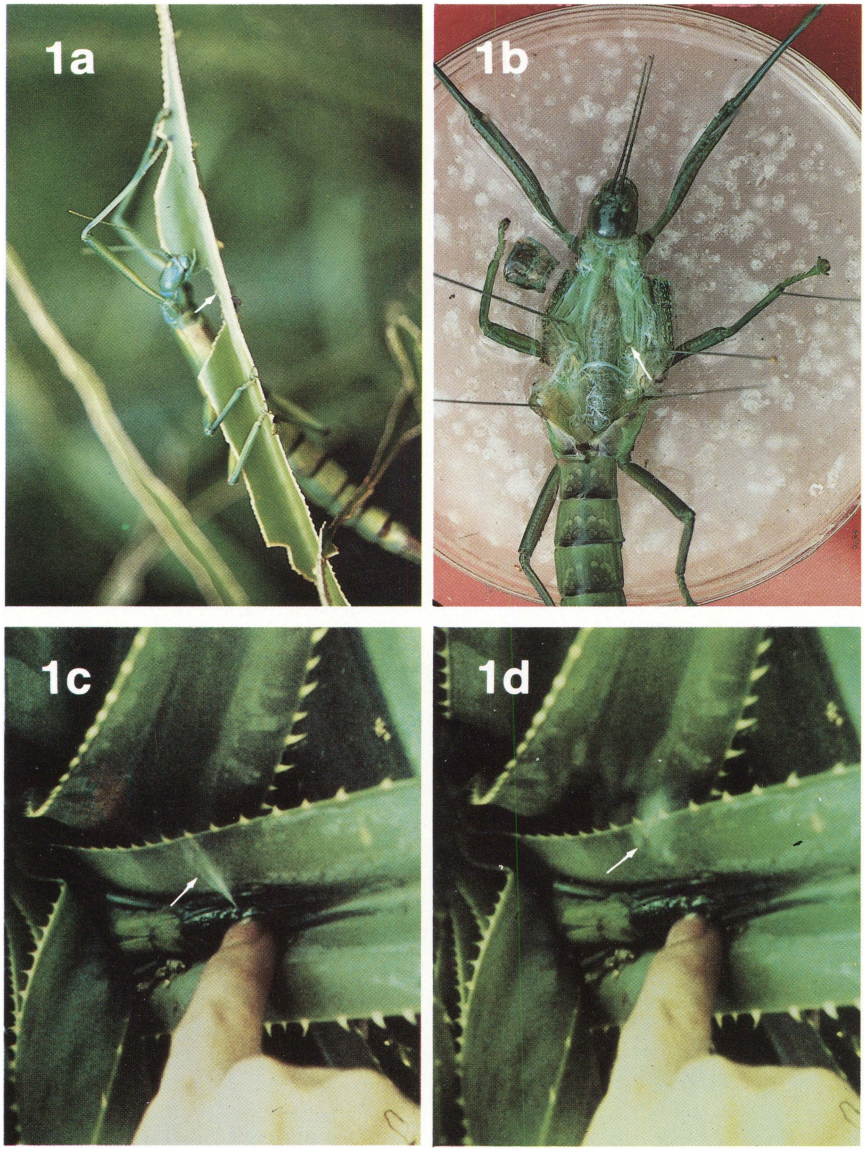


Fig. 1a. Female *Megacrania alpheus* Westwood feeding on screw-pine. The arrow shows the leaves that have just been eaten. The insect causes extensive damage to screw-pine in Taiwan.

b. The bilateral thoracic glands (arrow) are exposed when the dorsal thoracic cuticle is removed.

c and d. Motion picture sequence (2 frames per 1/24 second, Cannon Scopic 16mm movie camera macro-lens) showing a white discharge being sprayed when the insect was tapped on the thorax with finger.

*tectorius,* planted in the backyard of Academia Sinica. The insect was first disturbed by using a filter paper to knock its head. When the discharge had been sprayed on a clean filter paper in large enough quantity (collected from approximately 50 female adults), the milky secretion was then extracted with methylene chloride. After evaporation of the solvent, one $\mu$l of the liquid residue was injected into gas chromatography (Varian Aerograph Series 2800) under conditions described in Table 1. The major peak of the secretions were further analyzed by gas chromatography mass spectrometry (HP 5985A GC/MS system). Other measurements and the purification procedures were identical to those described by Lin and Chow (1980). The field experiment was carried out at O-luan-bi in the plantation of screw-pine, *Pandanus tectorius,* in June 1984 and July 1985.

Table 1. Retention times and peak areas of the different components of the defensive secretion of the stick insect.*

| GC | Columns | Components | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| OV-17(1) | Ret. time (min) | 2.37 | 5.97 | 7.21 | 8.04 | 10.54 |
| | Peak area (%) | (0.237) | (49.59) | (1.00) | (0.96) | (0.1) |
| OV-275(2) | Ret. time (min) | 4.37 | 9.37 | 12.69 | 14.50 | 15.62 |
| | Peak area (%) | (0.751) | (57.38) | (0.083) | (0.286) | (0.133) |
| OV-101 + | Ret. time (min) | 4.10 | 7.84 | 8.69 | 9.79 | 10.43 |
| OV-210(3) | Peak area (%) | (0.667) | (38.57) | (1.796) | (0.72) | (0.369) |

* Operating conditions: (1) 3% OV-17 on 100/120 Varaport, column temp. 85 - 250°C, 8°/min., carrier gas ($N_2$) flow rate 13 ml/min. (2) 15% OV-275 column temp. 80 - 200°C, 12°/min., carrier gas flow rate 13 ml/min. (3) 4% OV-101 + 6% OV-210, column temp. 80 - 230°C, 12°/min., carrier gas flow rate 30 ml/min. all columns were stainless steel, 1.6 m long and 3.2 mm ID.

In June 1984, two groups of 10 nymphs in the 6th instar were confined at 2 locations in O-luan-bi, one group as control and the other experimental. Each week the actinidine was removed from the experimental individuals by tapping the insect with fingers (Fig. 1c and 1d) to see if there was any difference between the control and the experimental group.

The same experiment was repeated in July 1985, but the secretion from the experiment group was sprayed in vicinity of the host plant leaves of the experiment group in order to see the residual effect of the secretion.

## RESULTS AND DISCUSSION

Based on the retention times and peak areas of 5 separated peaks on 3 different columns, one major component was identified. Therefore, the methylene chloride extract was further purified by preparative thin layer chromatography using silica gel (Merch Art No. 7747) as absorbent and a 5:3 mixture of hexane and ethyl acetate developing solvent. The bands with Rf values 0.25 - 0.35 were scraped off and extracted with ethyl acetate which led to recovery of pure major component B as shown in Table 1.

The mass spectrum of component B showed the molecular ion at m/z 147 indicating the $C_{10}H_{13}N$ constitution which was further confirmed by elemental analysis (cacd. for $C_{10}H_{13}N$: C 81.58%, H 8.90%, N 9.52%; found: C 81.61%, H

8.99%, N 10.01%). The major fragmentation peaks were m/z 147 ($M^+$, 54%), 146(30), 132(100), 131(18), 130(11), 117(42), and 77(12). The UV spectrum of B showed the maximal absorptions at 268 and 260 nm. The NMR spectrum ($CDCl_3$) showed the signals at $\delta$ 8.24 (2H, br.), 3.31 (1H, m, J = 7Hz), 2.80 (2H, m), 2.36 (1H, m), 2.24 (3H, s), 1.68 (1H, m), and 1.31 (3H, d, J = 7Hz). All spectral data coincided with those of actinidine (Auda et al. 1967; Caville et al. 1980). The structure was further confirmed by mixed melting point and comparisons of TLC, IR, Mass, and NMR spectra of component B and authentic actinidine picrate which was generously donated by S. Takahashi from Pesticide Research Institute, College of Agriculture, Kyoto University, Japan.

Actinidine, known to excite cats, was first isolated from the plant *Actinidia polygama* (Sakan et al. 1970), and was shown later to be present in other plants (Bellas et al. 1974).

In insects, actinidine has been reported as a defensive component of the rove beetle (Bellas et al. 1974), dolichoderine ants (Wheeler et al. 1977), and Argentine ants (Sakan et al. 1970) but not previously of stick insect. The biosynthesis pathway of actinidine was proposed to be of isoprenoid origin (Auda et al. 1967) or of an irridodial to actinidine by amination (Bellas et al. 1974) and the concomitant of irridodial and actinidine in some species of dolichoderine ants (Wheeler et al. 1977). On the other hand, the irridodial or its analogues, common defensive components of the known stick insects, does not exist in the extract of the defensive secretion of dissected bilateral thoracic glands of *M. alpheus*. Thus, the species may have the metabolic capability to convert irridodial to actinidine.

Mass spectrum of the minor component isolated previously (Chow and Lin 1983) showed the characteristic fragments of monobrominated compound at m/z (%) 178(12), 176(15), 164(27), 162(30), 150(54), 148(45), 137(100), 135(91), 97(82), 83(52), 69(93), 55(90), and 41(62). Although the presence of brominated compounds in seaweed has been reported (Faulkner 1977), its occurrence in land aminals is rare (Blum 1978, 1981). The analysis of our former sample by J. H. Tumlinson (Insect Attractants, Behavior and Basic Biology Research Laboratory, USDA, Gainesville, FL, USA) with chemical ionization mass spectrometry showed that the compound was 9-bromononanol with diagnostic peaks at m/z 225(M + 3), 223(M + 1), 207(M + 3 − $H_2O$) and 205(M + 3 − $H_2O$). Since we frequently use this compound to synthesize the sex pheromone of the tobacco cutworm, *Spodoptera litura* (Lin and Chow 1980) in the laboratory, we suspected the possibility of cross contamination. Hence, in order to clarify this, we recollected the fresh defensive secretion directly from the stick insects and, when these samples were analyzed by a GC-Mass, no brominated compound was obtained. Besides the confirmation of the presence of actinidine in component B, mass spectra of 2 minor components, A and C, was also obtained. The largest ion of the component A was m/z 126(42%) representing $C_8H_{14}O$. Additional fragments were observed at m/z (%) 111(2), 108(10), 98(8), 93(5), 83(61.4), 71(100), 55(60), 43(50), and 41(19). Compound C gave a molecular ion $M^+$, m/z 222, and peaks at m/z(%) 177(24), 163(29), and 149(100). Authentic compounds of the guessed structures such as 2-ethylcyclo-pentent-methanol, p,o, and m-diethyl phthalate have been compared with these minor components but no definitive results were obtained at this time.

Finally, we studied the function of this secretion. Since the insect reproduces by parthenogenesis, only the females and juvenile stages have been studied. All

juvenile stages beyond 2nd instar contained actinidine. In our field experiment to test the effect of secretion in 1984, after two months 8 of the 10 experimental insects were dead due to parasitism by cleopid wasps and ants, whereas the 10 control insects were alive and normal. On the contrary, in 1985, after two months of observation only one of the 10 experimental insects was dead as compared with 10 normal control insects. This indicates the defensive function of the secretion. Since the alkaloid actinidine has been not only identified in the pygidial gland of rove beetles but also demonstrated in an anal gland of two ant species in the genus *Conomyrma* as a defensive secretion (Blum 1981), actinidine could potentially be used as an insect repellent in the future.

## ACKNOWLEDGMENTS

We are grateful to Dr. Y. I. Chu, of the Department of Phytopathology and Entomology, National Taiwan University, for providing stick insects, to Dr. J. H. Tumlinson for CI MS measurement, and National Science Council, Taiwan, for financial support.

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