Studies on chemical ecology of the heteropteran scent gland components

Koji Noge*

Department of Biological Production, Faculty of Bioresource Sciences, Akita Prefectural University, 241–438 Kaidobata-nishi, Shimoshinjyo-Nakano, Akita, Akita 010–0195, Japan

(Received June 10, 2015)

Heteropterans, or true bugs, produce foul-smelling odors commonly composed of \( \alpha, \beta \)-unsaturated aldehydes and their derivatives such as 4-oxo-(\( E \))-2-hexenal that play important roles in chemical defense and communication. However, the defensive roles of each scent gland components are not well understood. In this study, repellent, feeding deterrent, toxic and antimicrobial effects of each scent gland component were evaluated using chemical identification and behavioral assays. (\( E \))-2-Hexenal and (\( E \))-2-octenal showed repellent activities against insect predators. (\( E \))-2-Octenal also functioned as feeding deterrent against the predators. Our predator behavioral assays revealed that OHE was deterrent and toxic but not repellent against the predators. This compound also induced permanent locomotive impairment in crickets that correlates with free thiol depletion. All of the \( \alpha, \beta \)-unsaturated aldehydes showed antimicrobial activities. Having diverse defensive components that function differently in a blend could fortify the heteropteran chemical defense system. © Pesticide Science Society of Japan

Keywords: Heteroptera, pheromone, chemical defense, 2-alkenals, ketoaldehydes.

Introduction

Heteropterans, or true bugs, are well known to discharge foul-smelling odors from scent glands when they are disturbed. These odors are known to be involved in chemical defense against predators and chemical communication among conspecific individuals.\(^ {1,2} \) Typically, these odors are composed of short-chain aliphatic aldehydes, alcohols and esters, alkanes, terpenes, and phenolics (Fig. 1). Of these secretory compounds, \( \alpha, \beta \)-unsaturated aldehydes, such as (\( E \))-2-hexenal, (\( E \))-2-octenal, (\( E \))-decenal, and 4-oxo-(\( E \))-2-hexenal (OHE), are frequently found from a wide variety of heteropteran species. These compounds originate in the dorsal abdomen in nymphs and in metathoracic scent glands in adults. It has been recognized that chemical components in nymphal secretion often change after metamorphosis. These chemical polymorphisms between nymphs and adults are possibly due to differences in predatory pressures during developmental stages. Yet, in spite of the advances in chemistry, the behavioral evidence supporting effects of each scent gland component on potential predators is still largely missing. Here we briefly introduce the roles of heteropteran scent gland components that show repellent, feeding deterrent, toxic and antimicrobial properties.

1. Repellent activity of heteropteran scent gland components\(^ {3} \)

In general, compounds shared across species and developmental stages are considered to function as nonspecific repellents or toxins. (\( E \))-2-Hexenal, (\( E \))-2-octenal and OHE were identified as the shared components of nymphs of Riportus pedestris (Alydidae) and Thasus acutangulus (Coreidae), and adults of Euschistus biformis (Pentatomidae) by using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Adults of E. biformis also emit two esters, (\( E \))-2-hexenyl acetate and (\( E \))-2-octenyl acetate, that have been previously described in the secretions of other heteropteran species. Predator behavioral assays using the Chinese mantids as an experimental model revealed that (\( E \))-2-hexenal, (\( E \))-2-octenal and (\( E \))-2-octenyl acetate significantly repelled the mantids compared to control treatment (hexane). When mantids were exposed to each compound, they turned in the opposite direction and moved away from the chemical sources. The compound blends also showed repellent activities against the mantids, but there was no additive or synergistic effects on predator’s behavior. Compound repellency is not likely related to the compound volatility, but to the affinity between each compound and the predator’s olfactory receptors. Future study should elucidate if

* To whom correspondence should be addressed.
E-mail: noge@akita-pu.ac.jp
Published online July 24, 2015
© Pesticide Science Society of Japan
there is a shared perception mechanism of repellents among distantly related predators. Some heteropteran species are known to have visual defensive strategies, for example, *R. pedestris* nymphs mimic the morphology and behavior of ants and *T. acutangulatus* nymphs are aposematically colored with vivid orange, yellow and black. These visual signals and repellents may synergize to enhance the defense system of heteropteran. On the other hand, \((E)-2\text{-hexenyl acetate}\) and \(OHE\) did not elicit dispersal behavior of mantids. These two components may have other biological activities.

2. **Feeding deterrent activity and toxicity of heteropteran scent gland components**

The presence of non-repellent compounds in the heteropteran secretions suggests functional variation among different compounds in a mixture. In order to evaluate the feeding deterrent effects of the heteropteran scent gland component, we investigated whether the California mantids oriented, attacked, and ate crickets treated with each compound. Mantids attacked and ate crickets treated with \((E)-2\text{-hexenal}\), \(\text{hexanal}\), \(1\text{-hexanol}\) and \(\text{hexyl acetate}\), while they did not consume crickets treated with \((E)-2\text{-octenal}\) and \(OHE\). These results suggest that \((E)-2\text{-octenal}\) is a bifunctional agent as repellent and feeding deterrent against insect predators. Mantids often died after being contact with crickets coated with \((E)-2\text{-hexenyl acetate}\) and \(OHE\), suggesting that this compound is deterrent and toxic but not repellent against the predators.

Nymphs of the giant mesquite bug (*Thasus neocalifornicus*: Coreidae) are brightly colored red with white spots. The nymphs secrete a mixture of \((E)-2\text{-hexenal}\) and \(OHE\), and they are chemically protected from insect predators. The adult bugs are large and cryptic. The adult secretions are composed of a series of saturated compounds composed of \(\text{hexanal}\), \(1\text{-hexanol}\) and \(\text{hexyl acetate}\) that did not affect mantid's feeding behavior. The differences in the defensive roles of components between nymphs and adults may reflect the differences in predator guilds and visual defensive strategies, and an adult-specific flying capability.

3. **Unique insecticidal activity of OHE**

Our predator behavioral experiments revealed that OHE showed different effects against insect predators compared to \((E)-2\text{-hexenal}\), a structurally related compound. However, it is still unclear how OHE and other \(\alpha,\beta\)-unsaturated aldehydes affect the physiology of insects. Thus, we evaluated the effects of these \(\alpha,\beta\)-unsaturated aldehydes using the house cricket as an experimental model. When the crickets were exposed to OHE, \((E)-2\text{-hexenal}\), \((E)-2\text{-heptenal}\), \((E)-2\text{-octenal}\) or \((E)-2\text{-hexen-1-ol}\), they became paralyzed, while \((E)-2\text{-pentenal}\), \((E)-2\text{-nonenal}\), \((E)-2\text{-hexene}\) and \(\text{hexanal}\) did not affect the crickets. The paralysis induced by \((E)-2\text{-hexenal}\), \((E)-2\text{-heptenal}\), \((E)-2\text{-octenal}\) and \((E)-2\text{-hexen-1-ol}\) were moderate and temporary, and the affected crickets were recovered when they were released from exposure and kept under fresh air. On the other hand, exposure to OHE impaired the cricket's locomotive ability resulting in permanent paralysis and death (Figs. 2 and 3). The hind legs were markedly affected by OHE, which became stretched. The
symptoms induced by OHE are similar but different from those of locusts treated with the insecticide, pymetrozine. These results suggest the 4-keto-(E)-2-aldehyde moiety may be critical in the permanent locomotive impairment of crickets. In the OHE treated crickets, the amounts of free thiols derived from both protein and non-protein sources were significantly lower than those of non-treated crickets. The \textit{in vitro} reaction of OHE with 1-butanethiol, a model of free thiol group, revealed that OHE could potentially react to any free thiol group. Treating crickets with \textit{N}-ethylmaleimide, a thiol inactivator, exhibited a similar symptom to that induced by OHE. These results suggest permanent locomotive impairment in crickets induced by OHE correlates with free thiol depletion.

4. Antibacterial activity of heteropteran scent gland components

\(\alpha,\beta\)-Unsaturated aldehydes found in the heteropteran secretions also showed antibacterial activities against both Gram-positive and Gram-negative bacterial species. Our assays using paper disk method revealed that OHE, (\(E\))-2-hexenal and (\(E\))-2-octenal showed dose-dependent antibacterial activities against four tested bacterial species including \textit{Pseudomonas} sp. that was collected on the host plant of a pentatomid, \textit{Dolycoris baccarum}. (\(E\))-2-Hexenyl acetate and (\(E\))-2-hexen-1-ol did not inhibit the bacterial growth, suggesting that the \(\alpha,\beta\)-unsaturated aldehyde moiety was required to show antibacterial activity. Of the active compounds, the activity of OHE was much higher than that of (\(E\))-2-hexenal and (\(E\))-2-octenal. This may be explained by the electrophilic reactivity of these unsaturated aldehydes based on the LUMO energy level. These results suggest that unsaturated aldehydes found in heteropteran scent glands protect the bugs against not only insect predators but also environmental bacteria.

Conclusion

In this study, we demonstrated some biological roles of heteropteran scent gland components based on chemical identification and behavioral assays. Specifically, OHE induced a unique permanent locomotive impairment in crickets compared to other \(\alpha,\beta\)-unsaturated aldehydes. Most of the heteropteran scent gland components are structurally simple, but their biological activities are not simple and rather profound. The diversity of the activities among structurally related compounds may be due to their chemical properties, such as electrophilic reactivity and penetration rate, physiology of the receivers and so on. Having diverse defensive components that function differently in a blend could fortify the heteropteran chemical defense system. Chemistry based multidisciplinary approach can lead to better understanding of the biological interaction between heteropterans and other organisms.

Acknowledgements

I thank Drs. Shigeru Tamogami and Makoto Abe (Akita Prefectural University), and Drs. Judith X. Becerra, Kathleen L. Prudic and Zakee L. Sabree, and Sarai Olivier-Espejel (University of Arizona).

References