J. Pestic. Sci., **31**(2), 159–160 (2006)

Society Awards 2006

(on prominent achievement)

Studies on insect behavior regulators*

Tetsu Ando**

Graduate School of Bio-Applications and Systems Engineering,
Tokyo University of Agriculture and Technology, Koganei, Tokyo 184–8588, Japan

(Accepted March 5, 2006)

In addition to conventional insecticides, damage by pest insects could be prevented by controlling their behavior using chemicals, such as pheromones. Lepidopteran sex pheromones with strong attraction are one of the most promising chemicals. Analytical methods for the identification of pheromones, their synthesis, application for agriculture, and biosynthetic studies concerned with pathways, inhibitors, and an activating endocrine system are reviewed. © Pesticide Science Society of Japan

Keywords: insect pheromones, behavior regulation, attraction, communication disruptants, biosynthesis regulators.

Introduction

Many insect behaviors are regulated by semiochemicals. The sex pheromone, which is secreted by a female moth for the benefit of a specific partner, plays an important role in reproductive isolation. Therefore, it is not surprising that the chemical structures of species-specific pheromones exhibit considerable differences. Lepidopteran sex pheromones have been identified from more than 560 species. Lepidoptera is the second largest insect group and includes nearly 150,000 described species, and thus it should be mentioned that our information is still rudimentary considering the species diversity. Other natural products, however, have not been investigated for so many different species as the lepidopteran sex pheromone. Female pheromones strongly attract male moths in a field; as a result, their use as a monitoring tool has been developed in integrated pest management programs. Furthermore, chemical communication between females and males is disrupted by permeating a field with synthetic pheromones. Recently, the biosynthesis of the pheromones has been actively studied in order to develop a new method to control the behavior of pest insects.

1. Synthesis, Identification, and Field Tests

The lepidopteran sex pheromones in the most predominant group (Type I) are composed of unsaturated C_{10}–C_{18} straight-chain compounds with a terminal functional group, such as bombykol produced by the silkworm moth (*Bombyx mori*). In addition, females in some evolved families produce C_{17}–C_{23} polyunsaturated hydrocarbons and *cis*-epoxy derivatives, constituting a second major group (Type II). By GC-EAD and GC-MS analyses, we have identified the pheromones of more than 30 species and found new Type I compounds with a 12-enyl, 4,6-dienyl, 5,7-dienyl, 11,13-dienyl, or 10,12,14-trienyl structure and novel Type II compounds with a *trans*-epoxy ring or two *cis*-epoxy rings.

Natural pheromones were identified by GC-EAD and GC-MS analyses of pheromone gland extracts with reference to data about standards, which had been systematically synthesized. Their chemical structures were confirmed by NMR, and a new empirical rule for ^{13}C signal assignment of Type I compounds with a conjugated dienyl system was identified. On EI-MS analysis, each diene showed characteristic fragment ions and the double-bond positions were determined without derivatization. MCPBA oxidation of (*Z*,*Z*)-6,9-dienes and (*Z*,*Z*,*Z*)-3,6,9-trienes, synthesized from linolic and linolenic acids, yielded mixtures of monoepoxy derivatives, Type II pheromones. Each positional isomer, which could be separated by MPLC, showed diagnostic fragment ions for structural determination on GC-MS analysis. HPLC with a chiral column accomplished optical resolution of the epoxy compounds and was utilized to determine the stereochemistry of natural pheromones.

Furthermore, synthetic pheromones and their analogs were supplied for random screening tests in a field. Their attractive activity was evaluated alone or in a mixture, and sex attractants were found for about 230 species in Japan and 20

---

* See Part II for the full Japanese article.

** To whom correspondence should be addressed.
E-mail: antetsu@cc.tuat.ac.jp

species in Vietnam.

2. Mating Disruption

Agricultural fields are very large and wind-blown; however, the permeation of a synthetic pheromone is possible, and several pheromones are being utilized as mating disruptants for more than 20 lepidopteran species. In order to spread applicable species, new disruptants were tested for the persimmon fruit moth (*Stathmopoda masinissa*) and the citrus leafminer (*Phyllocnistis citrella*), which secreted Type I pheromones with a dienyl structure. Each synthetic pheromone was dispensed into polyethylene tubes (50–100 mg/tube), which were placed in a field. Dispensers with the pheromone of *S. masinissa* (900 tubes/ha) effectively disrupted mating communication in a persimmon orchard, and reduced the number of fruit infected with the larvae, whereas the control of *P. citrella* by the disruption method did not succeed; the number of captured males vastly decreased in a citrus orchard permeated with the pheromone, but the population level did not diminish even with 1,300 dispenser tubes/ha. Trials of mating disruption by Type II pheromones, which have hardly been synthesized on a large scale, are very limited. A mixture of epoxydienes including the sex pheromone of the Japanese giant looper (*Ascotis selenaria cretacea*) and two positional isomers was examined in a tea garden. Mating of the tethered female was strongly inhibited by 1,000 dispenser tubes/ha, indicating the first successful formulation for the disruption of a geometrid pest.

3. Pheromone Biosynthesis and Its Regulation

The biosynthesis of Type I pheromones was first examined by the topical application of several ^{14}C- or ^{2}H-labelled compounds, and pathways starting from acetyl CoA in a pheromone gland were confirmed. For example, (*Z*)-7-dodecenyl acetate of *Macdunnoughia confusa* is synthesized from palmitic acid *via* Δ11-desaturation, and chain shortening by β-oxidation, reduction, and acetylation. Another experiment with *B. mori* revealed a unique two-step desaturation, Δ11 and Δ10,12, to form a 10,12-dienyl structure. In *Thysanopluia intermixta*, a 5,7-dienyl structure was formed by Δ5-desaturation of 7-monoenyl acid, indicating two different methods for the biosynthesis of conjugated dienyl systems.

Recent studies with *A. s. cretacea* showed that the biosynthetic processes for two pheromone types are quite different. The biosynthesis of the Type II pheromone, such as an epoxydiene of the geometrid species, starts from dietary linolenic acid, and its trienyl intermediate is produced outside of a pheromone gland, probably in oenocytes, and moves to the gland after binding to lipophorin; only epoxidation proceeds in the pheromone gland.

If pheromone biosynthesis can be blocked by an inhibitor, chemical communication between female and male moths will be interrupted and their chance of mating is expected to be very low. It appears promising that a chemical that prevents the desaturation step can be found because many lepidopteran pheromones include the C=C bond at a characteristic position and an inhibitor might specifically attack the insect enzyme system. Sterculic acid, 9,10-methyleneoctadec-9-enoic acid, is known to inhibit the Δ9-desaturation of octadecanoic acid in vertebrates. We investigated the syntheses and inhibitory activities of several cyclopropenes to define their structure-activity relationships in *B. mori*. The results suggested that Δ11-desaturation was blocked by 9,10- and 11,12-methylene compounds and the subsequent Δ10,12-desaturation by 11,12- and 13,14-methylene compounds.

The pheromone titer is synchronized with a light-dark cycle. The photo signal is received at the head and transported to the pheromone gland with the help of the hormone, which activates pheromone biosynthesis and is called a pheromone biosynthesis-activating neuropeptide (PBAN). PBAN is composed of about 30 amino acids, and the sequence has been determined for those from 10 lepidopteran species. On the other hand, pheromone production is terminated after mating. Experiments with *B. mori* showed that the pheromone gland of the mated female maintained its ability to synthesize bombykol but could not produce the pheromone due to the suppression of PBAN secretion from a suboesophageal ganglion *via* a neural signal for mating.

Conclusion

The diversity of species and their pheromone systems are of interest to many organic chemists, biochemists, and entomologists, who attempt to understand insect evolution chemically and biochemically in detail, and to apply their findings for plant protection. Recently, new research has produced results in the field of the perception of orders. In addition to pheromones, antennae recognize repellents, which prompt escape behavior. The neural transaction of attractants and repellents must be different, and if they were better understood, these interesting scientific subjects would become potentially important targets for pest control.