Isolation and Identification of Male Sex Pheromone Present
in the Wing Gland of *Achroia innotata obscurevittella*
RAGONOT (Lepidoptera: Pyralidae)

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(Received September 11, 1989; Accepted January 13, 1990)

The wing gland secretion in the male pyralid moth *Achroia innotata obscurevittella* RAGONOT consisted of 2 components, which were identified as nonanol and nonanal. Nonanol (96.8%) was present as a major component responsible for sexual attractancy of the virgin female moth, while nonanal (3.2%) was a minor component and indicated no activity alone.

INTRODUCTION

Achroia innotata obscurevittella RAGONOT is distributed widely in Japan. Its larvae inhabit not only the colonies of Japanese honeybee, *Apis cerana japonica* FABR., but also those of European honeybee, *A. mellifera* L. From early evening to midnight, adult *A. innotata* males display a characteristic wing-fanning (calling) behavior to attract females (OKADA, 1988). The same phenomenon is observable in 2 closely related species of Galleriinae which attack honeycomb: *Galleria mellonella* and *A. grisella*. Males of these species have a pair of well-developed wing glands located at the base of their forewings. Secretions of these glands are known as sex pheromones for the females of each; a mixture of nonanal and undecanal in *G. mellonella* (RÖLLER et al., 1968; LEYER and MONROE, 1973), and undecanal and (Z)-11-octadecenal in *A. grisella* (DAHM et al., 1971), respectively.

The wing gland components of *A. innotata* were considered to be like the sex pheromone of other related species, so secretion was collected by a Porapak-Q column (BYRNE et al., 1975). The active component of the sex pheromone was isolated and its structure was identified.

MATERIALS AND METHODS

Insects. *Achroia innotata obscurevittella* RAGONOT larvae were collected from unattended and deserted beehives at the University of Tsukuba at the end of May in 1988, and were reared in the laboratory at 25°C, 16L–8D. Feeding was on beehive or artificial diet. The diet consisted of a mixture of corn flour, honey, glycerol, dried yeast and water (4: 2: 2: 1), modified from DUTKY et al. (1962).

Collection of volatiles from male moths. Males were collected from rearing containers and transferred into a conical flask (500 ml), into which a piece of folded filter paper had

been placed as a foothold for males' wing-fanning. Purified and moistened air passing through an active charcoal and water-surface was introduced to the flask at a moderate flow rate (200 ml/min) using an aspirator. The out-stream of the air was passed through a Porapak-Q column (80–100 mesh, 0.6 g, 6 mm ID $\times$ 6 cm length). The trapped volatiles from the males were recovered by back-washing of the column with hexane (20 ml) every week. Each eluate was combined and kept in a refrigerator at -20°C until a workable amount of the material was accumulated.

Bioassay. Pheromone activity was monitored by the method reported by KUWAHARA (1980). When virgin females responded to the tip of a glass rod (2 mm dia. $\times$ 20 cm length, preliminarily impregnated by a candidate solution in hexane by displaying characteristic wing-fanning and subsequent searching and/or approaching behavior, the candidate solution was judged as active. The activity was expressed as the lowest concentration of the solution in hexane at which activity was observed. All assays were carried out at night and ambient temperature ($16\text{--}22^{\circ}\text{C}$).

Isolation of pheromone. Porapak-Q eluate (6.1 mg), after solvent evaporation, was chromatographed on a silica gel column (Wako-gel C-200, 600 mg, 6 mm ID $\times$ 6.5 cm in ht.), eluting stepwisely by a series of hexane-ether mixtures (100:0, 99:1, 95:5, 90:10, 80:20, 50:50, 0:100, by volume, each 10 ml). Each eluate was monitored by gas liquid chromatography (GLC) stated below and also by bioassay.

Analyses. Three types of GLC systems, each with different fused silica capillary columns and conditions were used as follows; CP-Sil 19 CB column (0.22 mm ID $\times$ 25 m, $df=0.18\text{ }\mu\text{m}$, Chrompack) using a Hitachi 263-30 GLC with a cooled-on-column injector system (OCI; OCI-3, SGE controlled by FC-2, Gasukuro Kogyo) at a temperature programmed condition ($60\text{--}250^{\circ}\text{C}$, $5^{\circ}\text{C}/\text{min}$), an FFAP column (0.25 mm ID $\times$ 25 m, $df=0.25\text{ }\mu\text{m}$, split ratio=100:1, Quadrex) using a Yanaco G-180FT GLC and CP-Sil 19 CB column using the other Hitachi 263-30 GLC with an inlet splitter (99:1), both running at 110°C . Kovats retention indexes (ETTRE, 1964) were calculated. Thin layer chromatography (TLC, E. Merck, Silica gel F₂₅₄, 1.5 cm $\times$ 5 cm) was also used by a mixture of hexane and ether (1:1) as the solvent. UV ray at 254 nm, iodine vapor, 2,4-dinitrophenylhydrazine solution in 2 N-HCl and 50%-sulfuric acid were used for TLC visualization. The following chemical reactions as functional group tests were carried out on the active fraction; alkaline hydrolysis, LiAlH_4 reduction, bromination and acetylation. Each product was subsequently subjected to bioassay and GLC analysis. Mass spectra (MS) were obtained by a Hitachi M-80B gas chromatograph-mass spectrometer at 70 eV using an Al-Clad fused silica capillary column ('400' methyl silicone, 0.25 mm ID $\times$ 25 m, $df=0.1\text{ }\mu\text{m}$, Quadrex) at 30 ml/min He flow rate and at 100°C . Proton magnetic resonance spectra (PMR) were measured by a JEOL FX-100 spectrometer in CDCl_3 using TMS as an internal standard.

RESULTS AND DISCUSSION

The Porapak-Q eluate (6.1 mg after evaporation of solvent, active at 100 ppm) gave 2 peaks by GLC; peak A (96.8%) and (3.2%). The activity was recovered from the silica gel column as 10% and 20% ether-hexane eluates. Combined active fractions (2.1 mg) gave one spot (R_f 0.24) by TLC and one slightly tailed peak (Peak A) by GLC (t_R 6.77 min on CP-Sil 19 CB, 11.66 min on FFAP and 9.90 min on OCI, respectively). The difference of retention indexes ($\Delta I=382$) obtained by CP-Sil 19 CB and

FFAP supported the presence of an alcoholic group, which was further confirmed by functional group tests. Among 4 reactions tested, acetylation only gave a product with a different t_R from the original (from 9.90 min to 12.41 min on OCI) and the product also lost its activity, while the other 3 did not affect its t_R and the activity. Therefore the compound was concluded to be a saturated alcohol.

MS indicated a profile of *n*-alkene and gave the biggest ion at m/z 126 ($M^{+} - H_2O$), suggesting the structure as nonanol ($C_9H_{20}O$). PMR indicated a *n*-alkanol as the structure, and gave the following chemical shifts: δ 0.88 (3H, broad triplet, $J=5.86$ Hz), 1.28 (ca. 14H, broad singlet), 1.50 (3H, quartet-like, $J=6.96$ Hz), and 3.64 (2H, triplet, $J=6.35$ Hz). The shift corresponding to one proton at 1.50 disappeared with addition of D_2O .

Commercially available nonanol (Nakarai Tesque) gave identical MS with those of the natural compound and also the same t_R on 3 GLC systems to the natural pheromone co-chromatographically. PMR of nonanol was also similar to the active fraction. Therefore the peak A was identified as nonanol.

Peak B was recovered in a 5% ether-hexane eluate from the SiO_2 column, suggesting an ester or a carbonyl compound. MS of peak B gave the biggest ion at m/z 98, indicative of $M^{+} - 44$, and corresponded to those of nonanal. Authentic nonanal was co-chromatographically identical to the peak B by 3 GLC systems (t_R 3.92 min on CP-Sil 19 CB, 2.49 min on FFAP and 7.58 min on OCI, respectively). Therefore peak B was identified as nonanal.

The wing gland secretion of *A. innotata* obtained by Porapak-Q column was consequently identified as a blend of nonanol (96.8%) and nonanal (3.2%). Authentic nonanol indicated the same activity as the isolated nonanol at 100 ppm, although nonanal alone had no activity. Synergistic function of nonanal against nonanol was not expected by the present assay because the activity of Porapak-Q trapped volatile gave the same activity (100 ppm) with nonanol itself. Therefore the male sex pheromone of *A. innotata* was concluded to be nonanol.

Male sex pheromone is commonly known among 7 species of Galleriinae, except in the case of the rice moth (*Corcyra cephalonica* STANTON), in which both male and female produce sex pheromones (ZAGATTI et al., 1987; HALL et al., 1987). The 3 species of Galleriinae infesting beehives use only *n*-alkyl and/or alkenyl compounds as their sex pheromones. The greater wax moth male emits a blend of nonanal and undecanal (RÖLLER et al., 1968; LEYRER and MONROE, 1973), both of which are active for females. The male lesser wax moth also uses (*Z*)-11-octadecenal as the active component, which is accompanied with inactive undecanal (DAHME et al., 1971). From male *A. innotata*, a mixture of nonanol and nonanal was identified. The former functioned as the sex pheromone.

In contrast male pheromones of 4 other species: the African sugar-cane borer, *Eldana saccharina* WALKER (KUNESCH et al., 1981; VIGNERON et al., 1982, 1984), the bumble-bee wax moth, *Aphomia sociella* L. (KUNESCH et al., 1987), the stored nut moth, *Paralipsa* (= *Aphomia*) *gularis* ZELLER (KUWAHARA, 1980), and the rice moth, *Corcyra cephalonica* STANTON (ZAGATTI, 1987) were a mixture of cyclic compounds and/or terpenoids, with no normal chain compounds known. Therefore, the 3 beehive pests belonging to Galleriinae each use different compounds biosynthetically available from beehives as their male pheromone.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. M. SASAKI, Tamagawa University and Dr. S. YOSHIMATSU, the National Institute of Agro-Environmental Sciences for identifying this species. We also thank Ms. H. YAMAMOTO for collecting the species in the field. This study was partly supported by a Research Project Program, University of Tsukuba.

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