(Z)-11-Hexadecenyl Acetate : A Sex-
Pheromone Component of the
Cabbage Armyworm Moth,
Mamestra brassicae LINNÉ¹

Y. HIRAI, H. KIMURA²

*Upland Farming Division, Hokkaido
National Agricultural Experiment
Station, Memuro, Kasaigun, Hokkaido
082, Japan*

K. KAWASAKI, Y. TAMAKI

*Division of Entomology, National
Institute of Agricultural Sciences,
Nishigahara, Tokyo 114, Japan*

(Received November, 28, 1977)

The cabbage armyworm, *Mamestra brassicae* LINNÉ (Lepidoptera : Noctuidae), is a serious economic pest of many crops and vegetables. The moth is widely distributed in the palearctic regions of the world. The presence of a sex pheromone in the female cabbage armyworm was suggested by SZENTESI et al. (1975). Field trapping of the cabbage armyworm by a series of synthetic compounds was conducted by ANDO et al. (1977). The isolation and identification of a sex pheromone component of the cabbage armyworm is described in this paper. The cabbage armyworm larvae were reared on an artificial medium (HIRAI et al., unpublished) at $23 \pm 2^\circ\text{C}$ under 16L-8D conditions. The sexes were separated at the pupal stage. Female pupae were placed in a polyethylene box ($27 \times 13 \times 8$ cm) for emergence, and female moths were transferred to a tin (17×15 cm) lined with filter papers and held in the rearing room for three days. The secretion from 3,200 virgin female moths absorbed by the filter papers was thoroughly washed out with methylene chloride. The filtrate was dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator at a temperature below 40°C to obtain 1.97 g of crude lipid. Activity was monitored by the EAG (electrocantennogram) method at each stage of isolation (TAMAKI et al., 1977).

Florisil (60—100 mesh) was used as an adsorbent for partial purification of the sex pheromone in the crude extracts according to a class-separating method for lipids (CARROLL, 1961). Hexane, 5% ether in hexane, 15% ether in hexane, 25% ether in hexane, 50% ether in hexane, and methanol were used in this order as eluting solvents.

Only the fraction eluted by 5% ether in hexane was potent with respect to EAG response (1.7 mV). Further purification of the active fraction was carried out with a column of silicic acid (Mallinckrodt, 100 mesh) impregnated with AgNO_3 . The AgNO_3 -silicic acid (AgNO_3 16.7%, w/w) was mixed with half its weight of Celite-545 (Johns-Manvill), and packed in a glass column as a slurry with hexane. Mixtures of ether and hexane were used for elution in which the ether concentration was increased in steps from 1 to 100%. Only the fraction eluted by 5% ether in hexane was potent with respect to EAG response (2.0 mV).

The active fraction was concentrated and examined by gas chromatography. A gas chromatograph (F & M Model 402) equipped with an effluent splitter and a hydrogen flame ionization detector was used. Column effluents were trapped by capillary glass tubes (30 cm, 1.5 mm i.d.) attached to the column outlet. The trapped fractions were bioassayed and then washed out from the glass tubes with hexane for chemical examination. EAG analysis of effluents collected from a column of 2% OV-1 (1.83 m, 3 mm i.d., 160°C) showed activity at two separate peaks in the chromatogram. Retention time relative to tetradecyl acetate was 1.22 (EAG, 1.9 mV) for the first active peak and 2.57 (EAG, 2.8 mV) for the second active peak. Both active fractions were injected into a column of 15% PEG-20M (1.83 m, 3 mm i.d., 170°C). The 1.22-peak of the OV-1 column showed a single clear peak at retention time of 1.12 relative to tetradecyl acetate by the PEG column. However, the EAG assay showed activity (EAG, 1.9 mV) at a retention time of 1.17 relative to tetradecyl acetate. The quantity of a possible component in the EAG active zone at 1.17 was too small for further examination. The 2.57-peak of the OV-1 column showed a single peak by PEG column at retention time of 2.33 relative to tetradecyl acetate, and EAG activity (2.8 mV) coincided with the 2.33 peak under these

¹ Appl. Ent. Zool. **13** (2): 136—137 (1978).

² Present Address: Hokkaido National Agricultural Experiment Station, Sapporo, Hokkaido 061-01.

conditions. The fractions corresponding to the 2.33-peak of the PEG column were pooled and then injected into a column of 2% OV-17 (1.83 m, 3 mm i. d., 220°C). The 2.33-peak of the PEG column showed two separate peaks at retention times of 1.06 and 1.40 relative to hexadecyl acetate by the OV-17 column; EAG activity (2.8 mV) coincided with the 1.06-peak under these conditions.

The active compound was examined by a gas chromatograph-mass spectrometer (Shimadzu-LKB 9000). Operating conditions were as follows: He flow rate, 30 ml/min; temperature of the ion source and the molecular separator, 290°C and 250°C; column, 2% OV-17, 2m; column temperature, 200°C. The mass spectrum of the compound showed an M^+ peak at m/e 282 and an $M^+ - 60$ peak at m/e 222, indicating a structure of hexadecenyl acetate.

The 2.33-peak of the PEG column was purified again by repeated fractionation by a gas chromatograph with the 2% OV-17 column at 220°C. The position of unsaturation was established by an ozonolysis (KLEIMANN et al., 1969) of the purified compound. The product was identified as 11-acetoxyundecanal (m/e , 43 [base], 83, 125, 185 [$M^+ - 43$]) by gas chromatograph-mass spectrometer. Thus the active compound is one of the two isomers of 11-hexadecenyl acetate. Geometry of the compound was determined by gas chromatography (Shimadzu GC-5A) with a glass capillary column of CHDMS (0.24 mm $\times$ 52 m, 170°C). The active compound was cochromatographed with a mixture (1:1) of (*Z*)- and (*E*)-11-hexadecenyl acetate. An increase in peak height was observed for the (*Z*)-isomer. Thus, the structure of the compound was established to be (*Z*)-11-hexa-

decenyl acetate.

A field test on the synthetic compound was conducted in a beet field in Memuro, Hokkaido, from August 11th to September 17th, 1977 to determine the attractiveness of (*Z*)-11-hexadecenyl acetate. Sticky traps (Takeda Chem. Ind., 40 $\times$ 26 $\times$ 8.5 cm) were used with 1 mg of synthetic (*Z*)-11-hexadecenyl acetate in rubber septum (Ao, Takeda, Chem. Ind.). A total of eleven male moths of the cabbage armyworm were trapped by three traps; a black light trap (20-W black light tube) caught four males and nine females during the same period. Ando et al. (1977) also determined the attractiveness of (*Z*)-11-hexadecenyl acetate for males of this species in the field by screening a series of synthetic compounds.

The authors are indebted to Dr. K. HONMA of the Upland Farming Division, Hokkaido National Agricultural Experiment Station and Dr. T. YUSHIMA of the National Institute of Agricultural Sciences for their helpful counsel. They are also grateful to Mr. H. TSUTSUI and Mrs. F. SATO for their assistance in rearing the insects.

REFERENCES

- ANDO, T., S. YOSHIDA, S. TATSUKI, and N. TAKAHASHI (1977) *Agric. Biol. Chem.* **41**: 1485—1492
CARROLL, K. K. (1961) *J. Lipid Res.* **2**: 135—141
KLEIMANN, R., G. F. SPENCER, F. R. EARLE and I. A. WOLFF (1969) *Lipids* **4**: 135—141
SZENTESI, Á., M. TÓTH, and A. DOBROVOLSZKY (1975) *Acta Phytopath. Acad. Sci. hung.* **10**: 425—429
TAMAKI, Y., K. HONMA and K. KAWASAKI (1977) *Appl. Ent. Zool.* **12**: 60—68