(1879) on *f. trapezoides*.

In the case of *A. japonica*, however, only one worm emerged from the cocoon of the two forms. The specimens of forms A and B have been found distributed in different habitats (NAKAMURA, 1972b).

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Sex Pheromone of *Spodoptera litura* (F.) (Lepidoptera : Noctuidae): Isolation, Identification, and Synthesis¹

Spodoptera litura (F.) is a serious pest of vegetable crops in the south-western district of Japan. Female moths of *S. litura* produce a potent sex pheromone which attracts a large number of male moths in the field. A sibling species, *Spodoptera littoralis* (BOISD.)², is a well-known pest on cotton plants in Egypt, and chemical properties of the sex pheromone of *S. littoralis* have been suggested as attributed to free alcohol and/or carbonyl compounds (ZAYED et al., 1963). Preliminary experiments on *S. litura*, however, indicated that the sex pheromone consisted of at least two unsaturated alcohol acetates (YUSHIMA et al., 1973). We report here the isolation, identification, and synthesis of two active components of the sex pheromone of *S. litura*. The isolation steps were monitored by a field bioassay (Yashima et al., 1973) and/or a laboratory bioassay (TAMAKI et al., 1973).

Abdominal tips of 50,000 virgin female moths were homogenized with methylene chloride, and 220 g of crude lipid was obtained. The crude lipid was saponified with 5% methanolic KOH, and the unsaponifiable matter obtained there-

from (20 g) was chromatographed on a column of Florisil (CARROLL, 1961). Only the fraction eluted by 25% ether in *n*-hexane possessed potent activity after acetylation with acetic anhydride and pyridine. The active material thus obtained was again chromatographed on a Florisil column. Five percent ether in *n*-hexane removed the active material (3.9 g), which was then chromatographed on a column of silicic acid impregnated with silver nitrate (Mallinckrodt, 100 mesh, containing 16.7% silver nitrate), and eluted successively with 1, 3, 5, 6, 10, and 50% ether in *n*-pentane, and subsequently eluted with ether. These seven fractions showed no biological activity when applied alone, but the combination of eluates in 10% and 50% ether in *n*-hexane showed a potent activity. The two eluates were examined by gas chromatography on glass columns packed with 15% PEGA (1.5 m, 180°C), 15% Carbowax-20M (1.0 m, 125°C), and 5% SE-30 (1.0 m, 160°C) on acid-washed Chromosorb W (60-80 mesh). The instrument used was an F & M model 402 equipped with an effluent splitter and a flame ionization detector.

Relative retention times (std.: C₁₄:₀OAc) of the active materials which emerged from the three types of columns were 2.04 (15% PEGA), 1.57 (15% Carbowax-20M), and 1.18 (5% SE-30) for the eluate in 10% ether in *n*-hexane (com-

¹ Appl. Ent. Zool. **8**(3): 200-203 (1973).

² Previously *Spodoptera (Prodenia) litura*. c.f., Distribution Maps of Insect Pest, Series A, Map No. 61 (1956), Map No. 61 (revised) and Map No. 232 (1967), Commonwealth Institute of Entomology, London.

pound A), and 1.65 (15% PEGA), 1.24 (15% Carbowax-20M), and 0.98 (5% SE-30) for the eluate in 50% ether in *n*-hexane (compound B). Quantities of compounds A and B at this step were 250 μg and 35 μg , respectively, from the 50,000 female abdominal tips. Repeated fractionation with gas chromatography on columns of 15% PEGA and 5% SE-30 enabled us to isolate compound A (97 μg) and compound B (12 μg). Neither compound A nor compound B alone conferred biological activity. Mixture of the two compounds in 1.0 to 0.12 ratio elicited characteristic copulatory attempts for 40 to 60% of male moths at a level as low as 1×10^{-5} μg of the two compounds in total.

Catalytic hydrogenation of both compound A and compound B gave myristyl acetate. The infra-red spectrum (Hitachi model 215) of compound A showed the following diagnostic peaks (ca. 50 μg , KBr-micropellet with beam-condenser, cm^{-1}): 2960 (CH_3 -), 2925 and 2850 ($-\text{CH}_2$ -),

1740 ($-\text{C}=\text{O}-$), 1240 ($\text{CH}_3\overset{\text{O}}{\underset{\text{O}}{\parallel}}\text{C}-\text{O}-$), 975 and 945 ($-\text{C}=\overset{\text{O}}{\underset{\text{O}}{\parallel}}\text{C}-$, conjugated). The mass spectrum (Shimadzu-LKB model 9000) of compound A showed a M^+ peak at m/e 252 and a $\text{M}^+ - 60$ peak at m/e 192 corresponding to an ion formed by the loss of acetic acid from the molecular ion of the acetate of a 14-carbon alcohol having two double bonds. The ultra-violet spectrum (Töshiba-Beckman model DB) of compound A (*n*-hexane sol.) showed a λ_{max} at 231–232 $\text{m}\mu$ ($\epsilon=29,000$), indicating a conjugated diene system. Reductive micro-ozonolysis (KLEIMAN et al., 1969) of compound A yielded 9-acetoxynonanal as the largest fragment. Thus, compound A is one of the four isomers of tetradeca-9,11-dien-1-ol acetate. All the four isomers were synthesized by the sequence in Fig. 1. Relative retention times (std.: $\text{C}_{14:0}\text{-OAc}$) from gas chromatographic analysis of the four isomers and compound A on a column of 15% PEGA (2.5 m, 180°C) were as follows: *cis*-9, *cis*-11-, 2.23; *cis*-9, *trans*-11-, 2.08; *trans*-9, *cis*-11-, 2.17; *trans*-9, *trans*-11-, 1.97; compound A, 2.08. Retention times of *cis*-9, *trans*-11-isomer also coincided to those of compound A on columns of 15% Carbowax-20M, 5% SE-30, and 2% OV-17. Results of a laboratory bioassay indicated that *cis*-9, *trans*-11-isomer elicited an apparent copulatory attempt by male moths when combined with

compound B. Therefore, compound A is *cis*-9, *trans*-11-tetradecadien-1-ol acetate.

compound A

compound B

The mass spectrum of compound B also showed a M^+ peak at m/e 252 and $\text{M}^+ - 60$ peak at m/e 192 indicating the structure of a doubly unsaturated 14-carbon alcohol acetate. The ultra-violet spectrum showed no significant absorbance between 210 and 320 $\text{m}\mu$. Reductive ozonolysis of compound B also yielded 9-acetoxynonanal. Gas chromatographic comparison of compound B and several authentic compounds revealed that the retention times of compound B coincided to *cis*-9, *trans*-12-tetradecadien-1-ol acetate on columns of 15% PEGA, 15% Carbowax-20M, 5% SE-30, 2% OV-1, 2% OV-17, and a capillary column of BDS (0.5 mm $\times$ 90 m). Relative retention times (std.: $\text{C}_{14:0}\text{-OAc}$) of the four isomers of tetradeca-9, 12-dien-1-ol acetate and compound B on a column of 15% PEGA (2.5 m, 180°C) were as follow: *cis*-9, *cis*-12-, 1.73; *cis*-9, *trans*-12-, 1.60; *trans*-9, *cis*-12-, 1.69; *trans*-9, *trans*-12-, 1.56; compound B, 1.61. Biological activity of *cis*-9, *trans*-12-isomer was verified by combination with compound A. Therefore, compound B is *cis*-9, *trans*-12-tetradecadien-1-ol acetate.

cis-9, *trans*-11-Tetradecadien-1-ol acetate has not thus far been reported as a natural product possessing biological activity. *cis*-9, *trans*-12-Tetradecadien-1-ol acetate, however, is a well known sex pheromone compound of several Lepidopterous insects (JACOBSON et al., 1970; KUWAHARA et al., 1971a,b; BRADY et al., 1971; BRADY and NORDLUND, 1971; BRADY and GANYARD, 1972).

Neither *cis*-9, *trans*-11-tetradecadien-1-ol acetate nor *cis*-9, *trans*-12-tetradecadien-1-ol acetate alone elicited copulatory attempts by male moths of *S. litura* at a level lower than 1×10^{-3} μg . A mixture of *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetradecadien-1-ol acetates in a 9 : 1 ratio, however, showed apparent biological activity (male response : 30%) at a total concentration of 1×10^{-6} μg of the two compounds. A preliminary

Fig. 1. Synthesis of four isomers of tetradeca-9, 11-dien-1-ol acetate.

experiment on various mixtures of *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetradecadien-1-ol acetates showed that the ratio of the two compounds is an important factor of potency of the sex pheromone as is the case of *cis*-9- and *cis*-11-tetradecen-1-ol acetates in *Adoxophyes fasciata* and *A. orana* (TAMAKI et al., 1971 a,b).

NOTES ADDED IN PROOF

We succeeded to identify the same two compounds, *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetra-

decadien-1-ol acetates, as active components of the sex pheromone of *Spodoptera littoralis* (BOISD.) using 3,000 abdominal tips of virgin females. A mixture of synthesized *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetradecadien-1-ol acetates was much more active than other 15 combinations of geometrical isomers. BR₅₀ (50% behavioural response) value of 20 : 1 mixture of the synthesized *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetradecadien-1-ol acetates was 10⁻⁷ μg in a laboratory bioassay. Details will be reported elsewhere.

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