Sex Pheromone of the Almond Moth and the Indian Meal Moth: cis-9, trans-12-Tetradecadienyl Acetate

Abstract. *Female moths of different species but belonging to the same subfamily produce an identical compound as their sex pheromone. The sex pheromone of the almond moth,* Cadra cautella *(Walker), and the Indian meal moth,* Plodia interpunctella *(Hübner), has been isolated and identified as* cis-*9,* trans-*12-tetradecadienyl acetate.*

The almond moth *Cadra cautella* (Walker) and the Indian meal moth *Plodia interpunctella* (Hübner) are serious economic pests of the stored grain, its products, and dried fruits; these pests are widely distributed in the Temperate and Tropical zones of the world. The existence of the female pheromone or pheromones in these moths has been demonstrated by several investigators (*1*), but the chemical nature of the pheromone has not been elucidated. Moreover it has been stated that sex pheromones of closely related pyralid species are not always specific to the species in their biological activity (*2*); in fact, males of the almond moth are attracted to the crude pheromone extract from females of the Indian meal moth, and vice versa (*3*).

We report here the isolation and identification of the sex pheromone of the almond moth and of the Indian meal moth, both of which have, upon elucidation, been shown to be *cis*-9, *trans*-12-tetradecadienyl acetate.

The pheromone of the almond moth. About 1,200,000 unmated females of the almond moth were collected within 24 hours after emergence according to the mass-rearing method for the FT strain (*4*) described previously (*5, 6*). Female bodies were immersed and extracted with methylene chloride. Evaporation of the solvent afforded a yellow viscous oil (1800 g; activity at 10^{-3} mg/ml). The activity of each fraction on the isolation process was monitored (*5*). Methanol (6 liters) was added to the concentrate with vigorous stirring, and the mixture was kept at −20°C for 24 hours. The yellow supernatant layer was separated by decantation from the inactive precipitate. The precipitate was also extracted four times as described above. The combined methanol solution was evaporated in a vacuum to give the crude pheromone as a yellowish brown oil (313 g, 10^{-4} mg/ml). The oil was chromatographed in four portions on silicic acid (640 g each, 100 mesh, Mallinckrodt); the solvents used successively were mixtures of *n*-hexane and ether (by volume) 100:0, 100:0.25, 100:0.5, 100:1, and methanol (6 liters each). The active fraction was eluted in a mixture of *n*-hexane and ether 100:0.5, or 100:1, or both (*7*). The active fraction (56.3 g) was separated further by repeating column chromatography three times under the following conditions: (i) Silicic acid (2000 g) was eluted with *n*-hexane:benzene (3:1, by volume), the yield of the active oil being 4.7 g (activity, 10^{-4} to 10^{-5} mg/ml); (ii) silicic acid (330 g) was eluted successively with *n*-hexane: ether (100:0.25, 100:0.5, and 100:1, by volume) and methanol, the yield being 193.5 mg (activity 10^{-6} to 10^{-7} mg/ml); and (iii) silicic acid (100 g) was eluted with *n*-hexane:benzene (3:1, by volume), the yield being 24.7 mg (activity 10^{-8} to 10^{-9} mg/ml). The resultant active oil was finally purified by column chromatography on silicic acid (10 g) impregnated with 15 percent silver nitrate with a mixture of *n*-hexane and ether as solvent, the ether portion of which was increased step by step. The active component was eluted with a mixture of *n*-hexane and ether (100:5, by volume). Evaporation of solvent from this eluate gave the pure sex pheromone as a colorless oil (yield 6.1 mg, activity 10^{-9} to 10^{-10} mg/ml) which, in gas-liquid chromatography (GLC) on a column (45 m by 0.25 mm inside diameter) coated with Ucon Oil LB-550X at 160°C, showed a single peak having a relative retention time of 1.167, with myristyl acetate as an internal standard (46.90 minutes).

The infrared spectrum of the isolated pheromone showed absorption bands at 3010 (shoulder), 2924, 2880, 1740, 1380, 1365, 1240, 965, and 720 cm^{-1} in 1 percent carbon disulfide, which suggests the presence of an *O*-acetyl group or groups and a *trans* double bond or bonds. The high-resolution mass spectrum indicated a molecular-ion peak at *m/e* 252.2059 (calculated value for $C_{16}H_{28}O_2$, 252.20893). Catalytic hydrogenation over paladium black and the subsequent alkaline hydrolysis of the pheromone (20 μg) gave myristyl acetate and myristyl alcohol, respectively, which were confirmed by cochromatography with authentic samples. Our results indicate that the pheromone molecule is a straight-chain C_{14} alcohol acetate with two double bonds. The nuclear magnetic resonance (NMR) spectrum of the pheromone ($CDCl_3$, 60 Mhz) was τ 4.57 (4H, -CH=CH-, multiplet); 5.95 (2H, $-CH_2-OCO-$, triplet); 7.27 (2H, $-C=C-CH_2-C=C-$, broad); 7.97 (3H, $-OCO-CH_3$, singlet); 7.8 to 8.1 (2H, $-C=C-CH_2-$, broad); 8.25 to 8.45 (5H, $CH_3-C=C-$ and $-CH_2-C-OCO-$), and 8.7 [10H, $-(CH_2)_5-$].

Therefore the structure of the pheromone is concluded to be 9,12-tetradecadienyl acetate. This conclusion is also supported by the following experimental result. On microozonolysis (*8*) of the pheromone (20 μg), ω-acetoxynonanal was identified as a product by GLC and also by GLC coupled with mass spectrometry.

To assign the geometrical structure, we synthesized four possible isomers: *cis-cis, cis-trans, trans-cis* and *trans-trans* (*9*). Of these isomers the *cis*-9, *trans*-12-dienyl isomer was identical to the natural pheromone with respect not only to physicochemical properties, such as NMR spectrum, and GLC as described, but also to biological activity.

The pheromone of the Indian meal moth. About 670,000 males and females of the Indian meal moth, which were reared on rice bran in the insectarium at 25° to 28°C, were collected within 24 hours after emergence. The preliminary test showed that no trouble was encountered on the purification of the sex pheromone produced by female moths, even when females were not separated from males before the extraction. Thus, both males and females (the sex ratio being about 1:1) were immersed and extracted with methylene chloride (18 liters).

The solvent was then filtered and removed, and the residual oil (980 g) was treated with methanol (four extractions, 6 liters total) according to the same procedure described for the almond moth for the separation of inactive lipids.

The exploratory experiment with a portion of the methanol-soluble oil (72.5 g) suggested the following preferred treatment. The oil was hydrolyzed in 4 percent methanolic potassium hydroxide (415 ml) at room temperature overnight and subsequently extracted with ether. The ethereal solution was washed with water, dried over

anhydrous sodium sulfate, and then evaporated.

The residual oil (10.0 g) was mixed with methanol (100 ml) and kept at −20°C overnight to separate insoluble inactive crystalline precipitates.

The methanol-soluble material (2.1 g) was acetylated according to a conventional method with acetic anhydride (7 ml) in pyridine (7 ml), to give an oil (2.2 g) which showed strong pheromone activity (10^{-5} mg/ml). The active oil was chromatographed twice on a silicic acid column, first with 100 g of silicic acid, eluted with a mixture of *n*-hexane and benzene (3:1, 7.5 liters), the active fraction (127.7 mg) being eluted from 2.8 to 6.0 liters; and second, with 20 g of silicic acid with successive elutions with mixtures of *n*-hexane and ether (by volume) 100:0 (100 ml), 100:0.25 (500 ml), and 100:0.5 (600 ml), the active fraction (38.7 mg) being eluted with an initial 300 ml of *n*-hexane:ether (100:0.5). As with the extract from the almond moth, the final purification was accomplished by chromatography on silicic acid (10 g) impregnated with 10 percent silver nitrate to give the pure sex pheromone as a colorless oil (500 μg; activity, 10^{-8} mg/ml).

The sex pheromone of the Indian meal moth was established as *cis*-9, *trans*-12-tetradecadienyl acetate by the following experimental data. Its behavior on GLC with the capillary column was identical to that of the synthetic compound, and the product of its microozonolysis was identified as ω-acetoxynonanal by GLC analysis with the authentic sample. In the laboratory bioassay, a response from the male *C. cautella* and the male *P. interpunctella* was elicited by both the isolated female pheromone and the synthetic compound.

During preparation of this report, it was learned that the sex pheromone of *Prodenia eridania* (southern armyworm), was identical to that of the almond moth and of the Indian meal moth (*10*). This result is not too surprising because Klun and Brindley reported the first instance where two lepidopterous insects of different taxonomic families were shown to respond sexually to the same compound (*11*).

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Sex Stimulant and Attractant in the Indian Meal Moth and in the Almond Moth

Abstract. cis-9, trans-*12-Tetradecadien-1-yl acetate was isolated from the female Indian meal moth,* Plodia interpunctella (*Hübner*), *and the female almond moth,* Cadra cautella (*Walker*). *It is the major if not the sole component of the sex stimulatory and attractant pheromone of female* Plodia. *It is present in the pheromone of the female* Cadra *along with at least one synergist.*

We report the isolation and identification of a sex pheromone produced by the female Indian meal moth, *Plodia interpunctella* (Hübner), and the female almond moth, *Cadra cautella* (Walker). These species are economically important pests of stored food products and are widely distributed throughout the world. The sex pheromone of *Plodia,* isolated from an extract of filter paper that had been in contact with nonmated females for 3 days, stimulates and attracts *Plodia* males. The sex pheromone of *Cadra,* isolated from an extract of the abdominal tips of 2-day-old nonmated females, incompletely stimulates and attracts *Cadra* males. In laboratory stimulatory tests (*1*) (Table 1) and attractancy tests (Table 2), *Plodia* males responded identically to each sex pheromone and to *cis*-9, *trans*-12-tetradecadien-1-yl acetate. *Cadra* males responded in the same manner to each compound, but in a different manner from that of *Plodia* males.

Initial experiments indicated that extracts of filter paper in contact with nonmated *Plodia* females for 3 days contained many times more sex pheromone as compared to extracts of abdominal tips of the same number of nonmated 3-day-old females. Moths were collected singly from the culture medium at the time of eclosion (*1*). About 19,000 nonmated females were held for 3 days in glass jars lined with two sheets of filter paper (190 mm in diameter) previously washed in *n*-hexane and dried. The papers were stored at −22°C in a mixture of hexane and ethyl ether (1 : 1) for 2 to 4 weeks, extracted once with the same mixture, and washed twice with ethyl ether. Combined extracts and washings were filtered through anhydrous sodium sulfate, concentrated with a rotary evaporator at 40°C, and chromatographed on a silicic acid column (2 by 43 cm; Unisil, 100 to 200 mesh, 37 g; Clarkson Chemical) that was eluted successively with 50 ml of hexane, 100 ml of a mixture of hexane and 2 percent ethyl ether, 100 ml of a mixture of hexane and 4 percent ethyl ether, 100 ml of a mixture of hexane and 6 percent ethyl ether, and 100 ml methanol. The active compound was eluted in the fraction eluted by hexane and 4 percent ether fraction.

The active fraction was concentrated under reduced pressure and purified by gas-liquid chromatography (GLC): 20 percent butane-1,4-diol succinate (Craig) on Chromosorb W (60 to 80 mesh); glass tubing 2 m by 4 mm (inside diameter); column temperature 190°C; N_2, 50 cm^3/min; retention time 15 to 18 minutes.

This fraction, in pentane, injected onto a column of 3 percent Apiezon L on Chromosorb W (100 to 120 mesh)