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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12 October 1970; revised 13 November 1970 ■

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)

of the same dimensions and under the above conditions, gave a symmetrical peak with a retention time of 12.3 minutes. The compound collected from Apiezon L gave a single symmetrical peak on gas chromatography on SE-30, OV-1, diethylene glycol succinate and on Carbowax 20M. Approximately 200 μg of the highly active sex pheromone was collected from the Apiezon column. *Plodia* males exhibited a typical excitatory response when exposed to this compound. No other fractions from silicic acid column chromatography or gas chromatography elicited a positive behavioral response nor did extracts of filter paper that had not been exposed to moths show a response.

The active compound was identified by mass, ultraviolet, and infrared spectra, and by chemical transformations. The mass spectrum showed the following diagnostic peaks [mass to charge (*m/e*); parent peak, P]: 252 (P), 192 (P-CH_3-COOH), 61 CH_3-$COOH_2^+$; the base peak was 43. The infrared spectrum (CS_2 solvent) showed the following diagnostic peaks (cm^{-1}): 3000 (C = *C-H*), 2926 (CH_2), 2853 (CH_2), 1732 (C=O), 1235 and 1035 (acetate C-O-C), and 962 (*trans* C=C). The ultraviolet spectrum (hexane) showed no absorption above 210 nm.

Saponification of the chromatographically pure pheromone resulted in loss of activity, and subsequent acetylation (*2*) produced a highly active material. Exposure to 5 percent bromine in CCl_4 caused rapid inactivation. Catalytic hydrogenation of 5 μg of chromatographically pure pheromone with platinum oxide produced a single inactive compound that gave the same gas-chromatographic retention times as tetradecyl acetate [on diethylene glycol succinate (DEGS), Apiezon L, OV-1, and SE-30].

The foregoing is conclusive evidence for a straight-chain, C_{14} acetate with two nonconjugated double bonds. It remained only to locate these bonds and determine the stereochemistry. At least one of the bonds is *trans*.

Microozonolysis (*3*) of the chromatographically pure pheromone in CS_2 at −78°C followed by treatment with triphenylphosphine yielded 9-oxononyl acetate, whose mass spectrum and GLC behavior (SE-30 and Carbowax 20M) were identical with those of a reference compound obtained by ozonolysis of *cis*-9-tetradecen-1-yl acetate. The aldehyde fragment from the hydrocarbon end of the pheromone molecule could not be located by GLC of the ozonolysis products when the ozonolysis was carried out in CS_2. Chromatograph quality pentyl acetate (Matheson, Coleman and Bell) has been reported (*3*) to be a good solvent for microozonolysis when acetaldehyde and propionaldehyde are expected products. However, samples of pentyl acetate (as received, a fractional distillation cut, and a GLC cut) all gave a peak on ozonization and treatment with triphenylphosphine that interfered with the acetaldehyde peak. *n*-Hexane (certified 99 mole percent pure, Fisher Scientific) on ozonization and treatment with triphenylphosphine appeared free of impurities that would interfere with acetaldehyde on Porapak Q. Acetaldehyde and propionaldehyde had retention times of 1.9 and 4.5 minutes, respectively, and *n*-hexane had a retention time of 16 minutes on Porapak Q (80 to 100 mesh); stainless steel tubing 1.5 m by 2.4 mm (inside diameter); column temperature 150°C, He, 15 cm^3/min. Microozonolysis of the chromatographically pure pheromone in *n*-hexane at −78°C followed by treatment with triphenylphosphine and chromatography on Porapak Q under the above conditions yielded acetaldehyde. The two double bonds, therefore, are located at the 9-carbon and the 12-carbon; and the sex pheromone is 9,12-tetradecadien-1-yl acetate, at least one of the double bonds being *trans*.

A compound, chromatographically and biologically identical with the sex pheromone from *Plodia* females, was isolated by the above procedures from the abdominal tips of 10,000 2-day-old nonmated female *Cadra* moths.

Recently, *cis*-9, *trans*-12-tetradecadien-1-yl acetate (compound **1**) was reported as one of two sex pheromones in the female southern armyworm (*4*). A synthesized sample of this compound, obtained from M. Jacobson (*4*), cochromatographed identically with the sex pheromone isolated from *Plodia* females (on DEGS, Apiezon L, Carbowax 20M, and SE-30), and from *Cadra* females (on DEGS and Apiezon L).

In the sex stimulatory bioassay, *Plodia* males responded identically to the pheromone isolated from *Plodia* females, to compound **1**, and to the pheromone isolated from *Cadra* females (Table 1). This response was almost identical to that obtained from extracts of tips of *Plodia* females containing about the same amount of *Plodia* pheromone used in the assay. *Cadra* males responded identically to compound **1**, to isolated *Cadra* pheromone, and to isolated *Plodia* pheromone (Table 1). However, the excitatory response generally was not accompanied by an orientation of males to the source of the test compound in contrast to an oriented and excitatory sexual response to extracts of tips of *Cadra* females. Surprisingly, exposure of *Cadra* males to higher concentrations (10^{-4} μg or more) of the pure sex pheromone isolated from *Cadra* or *Plodia* females or of compound **1** resulted in an immediate and unique inhibition of behavioral response in almost all males tested. In comparison, *Plodia* males are highly excited when exposed to 10^{-2} μg of compound **1**. Also, *Cadra* males are not inhibited but are highly excited in a typical manner by exposure to concentrations of silicic acid purified extracts of *Cadra* females containing about 100 times more of the *Cadra* active pheromone (about 10^{-2} μg) than is required to inhibit *Cadra* males when tested as a pure compound. Cross-stimulatory studies with extracts of *Plodia* and *Cadra* females have been reported (*5*).

Table 1. Sex stimulatory response of *Plodia* and *Cadra* males to *cis*-9, *trans*-12-tetradecadien-1-yl acetate (**1**) and the active sex pheromones of *Plodia* and *Cadra* females. Bioassays conducted with dilutions from GLC quantitated compounds as was described in (*1*) except that 10 μl containing desired amounts of each compound was placed on the rod. The result represents the number of picograms needed to elicit a response in 50 percent of the males from dose-response curves of two replications with 75 males per replication.

Male	Compound **1**	Sex pheromones	
		Plodia	*Cadra*
Plodia	0.02	0.04	0.03
Cadra	0.07	0.53	0.02

Table 2. Attraction of *Plodia* and *Cadra* males to *cis*-9, *trans*-12-tetradecadien-1-yl acetate (**1**) and the active sex pheromones of *Plodia* and *Cadra* females. Tests were conducted in an isolated and dark room (42.4 m^3). Each of four modified Frick traps were charged with 10 ng of compound in each test (one control trap). Each night 100 nonmated males were released. Results are an average of four to seven tests.

Male	Males trapped per night (%)		
	Compound **1**	*Plodia*	*Cadra*
Plodia	46	53	51
Cadra	27		25

The results given in the following paragraph support the conclusion that at least one other sex pheromone is present in *Cadra* females and is necessary to elicit a normal sex stimulatory response in *Cadra* males.

Another compound purified by GLC, isolated from tips of nonmated *Cadra* females, elicits no apparent behavioral activity in *Cadra* males. Yet this compound, combined in the natural ratio with the behaviorally active sex pheromone, causes a definite increase in orientation of males. As compared with extracts of *Cadra* females, the combined compounds effect the same degree of orientation and copulatory behavior in *Cadra* males observed. This is the first demonstration in a lepidopteran species of a second compound isolated from females that is inactive by itself, but is synergistic with a sex pheromone. This inactive compound may be a factor in isolating closely related species.

Attractancy tests were conducted as described in Table 2 and also as follows. About 5:00 p.m., 3 hours after nonmated males (2 days old) were released into the room, Stikem-coated traps were charged with test material on filter paper and an air stream was directed into each trap. Lights were then turned off, and captures were recorded next morning. The attracting property (as distinguished from the sexual stimulatory property) of compound **1** to *Plodia* males is the same as that of the active pheromones isolated from *Plodia* and *Cadra* females (Table 2), and is similar to but slightly less effective than that of crude extracts and live *Plodia* females. Initial results with single and combined fractions of a thin-layer chromatogram of an extract of *Plodia* females indicate the presence of a compound (or compounds) that slightly synergizes attraction to the behaviorally active sex pheromone. Compound **1** is as attractive to *Cadra* males as the active compound from *Cadra* females (Table 2). However, either compound is considerably less attractive to male *Cadra* than crude extracts of female *Cadra* containing about equal amounts of the behaviorally active sex pheromone.

We emphasize the distinction between our bioassays for sexual stimulatory response and for attraction. This distinction has unfortunately been ignored in many studies.

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19 October 1970; revised 10 December 1970 ■

Synaptic Adjustment after Deafferentation of the Superior Colliculus of the Rat

Abstract. *Eyes were removed from rats shortly after birth, when there are few formed synapses in the colliculus. It was found that synaptogenesis continues to give a near-normal ratio of terminals containing either spheroidal or flattened vesicles. After eye removal in adult rats, however, reinvasion of synaptic sites vacated by degenerate optic terminals occurs, with an incomplete return toward a normal proportion of synaptic types.*

Previous work has indicated that removal of afferent nerve fibers to a region of the central nervous system can cause compensatory sprouting of other pathways projecting to the region (*1*). The object of this study has been to investigate the degree of synaptic adjustment in the upper layers of the superior colliculus of the rat after removal of their major input—the input from the retina. Three experimental situations have been studied. In the first, one or both eyes were removed from adult animals, in which the normal synaptic patterns of the superior colliculus are well established. In the second situation, one or both eyes were removed from animals within the first postnatal day. At this time very few synapses of any kind have been formed in the colliculus. In the third case, eyes were removed at intermediate ages: 10, 14, and 24 days postnatal. At these times the full synaptic pattern has not yet been established, the main formation being within the 2-week period after the eyes are opened at 14 days postnatal.

Most of the animals were allowed to survive for 4 to 5 months; some of the enucleated adults were killed after 14 postoperative days. Of the animals with unilateral enucleation at birth, a few had second lesions as adults, either of the cortex or remaining eye, to see how much synaptic reorganization might be due to compensatory sprouting of the corticotectal or uncrossed retinotectal pathways. Comparison material for light microscopy from each series was obtained and stained with Fink and Heimer, neurofibrillar, and Nissl methods. All animals were perfused with paraformaldehyde and glutaraldehyde in phosphate buffer (*2*).

Attention is particularly directed to four main features: (i) synaptic vesicle morphology, (ii) serial synapses, (iii) morphology of synaptic contacts, and (iv) occurrence of synapses where the presynaptic element is dendritic. From previous work (*3*), in which buffered aldehyde fixatives of appropriate osmolarity were used, two main populations of terminals can be recognized; they contain either spheroidal synaptic vesicles (S terminals) or predominantly flattened vesicles (F terminals) (see Fig. 1a). With regard to serial synapses (where one terminal is presynaptic to another), the postsynaptic element in the superior colliculus always contains flattened vesicles, but the presynaptic element may be either an S or an F terminal. A synaptic density may have a pronounced postsynaptic thickening (asymmetric contact), or this may be absent (symmetric contact). Presynaptic dendritic profiles occur in the normal superior colliculus; they form F terminals with symmetric contacts.

Counts were taken of synaptic contact features from electron micrographs or by direct observation of electron microscope sections. The results are summarized in Table 1. Each of the experimental groups contained at least three animals, and, for each animal, counts were taken from several blocks of tissue. Each count was from a column extending from stratum zonale to the superficial region of the stratum