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# ISOLATION, IDENTIFICATION, AND SYNTHESIS OF A FEMALE SEX PHEROMONE OF THE NAVEL ORANGEWORM, *Amyelois transitella* (LEPIDOPTERA: PYRALIDAE)[1]

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**Abstract**—A sex pheromone of the navel orangeworm, *Amyelois transitella* (Walker), was obtained from ether rinses of the sex pheromone gland of calling females. The pheromone was isolated by means of liquid and gas chromatography and was identified as one of four possible geometrical isomers of 11,13-hexadecadienal by means of spectroscopic and microchemical methods. Synthesis and laboratory bioassay of all four isomers revealed that only the (*Z,Z*) isomer was biologically active. (*Z,Z*)-11,13-hexadecadienal elicited quantitatively similar activation and attraction responses by *A. transitella* males as did the natural product.

**Key Words**—Sex pheromone, navel orangeworm, *Amyelois transitella*, (*Z,Z*)-11,13-hexadecadienal, Pyralidae.

## INTRODUCTION

The navel orangeworm, *Amyelois transitella* (Walker), is a well-established primary pest of almonds in California for which no completely satisfactory method has been developed for monitoring adult populations. The principal methods that have been used to monitor adult activity for the purpose of timing chemical control measures include light traps (Madsen and Sanborn, 1962; Summers and Price, 1964) and traps baited with laboratory rearing

[1]Mention of a commercial or proprietary product in this paper does not constitute an endorsement of that product by the USDA. Accepted for publication February 28, 1979.

medium. The latter were reported by Rice (1976) and Rice et al. (1976) to be attractive to *A. transitella* females.

Proshold (1967) and Asoka Srinivasan (1969) studied the sex pheromone of *A. transitella* females and reported that traps baited with live females were attractive to males in commercial almond and walnut plantings. The lack of a synthetic sex pheromone has, however, limited the usefulness of population monitoring by this means. The availability of a synthetic sex pheromone for this species would allow more extensive use of this survey method than is presently possible and would be a useful adjunct to extant methods (light traps, oviposition attractants) of monitoring. In addition, the feasibility of using the synthetic pheromone or related compounds as disruptants of mating communication (Mitchell, 1975) could be explored.

This paper reports the isolation, identification, and synthesis of a sex pheromone of *A. transitella* that was obtained from diethyl ether rinses of the sex pheromone glands of laboratory-reared females.

## METHODS AND MATERIALS

Moths used in this study were reared in this laboratory at 26.7 ± 1° C and ca. 60% relative humidity under a reversed 14:10 light:dark photoperiod. Photophase and scotophase light intensities were >250 and ca. 0.3 lux, respectively. Details of the insect diet and rearing procedure are described elsewhere (Coffelt et al., 1978). Periodic introduction of new stock during the course of this study was made from eggs and larvae that were obtained from the Stored-Product Insects Laboratory, Fresno, California.

Details of the bioassay apparatus and procedures are described elsewhere (Coffelt et al., 1979). Briefly, two kinds of bioassays were conducted: In the first, males were placed individually in glass vials (22 × 53 mm) and the test sample placed within 2 cm of them; onset of locomotion and "wing-buzzing" within 15 sec of sample introduction was considered evidence of pheromone response. In the second bioassay, upwind movement (ca. 0.4 m) in Plexiglas tubes (apparatus described by Sower et al., 1973b) to within ca. 3 cm of the pheromone locus within 30 sec after sample introduction was taken as evidence of pheromone response. Male response to applicators treated with solvent only was ca. 10% in either bioassay.

Initial trials to obtain the pheromone from either solvent rinses of excised female abdominal tips, whole female bodies, or filter papers upon which females had been held yielded nonreproducible quantities as determined by bioassay. Subsequently, superficial diethyl ether rinses of excised pheromone glands of calling females (7-10th hr of scotophase) were made by the method described by Sower et al. (1973a). Quantitative bioassays of pheromone obtained by this method were reproducible, and the extracts were clean

enough for gas-liquid chromatography (GLC) without further purification.

All analytical and micropreparative GLC during the identification phase of the project were done on Varian model 2100 instruments that were equipped with $H_2$ flame ionization detectors. Instrument operating parameters were the same as those described by Coffelt et al. (1978).

## RESULTS

*Isolation.* A superficial pheromone gland rinse (30 females) was concentrated to ca. 4 $\mu$l and injected into a 1.8-m × 2-mm (ID) glass column packed with 3% OV-1 on 100/120 mesh Gas Chrom Q® column; the temperature was programed (6°C/min) from 100°C at injection to 220°C. The resulting chromatogram, shown in Figure 1A, revealed two detectable peaks in the

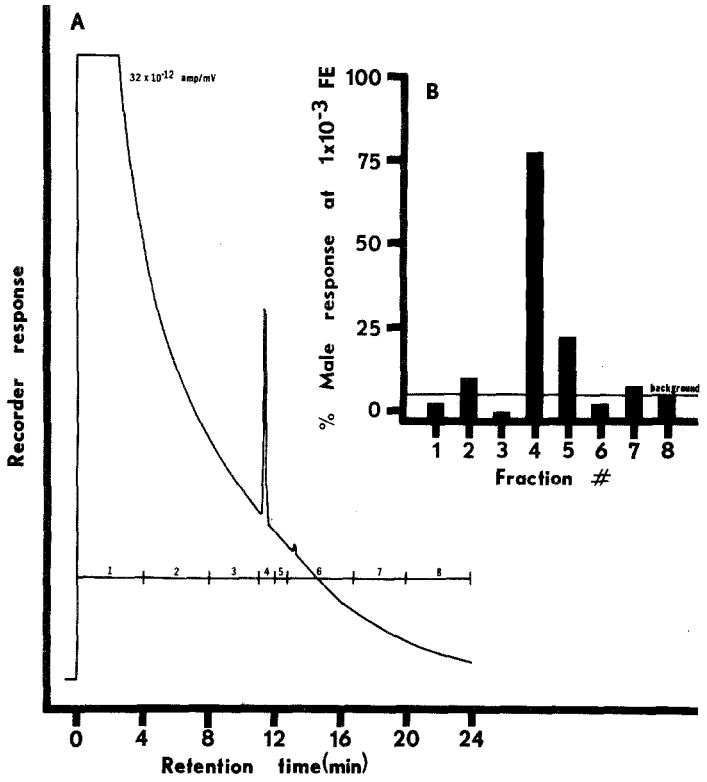


FIG. 1. (A) Gas chromatogram of superficial diethyl ether rinse of terminal abdominal tips of 30 calling *A. transitella* females. Instrument parameters as described in text. (B) Response of male *A. transitella* to micro-preparative GLC fractions as indicated in (A).

elution range of most previously identified lepidopteran sex pheromones. The major component, ca. 1.0–1.5 ng/female, had a retention time of 11.6 min (170° C) and a carbon number of 18.1 relative to normal paraffins. The minor component of the gland rinse eluted at 13.0 min (178° C) (carbon number 19.0 relative to normal paraffins) and was present in concentration of ca. 0.1 ng/female. A similar preparation (25 females) was injected as above, and the column effluent was collected in 1.0 ml of hexane at 0–4, 4–8, 8–11, 11–12, 12–13, 13–16.7, 16.7–20, and 20–24 min after injection. Each fraction was diluted to yield $1 \times 10^{-3}$ female equivalents (FE) / 5 $\mu$l and bioassayed (activation test). The results of this series of tests (5 replications) are shown in Figure 1B. More than 95% of the biological activity (based on additional bioassays of further dilutions) was contained in the 11 to 12-min GLC fraction, which corresponded to the retention time of the major component indicated in Figure 1A. Male response to the 11 to 12-min fraction was not significantly different from that recorded for an aliquot of the unchromatographed gland rinse over a range of concentrations from $1 \times 10^{-2}$ to $1 \times 10^{-4}$ FE. The 13.0 to 16.7-min fraction (fraction 6, Figure 1A) containing the minor component of the gland rinse was inactive.

Subsequently, similar gland rinses were collected from 5% Carbowax 20M on 100/120 mesh Chromosorb W®, column temperature 170° C, and 5% HiEff-1BP on 60/80 mesh Gas Chrom Q®, column temperature 140° C. In each case, one area of biological activity was found (Carbowax), 10.5–12.0 min; HiEff-1BP, 13.6–15.5 min). When the active fraction from either polar GLC column was recollected from the OV-1 column, the biological activity eluted only in the 11 to 12-min fraction shown in Figure 1A. No significant loss of biological activity (activation or orientation bioassays) was noted following GLC on any of the three columns used.

*Identification.* Pheromone was purified for mass spectral analysis as follows: Diethyl ether gland rinses from ca. 5000 calling females were stored in lots of 100 at −10° C until used. These extracts were combined, carefully concentrated under dry $N_2$ to near dryness, and taken back up in 0.5 ml of hexane. The extract was injected onto a 1.0-cm (ID) glass high-pressure liquid chromatographic (HPLC) column packed to a height of 50.0 cm with Poragel® 60A, 37–75 $\mu$m (Waters Associates). Forty 2.5-ml fractions were collected with hexane as the eluting solvent at a flow rate of 120 ml/hr. Biological activity (activation and orientation bioassays) appeared as a single band with an elution volume of 60–70 ml (fractions 24–28). Analytical GLC (OV-1) of each fraction showed one detectable peak with a retention time identical to that of the major component of the crude extract shown in Figure 1A. Analytical GLC runs of inactive Poragel 60A fractions did not contain this peak. Quantitative bioassays of pooled active fractions indicated no loss of biological activity relative to dilutions of an unchromatographed aliquot of the crude extract.

The combined active fractions from the Poragel 60A column were further purified by GLC on OV-1 (140° C isothermal). The biologically active fraction contained ca. 2-3 $\mu$g of a compound whose retention time (programed temperature run on OV-1) was identical to that of the major component shown in Figure 1A. Collection of this active fraction from the Carbowax 20M (170° C) column yielded a single apparently symmetrical peak (11.7 min) on both columns and had the same biological activity as the starting material.

A mass spectrum of the purified (>98%) compound was obtained with a Finnigan model 105C chemical ionization mass spectrometer that was equipped with a chromatographic inlet (Varian model 1400). The effluent of a 2.0 m × 2.3 mm (ID) column packed with 3% SE-30 on 100/120 Varaport® 30 was introduced directly into the ionization source. Methane served as the carrier and reagent gas. The resulting spectrum had a base peak at $m/e$ 95 and a molecular weight of 236 established by peaks at $m/e$ 237 (P+1), 265 (P+29), and 277 (P+41). A peak at $m/e$ 219 (P+1-18) and the character of the remaining portion of the spectrum suggested that the compound was an unbranched aldehyde or alcohol. Thus, the pheromone appeared to be either a 16-carbon triunsaturated alcohol or a diunsaturated aldehyde.

Hydrogenation (Pd on charcoal) of ca. 300 ng of the purified compound resulted in the loss of biological activity and the disappearance of the original peak (GLC on 3% OV-1 and 5% Carbowax 20M) and the appearance of a new peak with a retention time on both polar and nonpolar stationary phases identical to that of hexadecanal. This result, along with mass spectral data, suggested that the compound was a diunsaturated aldehyde.

Supportive evidence for an aldehyde (as opposed to alcohol) functionality was obtained from each of three independent tests. First, the pheromone eluted from silicic acid columns (Sower et al., 1973b) with standard aldehydes. Second, treatment of the pheromone with acetic anhydride in pyridine did not significantly reduce biological activity relative to untreated pyridine controls. Third, treatment of the pheromone with $NaBH_4$ resulted in a significant reduction in biological activity.

The retention times of the pheromone relative to hexadecanal on both polar and nonpolar GLC were much longer than expected for a hexadecadienal with two isolated double bonds. On OV-1, for example, the purified pheromone eluted after hexadecanal. This observation suggested that the two centers of unsaturation were conjugated, i.e., that the pheromone was a conjugated diene or an $\alpha,\beta$-unsaturated aldehyde. To confirm this, we obtained the UV spectrum of ca. 750 ng of GLC purified (~96%) pheromone in 200 $\mu$l of ethanol. Maximum absorbance was at 232 $\mu$m with an extinction coefficient of 18,100. This result confirmed the presence of a conjugated system in the compound, and suggested that the material was a conjugated diene rather than an $\alpha,\beta$-unsaturated aldehyde whose maximum absorbance would have been near 220 $\mu$m.

Microozonolysis (Beroza and Bierl, 1967) of ca. 1 μg of GLC purified (~96%) pheromone resulted in >99% loss of biological activity, disappearance of the original peak (OV-1 and Carbowax 20M), and the appearance of a new peak with a retention time on both columns identical to that of 1,11-undecanedial. The presence of the latter compound was confirmed by mass spectrometry. However, this compound would result from the ozonolysis of either 11,13- or 2,13-hexadecadienal.

The following experiment was conducted to unequivocally establish the correct positional isomer: Ca. 3 μg of GLC purified (~96%) pheromone was reduced in 100 μl of 1 molar ethanolic $NaBH_4$. This reaction product was acetylated by the addition of 200 μl of pyridine and 50 μl of acetic anhydride. The progress of each reaction was monitored by GLC. The final reaction product was ozonized as before.

The retention times of the major ozonolysis product were 11.6 and 10.0 min, on polar (5% Carbowax 20M; 175° C) and nonpolar (3% OV-1; 130° C) GLC substrates, respectively, and were identical to those of authentic 11-acetoxyundecanal. Also, the mass spectra of the ozonolysis product and 11-acetoxyundecanal were identical. This result fixed the position of the double bond nearest to the functional group at the 11 position, and along with the GLC and UV evidence for the presence of a conjugated system, indicated that the compound isolated from pheromone gland rinses of calling *A. transitella* was one of four possible geometrical isomers of 11,13-hexadecadienal.

*Synthesis and Purification of the 11,13-Hexadecadienal Isomers.* Although several methods exist that enable stereospecific construction of conjugated dienes, we employed a method that would generate mixtures thereby permitting some variation in isomer composition. The final purification of each isomer would be accomplished, in any case, by HPLC and/or preparative GLC. A de novo stereospecific approach for each geometrical isomer would only be time consuming. Also, purity could not be assured by using these previously established routes.

The method chosen is outlined in Figure 2. The key step, a Wittig condensation involving an 11-carbon aldehyde ether with an allylic ylid, afforded conjugated dienes of mixed geometrical composition. The ylids were prepared from a predominantly *cis*, or *trans* allylic phosphonium salt, and the base-solvent choices were varied to guide the isomer ratio somewhat. Mixtures were chosen to facilitate purification of individual isomers.

The tetrahydropyranyl ether (THP) of 10-undecen-1-ol was hydroborated and worked up oxidatively to produce primarily the mono-THP ether of 1,11-undecanediol which was contaminated to a degree (20%) by the diol itself. The mono-THP ether was further purified by fractional distillation (bp 150–156°, 0.02 mm). Pyridinium chlorochromate (PCC) that was buffered with sodium acetate was employed to yield the THP ether of 11-hydroxy-

undecanal, I (purified by gravity-flow liquid-solid chromatography; IR 1727 $cm^{-1}$).

Reduction of 2-pentyn-1-ol by either *syn* addition of hydrogen (P-2 nickel) or *anti* addition (lithium aluminum hydride in refluxing tetrahydrofuran, THF) produced (*Z*)-2-penten-1-ol and (*E*)-2-penten-1-ol, respectively. These were converted to the allylic bromides with phosphorus tribromide-pyridine and then to phosphonium salts with triphenylphosphine in benzene at room temperature. Although the reductions of propargylic alcohols and their conversions to allylic halides are highly stereoselective (Truscheit and Eiter, 1962), the conversions of the resulting allylic alcohols to bromides can be troublesome (Smith et al., 1978). In particular, *cis-trans* isomerization can occur during distillation. The bromides were therefore converted, without prior distillation, directly to phosphonium salts. The II-*Z* salt melted at 191–196°, its reported melting point is 144–145° (Truscheit and Eiter, 1962). The II-*E* salt melted at 170–173°, its reported melting point is 144° (Butenandt et al., 1962) and 178–180° (Truscheit and Eiter, 1962). The mixture melting point of our two preparations showed a distinct depression, 160–185°. The salts were converted to ylids by two methods (BuLi in THF-HMPA, NaH-DMSO) and allowed to react with the aldehyde I. The diene ethers III were converted to acetates IV for GLC analysis [Ultrabond I Carbowax, 2 m × 3 mm at 150° and SP-2340 capillary (WCOT) column, 45 m × 0.25 mm at 156°]. The acetates were further characterized by HPLC on a 20%

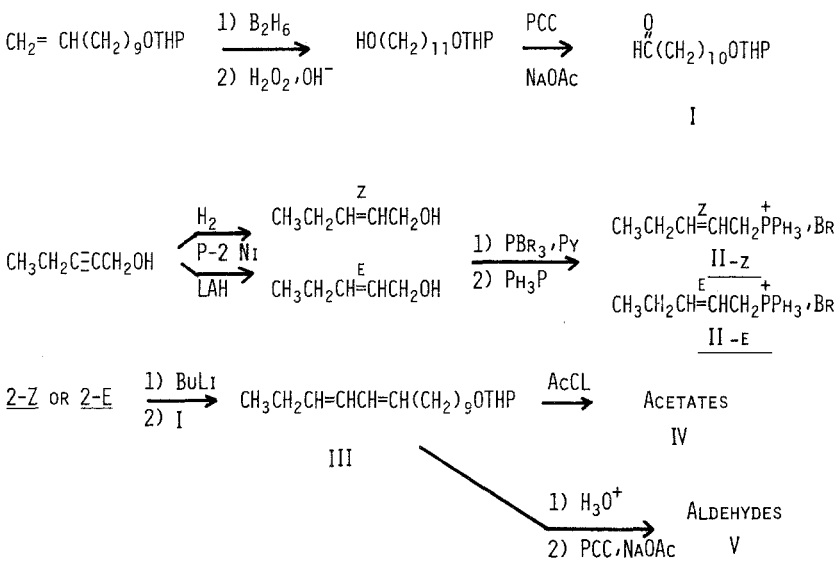


FIG. 2. Synthesis routes for geometrical isomers of 11,13-hexadecadienal.

$AgNO_3$-EF-54 column, 1.25 cm × 25 cm, 4:1 benzene-hexane at 590 psi. Diene ether preparations were chosen on the basis of the acetate analysis and were converted to aldehydes by (1) hydrolysis to the alcohols and (2) buffered PCC oxidation. The aldehydes were then collected by HPLC employing the column described. Since the 11-*Z*,13-*E* and 11-*E*,13-*Z* isomers were only partially separated by HPLC, collections were made from mixtures richer in one than the other by collecting the early or late portions of the unresolved doublet. The collected fractions were washed with aqueous sodium chloride, dried (sodium sulfate), and filtered. They were then concentrated in a slow stream of nitrogen and trapped after passage through a 4% OV-101 column, 2 m × 3.2 mm at 200° C. The aldehydes were then analyzed with the capillary column described above. This GLC analysis did not effect a complete separation of the *Z,Z* isomer from the (*E,Z*)-11,13-isomer. However, a judicious use of both HPLC and capillary GLC permitted a reliable assessment of the constitution of each collected aldehyde.

Because conjugated dienes have been previously determined as insect sex pheromones, a limited amount of chromatographic data concerning these materials has been published (Goto et al., 1975; Hall et al., 1975; Roelofs et al., 1971, 1974; Tamaki et al., 1973). These data deal with 7,9-dodecadien-1-ol acetate, 8,10-dodecadien-1-ol, and 9,11-tetradecadien-1-ol acetate. The pheromone of *A. transitella* is the second determination of a conjugated dienal, and we felt that inclusion of our chromatographic data for the synthesized diene acetates and aldehydes would be of value (Table 1). The estimated purities of the isomeric aldehydes are: (*E,E*); ⩾98%; (*E,Z*) 96%, contaminated with

TABLE 1. CHROMATOGRAPHIC PROPERTIES OF GEOMETRICAL ISOMERS OF 11,13-HEXADECADIENAL AND 11,13-HEXADECADIEN-1-OL ACETATE[a]

| Compound | HPLC[b] | UB-I-CW (GLC)[c] | SP2340 (GLC)[c] |
|---|---|---|---|
| (*E,E*) aldehyde | 1.00 | | 1.05 |
| (*E,Z*) aldehyde | 1.30 | | 1.03 |
| (*Z,E*) aldehyde | 1.24 | | 1.00 |
| (*Z,Z*) aldehyde | 1.52 | | 1.03 |
| (*E,E*) acetate | 1.00 | 1.69 | 2.24 |
| (*E,Z*) acetate | 1.23 | 1.57 | 2.19 |
| (*Z,E*) acetate | 1.17 | 1.50 | 2.09 |
| (*Z,Z*) acetate | 1.38 | 1.59 | 2.17 |

[a]Chromatographic columns as described in text.
[b]Retention volumes relative to (*E,E*) isomer.
[c]Retention times for aldehydes relative to (*Z,E*)-11,13 aldehyde isomer; for acetates relative to 1-hexadecanol acetate.

TABLE 2. ACTIVATION RESPONSE OF *A. transitella* MALES TO UNPURIFIED SEX PHEROMONE AND GEOMETRICAL ISOMERS OF 11,13-HEXADECADIENAL AT $1 \times 10^{-4}$ μg

| Treatment | Purity (%)[a] | % Male response (SE) |
|---|---|---|
| Unpurified pheromone[b] | | 90.0 (2.0) a[c] |
| *Z,Z* | ≥99 | 90.0 (3.2) a |
| *Z,E* | ~93 | 10.0 (2.0) b |
| *E,Z* | ~96 | 10.0 (4.8) b |
| *E,E* | >98 | 23.8 (6.0) b |
| All 4 isomers[d] | | 92.5 (2.5) a |
| Blank | | 12.2 (3.8) b |

[a]Purity determined by capillary GC; columns as described in text.
[b]Concentration determined by GLC as described in text.
[c]Means followed by same lower case letter are not significantly different at $P = 0.05$.
[d]$1 \times 10^{-4}$ μg of each isomer.

(*Z,E*); (*Z,E*) 93%, contaminated with (*E,Z*); and (*Z,Z*) ≥99%. Characteristic infrared bands were observed for the diene structures (Roelofs et al., 1974): (*E,E*), 986 $cm^{-1}$; (*Z,E*) and (*E,Z*), 945 and 979 $cm^{-1}$; (*Z,Z*), no absorption in this region of the spectrum.

Mass spectra of the synthesized isomers of 11,13-hexadecadienal were identical with that of the purified natural product.

*Laboratory Bioassays of Synthesized Isomers of 11,13-Hexadecadienal.* All bioassays (activation and orientation) were conducted as previously described by using hexane solutions of the four geometrical isomers of 11,13-hexadecadienal and unpurified pheromone gland rinse. Estimates of pheromone quantity in the latter solution were based on GLC of aliquots of the material. Unpurified pheromone was used as a standard for comparison of relative biological activity of the four isomers in all bioassays.

Initial trials with the synthetic compounds were made with $1 \times 10^{-4}$ μg in the activation bioassay apparatus. The results of this series of tests (8 replications) are shown in Table 2. Male response to the (*Z,Z*) isomer was the same as that recorded for an estimated equal quantity of the unpurified gland rinse. The response of *A. transitella* males to the remaining three isomers was not significantly different from male response to untreated pheromone dispensers. A mixture of the four compounds (1 : 1 : 1 : 1) had no significant effect upon male response relative to the (*Z,Z*) isomer alone (4 replications).

Subsequently, additional bioassays (activation, 8 replications; orientation, 12–15 replications) were conducted over a range of concentrations ($1 \times 10^{-4}$ to $1 \times 10^{-8}$ μg) with the unpurified gland rinse and (*Z,Z*)-11,13-hexadecadienal. The results of these bioassays are summarized in Figures 3

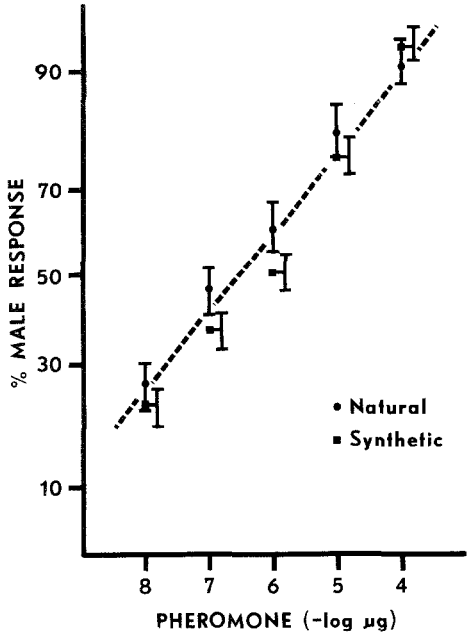


FIG. 3. Activation response of *A. transitella* males to (*Z,Z*)-11,13-hexadecadienal and unpurified pheromone. Background (control) response = 11.0 ± 1.9 (SE)%.

and 4, and indicate the qualitative and quantitative similarity of the response in both the activation and orientation bioassays. The quantitative similarity is evident from the *Y* values for each test material. The qualitative similarity is inferred from the generally similar slopes.

## DISCUSSION

The chemical and biological data presented in this paper are strong evidence that (*Z,Z*)-11,13-hexadecadienal is a pheromone of *A. transitella*. Because of the very small quantities of material produced by the female, the losses during extraction and purification (ca. 50%), and the similar gas chromatographic properties of the various isomers, especially the (*Z,Z*) and (*E,Z*), the presence in the natural pheromone of small amounts of the other three stereoisomers is not ruled out. The possibility that the complete pheromone may be a mixture of compounds with different functions cannot be established from our laboratory bioassay data. Determination of an optimum blend will, in all likelihood, be established only with field tests. Such tests are underway and the preliminary data (Curtis et al., unpublished) indicate significant attractiveness of the (*Z,Z*) isomer alone. Other isomers were inactive. Additional tests designed to determine the efficacy of different

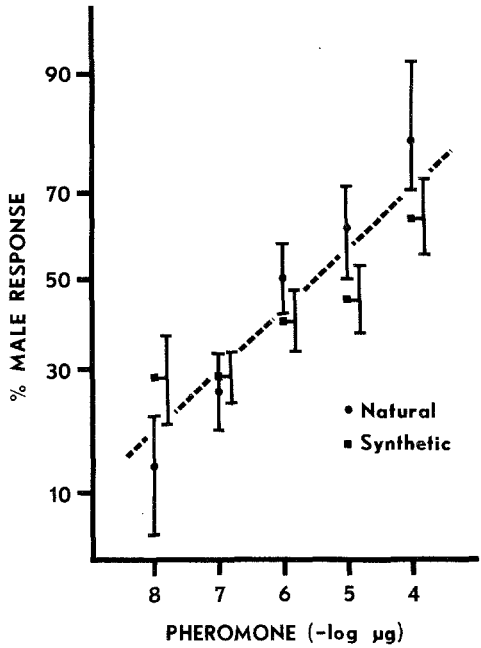


FIG. 4. Orientation response of *A. transitella* males to (*Z,Z*)-11,13-hexadecadienal and purified pheromone. Background (control) response = 9.4 ± 2.8 (SE)%.

substrates for releasing the synthetic pheromone and tests to evaluate the stability of the material are in progress.

A conjugated dienal (*E,Z*)-10,12-hexadecadienal was recently reported by Kasang et al. (1978) to be a sex pheromone for the silkmoth, *Bombyx mori* L., but the *A. transitella* pheromone is the first such compound to be reported as a pheromone based on behavioral data.

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## REFERENCES

BEROZA, M., and BIERL, B.A. 1967. Rapid determination of olefin position in organic compounds of microgram range by ozonolysis and gas chromatography. *Anal. Chem.* 39:1131-1135.

BUTENANDT, A., HECKER, E., HOPP, M., and KOCH, W. 1962. Sexual attractants of the silk moth. IV. Synthesis of bombykol and the *cis-trans* isomers of 10,12-hexadecadien-1-ol. *Justus Liebig's Ann. Chem.* 658:39-64.

COFFELT, J.A., SOWER, L.L., and VICK, K.W. 1978. Quantitative analysis of identified compounds in pheromone gland rinses of *Plodia interpunctella* and *Ephestia cautella* at different times of day. *Environ. Entomol.* 7:502-505.

COFFELT, J.A., VICK, K.W., SOWER, L.L., and MCCLELLAN, W.T. 1979. Sex pheromone behavior of the navel orangeworm, *Amyelois transitella*. *Environ. Entomol.* 8:587-590.

GOTO, G., SHIMA, T., MASUGA, H., MASUSKA, Y., and HIRAGA, K. 1975. A stereoselective synthesis of (*Z,E*)-9,11-tetradecadienyl-1-acetate, a major component of the sex pheromone of *Spodoptera litura*. *Chem. Lett.* 1975:103-106.

HALL, D.R., BEEVOR, P.S., LESTER, R., POPPI, R.G., and NESBITT, B.F. 1975. Synthesis of the major sex pheromone of the Egyptian cotton leaf worm, *Spodoptera littoralis*. *Chem. Ind.* 1 Mar, pp. 216-217.

KASANG, G., KAISSLING, K.E., VOSTROWSKY, O., and BESTMANN, H.J. 1978. Bombykal, a second pheromone component of the silkworm moth, *Bombyx mori* L. *Agnew. Chem.* 17:60.

MADSEN, H.F., and SANBORN, R.R. 1962. Blacklight traps help determine flights of codling moths and other deciduous fruit pests. *Calif. Agric.* 16:12-13.

MITCHELL, E.R. 1975. Disruption of pheromonal communication among coexistent pest insects with multichemical formulations. *BioSci.* 25(8):493-499.

PROSHOLD, F. 1967. The effects of gamma radiation on the navel orangeworm, *Paramyelois transitella* (Walker) (Lepidoptera: Phycitidae), and the feasibility of control with irradiated adults. Ph.D. dissertation, University of California, Berkeley. 130 pp.

RICE, R.E. 1976. A comparison of monitoring techniques for the navel orangeworm. *J. Econ. Entomol.* 69:25-28.

RICE, R.E., SADLER, L.L., HOFFMAN, M.L., and JONES, R.A. 1976. Egg traps for the navel orangeworm, *Paramyelois transitella* (Walker). *Environ. Entomol.* 5:697-700.

ROELOFS, W., COMEAU, A., HILL, A., and MILICEVIC, G. 1971. Sex attractant of the codling moth: Characterization with electroantennogram technique. *Science* 174:297-299.

ROELOFS, W., KOCHANSKY, J., and CARDÉ, R. 1974. *Trans*-7-*cis*-9-dodecadien-1-yl acetate. U.S. Patent 3,845,108. Oct 29, 1974. 3 pp.

SMITH, L.M., SMITH, R.G., LOEHR, T.M., DAVES, G.D., JR., DATERMAN, G.E. and WOHLEB, R.H. 1978. Douglas fir tussock moth pheromone: Identification of a diene analog of the principal attractant and synthesis of stereochemically defined 1,6-, 2,6-, and 3,6-heneicosadien-11-ones. *J. Org. Chem.* 43:2361-2366.

SOWER, L.L., COFFELT, J.A., and VICK, K.W. 1973a. Sex pheromone: A simple method of obtaining relatively pure material from females of five species of moths. *J. Econ. Entomol.* 66:1220-1221.

SOWER, L.L., VICK, K.W., and LONG, J.S. 1973b. Isolation and preliminary biological studies on the female-produced sex pheromone of *Sitotroga cerealella*. *Ann. Entomol. Soc. Am.* 66:184-187.

ASOKA SRINIVASAN, G.S. 1969. Sex pheromone of the navel orangeworm, *Paramyelois transitella* (Walker) (Lepidoptera: Phycitidae): Its biological and ecological significance. Ph. D. dissertation, University of California, Berkeley. 119 pp.

SUMMERS, F.M. and PRICE, D.W. 1964. Control of navel orangeworm. *Calif. Agric.* 18:14-15.

TAMAKI, Y., NOGUCHI, H., and YUSHIMA, T. 1973. Sex pheromone of *Spodoptera litura* (Lepidoptera:Noctuidae) isolation, identification and synthesis. *Appl. Entomol. Zool.* 8:200-203.

TRUSCHEIT, E., and EITER, K. 1962. Synthesis of four isomeric 10,12-hexadecadien-ols. *Justus Liebig's Ann. Chem.* 658:65-90.