Host-Seeking Stimulant for Parasite of Corn Earworm: Isolation, Identification, and Synthesis

Abstract. *13-Methylhentriacontane has been identified in the feces and larvae of the corn earworm,* Heliothis zea *(Boddie), as the major constituent that triggers the short-range host-seeking response of the parasite* Microplitis croceipes *(Cresson). This chemical, the first found that mediates the complex host-parasite relation, could upgrade present efforts to use parasites for insect control. Bioassay of closely related compounds indicated that the structural requirements for activity are remarkably specific.*

13-Methylhentriacontane (formula 1) has been found to direct the parasite *Microplitis croceipes* (Cresson) it its host, the corn earworm, *Heliothis zea* (Boddie), a pest of considerable economic importance. The chemical may be useful in suppressing the corn earworm with parasites.

$$CH_3(CH_2)_{11}\underset{|}{\overset{CH_3}{CH}}(CH_2)_{17}\,CH_3 \qquad (1)$$

The mechanism by which parasitic insects locate their host has been the subject of considerable study. Habitat location, host finding, host acceptance, and host suitability are involved in the process (*1*). Some investigators have suggested that the parasite first seeks a specific type of environment (*2*, *3*), after which the odor of the host is the primary stimulus guiding the parasite to its victim (*2*, *4*). The behavior of the parasite *Cardiochiles nigriceps* (Viereck) appears to be responsive to the host odor stimulus, because this insect begins directed searching movements to its larval host, *Heliothis virescens* (F.), upon crossing the "chemical trail" of the larva (*5*). The source of the chemical has been designated as the host's mandibular glands (*6*).

Thus, when a similar relation was established between *H. zea* and *M. croceipes* (*7*) and the host-finding substance was found to be present in varying degrees in the feces, salivary secretions, and hemolymph of the host, we extended the study to include the isolation, identification, and synthesis of the major component responsible for this activity. The chemical, 13-methylhentriacontane, is believed to be the first to be identified as a mediator in the complex relation between parasite and host insects.

Isolation of the active principle was monitored by the bioassay of Lewis and Jones (*7*). The parasite was placed in a petri dish on filter paper (9 cm in diameter), to which the sample was applied within a 0.25-cm^2 area. The characteristic positive response of the parasite to the active material consists of an intense search of the area surrounding the sample for a host larva, evidenced by rubbing the substratum with the antennae. Each parasite was allowed three approaches, responses being scored 3, 2, or 1 depending on whether the positive response resulted on the first, second, or third approach, respectively. Responses of insects were averaged to give the score for each compound at the concentration tested.

The active material was isolated as follows. Dry feces (100 g) of the corn earworm were homogenized with 500 ml of hexane and 10 g of sodium sulfate. The decanted liquid was then used to extract the residue in a Soxhlet apparatus for 2 hours. When the hexane extractive was chromatographed on a silica gel column (2.5 by 40 cm) (*8*), the active material was eluted with hexane within 25 ml, virtually at the solvent front. This chromatographic mobility signaled that the compound was a hydrocarbon. Since gas chromatography (*9*) gave 21 overlapping peaks, the active concentrate was chromatographed on a charcoal column (*10*). The six to eight peaks of the active fraction observed in gas chromatography (*9*) were reduced to three by rechromatographing on the charcoal column. Further purification was effected by preparative gas chromatography (*11*). The collected sample, although giving a single peak by gas chromatography on 5 percent OV 101 and 5 percent OV 210 (*12*), was still contaminated with the column bleed and had to be cleaned up by chromatography on a micro silica gel column (*13*). Yield of product was 1 mg.

Ozonolysis of the active material did not remove activity, and, therefore, indicated an absence of double bonds (*14*). The gas chromatographic retention index (Kovats method) of the active material was 3125 (*15*). If a branched C_{32} hydrocarbon was assumed, the ratio of the CH_3 to CH_2 and CH peaks in the nuclear magnetic resonance spectrum (CCl_4) (*15*) was consistent with one or more monomethylhentriacontanes or dimethyltriacontanes. The mass spectrum (*16*) of the active material showed the possible presence of at least five monomethyl compounds— the 15-, 13-, 11-, 9-, and 7-methylhentriacontanes—or similarly substituted dimethyltriacontanes; the only way to resolve the question was through synthesis. The 13-methylhentriacontane, believed to be the most abundant isomer from the pattern of mass spectral breakdown, was synthesized

Table 1. Response of female *Microplitis croceipes* to 150 ng of synthetic host-seeking stimulants.

Compound	Score*
9-Methylhentriacontane	0
11-Methylhentriacontane	.22
12-Methylhentriacontane	.48 a
13-Methylhentriacontane	2.32 c
15-Methylhentriacontane	1.68 b

* Scores not followed by the same letter are significantly different at the .05 level of probability. The 9- and 11-isomers are not included in the statistical comparison because only one replication (eight observations) was tested.

Table 2. Response of female *Microplitis croceipes* to 150 ng of synthetic host-seeking stimulants.

Compound	Score*
13-Methylhentriacontane	2.38
11-Methylhentriacontane	1.57
13-Methyltriacontane	2.00
13-Methyldotriacontane	1.67
9-Methyl-9-hentriacontene	1.78
11-Methyl-11-hentriacontene	1.11
12-Methyl-11-hentriacontene	1.33
13-Methyl-13-hentriacontene	1.78
15-Methyl-15-hentriacontene	2.00
13-Methyl-13-triacontene	1.25
13-Methyl-12-dotriacontene	1.67

* Each score based on two replicates (16 observations).

Table 3. Response of *Microplitis croceipes* to various mixtures of synthetic host-seeking stimulants.

Compound	Concentration (ng)	Score*
13-Methylhentriacontane	50	1.21 c
15-Methylhentriacontane	50	.63 ab
13- and 15-Methylhentriacontane	25 each	.39 a
13-Methylhentriacontane	100	2.49 e
15-Methylhentriacontane	100	1.76 d
13- and 15-Methylhentriacontane	50 each	.92 bc

* Scores not followed by the same letter are significantly different at the .05 level of probability.

from 2-tetradecanone and octadecyl bromide by a Wittig reaction and hydrogenation of the olefin product. (Other compounds were similarly synthesized.) Its retention index (3132) closely matched that of the active material (3125) and therefore ruled out the possibility of dimethyltriacontane.

The results of bioassays of the synthesized monomethyl constituents of the active material and the closely related 12-methyl analog are given in Table 1. 13-Methylhentriacontane was the most active compound, its activity being significantly greater than that of its analogs. Also, additional bioassays proved it to be active at the 50-ng level, which compared favorably with the activity of the isolated material. Table 2 gives the results obtained with other analogs and with the olefin intermediates used in the synthesis. Activity of the compounds generally depended on the closeness of the structure to that of 13-methylhentriacontane. Moreover, the compound was considered remarkably specific, especially for a saturated hydrocarbon, a class of compounds which are usually considered physiologically inert. Activity dropped significantly when the methyl was moved to an adjacent carbon atom or when the chain was shortened or lengthened by a single methylene group. Straight-chain C_{28}, C_{30}, and C_{32} hydrocarbons (not listed in the table) produced no response at any concentration.

Since the presence of compounds similar to 13-methylhentriacontane has been demonstrated in the cuticle of other species (*17*), the surface of third-, fourth-, and fifth-instar larvae (ten of each) was washed with hexane (3×10 ml). These fractions all elicited a positive response when they were bioassayed. The presence of the active material in the feces and salivary secretions may be explained by noting that the hind gut and salivary glands are of ectodermal origin and thus may secrete some of the same material as the epidermal cells of the integument. The fact that larvae consume their cast-off exuviae may also explain the presence of the compounds in the feces and in the hemolymph.

The possibility that the components of the isolated material were synergistic was tested by bioassaying 13- and 15-methylhentriacontane separately and together. The results (Table 3) do not indicate synergism, but imply a dilution effect. For example, the score of 0.92 for a mixture of 50 ng each of 13- and 15-methylhentriacontane is about halfway between the scores for 50 ng of the individual compounds.

We hope the findings of this study will stimulate further research in this important area of insect biochemistry and lead to a better understanding of insect behavior mechanisms.

13-Methylhentriacontane is also potentially valuable in biological control programs aimed at reducing the amount of pesticide pollution, because it may retain parasites in fields requiring protection from the corn earworm.

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7. W. J. Lewis and R. L. Jones, *ibid.*, in press.
8. Silica gel, J. T. Baker Chemical Co., No. 3405. The silica gel contained 3.5 percent water. Mention of a proprietary product in this paper does not constitute an endorsement of the U.S. Department of Agriculture.
9. A glass column (outside diameter, 6 mm; inside diameter, 4 mm; length, 120 cm) containing 5 percent OV 101 on 80 to 100 mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) was temperature programed at 5°C per minute between 200° and 300°C in an F&M model 700 instrument equipped with a flame ionization detector. Rate of flow of carrier gas (helium) was 85 ml/min.
10. Chromatography was conducted on a Darco KB (Atlas Chemical Industries, Inc., Wilmington, Del.) column (4 by 35 cm). Elution solvents were 500 ml of hexane followed by 2000 ml of 2 percent benzene in hexane. The active material emerged between 1500 and 1700 ml of the latter solvent.
11. The gas chromatograph and column described in (*9*) were fitted with a 10 : 1 splitter and operated isothermally at 250°C. The active material was collected on a stainless steel tube [21 by 0.6 (outside diameter) cm] packed with Gas Chrom Q and equipped with a male Luer fitting to permit its connection to the heated exit of the gas chromatograph. After the active material was washed from the tube with hexane, the solution was concentrated to 1 ml and rechromatographed in 10-μl amounts.
12. Conditions for gas chromatography on OV 101 were the same as those in (*9*). In the other chromatography, conditions were the same as in (*9*) except that the liquid phase was on OV 210.
13. A pentane solution of the sample was passed through a column (inside diameter, 5 mm) containing 0.5 g of silica gel (Baker, No. 3405) wet with pentane; the hydrocarbon was eluted with 3 ml of pentane.
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15. A column [3 mm (inside diameter) by 150 cm] containing 3 percent OV 1 on 80 to 100 mesh VarAport 30 (Varian Aerograph, Walnut Creek, Calif.) was used at 260°C with a helium flow rate of 15 ml/min.
16. We are grateful to Dr. J. M. Ruth of our Beltsville laboratory for the spectrum that was obtained on a CEC 21-110B spectrometer.
17. L. L. Jackson, *Lipids* **5**, 38 (1970); K. Tartivita and L. L. Jackson, *ibid.*, p. 35.
18. Journal series paper No. 999, University of Georgia College of Agriculture Experiment Station, Coastal Plain Station, Tifton.

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Latent Herpes Simplex Virus in Spinal Ganglia of Mice

Abstract. *Herpes simplex virus establishes a persistent, latent infection in spinal ganglia after mice have recovered from posterior paralysis. Infectious virus is replicated when these ganglia are explanted and maintained as organ cultures in vitro.*

The viruses of herpes simplex (HSV) and herpes zoster are considered to be classic examples of agents that induce latent infections in man [see reviews by Roizman or Fenner (*1*)]. Thus it is considered probable that, following initial infection, these viruses are maintained in some "quiescent" state from which they may periodically be reactivated to produce overt disease. Additional data from several sources suggest that the virus persists in sensory ganglia between these episodes of clinical disease. However, all the evidence supporting these statements is indirect. A direct demonstration that the viral genome can persist in sensory ganglia would be of central importance in establishing the validity of the entire concept. In this report, we show that HSV can induce a latent infection in the spinal ganglia of mice.

Four-week-old SJL mice (*2*) were inoculated in the left rear footpad with 4×10^3 RK_{13} cell plaque-forming units (PFU) (*3*) of HSV (*4*) according to the technique of Olitsky and Schlesinger (*5*). After inoculation in this manner, virus travels centripetally in the nervous system to the brain (*6*). In our experiments, about 80 percent of mice become paralyzed in one or both hind legs in 7 to 9 days. Of the mice paralyzed, about one half undergo a complete