tor levels in hypophysectomized male rats. This increase in receptor levels is preceded by a marked and sustained elevation of circulating rat prolactin levels. These observations taken together strongly suggest that the lactogen receptor is being induced by prolactin itself. They do not exclude a direct hepatic role for estrogen, although they do suggest that at least part of estrogen's inductive effect is through its capacity to stimulate an increase in circulating prolactin in the rat.

Several studies have emphasized that tissue receptor levels undergo modulation (*1, 3, 7*). This modulation may be an important way of regulating peripheral sensitivity to hormones. The repression of receptors by chronic elevation of hormone levels has been established for insulin and suggested for other hormones such as calcitonin (*8*). Our observations suggest that chronic elevation of hormone levels can induce receptors. Whether induction or repression occurs seems to depend, in part, upon the hormone and the tissue involved.

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Evidence for Origin of Insect Sex Pheromones: Presence in Food Plants

Abstract. *Compounds identified as sex attractant pheromones in a number of phytophagous insects were found in a variety of host plants. These agents vary in chemical composition in different plant species, which suggests that dietary factors may provide an evolutionary mechanism for diversification of certain insect species. A theoretical framework to explain this phenomenon is postulated on the basis of experiments with the oak leaf roller moth.*

Little is known about the origin or metabolic production of insect sex attractants. Other insect chemical signals such as aggregation pheromones are derived from plants; some insect defensive chemicals are also of extrinsic origin. For example, chemical communication in some species of bark beetles (*1*) is enhanced by synergistic aggregation substances found in host pine trees. Some insects may carefully sequester plant-produced chemicals and use them for their own defense (*2*). However, to our knowledge, the question of the origin of insect sex attractants remains unanswered. Moreover, the mechanisms by which these chemical signals contribute to species diversification (*3, 4*) are unclear. In this report, these questions are addressed in light of results in the study of insect pheromones in our laboratory, and a possible theoretical framework to explain these phenomena is proposed.

We have isolated and identified insect sex pheromones in a wide variety of host plants. The primary chemical communication system under investigation is the sex pheromone complex of the oak leaf roller moth (OLR), *Archips semiferanus* Walker (Lepidoptera:Tortricidae), an exceedingly destructive pest which has seriously damaged oak forests in the northeastern United States (*5*). A series of 21 isomeric tetradecenyl acetates (*6, 7*) were identified in the active sexual attractant fraction of the adult female; all isomers were active when tested on male OLR antennae by the electroantennogram (EAG) method (*8*). Seventeen of the 21 isomers proved attractive to OLR males when tested in field traps (*8, 9*).

During field testing, wild OLR male moths congregated near host oak trees and became sexually stimulated when near the leaves. Moreover, males frequently attempted to copulate with host leaves that had been damaged by larval feeding. The behavior was identical to the characteristic male sexual response to crude female extracts (*10*) and included extension and bending of the abdomen, rapid wing fluttering, projection of the hair pencils, and repeated brushing of these structures on the undersides of the leaves. This phenomenon suggested that the OLR pheromones could be present in the plant (*11*). In order to test this hypothesis, crude foliage extracts of several tree species were made by grinding fresh leaves, which were collected in areas having no visible insect defoliation (*12*), in redistilled spectrograde methylene chloride. The extracts were subjected to thin layer chromatography on silica gel with methylene chloride : hexane (50 : 50) as the developing solvent. A band having the same R_F value as standard 14-carbon unsaturated acetates and tetradecyl acetate was then eluted and rechromatographed on a nonpolar 5 percent SE-30 gas chromatographic (GC) column (*13*). A region having a retention time near that of standard tetradecenyl acetates was collected and subjected to computerized gas chromatography–mass spectrometry (GC-MS) (*14*) on 10 percent diethylene glycol succinate (DEGS). The GC-MS was programmed to scan only for discrete ions that were present in standard 14-carbon acetate standards. Our use of this technique, known as mass fragmentography or multiple ion detection, has been described (*6*). Analyses were made with the following diagnostic ions: *m/e* (mass to charge) 196, molecular ion of tetradecyl acetate (M_A) – $HOOCCH_3$; 194, molecular ion of tetradecenyl acetates (M_B) – $HOOCCH_3$; 166, M_B – $HOOCCH_3$ – C_2H_4; and 61, $H_2OOCCH_3^+$. Mass fragmentography of each extract was repeated with the same ions on a 5 percent SE-30 column to ensure against overlapping impurities. Optimum operating sensitivity of the GC-MS was in the range of 50 to 150 pg per component. If peaks having the same retention times (scan numbers) and the same quantitative ratios of these ions as standard tetradecyl acetate and the tetradecenyl acetates were observed,

total mass spectra were recorded for these components on both columns (Table 1) (*15*). All analyses were duplicated. The isolation procedure and subsequent mass fragmentography were also conducted on purified adult extracts from field males and females, extracts of laboratory females that were reared on alfalfa and wheat germ diets (*16*), extracts of wild OLR larvae, extracts of wild male and female pupae, and extracts of field OLR eggs.

Sample mass fragmentograms of the extracts of black oak leaves, apple leaves, and wild and laboratory-reared females are presented in Fig. 1 along with a few appropriate standards. (*Z*)-10-Tetradecenyl acetate (Fig. 1C) is a large component of the tetradecenyl acetate fraction of black oak (Fig. 1B), a major host tree for the OLR in areas where females were collected for pheromone extracts (Fig. 1A). This component is also one of the most active attractants of the tetradecenyl acetate isomers in field trapping tests on the OLR. Table 1 also indicates that all species of oak tested contain tetradecenyl acetates (*17*); however, in most cases, these isomers occur in complex mixtures (*18*). This is reflected in the complexity of the natural OLR pheromone, which was isolated from a large number of field females collected as larvae found feeding on a variety of host species. Tetradecyl acetate (Table 1) appears to be a common constituent in all tree species tested, including those not in the oak genus. Although this agent, to our knowledge, has not been identified as a primary pheromone in an insect, it may be involved in some way in the plant metabolism of the tetradecenyl acetates.

Previous reports on insect species in a variety of orders tend to indicate a direct relationship between the presence of pheromone and exogenous factors such as the type of host plant and other dietary factors. For example, laboratory-reared male boll weevils (*Anthonomus grandis*) (*19*) that were not provided access to cotton were less attractive than field males. Moreover, cotton plant extracts added to grandlure, a pheromone component of the boll weevil, increased attractiveness markedly (*20*). Bark and wood particles were required at the site of pheromone production of *Trypodendron lineatum* in order for attractive material to be produced by this ambrosia beetle (*21*). The New Guinea sugarcane weevil (*Rhabdoscelus obscurus*) would not release a sex attractant unless it was fed sugarcane (*22*). An insect more closely related to the OLR, the summer fruit tortrix moth (*Adoxophyes orana*) (*23*), produced considerably less pheromone when reared in the laboratory on a wheat germ diet; this phenomenon has also been reported for the gypsy moth (*Prothetria dispar*) (*24*). The most definitive evidence to date for the association of plant components and sex pheromones is a series of reports on danaid butterflies that assemble and feed on plants containing pyrrolizidine alkaloids (*25, 26*). The male sex pheromones of these insects were clearly identified by Meinwald *et al.* (*25*) as pyrrolizidinone or closely related heterocyclic compounds whose structures are similar to those of the host plant alkaloids.

Further evidence that the existence of the OLR sex pheromones in host plants was not a coincidence was obtained from laboratory feeding studies. Mass fragmentograms (Fig. 1D) of extracts of female OLR that had been successfully reared on semisynthetic diets (namely, wheat germ and alfalfa) on which other lepidopterans have been reared (*16*) did not show any detectable quantities of either tetradecyl acetate or tetradecenyl acetate isomers. Whether or not these compounds are present in the extracts, there is an obvious reduction in the quantity. In addition to the inability to detect these sexual messengers by analytical methods, crude extracts of laboratory-reared females did not attract or excite males in the laboratory or field nor were they active in EAG tests on field male antennae (*27*). However, when OLR were reared in the laboratory on a mixture of wheat germ and oak leaves, pheromone components were detected in adult female extracts by mass fragmentography (Table 1). These extracts were active when tested by the EAG method and in a laboratory flight chamber (*10*); wild males were also trapped by these extracts in field tests.

Components of the OLR pheromone complex were also found in OLR field larvae and pupae. Mass fragmentography of separated male and female pupal extracts showed that both sexes contained a mixture of tetradecenyl acetate isomers and that the complex was very similar to that of the adult

Table 1. Mass fragmentography elucidation of pheromone complex. A plus indicates presence of the chemical component or biological activity, and a minus indicates that the chemical was undetected in that extract or that biological activity was absent; NT, not tested; FC, laboratory flight chamber. All tests were duplicated.

Extract	Tetradecyl acetate		Tetradecenyl acetates		Biological activity on OLR ♂	
	5% SE-30	10% DEGS	5% SE-30	10% DEGS	EAG	FC
Red oak	+*	+*	+†	+†	+	NT
Black oak	+*	+*	+†	+†‡	+	NT
Scarlet oak	+*	+*	+†	+†§	+	NT
White oak	+	+	+	+	+	NT
Scrub oak	+	+	+	+	+	NT
Apple	+*	+*	+‖	+‖	+	NT
Red maple	+	+	−	−	+	NT
White pine	+	+	−	−	+	NT
Field OLR ♀ adult	+*	+*	+†	+†	+	+
Field OLR ♂ adult	Trace	Trace	Trace	Trace	NT	−
Lab OLR ♀ adult (wheat germ)	−	−¶	−	−	−	−
Lab OLR ♀ adult (alfalfa)	−	−	−	−	−	−
Lab OLR ♀ adult (wheat germ and oak leaves)	+	+	+	+	+	+
Field OLR ♀ pupae	+	+	+	+	NT	NT
Field OLR ♂ pupae	+	+	+	+	NT	NT
Field OLR larvae, fifth instar	+	+	+	+	NT	NT
Field OLR larvae, first instar, newly hatched#	+	+	+	+	NT	NT
Field OLR eggs#	+	+*	+	+†	NT	NT
Blank	−	−	−	−	−	−

* Total mass spectra and retention times were identical to that of standard tetradecyl acetate. † Total mass spectra were identical to those of standard tetradecenyl acetates. ‡ Total mass spectra and retention times of one component were identical to (*Z*)-10-tetradecenyl acetate. § Total mass spectra and retention times were similar to (*Z*)-3-tetradecenyl acetate. ‖ Total mass spectra and retention times of two components were identical to (*Z*)- and (*E*)-11-tetradecenyl acetate. ¶ Fragment ion *m/e* 61 was present but no *m/e* 196 was detected. # Eggs were collected in the spring before hatching; first instar larvae were deprived of food source.

female moths. This evidence suggests that females of the species may have an unknown mechanism for storing these chemicals, which ultimately reside in the pheromone gland. The males may relinquish the compounds upon emergence from the pupal case. Males of another insect species, *Itoplectis conquisitor*, contain the female sex pheromone upon emergence from pupae, but it is only present on their body surface for the first 24 hours (*28*). The presence of small amounts of these agents in OLR eggs and first instar larvae that were deprived of a food source is of considerable interest. Storage in the eggs of small amounts of these chemicals (possibly transferred via the pheromone gland of the adult female) could have adaptive benefit by serving as feeding cues to first instar larvae, thereby aiding in the selection of a host plant (*29*). The isolation of these chemicals in newly hatched first instar larvae could also explain how minute amounts of pheromone are commonly present in the first generation of insects reared in the laboratory on artificial diets.

The presence of (*Z*)- and (*E*)-11-tetradecenyl acetate (Fig. 1F) in extracts of apple leaves, as identified by mass fragmentograms (Fig. 1E) and by total mass spectra and GC-MS retention times, gives further credence to the theory that some insects may store their sexual chemicals from the host plant (*30*). More than a dozen lepidopteran species (*3*) utilize either of these isomers or specific mixtures of both compounds as sexual attractants. These insects feed on apples as their primary host and may be sequestering these chemicals directly from their food source. The fruit tree tortrix moth (*Archips podana*), an apple-feeding insect in the same genus as the OLR, cannot be laboratory-reared successfully on wheat germ without apple leaves; the pheromones were identified as a mixture of (*Z*)- and (*E*)-11-tetradecenyl acetate (*31*).

Initial evidence also suggests that the tedradecenyl acetate complex undergoes seasonal changes with the growth of the tree and that the pheromone complex may vary even within a given plant species. If this is a general variation, it raises serious questions about signal inconstancy. To what extent temporal and spatial variability of pheromone complexes within host plants contributes to reproductive substructuring of insect species into potentially isolated units remains to be elucidated. However, dietary synchrony could explain how pheromone complexes of some insects may vary throughout the feeding season (*32*). The apparent mystery governing the dramatic differences in pheromone content of isolated populations of the European corn borer (*Ostrinia nubilalis*) in Iowa, New York, and Europe could also be explained by differences in dietary chemicals (*3, 33*). On the other hand, similarities in dietary chemicals may explain instances of interspecies attraction in insects (*34*).

Previous field studies on the OLR indicate that apparently taxonomically identical males are attracted by different tetradecenyl acetate isomers on different days, and that peak trapping of males for a particular isomer may coincide with the development of the OLR on various host plants (*8*). For example, during the past two summers, (*Z*)-10-tetradecenyl acetate, a major constituent of black oak extracts, trapped a maximum number of OLR males 5 days after the maximum catch with (*Z*)-3-tetradecenyl acetate, identified in initial tests of scarlet oak extracts (*35*). Scarlet oaks in the areas of the tests bud several days earlier than the black oaks; thus, OLR feeding on black oak could develop more slowly than those on scarlet oak, which could result in temporal differences in the attraction of compounds in a mixed forest. This complicates potential survey and detection programs for this pest. However, one could envision insect control programs that would take advantage of these

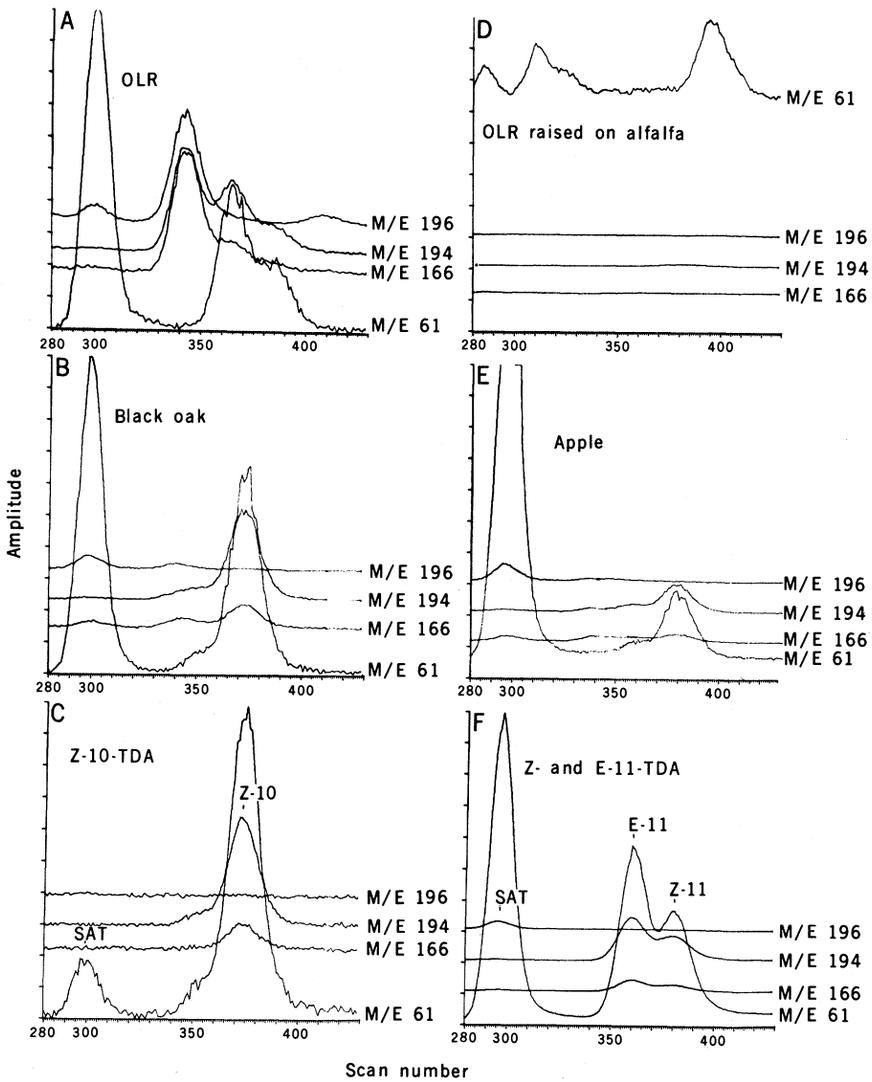

Fig. 1. Mass fragmentograms on 10 percent DEGS of purified pheromone extracts and authentic standards. (A) Extracts of virgin field female oak leaf rollers. (B) Black oak leaf extracts. (C) (*Z*)-10-Tetradecenyl acetate (Z-10-TDA) plus tetradecyl acetate (*SAT*). (D) Extracts of virgin female oak leaf rollers that were laboratory-reared on artificial alfalfa diet. (E) Apple leaf extracts. (F) (*Z*)- and (*E*)-11-Tetradecenyl acetates plus tetradecyl acetate.

factors—for instance, spraying microencapsulated synthetic pheromones on foliage during larval feeding, thereby ensuring subsequent incorporation of these signals in the sex attractant of the adult female. A second application of these chemicals in the adult stage might be highly successful in trapping males or disrupting communication.

In light of the data presented, we believe that host plant constituents govern in part the sex pheromones of the herbivore. It follows that the adult male may be programmed to select for a mate that conforms to certain materials it has contacted during feeding. The exact mechanism by which this could occur is not clear, for programming may require several generations. However, it is possible that upon emergence from the pupal case, the male codes or "imprints" the odor complex of the meconium. Once the pupal odor dissipates, the male spends the remainder of its adult life searching for the same complex of chemicals. This suggests that a key process in the perception of the pheromone complex is centralized and may occur at the brain. The EAG responses of OLR male antennae to a variety of substances, including a consistently prominent response to a substance that apparently does not attract males in the field (*8*), tends to support this hypothesis. In any case, it has been well documented in Hymenoptera (*36*) and Diptera (*37*) that insects have the ability to learn.

From an evolutionary standpoint, it is not suprising that some phytophagous insects may use unaltered chemicals from host plants for propagation of the species rather than develop mechanisms to synthesize certain sexual agents. In this regard, these chemical messengers may serve a dual role in a predetermined sequence: (i) to attract males to areas having suitable host plants; and (ii) to enable males to locate females, perhaps by a greater concentration of requisite chemicals being emitted from the abdomen of their mates.

Certain insects and perhaps other unrelated organisms utilize chemicals present in their food source to encourage reproduction. Diversification of insect species may be primarily due to the pheromone complexes available during evolution of host plants (*38*) rather than to the evolution of separate insect communication systems independent of changes in certain dietary chemicals. Whether sexual signals and other chemical communicants used by insects are mostly of plant origin or whether there are alternative mechanisms to produce these olfactory cues remains to be elucidated (*39*). In this report, only one concept focusing on the direct utilization by the insect of these plant chemicals is proposed. It is possible that they may function in more subtle ways, such as to cause physiological changes in the insect concomitant with the "induction" of pheromone biosynthesis. In any case, the chemical and biological mechanisms governing the continuance of insect species are exceedingly complex.

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15. In some cases, sufficient sample (1 to 5 ng) was not available for determination of total mass spectra. Blanks consisting of GC-collected sections from the SE-30 column were periodically analyzed. Mass fragmentograms and total mass spectra were determined on residues from evaporation of large amounts of methylene chloride and hexane. Neither solvent residues nor blanks contained any tetradecyl or tetradecenyl acetates.
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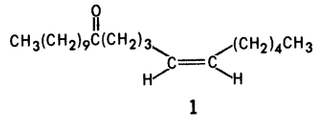
Douglas-Fir Tussock Moth: Sex Pheromone Identification and Synthesis

Abstract. *The sex pheromone of the Douglas-fir tussock moth* Orgyia pseudotsugata *(McDunnough) has been isolated and identified as* (Z)*-6-heneicosen-11-one. This compound and its* E *isomer have been synthesized and are highly potent in laboratory bioassays and field trials.*

The Douglas-fir tussock moth (DFTM) *Orgyia pseudotsugata* (McDunnough) (*1*) is a severe defoliator of fir forests of western North America (*2*, *3*). This insect is capable of dramatic population increases, which can result in severe damage to forest resources for a year or two before such populations subside (*2*). The availability of DFTM sex attractant (pheromone) for use in surveillance traps should prove invaluable, as such traps would constitute an early warning system for the detection of increasing moth populations, and would thus identify areas requiring intensified surveillance and possible containment measures.

The sex attractant of the DFTM has been isolated and identified as (*Z*)-6-heneicosen-11-one (**1**).



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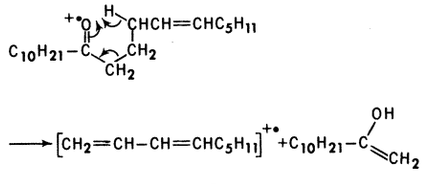
This structure, novel among known lepidopteran pheromones (*4*), has been confirmed by chromatographic, chemical, and spectrometric comparisons of the isolated attractant with synthetic **1**, and by moth attraction to the synthetic pheromone.

The isolation and structure elucidation was achieved by using the methylene chloride extract of 6000 female DFTM abdominal tips (each containing about 40 ng of pheromone). Functional groups present in the attractant molecule were established through chemical reactions in combination with bioassay. Alcohol and ester functional groups, two common structural features of known lepidopteran pheromones (*4*), were ruled out when treatment of the crude pheromone extract with alcoholic sodium hydroxide or acetic anhydride under reflux failed to destroy biological activity. Treatment of the crude extract with lithium aluminum hydride in dry ether destroyed the activity, which suggested the presence of an aldehyde, ketone, or epoxide function. A sample of the attractant extract in acetic acid heated at 105°C for 16 hours retained activity; this result excluded an epoxide function for the DFTM attractant, since (*Z*)-7,8-epoxy-2-methyloctadecane (disparlure), the attractant of the gypsy moth *Porthetria dispar* (*5*), reacted under these conditions to yield two products, presumably the two possible hydroxy acetates. Biological activity was destroyed when the DFTM attractant was treated with a standard 2,4-dinitrophenylhydrazine solution—a result consistent with the presence of a carbonyl group. The presence of one or more multiple bonds was inferred when pheromone activity was lost from samples which were catalytically hydrogenated, treated with ozone, or reacted with *m*-chloroperbenzoic acid.

A preliminary separation of the crude extract by "dry-column" chromatography (*6*) on alumina yielded an active fraction which, by gas-liquid chromatography [GLC, with a column 1.2 m by 6 mm (inner diameter) of 3 percent SE-30 on Chromosorb W, AW-DMCS], contained only two major components, one of which was biologically active. A mass spectrum of this purified pheromone exhibited a molecular ion at *m/e* (mass to charge) 308.3074 (the ratio calculated for $C_{21}H_{40}O$ is 308.3079); important fragment ions at *m/e* 169.1587 (calculated for $C_{11}H_{21}O$, 169.1592) and 167.1412 (calculated for $C_{11}H_{19}O$, 167.1435); and the base ion at *m/e* 124.1246 (calculated for C_9H_{16}, 124.1252). A mass spectrum of pheromone subjected to base-catalyzed deuterium exchange (*7*) indicated the presence of four exchangeable protons, consistent with a ketone functional group. The ions arising by the two modes of α-cleavage of the ketone attractant (*m/e* 167 and 169) and the deuterated analog (*m/e* 169 and 171) establish the position of the carbonyl at C-11. Conclusive identification of the carbon skeleton was obtained when catalytic reduction of the pheromone yielded a product that exhibited a mass spectrum and a GLC retention time identical to those of an authentic sample of 11-heneicosanone (*8*).

The base ion in the mass spectrum (*m/e* 124) arises via a McLafferty rearrangement (scheme 1) (*9*).



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Scheme 1

The fact that most of the charge is retained on the hydrocarbon ion at *m/e* 124 rather than on the oxygen-containing ion at *m/e* 184 (which retains 15 percent as much charge as the base ion) indicates that extra stabilization is associated with the olefin ion radical, possibly because of conjugation with a double bond at position 6, as shown for **2**. This initial suggestion that the double bond of the attractant molecule is at position 6 was confirmed when ozonolysis of the pheromone produced a keto aldehyde which exhibited a GLC retention time identical with that of the product of ozonolysis of 1-hexadecen-6-one (*10*).

To establish the stereochemistry about the C-C double bond of the natural attractant derivatives of the