tion of calcium ion to cone photoreceptors mimics illumination (*13*). These facts call attention to anatomical and physiological similarities between sarcoplasmic reticulum, photoreceptor disks, and microvilli, and raise the possibility of related mechanisms for the various kinds of excitation mediated by these organelles.

Although the cyclase system offers an attractive model for explaining photoreceptor function, there can as yet be no definitive statement concerning its role. Our earlier finding that frog rod outer segments contain cyclase regulated by light has been extended. (i) Cyclase inactivation is directly proportional to rhodopsin bleaching. (ii) A particulate phophodiesterase of high specific activity accompanies the cyclase in photoreceptors. (iii) Cyclic AMP and methylxanthines mimic the physiological effects of illumination in the compound eye of *Limulus*.

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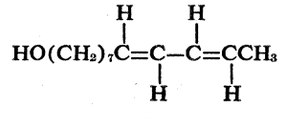
Sex Attractant of the Codling Moth: Characterization with Electroantennogram Technique

Abstract. trans-*8*, trans-*10-Dodecadien-1-ol is a sex attractant of the codling moth,* Laspeyresia pomonella. *Antennal responses (electroantennograms) to a series of monounsaturated compounds were used in determining the location and configuration of the two double bonds. The synthetic compound is very attractive to male codling moths in the field.*

The codling moth, *Laspeyresia pomonella* (Lepidoptera: Tortricidae: Olethreutinae), has long been considered a major worldwide pest of apple. Insecticide spray schedules could be drastically modified in several apple-growing areas if alternative methods of control could be found. Sex pheromone traps have successfully been used with another tortricid moth (*1*) for monitoring the presence and abundance of moths and for insect suppression in apple orchards. Similar uses of an attractant may well be feasible with the codling moth. Studies on the female sex pheromone gland and its extract have been carried out (*2*, *3*), but the structure of the pheromone compound still remained unknown. We have developed a technique with electroantennograms (EAG) which uses a small number of female glands and male antennae and allowed us to propose a structure for the codling moth sex attractant. The attractant compound is *trans*-8, *trans*-10-dodecadien-1-ol (*1*).



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Abdominal tips from 50 female codling moths (*4*) were excised and placed in methylene chloride. Portions of this crude extract were injected on both polar (5 percent cyclohexanedimethanol succinate on chromosorb Q, 170°C) and nonpolar (10 percent JXR on chromosorb Q, 190°C) gas chromatographic (GC) columns, and the column effluent was collected in 30-cm glass capillary tubes at 1-minute intervals. The tubes were assayed for activity by connecting one end to a 5-ml glass syringe containing 1 ml of air and inserting the other end into an airstream passing across an antenna affixed for EAG measurements (*5*). The syringe plunger was quickly depressed to send a 30- to 35-msec "puff" of air through the capillary tubing and over the antenna. Collected fractions containing active material elicited responses up to 5 mv, whereas inactive fractions gave responses of approximately 0.5 mv. More precise retention times were obtained by taking 0.5-minute collections in the area of interest. The retention time of the active component relative to dodecyl alcohol was 2.3 : 1 to 2.6 : 1 on the polar column, and 1.15 : to 1.25 : on the nonpolar column. The nonpolar column retention time indicates a molecular weight similar to that of dodecyl alcohol, while the comparatively long retention time on the polar column indicates that the active compound contains more functionality than dodecyl alcohol.

Evidence that the active compound contains an alcohol moiety, as was previously reported (*3*), was obtained by assaying several chemical reactions of the crude extract with the GC-EAG technique mentioned above. Crude extract treated with 0.5*N* methanolic potassium hydroxide still contained active material with a GC retention time identical to the active component in untreated extract, whereas, treatment with acetyl chloride eliminated the active component at this retention time. The above data suggest that the pheromone is a C_{12} alcohol, too polar to be monounsaturated, but with a molecular weight that most likely excludes an additional oxygen atom. At least two sites of unsaturation are suggested, with a strong possibility that conjugation accounts for the polarity indicated by the polar GC column retention time (*6*).

A number of synthetic chemicals were tested for electroantennogram responses by puffing 1 ml of air through a disposable pipette containing 80 μg of test chemical on a piece of filter paper (*5*). Antennal responses of male *Argyrotaenia velutinana* to a series of monounsaturated acetates and alcohols had shown that the standard that is identical to the sex pheromone always elicits the

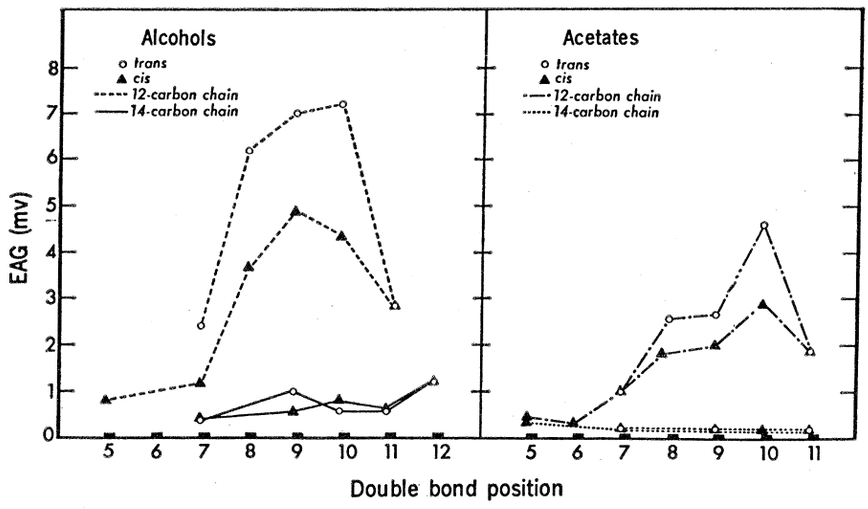

Fig. 1. Male codling moth antennal responses to various monounsaturated C_{12} and C_{14} acetates and alcohols (80 μg of test chemical on filter paper).

greatest EAG response (*5*). The responses are expressed quantitatively in millivolts and are very reproducible. Thus, the EAG is a bioassay that directly reflects the relative effectiveness of the series of geometrical and positional isomers. The same standards were used with various insects with known sex pheromone structures, such as *Grapholitha molesta* (*7*), *Trichoplusia ni* (*8*), *Choristoneura rosaceana* (*9*), *Polychrosis viteana* (*10*), and *Ostrinia nubilalis* (*11*), and in every case the standard identical to the sex pheromone elicits the greatest response. Closely related positional isomers of the same carbon chain length as the pheromone give stronger response than other standards —regardless of the differing volatilities of the various standards. Evidence that the codling moth pheromone may be a C_{12} alcohol was obtained from comparative responses of saturated C_{12}, C_{13}, and C_{14} alcohols and acetates. Dodecyl alcohol produced 1.5-mv responses, whereas all the other alcohols and acetates gave 0.3 mv (0.2 mv background). Results with a series of monounsaturated C_{12} and C_{14} acetates and alcohols (Fig. 1) show that C_{12} alcohols give strong responses and that *trans*-10-dodecen-1-ol produces the greatest response. *Trans*-8- and *trans*-9-dodecen-1-ol also gives good responses, unlike the pattern obtained from male antennae of the species listed above which respond to monounsaturated pheromones. In the last-mentioned case good responses are obtained at only one double bond position, while all the rest are greatly reduced. The EAG response pattern obtained with codling moth antennae suggests that the *trans*-8 double bond is an important feature of the pheromone structure as well as the *trans*-10, indicating that the pheromone may contain the conjugated system *trans*-8, *trans*-10.

It is reasonable to find strong responses from *cis*- and *trans*-9-dodecen-1-ol, since unsaturation in the 9-position can probably interact with the binding sites of the 8- and 10-position double bonds. This same pattern of response was also obtained when silkworm (*Bombyx mori*) antennae were subjected to monounsaturated C_{16} alcohols (*12*). The silkworm pheromone is *trans*-10, *cis*-12-hexadecadien-1-ol

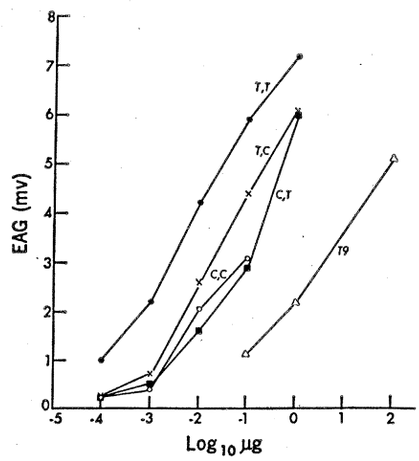

Fig. 2. Male codling moth antennal responses to various concentrations on filter paper of the attractant, *trans*-8, *trans*-10-dodecadien-1ol (T,*T*),, the geometrical isomers of the attractant—*trans*-8, *cis*-10 (T,*C*), *cis*-8, *cis*-10 (C,*C*), *cis*-8, *trans*-10 (C,*T*)—and of *trans*-9-dodecen-1-ol (*T9*).

(*13*), so it was not surprising to find that *trans*-10- and *cis*-12-hexadecen-1-ol give stronger responses than *cis*-10- and *trans*-12-hexadecen-1-ol and that compounds with unsaturation in other positions produce much weaker responses. Once again, however, strong responses were elicited by the compounds with unsaturation in the intermediary position—in this case *cis*- and *trans*-11-hexadecen-1-ol (*14*). Another species with which monounsaturated standards could have been used to predict the position and unsaturation of a diunsaturated sex pheromone is *Plodia interpunctella*. In this case *cis*-9-tetradecenyl acetate and *trans*-9-tetradecenyl acetate both give the greatest antennal response of all standards, consistent with the sex pheromone structure, *cis*-9, *trans*-12-tetradecadienyl acetate (*15*).

The above data suggest that a codling moth attractant structure may be *trans*-8, *trans*-10-dodecadien-1-ol (**1**). All four geometrical isomers were synthesized for comparisons of biological activity. Wittig reaction (*16*) of the triphenyl phosphonium salt of *trans*-1-bromo-2-butene (*17*) in dimethylformamide with excess sodium methoxide and methyl 8-oxooctanoate (*18*), and reduction of the resulting acid with Red-al (*19*) gave the *trans, trans* isomer **1** (75 percent) and the *cis*-8, *trans*-10 isomer (25 percent). The mixture was converted to *trans, trans* by isomerization with iodine and sunlight (*20*).

The *trans*-8, *cis*-10 isomer was similarly prepared from 1-bromo-2-butene. After Red-al reduction, the alkyne was reduced by hydrogenation with Lindlar catalyst to give *trans*-8, *cis*-10 and *cis*-8, *cis*-10 isomers. The mixture was acetylated and separated by thin-layer chromatography on silver nitrate–impregnated silica gel developed with benzene. The *trans*-8, *cis*-10 acetate was scraped from the plate and saponified. The *cis*-8, *trans*-10 isomer was similarly synthesized from the triphenylphosphonium salt of methyl 8-bromooctanoate (*18*) and *trans*-crotonaldehyde. Final purification was made by collection from the polar GC column. Wittig reaction of the triphenylphosphonium salt of methyl 8-bromooctanoate and tetrolaldehyde (*21*), followed by Red-al reduction and hydrogenation with Lindlar catalyst gave the *cis-cis* isomer.

The infrared and ultraviolet absorption spectra of the four isomers are similar to those shown for the isomers

of 10,12-hexadecadien-1-ol (*20*). Three of the synthetic isomers, those excluding *cis*-8, *trans*-10 (7.3 minutes at 173°C), possess identical retention times, 7.75 minutes at 173°C on the polar GC column. Retention time of the crude extract active component was again determined by measuring EAG responses of 1-minute collections and was found to be identical to that of the three synthetic isomers possessing a common retention time. This is strong evidence that one of the three isomers may be similar to the pheromone compound, since conjugation with the double bond in the 10-position would produce compounds with longer retention times than other doubly unsaturated C_{12} alcohols. The EAG responses to the four synthetic isomers for a series of concentrations give curves (Fig. 2) that are very similar to those described for the geometrical isomers of the silkworm attractant (*22*), except that in our study the *trans-trans* isomer produces the strongest responses. The *trans-trans* EAG response trace is also distinguished from the others by exhibiting a much slower return to baseline. Good EAG responses with the *trans-trans* isomer at a concentration of 10^{-10} g provide additional evidence that this compound may be the codling moth sex pheromone. The antennal responses are logarithmic with concentration, making it necessary to use over 2000 times more *trans*-9-dodecenyl acetate than compound **1** to elicit a 4-mv response (Fig. 2).

Intense sexual stimulation is elicited in laboratory bioassays with very low concentrations of the synthetic compound **1**, although many chemicals produce stimulation in the laboratory but do not attract males in the field. Field tests with synthetic compound **1** showed it to be very attractive to male codling moths. Preliminary tests were conducted by releasing male codling moths in an apple orchard. Test chemicals (1 μl) were placed on rubber septa located in Sectar insect traps (*23*). The *trans-trans* isomer was very attractive to male moths (19 males per trap per night), as was the reaction mixture containing *trans-trans* (75 percent) and *cis*-8, *trans*-10 (25 percent) (15 males per trap per night), while the *trans*-8, *cis*-10 isomer attracted no males. A mixture of synthetic isomers was acetylated and separated by thin-layer chromatography on silver nitrate–impregnated silica gel developed with benzene. Several fractions were scraped from the plate, hydrolyzed, and placed in a trap on dental wicking. Only the top scraping containing the *trans-trans* isomer was attractive to males in the field. Field studies in Australia showed that a rubber septum containing 1 μl of attractant was attractive for over a month and out-caught the usual port wine lure pots (58 males per trap to 4 males per pot).

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Circadian Rhythm: Population of Interacting Neurons

Abstract. *The circadian rhythm in the frequency of compound action potentials recorded from the isolated eye of* Aplysia *is a consequence of interactions among the cells of the retinal population. As the population number is reduced to a critical 20 percent, progressively shorter circadian periods and ranges are expressed. Below the critical number, the population oscillates at ultradian frequencies.*

Many physiological and biochemical processes in both plants and animals exhibit fluctuations in output that occur with a circadian (about a day) periodicity (*1*). These circadian rhythms are not just a response to fluctuating environmental stimuli but are due to internal endogenous oscillators whose phases are influenced by environmental stimuli. Many circadian activity rhythms in animals are thought to be controlled by neuronal oscillators in the central nervous system, probably in the brain (*2*). One fundamental question regarding neuronal oscillators, and circadian oscillators in general, is whether the observed circadian oscillations are caused by a single master oscillator or by interactions within a population of oscillators (*3*). Two circadian rhythms in neuronal activity have been described in *Aplysia*. A single neuron within the central nervous system has been shown to have a circadian rhythm of spike frequency (*4*), and a circadian rhythm in the frequency of compound action potentials in the isolated eye of this animal has been characterized (*5*, *6*).

We now show that the circadian rhythm in the isolated eye of *Aplysia* is a consequence of interaction among a population of endogenously active neurons of the eye. Eyes removed from *Aplysia californica*, subjected to light-dark cycles of 12 hours of light (200