lack of response to glutathione, γ-aminobutyric acid, and *N*-acetylneuraminic acid. Glycine, threonine, valine, and leucine apparently repulsed the snails (Table 3).

Snail conditioned water (*7*) and a water-soluble extract of lyophilized snail tissues (1 g per 10 ml of distilled water, incubated for 3 hours at 23°C, and then centrifuged at 27,000*g* for 20 minutes) also attracted *B. glabrata*. These materials contain amino acids (*7*), but the presence of other molecules that may serve as pheromones must still be considered. It is of interest that we have observed an apparent increase in mating in *B. glabrata* in the presence of proline, but quantitation of this response is not yet complete.

Our experiments indicate that the amino acids glutamate and proline, snail conditioned water, and perhaps other unidentified molecules serve as chemical signals for and between individual *B. glabrata*. Jahan-Parwar (*9*) reported that glutamate is the main attractant in seaweed for the sea slug *Aplysia*, and also suggested that proline may activate its reproductive processes.

Although polyglutamate did not provide an effective attractant in our relatively short (1 hour) experiments, it merits further study as a source of glutamate since it could perhaps be used to release glutamate slowly through natural hydrolysis in controlled-release molluscicides. Combinations of Mg^{2+} and Ca^{2+} with glutamate and proline should be explored as sources of controlled-release attractants since we now know that such combinations affect three aspects of the schistosome life cycle. Miracidia respond to amino acids (*7*) and to Mg^{2+}/Ca^{2+} ratio (*10, 11*), and cercariae respond to glutamate (*6*) as do the snail vectors used in our experiments. Starved snails also readily find chalk in our experimental design, and Ca^{2+} and Mg^{2+} are often used in the production of controlled-release products (*2*).

An ideal molluscicide would release no poison into the environment, and would contain a slow-release attractant or chemical stimulant that attracted the snail to its surface or induced the snail to ingest a particle. If the particles could be coated with cellulose, as suggested by Lewin (*12*), then they might be digested only by snails or other organisms possessing cellulase. For areas with a high incidence of schistosomiasis, molluscicides could be designed to release poison slowly so that they would kill not only the adult snails but also the parasite's larval stages. These larval stages might also futilely expend their energies attacking the chemical charade which mimics the host.

Our experiments indicate that it might be possible to include relatively inexpensive attractants in controlled-release molluscicides which may also serve as schistosome larvicides.

LESLIE S. UHAZY
RICHARD D. TANAKA
AUSTIN J. MACINNIS
Department of Biology, University of California, Los Angeles 90024

References and Notes

1. F. J. Etges, *Am. J. Trop. Med. Hyg.* **12**, 701 (1963).
2. N. Cardarelli, *Controlled Release Molluscicides* (Environmental Management Laboratory Monograph, University of Akron, Akron, Ohio, 1977).
3. F. J. Etges, *Am. J. Trop. Med. Hyg.* **12**, 686 (1963).
4. E. H. Michelson, *ibid.* **9**, 480 (1960).
5. C. R. Townsend, *Anim. Behav.* **21**, 544 (1973).
6. A. J. MacInnis, *Nature (London)* **224**, 1221 (1969).
7. ———, W. M. Bethel, E. M. Cornford, *ibid.* **248**, 361 (1974).
8. G. W. Snedecor, *Statistical Methods* (Iowa State College Press, Ames, 1956).
9. B. Jahan-Parwar, in *Olfaction and Taste*, D. A. Denton and J. P. Coghlan, Eds. (Academic Press, New York, 1975), vol. 5, pp. 133–139.
10. H. H. Stibbs, E. Chernin, S. Ward, M. L. Karnovsky, *Nature (London)* **260**, 702 (1976).
11. G. M. Sponholtz and R. B. Short, *J. Parasitol.* **62**, 155 (1976).
12. R. A. Lewin, U.S. Patent Application No. 30141/61, 1961.
13. J. Folch, M. Lee, G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
14. I. Smith, *Chromatographic and Electrophoretic Techniques* (Wiley, New York, 1969), vol. 1.
15. E. Nakano and S. Yamamoto, *Dev. Biol.* **28**, 528 (1972).
16. We thank R. Rodriguez, D. Kebo, and R. Kim for technical assistance. This work was supported by the Edna McConnell Clark Foundation grant 276-0016 and NIH grant AI 13228 to A.J.M.

30 March 1978; revised 14 June 1978

Chemoreceptors in Lepidoptera: Stereochemical Differentiation of Dual Receptors for an Achiral Pheromone

Abstract. *The racemate and optically pure enantiomers of 9-(2-cyclopenten-1-yl)nonyl acetate have been synthesized and shown to mimic certain biological properties of (Z)-11-tetradecenyl acetate. European corn borers and red-banded leaf rollers respond differently to the racemate and to the enantiomers in precopulatory behavior bioassay. The responses demonstrate the presence of two stereospecific chemoreceptors, show the chiral character of these receptors, and define the conformation of carbon atoms 10 to 14 of (Z)-11-tetradecenyl acetate in these receptors.*

The European corn borer and the red-banded leaf roller use (*Z*)-11-tetradecenyl acetate in two quite distinct pheromone systems, sex attraction and precopulatory behavior (*1*). Male sex attraction is dependent on specific ratios of the (*Z*)- and (*E*)-11-tetradecenyl acetates (*2*).

Definition of the conformation of (*Z*)-11-tetradecenyl acetate in the pheromone chemoreceptor has been of considerable interest to us. In view of the infinite number of conformations possible for (*Z*)-11-tetradecenyl acetate, the problem initially seems impossible. We now present our approach to the solution of the problem of defining the conformation of the pheromone as it interacts with the chemoreceptors of male European corn borer and red-banded leaf roller moths. Our results show that the chemoreceptor systems of the two moths are different, that the precopulatory behavior system has two stereospecific chemoreceptors, and that both chemoreceptors for the achiral pheromone are chiral. The conformation of carbon atoms 10 to 14 of (*Z*)-11-tetradecenyl acetate in each chemoreceptor is defined.

The European corn borer and the red-banded leaf roller are capable of detecting the methyl group at position 14 in (*Z*)-11-tetradecenyl acetate (*3*). Starting from this observation, it is possible to design experiments that explore the conformation of the olefinic region of the molecule (carbon atoms 10 to 14). One possible conformation is particularly easily tested, that is, that in which the C-14 methyl group is approximately in the plane defined by carbon atoms 10 to 13. This conformation can be mimicked by the cyclic system (Fig. 1A) formed by removal of hydrogen atoms from carbon atoms 10 and 14. This change also introduces an asymmetric center at position 10.

Racemic 9-(2-cyclopenten-1-yl)nonyl acetate was synthesized by coupling 2-(2-cyclopenten-1-yl)ethyl tosylate and the Grignard reagent from 7-bromo-1-heptyl 2-tetrahydropyranyl ether, removal of the tetrahydropyranyl protecting group, and acetylation. The same sequence starting with optically pure (+)-(*S*)-2-(2-cyclopenten-1-yl)ethyl tosylate (*4*) gave (+)-(*R*)-9-(2-cyclopenten-1-yl)nonyl acetate chemical purity > 99.5 percent, $[\alpha]_D^{24} = +70.8° \pm 0.7°$ (C = 3.18, $CHCl_3$), > 99.9 percent optical purity (*5–8*). The enantiomer was synthesized by a different procedure. Coupling of optically pure (−)-(*S*)-2-cyclopenten-1-ylmethyl tosylate (*9*) and the Grignard reagent from 8-bromo-1-octyl

0036-8075/78/0908-0926$00.50/0

2-tetrahydropyranyl ether, removal of the tetrahydropyranyl protecting group, and acetylation gave (−)-(*S*)-9-(2-cyclopenten-1-yl)nonyl acetate chemical purity > 99.5 percent, $[\alpha]_D^{25} = -70.7° \pm 0.4°$ (C = 3.18, $CHCl_3$), > 99.9 percent optical purity (*6*, *7*). The second procedure starting with (+)-(*R*)-2-cyclopenten-1-ylmethyl tosylate gave (+)-(*R*)-9-(2-cyclopenten-1-yl)nonyl acetate ($[\alpha]_D^{25} = 70.0° \pm 0.6°$), which had biological properties identical to those of the same enantiomer prepared by the first route. This fact eliminates trace contamination in the reactants as a problem in the bioassay.

Results of bioassays (*10*, *11*) with the enantiomers of 9-(2-cyclopenten-1-yl)-nonyl acetate, the racemate, and (*Z*)-11-tetradecenyl acetate are presented in Table 1. Data for the European corn borer show that the (−)-(*S*)-9-(2-cyclopenten-1-yl)nonyl acetate is as effective in elicitation of precopulatory behavior as the natural sex pheromone [(*Z*)-11-tetradecenyl acetate]. The (+)-(*R*) enantiomer is much less effective, and the racemate is intermediate in activity between the enantiomers. The red-banded leaf roller responses are quite remarkably different. The enantiomers show equal activity, and the racemate is significantly more active than either enantiomer. These data are reminiscent of the data obtained with enantiomers of the chiral aggregation pheromone of *Gnathotricus sulcatus* (*12*) in which an enantiomeric blend was required to attract the beetle. The greater activity of the racemate in the red-banded leaf roller requires the existence of two stereospecific chemoreceptors, one primarily (or exclusively) sensitive to the (+)-(*R*)-9-(2-cyclopenten-1-yl)nonyl acetate and one sensitive to the (−)-(*S*) enantiomer. The greater activity of the racemate is a consequence of reinforcing signals from two chemoreceptors. The European corn borer data are consistent with the presence of a single stereoselective chemoreceptor.

The possible differences in the conformation of (*Z*)-11-tetradecenyl acetate in the two receptors can be defined by two models. Either the double bonds are in the same position in both chemoreceptors and the C-10 stereochemistry is inverted (model 1, Fig. 1B) or the stereochemistry at C-10 is maintained, and the difference lies in the double bond position (model 2, Fig. 1C). Model 1 is not tenable for the natural pheromone. The achiral pheromone has two hydrogen atoms attached to C-10, and the configurational identity is lost. Model 2, however, makes perfect sense for the natural pheromone. In model 2 (Fig. 1C), the two possible arrangements correspond to two sensible conformations of the natural pheromone, which differ in the coiling of the carbon chain. In a chiral receptor, these conformations become chiral; that is, the interactions with the receptor are diastereomeric. The prochiral character of (*Z*)-11-tetradecenyl acetate is thus used to good advantage in the insect chemoreceptor system. The red-banded leaf roller has evolved at least two chemoreceptors (one in common with the European corn borer) that accommodate different conformations of the achiral (but prochiral) pheromone. This striking result suggests that the insect chemoreceptor systems that are known to sense ratios of different geometric isomers (*2*) may, in general, use

Table 1. Sex stimulation assay results with European corn borer and red-banded leaf roller males (*10*, *11*). Numbers followed by the same letter are not statistically different from each other, on the basis of contingency table analyses.

Stimulus (500 ng)	Mean percent male response	
	European corn borer	Red-banded leaf roller
(*Z*)-11-Tetradecenyl acetate	64a	90d
(±)-9-(2-Cyclopenten-1-yl)nonyl acetate	44b	93d
(+)-(*R*)-9-(2-Cyclopenten-1-yl)nonyl acetate	16c	67e
(−)-(*S*)-9-(2-Cyclopenten-1-yl)nonyl acetate	65a	67e

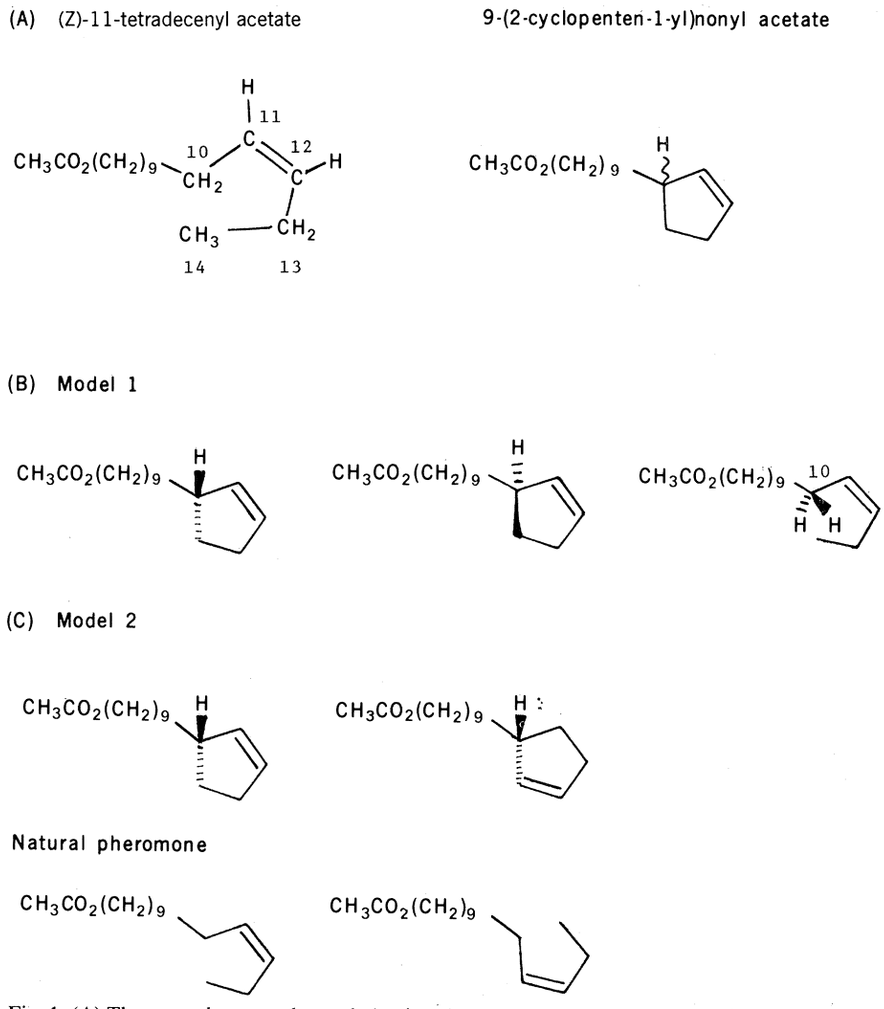

Fig. 1. (A) The natural precopulatory behavior pheromone (*Z*)-11-tetradecenyl acetate, and the cyclic mimic 9-(2-cyclopenten-1-yl)nonyl acetate. (B) Model 1 shows the enantiomers as they would appear in different receptors which maintain the double bond position constant and differ in the configuration at C-10. This model is meaningless for the natural pheromone, which has two hydrogens at C-10. (C) Model 2 shows the enantiomers in receptors that maintain the configuration at C-10 and place the double bond in different positions. This model makes sense for the natural pheromone, which simply coils differently in the two chiral receptors.

ratio discrimination based on two or more receptor systems even when detecting a single chemical compound. The adaptive advantage for the insect is clearly specificity in detection. The greater the number of specific conformations of a single molecule required to satisfy the different chemoreceptors, the lower the probability that an incorrect molecule will satisfy the chemoreceptor requirements. We consider it probable that other highly selective chemical communication systems, such as hormones, also use multiple chemoreceptor systems to achieve a high degree of chemical specificity.

ORVILLE L. CHAPMAN
Department of Chemistry, University of California, Los Angeles 90024

JEROME A. KLUN
Organic Chemical Synthesis Laboratory, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, Maryland 20705

K. C. MATTES
ROBERT S. SHERIDAN
Department of Chemistry, University of California, Los Angeles

STEFANO MAINI
Instituto di Entomologia, Universita di Bologna, Bologna, Italy

References and Notes

1. J. A. Klun and T. A. Brindley, *J. Econ. Entomol.* **63**, 779 (1970); J. A. Klun and J. F. Robinson, *ibid.*, p. 1281; W. L. Roelofs and H. Arn, *Nature (London)* **219**, 513 (1968).
2. J. A. Klun, O. L. Chapman, K. C. Mattes, P. W. Wojtkowski, M. Beroza, P. E. Sonnet, *Science* **181**, 661 (1973).
3. J. A. Klun, O. L. Chapman, K. C. Mattes, M. Beroza, *Environ. Entomol.* **4**, 871 (1975); O. L. Chapman, J. A. Klun, K. C. Mattes, M. Barry, in *New Methods in Environmental Chemistry and Toxicology*, F. Coulson, F. Korte, M. Goto, Eds. (International Academic Printing, Totsuka, Tokyo, 1973), p. 163.
4. The 2-(2-cyclopenten-1-yl)ethyl tosylate was prepared from optically pure 2-(2-cyclopenten-1-yl)ethanol (*5*). The syntheses are described in (*6*).
5. K. Mislow and I. V. Steinberg, *J. Am. Chem. Soc.* **77**, 3807 (1955).
6. O. L. Chapman, K. C. Mattes, R. S. Sheridan, J. A. Klun, S. Maini, *ibid.* **100**, 4878 (1978).
7. A plot of specific rotation as a function of molecular weight for the well-characterized acids of chaulmoogra oil and their esters is linear (*6*). Even (+)-(*S*)-2-cyclopenten-1-yl acetic acid and (+)-(*S*)-2-(2-cyclopenten-1-yl)ethanol fall on the plot. Linear regression analysis (*6*) of these data gives a predicted rotation for (+)-(*R*)-(2)cyclopenten-1-yl)nonyl acetate of +68.6°. The data plotted are from (*5*) and (*8*). The linearity of the plot is a consequence of the fact that the rotation in the 2-cyclopenten-1-yl system depends only on the molecular weight of the side chain when $n \geq 1$.
8. H. I. Cole and H. T. Cardoso, *J. Am. Chem. Soc.* **61**, 2349 and 2351 (1939).
9. Resolution of 2-cyclopentenecarboxylic acid was accomplished by using the enantiomers of α-phenethylamine. The (+)-(*R*)-cyclopentenecarboxylic acid, $[\alpha]_D^{25} = +262°$, was obtained with (+)-α-phenethylamine. Use of (−)-α-phenethylamine gave the (−)-(*S*) enantiomer. The syntheses are described in (*6*).
10. All compounds used in sex stimulation bioassays were analyzed on a glass open tubular column (60 m by .05 mm) coated with SP-1000 (J & W Scientific). The chromatography indicated that the compounds were chemically pure. The details of the syntheses, resolutions, and characterizations have been described (*6*).
11. Pupae of the European corn borer (Iowa) and the red-banded leaf roller were isolated individually. As the adult moths emerged, males were selected from the culture and placed in respective cages for the bioassay. The males were conditioned for 96 to 120 hours at constant light, 80 percent relative humidity, and 27°C. The bioassay consisted of exposure of a set of ten males in a screened cage positioned in a 20° to 22°C airflow (1.5 to 1.8 m/sec) to 0.5 μg of compounds for 30 seconds on the tip of a glass rod held 4 to 5 cm upwind of the caged moths. The number of males that responded to the stimulus with wing vibration, extension of genitalia, and clasper responses in the 30-second exposure period were recorded. The assays were conducted in a randomized complete-block design with 30 replicates; each set of ten males was exposed to only one stimulus, and then the moths were discarded. Variation of the amount of stimulus (nanograms to micrograms) did not alter qualitatively the relative response to (+)-(*R*)-, (−)-(*S*)-, and (±)-9-(2-cyclopenten-1-yl)nonyl acetate.
12. J. H. Borden, L. Chong, J. A. McLean, K. N. Slessor, K. Mori, *Science* **192**, 894 (1976).
13. The research at the University of California was supported by a grant from the Mobil Foundation. R.S.S. was supported by a predoctoral fellowship from the National Science Foundation. J.A.K. was formerly at Corn Insects Research Unit, Ankeny, Iowa 50010. This is journal paper No. 9070 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, project No. 2183.

9 February 1978; revised 9 May 1978

Regulative Interactions Between Cells from Different Imaginal Disks of *Drosophila melanogaster*

Abstract. *The regulative behavior of cells from the imaginal wing disk of* Drosophila melanogaster *can be modified by interaction with cells from different disk types. Both thoracic and nonthoracic disks are able to interact, but there are major differences in the effectiveness of interaction. The finding lends experimental support to the idea that cells in different fields within the same organism use the same mechanism for specifying positional information. A similar conclusion has been reached by Wilcox and Smith based on studies of the mutation* wingless.

The spatial patterns of differentiation which arise during animal development are thought to be generated by cell interactions that occur before differentiation. These interactions appear to assign different states (positional information) to cells according to their locations relative to other cells in the same cell population (*1*). Although Wolpert (*1*) proposed some time ago that the mechanism for specifying positional information may be the same for different fields, it has only recently been shown (*2*) that pattern formation and regulation (a term we use to designate developmental responses to surgical intervention) in several different systems can be understood in terms of a single set of rules for cell behavior. In the experiments reported here, we tested the idea that cells in different imaginal disks of *Drosophila* larvae use the same kind of cellular signals in establishing

Table 1. Structures derived from 02 wing fragments (identified by wild-type genotype) mixed with irradiated (15,000 R) genetically marked whole disks and wing disk fragments and cultured for 7 days in adults before transfer to larvae for metamorphosis. Occurrence of structures below the axillary cord indicates regeneration.

	Wing disk fragment 02 mixed with										
	Wing* 02	Wing* 68	Wing	Antenna	Eye	Leg			Haltere	Genital	
						1	2	3		Male	Female
N†	61	15	81	68	58	43	41	37	64	44	53
Notum	61	15	80	68	58	42	41	31	64	44	53
Tegula	7	10	44	30	14	21	11	9	34	11	19
Axillary cord	3	11	42	12	2	21	11	14	29	10	1
			Regeneration								
Costa	1	1	31	9	4	8	4	7	12	4	3
Triple row	1		14	5	2	7	1	4	5	3	1
Double row		2	20	7	2	7	5	6	6	1	1
Posterior row		6	37	15	1	18	8	12	25	8	3
Alar lobe		8	40	12	4	20	9	13	27	6	4
Dorsal radius		1	51	15	6	23	16	14	42	8	1
Ventral hinge		2	14	1	3	9	1		2	4	
Wing blade	1	6	69	25	8	30	18	19	53	12	7
Regenerated											
Number	1	8	71	28	9	34	22	19	54	12	7
Percentage	2	53	88	41	16	79	53	51	84	27	13

*Data from (*9*) are incorporated. †*N* = total number of mixtures examined.

0036-8075/78/0908-0928$00.50/0