duckling survive an infection with the strain of *P. lophurae* used here. Occasionally, typical symptoms of the spleen necrosis virus have been observed. This agent can be transmitted to healthy ducklings by inoculation of small volumes of plasma; even though this virus does slow down normal development of *P. lophurae*, it also invariably kills infected ducklings (*7*). The possibility that the observed protection was due to any other infectious agent transferred with immune globulin was eliminated experimentally. Six 8-day-old ducklings were each inoculated with 0.5 ml of plasma from each of six immunized ducks. When challenged with an infective dose of *P. lophurae*, all six ducklings showed the expected course of parasitemia and died. However, globulin fractions of serums from three of the same six immunized donor ducks protected recipient ducklings from *P. lophurae* infection. When plasma (0.5 ml) from each of the three protected ducklings was inoculated into three other ducklings, the course of infection with *P. lophurae* was not altered.

The HRP from *P. lophurae* is the only chemically characterized protein from malaria parasites. A similar protein was demonstrated in *P. falciparum* (*8*), but the possible immunological cross-reactivity of the proteins from these two species remains to be determined. The finding of cross-reactivity could lead to a possible synthetic malaria vaccine. Even though through the use of crude antigens it has been concluded that protection against malaria is species-specific, the possibility of immunological cross-reactivity to a purified antigen cannot be ruled out without experimental evidence. In addition, the rather peculiar amino acid composition of HRP could lend itself to immunological alteration through chemical manipulation.

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References and Notes

1. "Developments in Malaria Immunology," *WHO Tech. Rep. Ser.* (No. 579) (1975).
2. A. Zuckerman, *Exp. Parasitol.* **42**, 374 (1977).
3. A. Kilejian, *J. Biol. Chem.* **249**, 4650 (1974).
4. ———, *J. Protozool.* **23**, 272 (1976).
5. ———, in *Biochemistry of Parasites and Host-Parasite Relationships*, H. van den Bossche, Ed. (North-Holland, Amsterdam, 1976), p. 441.
6. R. F. Itzhaki and D. M. Gill, *Anal. Biochem.* **9**, 401 (1964).
7. W. Trager, *Proc. Soc. Exp. Biol. Med.* **101**, 578 (1959).
8. A. Kilejian and J. B. Jensen, *Bull. WHO* **55**, 191 (1977).
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Schistosoma mansoni: Identification of Chemicals That Attract or Trap Its Snail Vector, *Biomphalaria glabrata*

Abstract. *A new bioassay for chemical attractants of aquatic snails demonstrated that* Biomphalaria glabrata *could be attracted to or trapped in the vicinity of homogenates of lettuce. Fractionation of homogenates revealed the amino acids glutamate and proline as the primary attractants. Attraction was specific for the* L *form of glutamate. Proline appeared to stimulate reproductive activity. Glutathione, γ-aminobutyric acid, and a number of other compounds had no effect. Extracts of lyophilized snail tissue also attracted other snails and may thus contain pheromones. These results permit formulation and testing of controlled-release attractants designed to overcome the repellant effects of slow-release molluscicides, as well as the design of stimulants to be used with no-release poisons.*

Molluscicides targeted at snail vectors of important human pathogens such as schistosomes frequently repel the snail from areas containing lethal doses (*1*). The advent and success of slow- or controlled-release technology for dispensing pesticides has provided new and exciting possibilities for developing baits, attractants, or other chemostimulants which will enhance the efficacy of these formulations (*2*). Attraction or trapping of *Biomphalaria glabrata*, the snail host for *Schistosoma mansoni*, by various substances, especially food sources, has been reported (*2–5*). But specific chemicals emanating from food sources or the snails themselves, which can attract or trap the snail, have not been identified. We report herein the isolation and identification of chemicals from a food source and from snails which attract or trap *B. glabrata*. Fractionation of lettuce revealed that the portion containing free amino acids was the primary stimulant.

Table 1. Responses of *B. glabrata* to Romaine lettuce homogenate and fractions in 1 percent ionagar or on filter paper. Homogenates were prepared from 50 g (wet weight) of the leafy portion of lettuce in 200 ml of double-distilled water by blending at low speed in a Waring blender in 3- to 5-second bursts. The homogenate was filtered through cheese cloth, precipitated with 5 percent (weight to volume) trichloroacetic acid (TCA), stirred 1 hour at 0°C. After centrifugation at 500*g* for 20 minutes at 5°C, the supernatant was extracted five or six times with diethyl ether. The aqueous layer was saved for recovery of sugars, amino acids, and organic acids. The ether layer was removed and evaporated under N_2, and residual TCA was removed by chloroform partitioned against double-distilled water and subsequent evaporation of the chloroform. The resulting oil was tested as the lipid fraction. Total lipids were extracted also by the method of Folch *et al.* (*13*). Sugars, amino acids, and organic acids were recovered from the aqueous layer by chromatography with Dowex-50 followed by *O*-(diethylaminoethyl) (DEAE)-cellulose. The loaded Dowex was washed with 0.01*N* HCl to elute organic acids and sugars subsequently separated on DEAE. Amino acids were eluted from the Dowex with 4*N* NH_4OH. Sugars were recovered from the DEAE void volume by washing with double-distilled water (*p*H 6.4), and organic acids were eluted with 4*N* formic acid. These fractions were reduced in volume by flash evaporation, and were then lyophilized and reconstituted in spring water (Arrowhead Puritas). The purity of each fraction was ascertained by thin-layer chromatography (*14*). Percentage recoveries were measured by using ^{14}C-labeled glycine, lactate, and glucose as internal standards. The recoveries were 62, 45, and 57 percent, respectively, for amino acids, organic acids, and sugars. The constituents of the amino acid fraction are shown in Table 2. The organic acid fraction contained malate and citrate, the sugar fraction contained fructose and glucose. No attempt was made to identify amino sugars or vitamins. Any cross-contamination of fractions or presence of minor components was below resolution by thin-layer chromatography. The ionagar blocks measured 4 by 1.5 by 1.5 cm; the amino acid fraction was reconstituted to an equivalence of 10 m*M* glutamate, the organic acid fraction to 9 m*M* malate plus citrate, and the sugar fraction to 10 m*M* glucose plus fructose. Lipids were dissolved in absolute ethyl alcohol and portions of these were allowed to evaporate on filter paper (Whatman No. 4).

Experimental groups		No. of replicates*	Responses	
Attractant	Control		Attractant	Control
None	None	13	20.9 ± 2.3 (14.3)†	20.5 ± 2.5 (14.2)
Ionagar	Ionagar	25	25.2 ± 1.1 (18.7)	27.5 ± 0.9 (21.7)
Homogenate	Ionagar	2	36.3 ± 1.2 (35.0)‡	7.1 ± 7.1 (3.0)
Amino acids	Ionagar	4	34.5 ± 2.1 (32.3)‡	24.9 ± 1.7 (18.0)
Organic acids	Ionagar	5	30.9 ± 3.3 (27.0)	25.9 ± 0.6 (19.2)
Sugars	Ionagar	5	24.1 ± 1.9 (20.2)	26.5 ± 2.2 (17.0)
Lipids	Filter paper	4	19.1 ± 4.5 (12.0)	24.2 ± 2.0 (17.0)
Folch lipids	Filter paper	5	27.9 ± 1.6 (22.2)	25.1 ± 1.7 (18.2)

*Ten snails per replicate. †Mean ± standard error of transformed values (actual percentages). ‡Statistical significance $P < .05$.

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Subsequent experiments demonstrated that glutamate and proline are the major entities in food and possibly snail conditioned water which attract or trap *B. glabrata*. These results indicate that it should be possible to formulate slow- or no-release molluscicides coupled with controlled-release attractants. The attractants might be effective not only for snails but also for the larval stages of schistosomes, which likewise respond to amino acids (*6, 7*).

Observations of *B. glabrata* in the laboratory indicated that the snail had a preference for moving to and feeding on cut rather than uncut edges of lettuce. This suggested that the snails were attracted to diffusible components released from the cut edge. Experiments were performed to identify the chemical nature of this stimulus and to assess its effect on *B. glabrata* under controlled conditions.

Biomphalaria glabrata were maintained as reported previously (*6*). Bioassays were conducted in white, enamelware pans (29 by 18 by 5 cm) containing 900 ml of aquarium water at 22.5° to 23.5°C in a room maintained at a constant temperature. Aquarium water conditioned by a resident population of snails was used so that any attractant tested would have to override substances released by snails. Overhead fluorescent lighting was constant. Stainless steel pins centrally positioned at both ends of the pan were used to retain experimental or control material. The positions of the experimental and control material were alternated in each replicate. A grid of 10 by 5 equal units was ruled on the bottom of each plan. To begin an experiment, ten snails (8 to 15 mm in diameter) that had been starved for 24 hours were placed on the central line of each pan, then control and experimental materials were positioned. Locations of snails were recorded within the grid every 5 minutes for 60 minutes (12 observations).

The data presented are based on the numbers of snails observed in the end zones of the pans (that is, zones containing control or experimental material) as a proportion of the total observations of snail locations. Attractability (or trapping) was considered positive when statistical evaluation by the method of paired comparisons (*8*) (differences in means tested by Student's *t*-test following transformation of the proportions by the arc sine of the square root of the percentage) yielded a $P < .05$. This simple procedure permits the use of large numbers of animals and replicates, and gives consistently reliable results with control or experimental materials essential to screen large numbers of fractions or chemicals. This protocol does not necessarily separate a directed response, properly called attraction, from trapping within the effective zone of the stimulus following random entry. Henceforth, where we use the term attraction, it may be construed to include trapping, but the end result is the same in that the snails end up in the vicinity of the bait.

Table 2. Comparison of amino acids in the purified amino acid fraction (see Table 1) and a fresh, unfractionated homogenate of Romaine lettuce. The amino acids in an unfractionated homogenate were obtained from 10 g of lettuce in 100 ml of distilled water by the method of Nakano and Yamamoto (*15*). The amino acids were determined with a Beckman model 120 automated analyzer. They are reported as a percentage of total ninhydrin-positive amino acid, because absolute quantities varied somewhat depending on the portions of lettuce used, how it was prepared, and its water content.

Amino acid	Percentage of total ninhydrin-positive material	
	Fractionated	Unfractionated
Glutamate	21.00	20.40
Aspartate	14.60	15.70
Asparagine and glutamine	13.80	15.90
Serine	9.54	9.60
Proline	7.76	8.16
Alanine	5.69	4.44
Valine	4.66	4.32
Phenylalanine	3.67	3.36
Leucine	3.57	3.60
Threonine	3.47	3.60
Isoleucine	2.64	2.40
Lysine	1.43	1.08
Histidine	0.94	0.96
Tyrosine	0.84	0.72
Glycine	0.70	0.84
Tryptophan	1.11	0.24
Methionine	0.12	0.12
Arginine	4.47	4.56

Homogenates of Romaine lettuce (*Lactuca sativa* var. *longiafolia*) were prepared and fractionated (see Table 1). Bioassay of these homogenates when they were incorporated into ionagar (Consolidated Laboratories) (Table 1) revealed significant attraction by the homogenate and the amino acid fraction. No attraction occurred with the organic acid or sugar fractions. The lipid fractions, tested on filter paper, were not attractive. These results clearly indicated that the major attracting or trapping factor resided in the amino acid fraction.

The homogenate and the amino acid fraction were analyzed for constituent amino acids (Table 2). Glutamate, asparagine-glutamine, aspartate, serine, and proline were the major components. Subsequently, known amino acids from commercial sources were also incorporated into ionagar and bioassayed (Table 3). Glutamate and proline were revealed as the most important molecules in attracting the snails, and the best results occurred at an initial concentration of 10 m*M* in the agar. Higher and lower concentrations were less effective. The response was specific for the L isomers. Table 3 contains only data that were statistically significant. Other experiments had indicated some positive attraction by Mg^{2+} but not Ca^{2+} ions (as chlorides), and addition of 10 m*M* $MgCl_2$ to a homogenate of lettuce enhanced the positive effect of the homogenate (Table 3). No positive responses were detected to polyglutamate (20 mg/ml), aspartate, serine, glutamine, asparagine, glutamine plus asparagine, phenylalanine, alanine, isoleucine, lysine, histidine, tyrosine, methionine, tryptophan, or arginine, all at 10 m*M*. The snails showed a similar

Table 3. Responses of *B. glabrata* to various chemicals in 1 percent ionagar. Chemicals giving no statistically significant response are listed in text.

Experimental groups		No. of replicates*	Responses	
Attractant	Control		Attractant	Control
None	None	13	20.9 ± 2.3 (14.3)†	20.5 ± 2.5 (14.2)
Ionagar	Ionagar	25	25.2 ± 1.1 (18.7)	27.5 ± 0.9 (21.7)
Glutamate	Ionagar	15	32.2 ± 1.7 (28.9)‡	23.0 ± 2.2 (16.7)
Proline	Ionagar	6	31.0 ± 2.4 (27.0)‡	21.7 ± 2.4 (14.3)
Proline and glutamate	Ionagar	3	42.3 ± 2.9 (45.3)‡	18.5 ± 4.3 (11.0)
Valine	Ionagar	5	20.3 ± 2.5 (12.6)	27.5 ± 1.4 (21.4)‡
Threonine	Ionagar	5	22.3 ± 2.2 (14.8)	30.5 ± 3.9 (26.6)‡
Leucine	Ionagar	5	21.7 ± 2.6 (14.2)	31.0 ± 0.9 (26.6)‡
Glycine	Ionagar	5	22.9 ± 2.8 (15.8)	34.1 ± 1.9 (31.6)‡
Lettuce homogenate plus 10 m*M* $MgCl_2$	Ionagar	4	52.7 ± 4.9 (62.8)‡	15.1 ± 2.9 (7.5)

*Ten snails per replicate. †Mean ± standard error of transformed values (actual percentages). ‡Statistical significance $P < .05$.

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.

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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).
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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

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