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SPECIFICITY OF LABORATORY TRAIL FOLLOWING BY THE ARGENTINE ANT, *Iridomyrmex humilis* (Mayr), TO (*Z*)-9-HEXADECENAL, ANALOGS, AND GASTER EXTRACT

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Abstract—In laboratory trail-following bioassays of Argentine ant workers, *Iridomyrmex humilis* (Mayr), the geometric isomer, (*E*)-9-hexadecenal, of the trail pheromone component (*Z*)-9-hexadecenal elicited insignificant trail following as did the potentially more stable formate analogs, (*Z*)-7-tetradecenyl formate, (*E*)-7-tetradecenyl formate, and tetradecyl formate. Further, in direct choice tests, workers showed no preference for gaster extract trails (0.002 ant equiv/cm) over trails of (*Z*)-9-hexadecenal (0.2 ng/cm). Moreover, a 10-fold increase in synthetic trail concentration to 2.0 ng/cm caused (*Z*)-9-hexadecenal trails to be significantly preferred over gaster extract trails by trail-following ants.

Key Words—Argentine ant, *Iridomyrmex humilis* (Mayr), Hymenoptera, Formicidae, trail-following bioassay, (*Z*)-9-hexadecenal, geometric isomer, analogues, choice tests.

INTRODUCTION

Recently, (*Z*)-9-hexadecenal (*Z*9–16:Ald) was isolated and identified from the ventral glands of Argentine ants, *Iridomyrmex humilis* (Mayr) and, from preliminary tests of its "aggregating" effects of workers, implicated as part of this species' trail pheromone (Cavill et al., 1979, 1980). Van Vorhis Key and Baker (1981) demonstrated that intense and prolonged trail following is elicited by this compound alone, and not by several analogs. Although trail following levels to *Z*9–16:Ald were equivalent to crude gaster extract trails, direct choice tests between natural and synthetic trails were not performed.

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Because of the possibility of using Z9–16:Ald to modify *I. humilis* trail-following behavior in urban settings and in the field where this ant is a pest of citrus, we sought to determine its competitiveness with gaster extract trails and to see whether several formate analogs, potentially more stable than the aldehyde pheromone, would elicit comparable levels of trail following.

METHODS AND MATERIALS

Chemicals. Z9–16:Ald and (Z)-7-hexadecenal (Z7–16:Ald) were obtained from the Controlled Release Division of Albany International Corporation. Purity of these compounds was determined by gas–liquid chromatography (GLC), using 10% XF-1150 (50% cyanoethyl methyl silicone) on Chromosorb W, AW-DMCS, 100/120 mesh (2.5 × 2 mm), at 150° C and a carrier flow rate of 25 ml/min. The Z7–16:Ald was greater than 98%, and the Z9–16:Ald greater than 97% free of other volatile impurities. (Z)-7-tetradecenyl formate (Z7–14:Form), (E)-7-tetradecenyl formate (E7–14:Form), and tetradecyl formate (14:form) were provided by W. Roelofs and M. Gieselmann at the Geneva, New York, Agricultural Experiment Station. The Z7–14:Form was greater than 94%, the E7–14:Form greater than 91%, and the 14:Form greater than 93% free of other volatile impurities, determined by GLC, using 3% OV-101 (methyl silicone) on 100/120 mesh, acid-washed Chromosorb W-DMCS on a 2-m × 2-mm column at 170° C and a carrier flow rate of 40 ml/min. (E)-9-Hexadecenal was provided by L.K. Gaston and M.M. Pope (University of California at Riverside) and was greater than 96% free of other volatile impurities (<0.2% Z9–16:Ald; <0.2% 16:Ald), determined by GLC using 10% Silar 10C (3-cyanopropyl silicone) on acid-washed Chromosorb W, 100/120 mesh, on a 2.8-m × 2-mm column. The retention time of E9–16:Ald was 8.07 min at 170° C and a 25 ml/min carrier flow rate.

General. Colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr), collected near Riverside, California, were maintained in the laboratory as described previously (Van Vorhis Key et al., 1981). Immediately prior to bioassay periods, colonies were provided with new food sources in enclosed dishes attached to the nest boxes by flexible tubing. Once recruitment had been initiated through the tubing, individual unfed ants which had been recruited to the food source but had not yet arrived were redirected and introduced onto the experimental trail directly from the natural trail inside the tubing.

Analog Activities. Application of trails, preparation of gaster extracts, and bioassay procedure were performed as described previously (Van Vorhis Key et al., 1981). Circular trails (50.7 cm circumference, 2 mm wide) were constructed by siphoning diluted solutions onto revolving filter paper disks

(Whatman No. 1, 24 cm diam). These disks were then placed under a glass plate held 3 mm above the disk by a spacer ring. The time each ant spent within 15 mm of the center of the trail and the number of times it entered this area (approaches) were recorded during a 2-min period after introduction of a single recruited worker onto the trail. Trail-following continuity (sec following/approaches) was then calculated for each ant and mean trail-following continuities were compared using Duncan's new multiple-range test on $\log_{10}$-transformed data.

Choice between Synthetic and Gaster Extract Trails. Two circular trails, 14 cm in diameter with their centers 6.8 cm apart, were applied to a 24-cm-diam filter paper disk. The trails were 44 cm long and contained dosages equivalent to the previously used 50.7-cm trails (Van Vorhis Key et al., 1981; Van Vorhis Key and Baker, 1981) (Figure 1).

Trails of 100 ng-eq (2.0 ng/cm) *Z*9–16:Ald were paired with trails of either 10 ng/eq (0.2 ng/cm) or 1 ng/eq of (0.02 ng/cm) *Z*9–16:Ald, 0.1 ant-eq of gaster extract trails (0.002 ant-eq/cm), or solvent trails. Trails of 10 ng-eq *Z*9–16:Ald were also paired with either 1 ng-eq trails of *Z*9–16:Ald, with gaster extract trails or with another 10 ng-eq trail. Trail-following ants choosing the dashed-lined paths in Figure 1 would be making turns of approximately: (a) 60°, (b) 60°, (c) 120°, and (d) 0°.

Recruited, unfed ants were individually introduced randomly in equal numbers onto one of the two trails in each pair (except solvent trails) and, from video tapes of the assays, the choice each ant made at each choice point was determined. Trail pairs were assayed in a complete-block design, and individual ants contributed no more than 3 replicates of choice to the experiment. To be scored as trail following, ants had to be within 0.25 cm of the trail center for 2 cm before and after the choice point. Trail preference was determined using chi-square comparisons between actual choices and expected values from ants choosing between two 10-ng trails. The numbers of

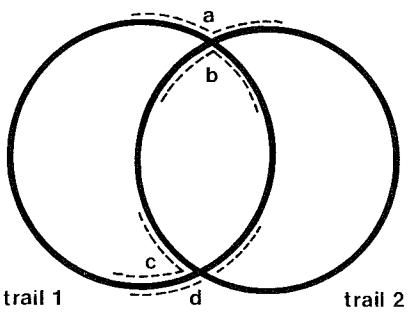

FIG. 1. Schematic of the arrangement of circular trails for trail choice tests. Dashed lines indicate possible paths of trail-following ants; required turning angles upon taking these routes are (a) 60°, (b) 60°, (c) 120°, and (d) 0°.

TABLE 1. FREQUENCIES OF TRAIL SWITCHING AT CHOICE POINTS BETWEEN TRAILS OF DIFFERENT CONCENTRATIONS OF *Z*9–16:ALD AND GASTER EXTRACT TRAILS OF 0.1 ANT-EQUIVALENT

Trail 1	Trail 2	% switching from trail 1 to trail 2 (*N*)	% switching from trail 2 to trail 1 (*N*)
100 ng-eq	solvent	0.4%[a] (1/256)[b]	100% n.s. (1/1)
100 ng-eq	1 ng-eq	7.8% (10/129)	95.4% (21/22)
100 ng-eq	10 ng-eq	6.3% (7/111)	86.7% (13/15)
100 ng-eq	0.1 ant-eq gaster extract	0% (0/47)	100% n.s. (2/2)
10 ng-eq	1 ng-eq	8.7% (21/242)	97.8% (44/45)
10 ng-eq	0.1 ant-eq gaster extract	42.8% n.s. (24/56)	52.4% n.s. (22/42)

[a] Asterisk indicates a significant deviation from the expected frequency of trail choice based on actual choices between two identical 10 ng-eq trails, determined using chi-square analysis ($P < 0.005$).

[b] Numbers in parentheses refer to fraction of ants following one trail which switched to following the other. Equal numbers of ants were introduced to each trail.

choices observed for each trail pair are listed in Table 1 and range from 49 to 187.

Validity of the experimental design and of the analysis technique was assessed by comparing the frequency with which ants switched from following one 10 ng-eq trail to following the other (41%: a + b + c in Figure 1); 59% remained following the same trail (d in Figure 1; $N = 226$). These percentages were then used to calculate the estimated values for chi-square comparisons with data from other pairs of trails.

RESULTS

Analog Activity. Among the compounds tested, only one, the known trail pheromone component, *Z*9–16:Ald, elicited significant trail following, and the activity of 10 ng-eq trails was equivalent to that of 0.1 ant-eq of gaster extract (Figure 2). Interestingly, the geometric isomer, *E*9–16:Ald, evoked no significant trail following at this dosage, and therefore behavioral and probably receptor specificity extends to geometric as well as positional isomers (Van Vorhis Key and Baker, 1981). Although ants tended to spend more time near *Z*7–16:Ald trails than near formate or solvent trails, *Z*7–16:Ald also did not elicit significant trail following in this experiment. Perhaps most surprising was the lack of trail following to the formates, especially to *Z*7–14:Form, the closest structural mimic to *Z*9–16:Ald we

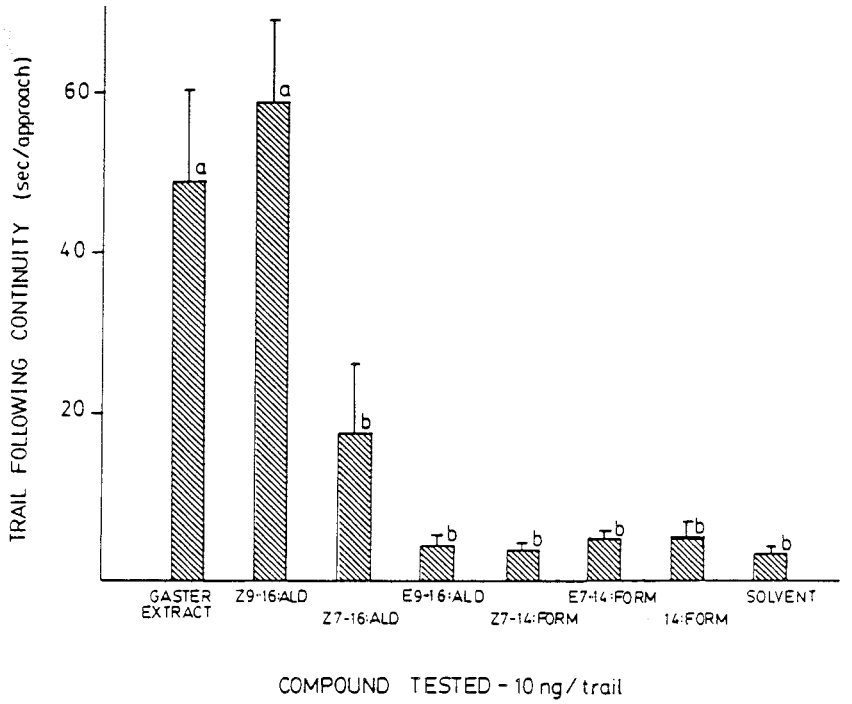

FIG. 2. Trail-following continuities elicited by 10 ng-eq trails of various analogs of Z9-16: Ald, 0.1 ant-equivalents of gaster extract and solvent trails. Means not followed by the same letter are significantly different according to Duncan's new multiple-range test ($P < 0.01$; $N = 20$).

tested. This demonstrates further the high specificity of the trail-recruitment system of this ant.

Trail Choice. When at the intersections of two Z9-16: Ald trails, Argentine ant workers consistently chose the more concentrated of the two in reciprocal choices (Table 1). These preferences deviated significantly from the expected 59%:41% choice frequencies of ants either remaining on, or switching to, respectively, identical 10 ng-eq trails. As expected from earlier work (Van Vorhis Key and Baker, 1981), 10 ng-eq Z9-16: Ald and 0.1 ant-eq of gaster extract trails were chosen equivalent numbers of times, and at frequencies not significantly different from those expected between two 10 ng-eq trails. However, a 10-fold increase to 100 ng-eq in the Z9-16: Ald trail allowed it to outcompete the gaster extract trails in choice tests with workers always choosing the synthetic over the gaster extract trails. The equality of the 10 ng-eq Z9-16: Ald and 0.1 ant-eq gaster extract trails was demonstrated by the equivalent reciprocal crossing from one trail to the other. That such

crossings were equally likely implies that the higher concentration of Z9-16:Ald in the synthetic trail compared to gaster extract (Van Vorhis Key and Baker, 1981) can compensate for the greater chemical diversity of the gaster extract trails (Cavill et al., 1979, 1980).

DISCUSSION

Argentine ant workers follow trails of Z9-16:Ald, and not other positional isomers of aldehyde analogs of different chain length, although in a previous experiment using a less discriminating bioassay Z7-16:Ald did show substantial activity (Van Vorhis Key and Baker, 1981). The lack of following to the opposite geometric isomer, E9-16:Ald, now demonstrates further the specificity of trail following to only Z9-16:Ald. Similar specificity of response by ants is known for trail-following *Atta texana* workers (Sonnet and Moser, 1972, 1973; Caputo et al., 1979), and for alarm responses by *A. texana* and *Atta cephalotes* (Riley et al., 1974), *Pogonomyrmex barbatus*, and *P. badius* (Benthuysen and Blum, 1974; Blum et al., 1971).

Perhaps even more interesting than the lack of trail following after changes in chain length, position of the double bond, or geometric configuration of that bond, was the lack of following to Z7-14:Form, probably the compound most structurally similar to Z9-16:Ald of all those we tested. Here, the substitution of an oxygen atom for a carbon at a single point in the chain was enough to eliminate trail-following response. The other tetradecenyl formates tested were also behaviorally inert, but this was expected due to their similarities to the inactive E9-16:Ald and 16:Ald.

Interest in formates as behaviorally active substitutes for aldehydes has been shown by workers in the field of moth sex pheromones (Mitchell et al., 1975, 1976; Cross et al., 1980; Caro et al., 1980). Formates should be more stable than aldehydes when applied as mating disruptants in slow-release field formulations and, therefore, could be more efficacious over longer time spans than similarly applied aldehydes. The behavioral inactivity of Z7-14:Form, however, indicates that this formate will not be acceptable as substitute for Z9-16:Ald in Argentine ant field-behavior-modification programs.

The Z9-16:Ald trails were competitive with gaster extract trails in trail-choice experiments, and the ants even showed a significant preference for 100 ng-eq Z9-16:Ald trails over trails of 0.1 ant-eq of gaster extract. That trail following ants "choose" to leave tubing containing naturally deposited trails to follow experimental trails of Z9-16:Ald is further evidence of this compound's activity. These results are encouraging for possible field use of this compound in bait-finding and pick-up schemes. Further work is now being conducted to determine the competitiveness of Z9-16:Ald in the field with naturally deposited trails.

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