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TRAIL PHEROMONE OF THE LEAF-CUTTING ANT, *Acromyrmex octospinosus* (REICH), (FORMICIDAE: MYRMICINAE)

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Abstract—The most active component of the trail pheromone of the leaf-cutting ant, *Acromyrmex octospinosus*, is methyl 4-methylpyrrole-2-carboxylate (I). Two pyrazine isomers (II) and (III) are present but inactive.

Key Words—Trial pheromone, leaf-cutting ant, *Acromyrmex octospinosus*, Hymenoptera, Formicidae, Myrmicinae, methyl 4-methylpyrrole-2-carboxylate, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, ant.

INTRODUCTION

Leaf-cutting ants of the genera *Atta* and *Acromyrmex* use trail-marking pheromones while foraging. The most active components of the pheromones of three species were identified as methyl 4-methylpyrrole-2-carboxylate (I) for *Atta texana* (Tumlinson et al., 1971) and *A. cephalotes* (Riley et al., 1974), and 3-ethyl-2,5-dimethylpyrazine (II) for *A. sexdens* (Cross et al., 1979), which also contained the pyrrole (I). The source of these compounds is the poison gland reservoir.

In the present study, we report that the most active component of the trail pheromone of *Acromyrmex octospinosus* (Reich) is the pyrrole (I); also present are the pyrazine (II) and its isomer, 2-ethyl-3,5-dimethylpyrazine (III).

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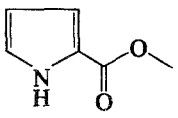

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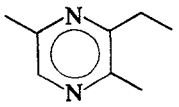

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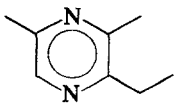

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METHODS AND MATERIALS

Acromyrmex octospinosus worker ants were obtained from nests in and near citrus and cacao plantations in Trinidad, West Indies, in August 1975. The abdomens of 120 g of ants (approximately 8000 ants) were removed and macerated in methylene chloride (CH_2Cl_2); the filtered solution was concentrated by distillation of the CH_2Cl_2 through a short packed column at atmospheric pressure, and the concentrated extract was subjected to short-path distillation (96°, 25 mm) to give a distillate that elicited a strong trail-following response in the laboratory bioassay (Cross et al., 1979).

Trail-marking deposits were accumulated from laboratory colonies of *A. octospinosus* at the University College of North Wales by allowing the ants to pass through an enclosed Plexiglas maze between the nest and the foraging area. Each nest had a fungus garden volume of 500–600 cc. A polythene tube, 2.5 cm in external diameter, led from the nest box into one end of the maze, which was a Plexiglas box 24 × 24 × 3.5 cm deep fitted with a series of internal partitions 2 cm apart running alternately from one side to within 3 cm of the other side. To traverse the maze, the ants had to take a zigzag path 2 m long. At the other end of the maze, a polythene tube led to a feeding box where privet leaves were provided as forage. The lid of the maze was removed, and 10.0 g of purified Porapak Q (Byrne et al., 1975) were poured onto the central section of the floor of the maze, to give a trail over Porapak 1.0 m long. After 50 days, the Porapak Q from two such mazes connected to two nests was extracted with 250 ml pentane in a Soxhlet apparatus for 24 hr, and the solvent was distilled through a short, packed column to leave 700 μl of a biologically active solution.

The distillate and the concentrated Porapak extract were fractionated by GC on the following glass columns: column A, 2.44 m × 4 mm ID, 5% SE-30 on Chromosorb G 50–80 mesh; column B, 6.10 m × 4 mm ID, 4% Carbowax 20 M on Chromosorb G, 50–80 mesh; column C, 1.53 m × 4 mm ID, 5% FFAP on Varaport 30, 80–100 mesh; column D, 6.10m × 4mm ID, 4% NPGA on Chromosorb G, 80–100 mesh; column F, 6.10 m × 4 mm ID, 5% Apiezon L on Chromosorb G, 80–100 mesh; column G, 6.10 m × 4 mm ID, 5% FFAP on Varaport 30, 80–100 mesh.

Mass spectra (electron impact) were obtained on a Hitachi RMU-6 and on a Finnegan GC-MS 3000. Fractions from the Varian 2700 chromatograph were collected in glass capillary tubes (30 cm × 2 mm OD) in a thermal-

gradient splitter/collector (Brownlee and Silverstein, 1968). Methyl 4-methylpyrrole-2-carboxylate (I) was obtained from Dr. P.E. Sonnet. A sample containing a 1:1 mixture of 3-ethyl-2,5-dimethylpyrazine (II) and 2-ethyl-3,5-dimethylpyrazine (III) was obtained as a gift from Pyrazine Specialties, Atlanta, Georgia; the isomers were separated on column F.

Laboratory bioassays were carried out as described by Robinson et al. (1974) and Cross et al. (1979).

RESULTS

Isolation and Identification. Fractionation of the concentrate of the Porapak extract on column A (60 ml He/min. 75° for 12 min, programed at 2°/min to 200°) gave a very active fraction (2) collected at 33–58 min. Slight activity was found in the other fractions. Fraction 2 was fractionated on column B (60 ml He/min, 155° for 24 min, programed at 4°/min to 190°). The most active fraction (2-8, 36–43 min) was fractionated on column C (60 ml He/min, 130°), and an active compound (2-8-4) eluting as a single symmetrical peak at 53 min was collected and identified as methyl 4-methylpyrrole-2-carboxylate (I) by congruence of the mass spectrum with that of an authentic sample (Tumlinson et al., 1972) and by coinjection with the authentic sample on columns B and C. In this way, approximately 20 μg of I was isolated from the deposits of ants foraging for 50 days from two laboratory nests.

The pyrazine that was found in *Atta sexdens* (Cross et al., 1979) was sought through the following fractionation sequence: Fraction 2-1 (0–6 min) from column B (60 ml He/min, 155° for 24 min, programed at 4°/min to 190°) gave fraction 2-1-4 (22–29 min) on column C (60 ml He/min, 90° for 6 min, programed at 2°/min to 150°), which gave fraction 2-1-4-2 (29–34 min) on column D (60 ml He/min, 115°), which in turn gave two peaks [55.5 min (II) and 60.5 min (III) on column G (60 ml He/min, 80°)]. These compounds were identified as II and III by coinjection with authentic samples on column G, and by congruence of the mass spectra with those of authentic samples [II and III, M = 136 (70%), M − 1 = 135 (100%)]. The ratio of II to III was 1:3 (54 ng–158 ng).

The short-path distillate from approximately 8000 ant abdomens yielded approximately 5 μg of the pyrrole (I) following the above fractionation procedure, but the presence of the pyrazines (II and III) could not be confirmed because of small amounts.

Bioassays. Acromyrmex octospinosus responded to the synthetic pyrrole (I) over a range of 4.0 pg/μl to 4.0 ng/μl in a laboratory bioassay; maximum response was at about 0.4 ng/μl (Robinson et al., 1974). In the present study, the GC fraction containing I gave a positive response over the three concentrations tested from 6 ng/μl to 0.06 ng/μl, with the highest response at

the highest dilution; presumably at higher concentrations, the fraction had other effects on the ants that interfered with the bioassay. Responses to the pyrazines II and III, alone or in combination, and to the fraction containing them were negative.

DISCUSSION

It is an oversimplification to ascribe the complex social behavior of trail following solely to the compound identified as the most active, satisfying though it may be to elicit this response in the laboratory and in the field with a defined chemical. In a recent study, Robertson et al. (1980) described the complex interactions of attractants, activators, synergists, and antitrailants in the trail following of the Argentine ant, and also noted that visual clues and physical contact with fellow trailers are involved.

In the present study, the role of methyl 4-methylpyrrole-2-carboxylate (I) in *Acromyrmex octospinosus* is clear, and its presence explains the earlier empirical observation by Robinson et al. (1974) that this ant would follow a trail of synthetic I. The role of the pyrazines II and III is less clear; The fractions containing II and III did not show significant activity, and we could not confirm our reported result that a 1:1 mixture (synthetic sample) elicited a response from *A. octospinosus* at a very high concentration (Cross et al., 1979). At any rate, such high concentrations have no biological significance. It is interesting to note the presence of II as the most active component of the pheromone of *Atta sexdens* together with the absence of III and the presence of I as an inactive compound in that ant. Certainly, sympatric species of many insects discriminate between species by recognizing specific blends of the same compounds that are shared and function both as pheromones and allelochemics (for example, Tamaki, 1977; Silverstein, 1977, Blum, 1979; Birch et al., 1980), and it has been shown that ants can distinguish their own trail from those of other species despite the commonality of components (Robinson et al., 1974; Cross et al., 1979).

Use of an absorbent such as Porapak Q, to accumulate trail pheromones from a limited number of ants, appears to be a useful procedure given a laboratory bioassay to track active compounds. We accumulated more of the compounds of interest from two nests than from the approximately 8000 ants laboriously harvested in the field and dissected.

We had hoped that a practical method of ant control might result from a pheromone-enhanced toxic bait. However, Robinson and Cherrett (1978) and Robinson et al. (1982) have shown that, although pick-up of filter paper disks in the laboratory was enhanced by the addition of synthetic I, adding it to citrus pulp bait did not produce worthwhile additional pick-up in the field. They suggested that this was due to a lower sensitivity to given concentrations of pheromone on baits in the field than in the laboratory, to the pheromone's

role being restricted to that of an attractant so that other chemicals are needed to induce pick-up of the bait, and to competition from naturally occurring "food odors" in the bait particles, which act as attractants in their own right. They concluded that the pheromone would not be a cost-effective addition to current baits, although it might be a worthwhile addition to the synthetic baits now being developed (Jutsum and Cherrett, 1981).

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