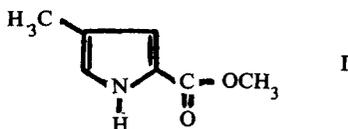


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Identification of the Trail Pheromone of a Leaf-cutting Ant, *Atta texana*

THERE are numerous reports of the isolation and identification of insect sex pheromones, and recently a termite trail-following pheromone was isolated and identified¹, but this is the first isolation and identification of an ant trail pheromone to be reported. We have identified the major volatile component of the trail marking substance laid down by the town ant, *Atta texana* (Buckley), as methyl 4-methylpyrrole-2-carboxylate (I).

The worker ants of *Atta texana*, whose trail marking substance contains both volatile and nonvolatile components², readily followed trails made by us with synthesized (I).



The whole bodies of 3.7 kg of worker ants of mixed sizes obtained from Grant Parish, Louisiana, were macerated in methylene chloride and the soluble material was distilled in a short-path still onto a condenser (cooled with dry ice) at 90° C and 0.05 mm Hg. The trail-following response³ of the minor workers from a laboratory colony was used to monitor this and all subsequent isolation steps. The active nonvolatile material was saved for future study, and the volatiles were used for this investigation. The active distillate was fractionated by gas chromatography. Successive vapour phase chromatography (VPC) of the major active component on four columns (SE-30, diethylene glycol succinate, Carbowax 20 M, and silicone DC QF-1) yielded 150 µg of a single compound. This very potent trail pheromone, when rechromatographed on the columns, gave a single symmetric peak on each column. Four other VPC fractions, which have not been purified or identified, are active at about ten times higher concentrations than the major active compound and are present in about five to twenty times smaller amounts.

On the basis of mass, infrared, and nuclear magnetic resonance spectra, we assigned structure (I) to the pheromone, which was confirmed by congruence of these data and of VPC retention times with those properties of an authentic synthetic sample⁴.

Potency of the synthesized (I) was evaluated by describing on slick cardboard sheets, 50 cm circumference circles with serially diluted 10 µl. chloroform solutions. Twelve minor workers from a laboratory colony were then released into the centre of the circle. The lower threshold of detection was 0.08 pg/cm (3.48×10^8 molecules/cm), about the same as that recorded for *R. virginicus*¹. Thus only 0.33 mg would be required to draw a detectable trail around the world. Strong responses were obtained from 0.8, 8.0, and 80.0 pg/cm, but 0.8 ng/cm produced some repellency. Minor workers were completely repelled by concentrations of 8.0 ng/cm and above. Volatility of the pheromone was demonstrated by placing the minor workers on plastic sheets 1 mm above the trail². Workers detected the

odour at a concentration of 80 pg/cm and strongly responded at 0.8 ng/cm described on the trail below the plastic sheet.

Medium and large workers were then tested on natural trails leading from nests in the field. They readily followed a 2.7 pg/cm trail on a 15 cm strip of cardboard placed across an "erased" portion of the trail. They detected another trail made by dribbling 4.0 pg/ μ l. on the sand at a 45° angle to the field trail; response was strong at 40.0 pg/ μ l.

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