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BEHAVIORAL RESPONSES TO AN ALKYLPYRAZINE FROM THE MANDIBULAR GLAND OF THE ANT *WASMANNIA AUROPUNCTATA*

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SUMMARY

Mandibular glands of the ant, *Wasmannia auropunctata*, contain 2,5-dimethyl-3-isopentylpyrazine, a compound which attracts nestmates to disturbed workers. During interspecific aggressive encounters, this small ant may utilize the alkylpyrazine as a repellent as well as disabling opponents with its potent sting venom. Alkylpyrazines have been previously identified as cephalic products from ants of the subfamilies Ponerinæ, Formicinæ, and Dolichoderinæ but this is the first report of an alkylpyrazine from the mandibular glands of a member of the Myrmicinæ.

ZUSAMMENFASSUNG

Verhaltensreaktionen auf ein aus den Kieferdrüsen der Ameise, Wasmannia aurapunctata, **stammendes Alkylpyrazin**

Die mandibularen Drüsen der Ameise *Wasmannia auropunctata* enthalten 2,5-dimethyl-3-isopentylpyrazin, eine Verbindung mit der andere gleich-artige Nestbewohner von gestorten Arbeitern herbeigelockt werden. Während einer aggressiven interspezifischen Begegnung kann die kleine Ameise das Alkylpyrazin als ein Abstossungsmittel benutzen und ihren Opponenten noch dazu mit ihrem wirkungsvollen giftigen Stich untauglich machen. Alkylpyrazine sind schon früher in Vertreten der Unterfamilien Ponerinæ, Formicinæ und Dolichoderinæ gefunden worden, jedoch ist dies der erste Bericht über Alkylpyrazine in den mandibularen Drüsen einer Art der Myrmicinæ.

INTRODUCTION

Among social insects, small size need not be equated with defensive inadequacy. What many diminutive ant species lack in stature, they more

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than adequately compensate for with remarkable chemical arsenals. The diminutive thief ant, *Solenopsis (Diplorhoptrum) fugax,* for example, is able to plunder brood from the nests of much larger species by repelling them with a potent pyrrolidine-dominated secretion (HOLLDOBLER, 1973; BLUM *et al.*, 1980), and other small species such as *Monororium pharanois* are similarly chemically endowed (HOLLDOBLER, 1973).

Another Lilliputian, yet highly successful ant, is *Wasmannia auropunctata* (Roger), whose workers measure approximately 1.5 mm in length. This myrmicine originates from the Neotropics and has been introduced into the U.S. in Florida, nesting in the soil or opportunistically under tree bark and in other cavities. Often forming huge, diffuse colonies in citrus groves, *W. auropunctata* forages openly for homopteran honeydew, insects, and other materials (SMITH, 1965), with the workers moving at a notably slow pace along extensive trail systems (SPENCER, 1941).

Very little is known of either the defensive behavior or alarm communication of this ant. It is sometimes the only ant species foraging in heavily infested trees, suggesting an ability to repel potential competitors (SPENCER, 1941). It is not unlikely that the venom of *W. auropunctata* contributes to its defensive capabilities, especially since the sting of this diminutive ant is reported to be inordinately painful to humans (WHEELER, 1929).

To elucidate further the defensive and communicative capabilities of this species, we studied the responses of *W. auropunctata* workers to stressed nestmates and to aggressive treatment by other ants. Establishing that the mandibular gland secretion plays an important pheromonal role in such situations, we investigated its chemical composition.

MATERIALS AND METHODS

Several small colonies of *W. auropunctata* were collected in Key West, Florida and housed in artificial nests in the laboratory. Each nest, consisting of a pair of plastic petri dishes with moistened Castone® flooring, was situated in a large plastic box which served as a foraging arena. A coating of Fluon® prevented the ants from scaling the arena walls and the colonies were regularly fed minced insects and 50 % honey water.

Observations were made on reactions of *W. auropunctata* foragers to "stressed" nestmates. Stress was induced by pinching the legs of a worker with forceps and holding it just out of reach of its foraging sisters. To determine sources of attractiveness in the workers' bodies, ants were anesthetized with nitrogen, trisected into head, thorax and gaster, and after being crushed between two filter paper discs (6 mm diameter), the filter paper sandwiches were introduced into the arena. The treated discs and a pair of empty, control discs were positioned 5 cm from one another and an equal distance from the nest with their order randomized in 12 replicates. At three minutes, the number of foragers at each disc was noted. Similar disc-tests compared the effects of extracts of body parts with synthetic material and solvent controls.

Individual *W. auropunctata* workers were also placed in the foraging arenas of colonies of *Solenopsis invicta* and *Monomorium minimum.* The distributions of *W. auropunctata* and the other two species overlap in southern Florida and they are likely to encounter one another frequently in the field.

Extracts of macerated *W. auropunctata* body parts were prepared in methylene chloride, carbon disulphide, acetone or ether, and volatiles were analyzed on a LKB-9000 gas chromatograph-mass spectrometer (GC-MS). OV-1 and OV-17 columns were used with column temperatures programmed from 30 to 200°C/8° per minute. Some extracts were treated with diazomethane and analyzed for the presence of methyl esters by GC-MS.

RESULTS

In both the field and the laboratory, *W. auropunctata* workers move at a sluggish rate. This may have partly been illusory, stemming from the small size of the workers, but even on uncomfortably hot afternoons, when entire colonies were suddenly unearthed, the ants moved relatively slowly. However, the introduction of stressed workers into foraging arenas resulted in foragers moving somewhat more rapidly and usually toward the stimulus. Ants were attracted from as far as 2-3 cm and quickly accumulated beneath the captive ant. They frequently probed upward, reaching with forelegs, antennae and open mandibles. Crushed, immobile workers proved equally effective stimuli but bits of filter paper or aged worker corpses did not. Subsequent experiments demonstrated that the foragers' responses were predominantly mediated by olfactory cues.

Foragers were attracted to the head significantly more than to other body parts ($p < .05$, ANOVA). Three minutes after introduction, 4.42 ants (mean $\pm$ S.E. of 0.40) were at the crushed heads but only 1.33 ($\pm$ 0.47) were at the thorax, 1.17 ($\pm$ 0.32) at the gaster and 1.33 ($\pm$ 0.31) at controls. Behaviors such as opening of mandibles and biting or tugging at paper discs occurred occasionally with all body parts but only rarely with controls. Stinging was never observed nor did workers display overt aggression towards one another.

A single volatile compound was detected in methylene chloride extracts of 150 workers. The compound possessed a base peak at m/z (mass/charge) 122 and additional peaks at m/z 178, 177, 163, 149, 136, 135, 123, 121, 108, 107, 80, 53, 51, 42, 41, and 40. This spectrum is identical to that reported for either 2,5-dimethyl-3-(3-methylbutyl)-pyrazine (2,5-dimethyl-3-isopentylpyrazine) or 2,5-dimethyl-3-n-pentylpyrazine (STENHAGEN *et al.*, 1974 a). Direct comparison and coinjection (2 m $\times$ 2 mm i.d. column packed with 4 % OV-1 on Gas ChromQ ; 70° C isothermal) revealed identical retention times for the former alkylpyrazine and the natural material. The retention time of 2,5-dimethyl-3-n-pentylpyrazine was different from that of the natural product.

2,5-Dimethyl-3-isopentylpyrazine was detected in carbon disulphide extracts of the heads of 50 workers, but no violatiles could be detected in extracts of their gasters. This pyrazine was demonstrated to be present in extracts of 40 dissected mandibular glands.

Initially, we estimated that each ant contained approximately 2 ng of alkylpyrazine. However, treatment of cephalic extracts with diazomethane revealed that individuals could contain more than 100 times as much of this compound. Significantly, substantial amounts of free fatty acids were also detected in extracts of the head and mandibular glands. Fatty acids form weak nonvolatile salts with 2,5-dimethyl-3-isopentylpyrazine (a base), whereas methylation of the fatty acid frees more of the base for detection by GLC.

Filter paper discs impregnated with doses of alkylpyrazine in the 1 ant equivalent range elicited increased locomotory rates among foragers, a response similar to that shown to cephalic extracts, but not to controls. One minute after placement in the foraging arenas, 12.1 (mean $\pm$ S.E. of 1.1) ants were at discs treated with head extracts, 5.4 $\pm$ 0.7 were at alkylpyrazine baits, and 0.1 $\pm$ 0.1 were at controls. The compound was never more than two thirds as effective as extracts from a single head. A dose of 10 to 20 ant equivalents did not enhance the attractiveness of the alkylpyrazine-treated discs.

W. auropunctata workers displayed a remarkable variety of behaviors during encounters with other ants. These ranged from lunging for and stinging the first alien worker encountered to tucking in their legs and antennae, sitting immobile, and permitting foreign workers to pick them up between their mandibles. The latter, apparently submissive behavior, usually terminated with the smaller workers being deposited and escaping unharmed.

The sting of *W. auropunctata* appeared to be a particularly effective weapon; *M. minimum* workers succumbed within seconds of being stung. Some aggressive encounters ended rather abruptly. *S. invicta* workers, which had been struggling with those of *W. auropunctata,* suddenly released their diminutive foes, staggered away, and groomed their appendages for prolonged periods. Furthermore, *M. minimum* foragers, which had surrounded a *Wasmannia* worker, stopped antennating or probing it with their mandibles and disbanded even though the smaller ant made no obvious movements.

These observations suggested that *Wasmannia* is chemically defended during interspecific encounters, perhaps utilizing the mandibular gland secretion as a repellent. We tested the repellency of 2,5-dimethyl-3-isopentylpyrazine by applying it to pieces of mealworms *(Tenebrio molitor)* and monitoring acceptance by workers of *M. minimum.* Less than onethird as many *M. minimum* foragers fed at mealworms treated with 5 ant equivalents of alkylpyrazine than at mealworms that received only acetone solvent (mean $\pm$ standard error, 3.00 $\pm$ 1.36 versus 9.57 $\pm$ 1.23). Fewer ants contacted the treated baits than controls, and those which did usually withdrew rapidly.

DISCUSSION

Alkylpyrazines have been found in other species of ants, but this is the first report of these compounds as cephalic products of the Myrmicinæ, a subfamily whose mandibular gland secretions are dominated by ethyl ketones (BLUM and HERMANN, 1978). Ponerines have provided the majority of examples, with one or more such compounds identified in species in 5 genera (WHEELER and BLUM, 1973 ; DUFFIELD *et al.*, 1976 ; LONGHURST *et al.*, 1978). Alkylpyrazines have also been detected in *Calomyrnex spp.* (BROWN and MOORE, 1979), an Australian member of the subfamily Formicinæ, and in *Iridomyrmex humilis* (CAVILL and HOUGHTON, 1974), a dolichoderine. Interestingly, in the three previous cases where only a single pyrazine was detected as a mandibular gland product (DUFFIELD *et al.*, 1976 ; WHEELER and BLUM, 1973), the compound was always 2,5-dimethyl-3-isopentylpyrazine and the results with *W. auropunctata* are in keeping with this pattern. The recurrent appearance of alkylpyrazines in widely diverse taxa suggests that these compounds play an important role in the biology of many ant species.

The mandibular glands have usually proven to be the source of formicid alkylpyrazines and the compounds have generally been found to elicit intraspecific alarm. For example, LONGHURST *et al.* (1978) determined that workers of the ponerine *Odontomachus troglodytes* respond to 3 of their 4 alkylpyrazines with behaviors ranging from increased alertness to outright attack. An exception may exist in *Iridomyrmex humilis*, however, since ROBERTSON (pers. comm. in CAVILL and HOUGHTON, 1974) could not demonstrate an alarm function for the alkylpyrazines in workers of this dolichoderine species. The utilization of a pyrazine derived from the poison gland, as a trail pheromone by *Atta sexdens rubropilosa* (CROSS *et al.*, 1979), also demonstrates that this class of compounds is produced in abdominal glands of at least one formicid species.

Our results show that 2,5-dimethyl-3-isopentylpyrazine excites and attracts *W. auropunctata* workers, but it less effectively holds workers near the source of emission than stressed ants or extracts of heads. Presumably the synthetic alkylpyrazine is more volatile than the natural product which is present in admixture with lipids in the prepared extracts. It is possibile that workers are drawn to the source by the alkylpyrazine, but remain in the vicinity due to their eventual detection of less volatile compounds.

WHEELER and BLUM (1973) determined that alkylpyrazines from *Odontomachus spp.* can repel *Solenopsis invicta* workers and suggested that these compounds may serve both alarm and defensive functions. The alkylpyrazine in *W. auropunctata* also appears to enhance its defenses, based on the outcome of interspecific aggressive interactions and the compound's adverse effect on workers of *Monomorium minimum*. If fatty acids are components of the mandibular gland secretion, they could act as a spreading agent, analogous to aliphatic hydrocarbons which spread formic acid from the venom of ants onto the cuticle of their foes (REGNIER and WILSON, 1968). Further work is needed to clarify this point.

The sting of *W. auropunctata* is a highly effective weapon, lethal against other ants, and capable of inflicting painful burning sensations in humans.

The pain is reminiscent of that produced by alkaloidal fire ant venoms (MacConnell *et al.*, 1974), but our chemical analyses did not reveal any detectable volatiles in the gasters of *W. auropunctata.*

We thus envision *W. auropunctata* as being equipped with a tripartite defense system : In aggressive encounters it can both employ its formidable venom and simultaneously bite or apply an irritating secretion with its mandibles. Thirdly, reinforcements can be recruited as volatiles from the defensive secretion are detected by nearby nestmates. Such a system requires field tests for confirmation, but it can be predicted to be particularly advantageous in a species which mass-forages, as *W. auropunctata* is known to do (Spencer, 1941). It may also help to explain the peculiar reluctance of *W. auropunctata* workers to disperse and become less obtrusive when their nests are suddenly exposed. Their limited locomotory capabilities could be effectively compensated for by remaining in groups and aggregating their potent stinging and repellent defenses.

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