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PYGIDIAL GLAND OF *Azteca* NR. *bicolor* AND *Azteca chartifex*: MORPHOLOGY AND CHEMICAL IDENTIFICATION OF VOLATILE COMPONENTS

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Abstract—The large paired reservoirs of the pygidial gland of *Azteca* nr. *bicolor* and *A. chartifex* represents the largest exocrine structure in the abdomen. The glands produce a secretion with a strong smell, which the ants release when they are disturbed. Analyses of the secretions by gas chromatography–mass spectrometry (GC-MS) revealed a mixture of iridoids and ketones. *A.* nr. *bicolor* contains 2-heptanone (8%) and a mixture of three iridodial isomers, with *trans-trans*-iridodial as the major component (32%). *A. chartifex* contains 6-methyl-5-hepten-2-one (13%) and the three isomeric iridodials with *cis-trans*-iridodial as the principal component (32%).

Key Words—*Azteca* spp., morphology, iridoids, pygidial glands, gas chromatography–mass spectrometry.

INTRODUCTION

Azteca ants (Dolichoderinae) occur exclusively in the neotropics, being distributed from Mexico to the north of Argentina (Harada and Benson, 1988). All species of *Azteca* are arboreal. Some species have a symbiotic relationship with

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certain trees of *Cecropia* sp. while others construct carton nests in trees and shrubs.

The species of *Azteca* that possess a symbiotic relationship with *Cecropia* trees have their nests located in cavities inside the branches and trunk of the tree and feed mainly on special structures called Müllerian bodies, produced by the *Cecropia*. The ants in return keep the tree clear of any invading invertebrates. They swarm out to attack any small herbivores. The Kayapó Indians of Brazil use these ants to protect their cultivated crops from pests, such as leaf-cutting ants (Overall and Posey, 1990).

Ants use volatiles from exocrine glands for communication (Attygalle and Morgan, 1984). In dolichoderine ants, two glands are responsible for the production of volatiles involved in their communication system: Pavan's gland, situated between the 6th and 7th abdominal sternites, is the source of the trail pheromone (Wilson and Pavan, 1959). The pygidial gland occurs between the 6th and 7th abdominal tergites. In dolichoderine ants it is very large, and has sometimes been confusingly called the "anal gland" (Pavan and Ronchetti, 1955).

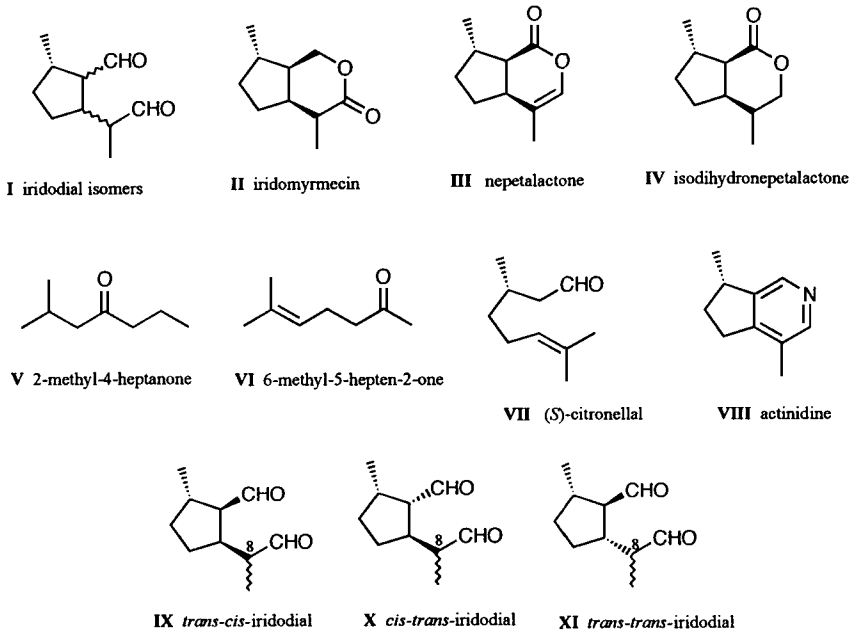
The secretion from the pygidial glands of dolichoderine ants is generally characterized by a mixture of iridoids (I–IV) and ketones (Figure 1). The iridoids seem to be used for defense, being repulsive to a number of insects whereas the ketones; for example, 2-methyl-4-heptanone (V) and 6-methyl-5-hepten-2-one (VI), elicit alarm behavior in conspecific individuals (Hölldobler and Wilson, 1990).

Knowledge of the chemical nature of volatile components present in the secretion from the pygidial glands of *Azteca* ants has until now been restricted to three species, *A.* nr. *nigriventris*, *A.* nr. *instabilis*, and *A.* nr. *velox* (Wheeler et al., 1975). Iridodial and cyclopentyl ketones (2-acetyl-3-methylcyclopentene, *cis*-1-acetyl-2-methylcyclopentane, and 2-methylcyclopentanone) were identified in the pygidial gland of these species (Wheeler et al., 1975).

In this paper, we report on the chemical characterization of the volatile compounds from the pygidial gland secretions of two *Azteca* species: *Azteca* nr. *bicolor* and *A. chartifex*. With the identification of the compounds present in the exocrine secretions of these species, we hope to increase knowledge of the chemistry of volatiles present in the secretion from these glands. Their secretions may help in chemical taxonomy and diagnosis of the species, since there are no well-defined morphological characters that differentiate the species (Harada, 1982).

METHODS AND MATERIALS

Live ant colonies of *A.* nr. *bicolor* (Formicidae: Dolichoderinae) were collected from a *Cecropia peltata* tree near the Federal University of Alagoas



I iridodial isomers II iridomyrmecin III nepetalactone IV isodihydronepetalactone

V 2-methyl-4-heptanone VI 6-methyl-5-hepten-2-one VII (*S*)-citronellal VIII actinidine

IX *trans-cis*-iridodial X *cis-trans*-iridodial XI *trans-trans*-iridodial

FIG. 1. Structures of compounds commonly encountered in the pygidial gland secretion of dolichoderine ants.

(Maceió-Alagoas, Brazil) and colonies of *A. chartifex* were collected in the Amazon region and sent to Maceió where the colonies of the two species were kept in the laboratory. Whole abdomens of *A. chartifex* were removed and sealed in soft glass capillary tubes (15 mm × 1.8 mm ID × 0.2-mm wall thickness) (Morgan, 1990), and pygidial glands of *A.* nr. *bicolor* were dissected under a binocular microscope with the aid of a pair of fine forceps and also sealed in soft glass capillary tubes.

For morphological studies, abdominal tips of *A.* nr. *bicolor* were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. After postfixation in 2% osmium tetroxide, they were dehydrated in acetone and embedded in Araldite, Semi-thin sections of 0.5 μm were stained with methylene blue and thionine.

Gas chromatography–mass spectrometry (GC-MS) was conducted with a Hewlett Packard 5890 gas chromatograph directly coupled to a 5970 B Mass Selective Detector (quadrupole mass spectrometer with 70 eV electron impact ionization). The system was controlled by, and data accumulated on, a Hewlett Packard Series 300 computer with HP 59970 C ChemStation. Mass spectra were scanned from *m/z* 35 to *m/z* 550. Scan rate was about 2.4 sec/scan.

Samples of *A.* nr. *bicolor* were chromatographed on an immobilized polydimethylsiloxane phase coated in a fused silica column (12 m × 0.32 mm ID × 0.32-μm film thickness). Samples of *A. chartifex* were chromatographed on a different capillary column coated with the same phase, but of a different length (14 m × 0.32 mm ID × 0.32-μm film thickness). Helium was used as carrier gas at 1 ml/min. The sample was heated in the injector to 150°C for 2 min before crushing the capillary as described by Morgan and Wadhams (1972). The oven was programmed from 30°C at 8°C/min to 200°C. The split vent was closed before crushing the sample and reopened 30 sec later.

RESULTS

The paired pygidial gland of *A.* nr. *bicolor* consists of two large reservoirs that open between the 6th and 7th abdominal tergites. On each side there is a cluster of a few tens of secretory cells (Figure 2). These rounded secretory cells with a diameter of approximately 10 μm open into the lateral wall of the reservoir through an accompanying narrow duct, according to the type-3 secretory cells in the classification of Noirot and Quennedey (1974). The reservoirs have a diameter of approximately 100 μm and represent the major exocrine structure in the abdomen. They are characterized by a wrinkled lining of flattened epithelial cells. The general organization of the pygidial gland is similar to that of other dolichoderine ants (Dazzini Valcurone and Fanfani, 1982; Billen, 1986). Because of their large size, much secretion can be stored in the gland's reservoir ready for release in an encounter.

Chemical analysis of the pygidial gland contents of *A.* nr. *bicolor* and *A. chartifex* revealed a mixture of iridoids and oxygenated compounds (Figure 3A and B). The number above each peak corresponds to the compounds listed in Table 1, identified by interpretation of their mass spectra. Identification of 2-heptanone, 6-methyl-5-hepten-2-one, isopulegol, *cis-trans*-iridodial (X), *trans-trans*-iridodial (XI), and *trans-cis*-iridodial (IX) were all confirmed by injection of synthetic standards into the GC-MS. Iridodial was synthesized by Dr. Neil J. Oldham according to the method of Clark et al. (1959). This method gives mainly the *trans-trans*-iridodial (Oldham, 1994). Based on Oldham (1994), who showed that the elution order of iridodial isomers from a nonpolar phase capillary column gas chromatography is *cis-trans-*, then *trans-trans-*, and *trans-cis-*, it was possible to identify the relative stereochemistry of the natural iridodials. Each of these compounds can exist as two epimers at C-8; only one epimer of each pair was present in both species, but the order of elution of these epimers is not known, so the stereochemistry of the C-8 methyl group is not known. 2-Acetyl-3-methylcyclopentene, 2-formyl-3-methylcyclopentaneacetaldehyde, 2-formyl-3-methylcyclopenteneacetaldehyde, 2-(3-methylcyclopentyl)propio-

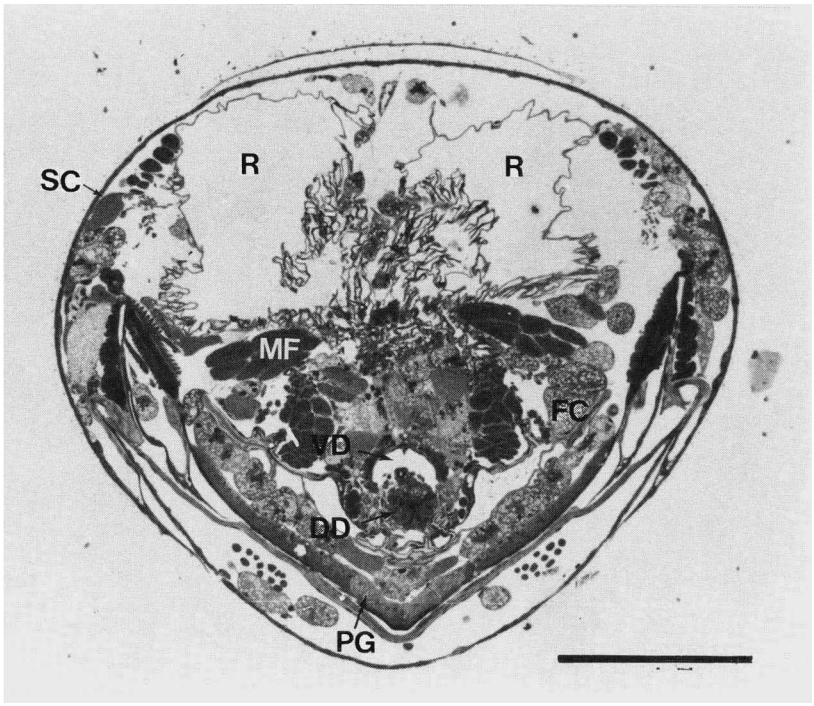


FIG. 2. Semi-thin cross section through the abdominal tip of an *Azteca* nr. *bicolor* worker, showing the large paired reservoirs (R) of the pygidial gland. DD: Dufour gland duct, FC: fat cells, MF: muscle fibers, SC: secretory cells pygidial gland, PG: Pavan's gland, VD: venom gland duct (scale bar 100 μm).

naldehyde, and an isomer of nepetalactol were identified by comparison of their mass spectra with those published in the literature. Actinidine (VIII) was also found to be present in the gland contents from three abdomens of *A. chartifex*, in which the amounts of iridodials were considerably reduced. As we have found that actinidine can be formed by heating iridodials with amino acids and we were in this case using whole abdomens for chromatography, these three specimens were omitted from consideration.

Although the iridodial isomers are common to both *A.* nr. *bicolor* and *A. chartifex*, the composition of the gland contents are species-specific. *Trans-trans*-iridodial (32%), and *cis-trans*-iridodial (21%) are the principal components in the gland of *A.* nr. *bicolor*. *Cis-trans*-iridodial (32%), and *trans-cis*-iridodial (27%) dominate the contents of the pygidial gland of *A. chartifex*. 6-Methyl-5-hepten-2-one and a small amount of isopulegol are also present in

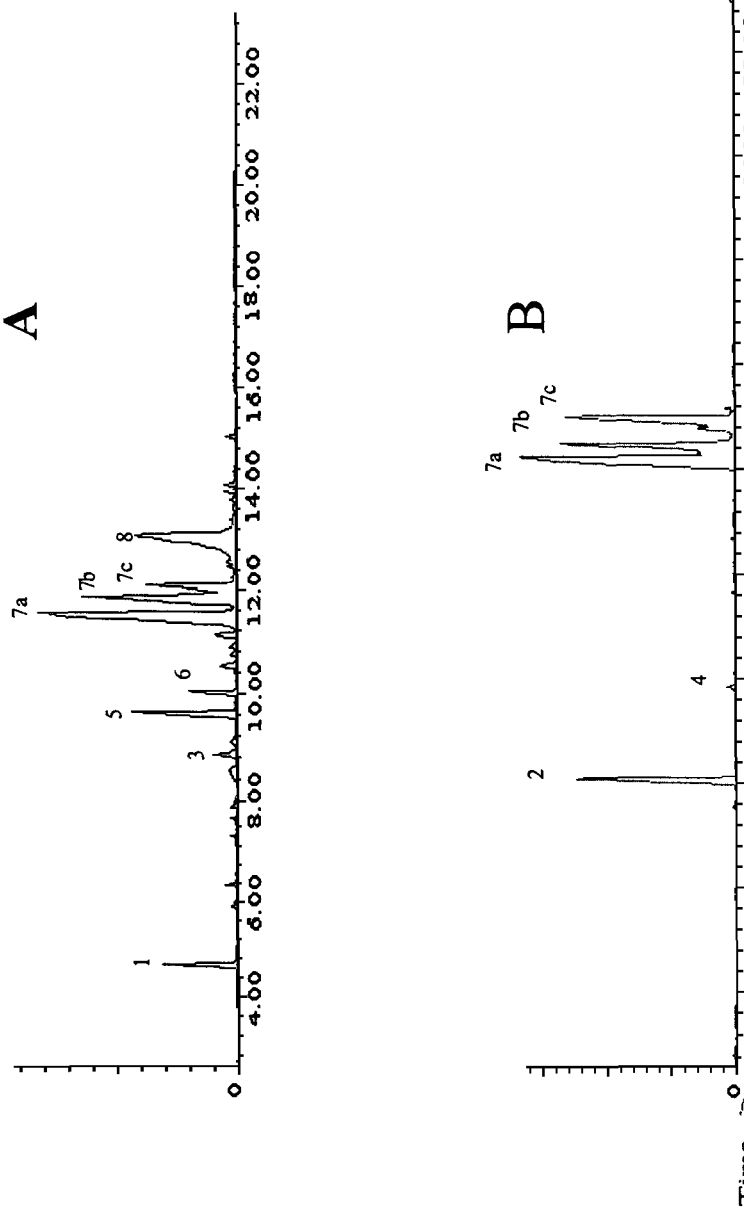


FIG. 3. Total ion chromatograms showing the volatiles from the pygidial gland of (A) *Azteca* nr. *bicolor* worker and (B) *A. chartifex* worker. The number above each peak corresponds to those compounds named in Table 1. Retention times were altered by use of two capillary columns with a similar stationary phase but different lengths between two sets of data.

TABLE 1. MEAN PERCENTAGE OF VOLATILES PRESENT IN PYGIDIAL GLANDS OF *Azteca* nr. *bicolor* ($N = 10$) AND *A. chartifex* ($N = 8$)

Peak number in Figure 3A	Peak number in Figure 3B	Compound	*A.* nr. *bicolor* (% ± SD)	*A. chartifex* (% ± SD)
		2-Acetyl-3-methylcyclopentene	0.2 ± 0.6	
1		2-Heptanone	7.5 ± 4.9	
	2	6-Methyl-5-hepten-2-one		13.1 ± 4.4
		2-Formyl-3-methylcyclopentane-acetaldehyde	trace[a]	
3		2-Formyl-3-methylcyclopentene-acetaldehyde	1.7 ± 2.9	0.2 ± 0.4
		2-Methyl-1-cyclopentene-carboxaldehyde		0.2 ± 0.7
		2-(3-methylcyclopentyl)propion-aldehyde	0.9 ± 1.2	
	4	Isopulegol		0.3 ± 0.4
5		Unknown 1	0.3 ± 0.8	
		Unknown 2	0.3 ± 0.6	
6		Unknown 3	8.4 ± 4.3	
		Unknown 4	1.5 ± 2.0	
7a	7a	*cis-trans*-Iridodial	21.4 ± 11.8	31.8 ± 13.0
7b	7b	*trans-trans*-Iridodial	31.7 ± 10.0	27.1 ± 10.6
7c	7c	*trans-cis*-Iridodial	15.0 ± 9.2	26.6 ± 10.4
		Isomer of nepetalactol	4.2 ± 3.5	trace
8		Unknown 5	6.8 ± 6.7	

[a]Less than 0.1%.

A. chartifex. The glands from *A.* nr. *bicolor* do not contain isopulegol; however, 2-heptanone and four unidentified compounds were present as minor components. The mass spectra of these unidentified compounds show a *m/z* 43 ion as a base peak, which suggests that they are methyl ketones; however, the mass spectra were otherwise too featureless to be able to identify these compounds.

Volatile ketones from the pygidial gland of *A.* nr. *velox* and *A.* nr. *nigriventris* species (Wheeler et al., 1975) produced typical alarm behavior in both species. Nonetheless, in contrast with Wheeler et al. (1975), we found with live colonies of *A.* nr. *bicolor* that few workers were attracted to the filter paper containing diluted or concentrated solutions of pygidial gland contents and synthetic 2-heptanone. Moreover, the attracted ants did not show any sign of alarm behavior.

DISCUSSION

Iridoids and ketones in the pygidial glands of dolichoderine ants are characteristic of this subfamily. The most common iridoids encountered are iridodial and isodihydronepetalactone (IV) (Attygalle and Morgan, 1984). These compounds are thought to be derived from (*S*)-citronellal (VII). 2-Heptanone, 2-methyl-4-heptanone, and 6-methyl-5-hepten-2-one are also common pygidial gland components.

Besides their occurrence in the pygidial glands of dolichoderine ants, iridoids are also present in plants and some other insects. In plants, they function either as feeding or olfactory attractants and in insects they serve as defensive compounds (Harborne, 1993). On the other hand, the ketones, especially 2-heptanone and 6-methyl-5-hepten-2-one, were found to function as very effective allomones for cockroaches in the genera *Palyzosteria* and *Neostylopiga* (Wallbank Waterhouse, 1970; Trave and Pavan, 1956), as well as for beetles in the genus *Dyschirius* (Moore and Brown, 1979).

Although the functions of the compounds from the pygidial gland of *A.* nr. *bicolor* and *A. chartifex* remain unknown, we suggest that, like cockroaches and beetles, the ants may use some or all of them as allomones against herbivores.

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