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Volatile components from the mandibular glands of the turtle ants, *Cephalotes alfaroi* and *Cephalotes cristatus*

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A B S T R A C T

GC–MS analysis of whole head extracts from the turtle ants, *Cephalotes alfaroi* and *Cephalotes cristatus*, showed that 4-heptanone and 4-heptanol were the major volatile components in the mandibular glands. 4-Heptanone and 4-heptanol have rarely been identified in mandibular gland secretions from other ant genera. Thus, these compounds may be chemotaxonomic markers for the genus *Cephalotes*, since they have been identified in the mandibular glands from all members of this genus that have been investigated to date. Minor components identified in the whole head extracts of these ants were 3-methyl-1-butanol, 3-heptanone, 3-hexanol, 2- and 3-methylbutanoic acids, 2-methyl-4-heptanone, 2-phenylethanol and phenol. To our knowledge, this is the first time that 2-methyl-4-heptanone and phenol have been reported in the mandibular gland secretion from any Formicid.

1. Introduction

Ant mandibular gland secretions were among the first semiochemicals to be identified. Over the years, many different chemicals have been documented from these glands (Ali et al., 1987; Attygalle and Morgan, 1984; Bellas and Hölldobler, 1985; Bestmann et al., 1988; Blum, 1969, 1981; Blum and Hermann, 1978a,b; Bradshaw et al., 1979: Cammaerts et al., 1983, 1987; do Nascimento et al., 1993; Hölldobler and Wilson, 1990; Hughes et al., 2001; Jackson and Morgan, 1993; Jones et al., 2004; Keegans et al., 1991, 1992; Morgan et al., 1999, 2006, 2008; Oldham and Morgan, 1993; Oldham et al., 1994; Olubajo et al., 1980; Parry and Morgan, 1979; Voegtle et al., 2008; Wood, 2005; Wood and Chong, 1975; Wood et al., 2002, 2006). Ketones and their corresponding alcohols are reported as the major components in many of these secretions. In almost all cases, the carbonyl or corresponding hydroxy group of these compounds is either at the 2- or 3-position of the carbon chains. There are few reports of ketones and alcohols at the 4-position of the carbon chains from ant mandibular gland secretions.

4-Heptanone and 4-heptanol were identified in the mandibular gland extracts of the turtle ants (Myrmicinae), *Cephalotes varians* and *Cephalotes pusillus* (Olubajo et al., 1980; Morgan et al., 2006). The only other ant species reported to contain both 4-heptanone and 4-heptanol in its mandibular glands is the unrelated African Formicine, *Polyrhachis schistacea* (Brand and Lindner, 1989). Finally, 4-heptanone along with 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol has been reported from the mandibular glands of the weaver ant (Formicinae) *Polyrhachis simplex* (Hefetz and Lloyd, 1982).

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Ants of the genus *Cephalotes* are large, attractive, heavily armoured and arboreal residents of the high rain forest canopies in the Neotropics. Because of the inaccessible locations that many members of this genus inhabit, it is not surprising that only two previously noted members of the genus *Cephalotes* have been investigated to date (Olubajo et al., 1980; Morgan et al., 2006). We examined whole head extracts from two additional members of this genus, *Cephalotes alfaroi* and *Cephalotes cristatus*. Like the previous investigations on members of this genus, we found that the major components in the mandibular gland secretions of *C. alfaroi* and *C. cristatus* were 4-heptanone and 4-heptanol. Unlike previous reports on *Cephalotes*' mandibular glands, we report minor compounds in the whole head extracts from *C. alfaroi* and *C. cristatus*.

2. Materials and methods

Workers of the arboreal turtle ants, *C. alfaroi* and *C. cristatus* were collected at La Selva Biological Station, Heredia Province, Costa Rica (10° 25′ 54″ N, 84° 00′ 13″ W) and were identified by TM using online taxonomic resources (Longino, 2009). *C. alfaroi* workers were individually collected from trees, placed in resealable plastic bags, and then put in a freezer for over 1 h to immobilise the ants. The heads from 25 worker ants were excised and placed in glass vials with Teflon-lined stoppers containing 0.5 mL of dichloromethane (CH_2Cl_2), which was stored at −20 °C for later analysis. Likewise, two additional collections of 25 *C. alfaroi* worker ant heads were made (the three collections were analyzed separately and the results averaged.) Finally, a single collection of 30 *C. cristatus* worker ant heads was made using the same procedure.The CH_2Cl_2 from these extractions was analyzed without any further manipulation. Decapitation and storage of ant heads in solvent is a standard method for the extraction of the mandibular gland secretions of ants when they cannot be analyzed immediately (Wood, 2005; Wood et al., 2002, 2006). A recent study showed no difference between whole head extracts and pure mandibular secretions of the congeneric *C. pusillus* (Morgan et al., 2006).

GC–MS analyses were performed on the CH_2Cl_2 extracts in a splitless mode (0.5 min), using a Hewlett–Packard GCD Plus fitted with a 30 m × 0.25 μm cross-linked phenyl methyl silicone capillary column (HP-5MS). The gas chromatograph was programmed so the oven temperature was kept at 40 °C for 4 min, then increased to a final temperature of 325 °C at a rate of 30 °C/min and held at this temperature for 5 min. Impurities in control solvent samples and sample constituents less than 0.1% of the total ion current (TIC) were not reported. Initial identification of secretion components was done by comparison to published electron impact - mass spectra (EI-MS) in the National Institute of Standards and Technology (NIST) 1998 computerised mass spectral library, and confirmed by comparison of spectra and retention times to those of commercially available standards, except 2-methyl-4-heptanone that was prepared synthetically (de Jong and Feringa, 1991).

3. Results

The major compounds in the mandibular glands of *C. alfaroi* and *C. cristatus* were 4-heptanone and 4-heptanol (Table 1). Previous reports of 4-heptanone and 4-heptanol in mandibular glands of *C. varians* and *C. pusillus* did not identify any additional components (Olubajo et al., 1980; Morgan et al., 2006). In our study, we found 3-methyl-1-butanol, 3-heptanone, 3-hexanol, 2- and 3-methylbutanoic acids, 2-methyl-4-heptanone, phenol, and 2-phenylethanol as minor components in the whole head extracts (Table 1).

4. Discussion and conclusion

Ant mandibular gland secretions often function as alarm pheromones, as they typically elicit aggressive behaviour. Ants of the genus *Cephalotes* are generally unaggressive and frequently use a passive defence that includes stopping and lying flat on the surface when molested. Several studies have reported on the defencive behaviour of *C. varians,* a species that ranges from the Florida Keys to the Bahamas, Cuba and Jamaica. Wilson (1976) reported an ethogram, a complete catalogue of the behavioural patterns of this species. Another study describes the behaviour of *C. varians* workers in response to mandibular

Table 1
Volatile compounds in *Cephalotes* mandibular glands as % of the total ion current (TIC).

Compound	Retention time (min)	*C. alfaroi*[a]	*C. cristatus*[b]
3-methyl-1-butanol	3.45	–	0.7
3-Hexanone	4.54	1.5	0.1
3-Hexanol	4.70	1.5	0.1
3-Methylbutanoic acid	5.69	0.4	–
2-Methylbutanoic acid	5.74	0.4	–
4-Heptanone	5.86	87.4	49.3
4-Heptanol	5.99	6.3	46.9
2-Methyl-4-heptanone	6.34	–	0.3
Phenol	6.86	2.6	1.9
2-Phenylethanol	7.97	–	0.7

[a] Average of 3 replicate analyses of pooled extracts from 25 workers.
[b] Single sample analysis of pooled extracts from 30 worker.

gland chemicals (Olubajo et al., 1980). When worker ants were exposed either to crushed ant heads, 4-heptanone, 4-heptanol, or a mixture of these chemicals, a non-aggressive response was elicited. The ants were reported to stop moving and back up when exposed to these chemical cues. 4-Heptanone has been tested for alarm behaviour with *Pogonomyrmex badius* (Blum et al., 1971) and *Atta texana* (Moser et al., 1968), ants that utilise 4-methyl-3-heptanone as an alarm pheromone. In both cases, 4-heptanone was weakly active and only elicited alarm behaviour at high concentrations. Thus, it is possible that ant species, which use alarm pheromones with a ketone functional group in the 2- or 3-position, are unable to detect chemical cues from *Cephalotes* spp.

A major difference between 4-heptanol and mandibular gland alcohols with the hydroxy group in the 2- or 3-position, is that it is achiral. Mandibular gland alcohols with the hydroxy group in the 2- or 3-position are chiral, and it has been shown that ants produce enantiomerically pure compounds. As an example, *Crematogaster castanea* and *Crematogaster liengmei* make only (*S*)-(+)-3-octanol (Brand, 1985) and (*S*)-2-methyl-1-hexanol is the only isomer produced by *Cataglyphis bicolor* (Agosti et al., 1996).The lack of chirality may be important to the semiochemistry of 4-heptanol.

The minor compounds, except phenol and 2-methyl-4-heptanone, have frequently been reported in ant mandibular gland secretions. 2-Methyl-4-heptanone has been reported from the anal (pygidial) glands of *Tapinoma simrothi* (Hefetz and Lloyd, 1983) and *Tapinoma nigerrimum* (Trave and Pavan, 1956). Phenol has been reported from the Dufour's gland of several *Myrmecocystus* spp. (Lloyd et al., 1989) and the poison gland of *Camponotus socius* (Kohl et al., 2001). To our knowledge, this is the first time that 2-methyl-4-heptanone and phenol have been reported in the mandibular gland secretion from any Formicid.

Finally, if it is found that other members of genus *Cephalotes* also have high concentrations of 4-heptanone and 4-heptanol in their mandibular gland secretions, these compounds may be chemotaxonomic markers for the genus.

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