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Mandibular gland chemistry of four Caribbean species of *Camponotus* (Hymenoptera: Formicidae)

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Abstract

The volatile components of whole-body extracts of males, females and workers were analyzed in four species of Neotropical ants in the formicine genus, *Camponotus*. The species, *C. kaura*, *C. sexguttatus*, *C. ramulorum* and *C. planatus*, represent three different subgenera. Volatile mandibular gland components were found only in male extracts in three of the species. In *C. ramulorum*, volatile components were found in male and female reproductives and workers. 3,4-Dihydro-3,5-dihydroxy-6-methylpyran-4-one and octanic acid were found in different sets of three of the species. Methyl 6-methyl salicylate was found in two species and the isocoumarin, mellein, was found in a third species. The significance of the mandibular gland secretion for formicid systematics is discussed. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Mandibular gland; Formicidae; *Camponotus* species; Carpenter ant; Ant

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1. Introduction

The number of components found in mandibular gland secretions of ants is much fewer than the number of components found in Dufour's glands. In general, the components of mandibular glands are relatively constant within a genus or subgenus. For example, mandibular gland secretions of *Pogonomyrmex* species contain 4-methyl-3-heptanone and the corresponding alcohol (McGurk et al., 1966). In the *pallidefulva* group of *Formica* (Formicinae), mandibular gland secretions are dominated by 6-methyl-5-hepten-2-one (Duffield et al., 1977); in *Odontomachus* (Ponerinae) species, the secretions contain mixtures of dimethyl alkyl pyrazines (Wheeler and Blum, 1973); in Nearctic species belonging to the genus *Crematogaster* (Myrmicinae), the secretions are dominated by 3-octanone (Crewe et al., 1972).

In contrast to these genera, the composition of the mandibular gland secretions of *Camponotus* species exhibit qualitative differences in the volatile components among species. Brand et al. (1973a,b) demonstrated caste-specific mandibular gland secretions in five species of Nearctic *Camponotus*. Secretions contained methyl 6-methyl salicylate, 2,4-dimethyl-2-hexanoic acid, methyl anthranilate, mellein and 10-methyl dodecanoic acid. An unknown component was later identified as E-6-(1-pentenyl)-2H-pyran-2-one, which had also been found in *Solenopsis wagneri* queens (Jones and Fales, 1983), as *S. invicta*. Initially, it appeared that mandibular gland secretions were restricted only to the males in *Camponotus*. Subsequent to the initial reports of Brand et al. (1973a), Duffield and Blum (1975a,b) demonstrated 3-octanone and 3-octanol in the mandibular gland secretions of both workers and reproductives of *Camponotus schaefferi* (Whr.). Lloyd et al. (1975) identified 10 volatile components in the male mandibular gland secretions of the Californian *Camponotus clarithorax* Emery. This species of carpenter ant contained the greatest number of volatile mandibular gland components in any species of *Camponotus* investigated to date.

Duffield (1976), studying the chemistry of the volatile mandibular gland secretions of 30 species of *Camponotus*, demonstrated that some species exhibited volatile components in the mandibular gland secretions of male and female reproductives as well as workers. In other species, these secretions were restricted to males only. In many of the species, the composition of the mandibular gland secretions paralleled their subgeneric groupings. In other species, such as *C. clarithorax*, the secretions were found to be unique.

Since these earlier investigations, there have been additional papers published on the volatile mandibular gland chemistry of *Camponotus* including Blum et al. (1987, 1988) and Duffield et al. (1988).

It is now clear that *Camponotus* species exhibit considerable variation in the chemical composition of their mandibular gland secretions. Differences exist not only among species but also between males, females and workers. This report adds to our understanding of the chemistry of the volatile mandibular gland components of four Neotropical species of *Camponotus*.

2. Materials and methods

2.1. Collection of data

Camponotus kaura Snell. and Torres were collected from Guaynabo, Puerto Rico, May 21–25, 1995. Colonies of *C. ramulorum* Whr. were collected at Playa Jibacoa, North Havana Province, Cuba, June 6, 1997 and of *C. sexguttatus* (Fabr.) from Guaynabo, Puerto Rico, December 15, 1994. *Camponotus planatus* Roger was collected 3 km west of Santa Cruz al Norte (Cuba) June 7, 1997.

Data from a second collection is based on material collected from mangrove trees in Key Largo, FL USA, summer 1973. Live colonies were maintained in the laboratory in modified wooden “trap nests” (Krombein, 1967) until male and female reproductives were produced. Individuals were then sacrificed for chemical analyses. Heads were removed individually using two pairs of forceps. Pooled head extracts were prepared for male, female reproductives and workers of each species.

2.2. Chemical analysis

Spectral-grade methylene chloride or methanol was used to extract the heads. The solvent was drawn off each extract and dried with sodium sulfate. Each sample was concentrated by room evaporation and analyzed on a Shimadzu QP-5000 gas chromatograph-mass spectrometer equipped with a 30 m × 0.25, RTX-5 column temperature programmed from 60 to 250°C at 10°/min. Compounds were identified by comparison of their mass spectra with a data base of spectra (NIST/EPA/NIH Mass Spectral Database: version 4.0; National Institutes of Standards and Technology, Gaithersburg, MD, 1992) and with those reported in the literature.

2.3. Voucher specimens

Representative material of the four species collected in Cuba and Puerto Rico is deposited in the entomology collections at the Natural History Museum of Los Angeles County. Accession numbers for this material are: *C. kaura* (95-V-25-[03–05]); *C. ramulorum* (97-VI-06-[01–06]); *C. sexguttatus* (94-XII-15-12) and *C. planatus* (97-VI-15-02). A series of *Camponotus planatus* collected in Florida is deposited in the entomology collections at the Natural History Museum, University of Georgia, Athens, GA, with the accession number 73-IV-15-02 (Snelling and Torres, 1998).

3. Results

3.1. Male samples

Compound [2] was detected in the male head extracts of three of the four species investigated (Table 1). The mass spectra of compound [2] gave a molecular ion and a

Table 1

Volatile components in male mandibular gland secretions of four Caribbean species of *Camponotus* ants^a

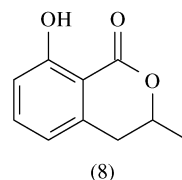
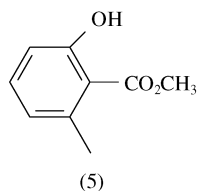
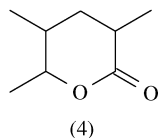
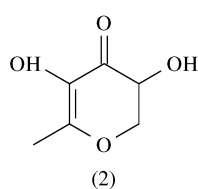
Species/volatile compounds	1	2	3	4	5	6	7	8	9
<i>C. kaura</i>	–	+	tr	+	+	–	–	–	–
<i>C. Sexguttatus</i>	–	–	+	–	–	+	+	–	–
<i>C. ramulorum</i>	+	tr	–	–	–	–	+	tr	+
<i>C. planatus</i> (Cuba)	–	tr	+	–	+	–	–	–	–
<i>C. planatus</i> (USA)	–		+		+				

^a + = present, – = absent, tr = trace; volatile compounds: 1 = methyl ester, 2 = 3,4-dihydro-3,5-dihydroxy-6-methylpyran-4-one, 3 = octanoic acid, 4 = 2,4-dimethyl-5-hexanolide, 5 = methyl 6-methyl salicylate, 6 = unknown, 7 = unknown, 8 = mellein, 9 = dodecanol.

base peak at m/z 144 and major ions at m/z 115 (4), 101 (52), 73 (24), 72 (27), 44 (63), and 43 (99). The mass spectrum was identical to that reported for 3,4-dihydro-3,5-dihydroxy-6-methylpyran-4-one (Haak et al., 1996).

Methyl 6-methyl salicylate [5] (methyl 2-hydroxy-6-methyl benzoate) was detected in male head extracts of two species, *C. kaura* and *C. planatus* (Table 1) and gave a well-defined mass spectrum. The ester exhibits a molecular ion at m/z 166 with strong signals at m/z 134 (loss of $-\text{CH}_3\text{OH}$), 106 (loss of $-\text{HCOOCH}_3$), 78 and 77 (aromatic ring). The mass spectrum was congruent with those from an authentic sample methyl 6-methyl salicylate.

A trace of the isocoumarin, mellein (3,4-dihydro-8-hydroxy-3-methylisocoumarin) [8], was found in the male head extracts of *C. ramulorum* (Bestmann, 1992). The mass spectrum is quite characteristic and exhibits a molecular ion at m/z 178 and major fragments at m/z 160, 134, 78, 77. The mass spectrum was identical to that of an authentic sample of mellein.



Male head extracts of three of the species contained the same compound. The mass spectrum exhibited a small molecular ion at m/e 144. Large fragments at m/e 60 and m/e 73 ($(-\text{CCH}_2)_2 \text{COOH}$) indicated that this compound was an aliphatic acid. Direct comparison showed that this compound had a gas chromatographic retention time and mass spectrum indistinguishable from *n*-octanoic acid [3]. The methyl ester of this acid was also detected, and was most likely an artifact formed from the solvent in which the ant heads were extracted.

In *C. kaura*, a compound whose mass spectrum was identical to that reported for 2,4-dimethyl-5-hexanolide [4] was detected (Bestmann et al., 1995), and traces of it were found in the other species. One sample of the males of *C. sexguttatus* contained two unidentifiable compounds, which appeared as trace components in the other extracts. The first [6] had a mass spectrum characterized by intense ions at

$m/z = 68(100)$, and $97(90)$ with no discernable parent ion. The second [7] had a mass spectrum: $m/z = 183(1)$, $182(1)$, $164(1)$, $111(80)$, $83(40)$, and $82(100)$. Because of the small amounts of material and the complexity of the mixture, further attempts at identification of these compounds were unsuccessful.

Head extracts of female reproductives and workers of *C. kaura*, *C. sexguttatus* and *C. planatus* contained only the characteristic structural lipids including the long-chained fatty acids (palmitic, stearic and oleic acids) and odd numbered *n*-alkanes (C_9 – C_{17}). In addition to these, head extracts of worker and female reproductives of *C. ramulorum* showed three additional compounds. These compounds were identified based on comparison of their mass spectrum to that of authentic standards as dodecanol, and decyl and dodecyl acetates.

4. Discussion

Unlike many other genera of ants in which the chemistry of the mandibular gland secretions of both workers and reproductives have been analyzed, several genera belonging to the Formicinae have been shown to exhibit male-specific mandibular gland secretions. These include *Acanthomyops* and *Lasius* (Law et al., 1965), *Camponotus* (Brand et al., 1973a, b; Duffield, 1976) and *Oecophylla* (Bradshaw et al., 1979). Compared to *Lasius*, *Camponotus* is one of the largest genera of ants in the world with over 1000 described species (Bolton, 1995a, b).

Many *Camponotus* species have well-developed mandibular glands (Blum et al., 1988). They are unique compared to other species of ants because many of the species exhibit volatile, multi-component mandibular gland secretions found in the males and absent in female reproductives and workers (Duffield, 1976).

Methyl 6-methyl salicylate appears to be a relatively common natural product of ant exocrine secretions. It has been documented in the male mandibular gland secretions of more than 20 species of *Camponotus* (Brand et al., 1973a, b; Duffield, 1976; Blum et al., 1987). It has also been reported in the mandibular gland exudates of the ponerine ant, *Gnamptogenys pleurodon* (Duffield and Blum, 1975a, b) and the worker mandibular gland secretions of *Polyergus* species from Georgia (Duffield, 1976).

More recently methyl 6-methyl salicylate has been identified as a major component of the trail pheromone of *Tetramorium impurum*. The trail pheromone in this species is released by the poison gland (Morgan et al., 1990).

The isocoumarin, mellein, is a well-known fungal metabolite of *Aspergillus* species. It was first isolated and identified in ants by Brand et al. (1973a, b) in male mandibular gland secretions of several *Camponotus* species. Although widely distributed in Nearctic *Camponotus* (Duffield, 1976), it is not common in other subfamilies of ants. Mellein has been identified in the Australian ponerine, *Rhytidoponera metallica* (Brophy et al., 1981).

In the last few years, studies of the chemistry of the rectal gland secretions in a number of formicine genera have not only documented the presence of mellein but have shown that it is one among a number of components present. It has been

identified as one of the rectal gland trail pheromone components in *Camponotus silvicola* and *C. rufipes* (Ubler et al., 1995). (R)-(–)-Mellein has been shown to be one of several components of the trail pheromone of *Lasius fuliginosus* (Kern et al., 1997).

In addition, mellein has been identified in soldier head extracts of the termite *Cornitermes* (Termitidae) (Blum et al., 1982); the anal secretions of the thrip, *Haplothrips leucanthemi* (Blum et al., 1992); and in the hairpencil secretions of male Oriental fruit moths (Baker et al., 1981; Nishida et al., 1982). It is also present in the male hairpencil secretions of the giant danaine butterfly, *Idea leuconoe* (Nishida et al., 1996) and in the wing glands of male bumblebee wax moths, *Aphomia sociella* (Kunesch et al., 1987).

Compound [4] has been identified in the rectal glands of *Camponotus herculeanus* where it functions as a component of the trail pheromone (Bestmann et al., 1995). This compound has been detected in the male mandibular gland extracts of *Calomyrmex* (Brown and Moore, 1979) and *Camponotus thoracicus fellah* (Lloyd et al., 1984). In this study, we did not determine whether this compound is a mandibular gland component or a rectal gland contaminant.

The male-specific mandibular gland secretions of *Camponotus herculeanus* appear to be utilized as a sex pheromone. Males leave the nest and take flight before the female reproductives. The male pheromone is released as the males initiate flight during the swarming process. Upon detecting the pheromone, the female reproductives exit the nest and also take flight.

When the mandibular glands are present in male and female reproductives as well as workers, the mandibular gland secretions appear to have a different role in the biology of *Camponotus*. In this case, the mandibular gland secretions function in alarm releasing behavior. In comparison to the male-specific secretions, the alarm releasing secretions are dominated by simple ketones and alcohols (Duffield and Blum, 1975a, b; Blum et al., 1982).

The species investigated in this report belong to three different *Camponotus* subgenera. *Camponotus kaura* and *C. ramulorum* belong to the subgenus *Tanaemyrmex* while *C. sexguttatus* belongs to *Myrmosphincta*. *Camponotus planatus* belongs to the subgenus *Myrmobrachys*. Nothing in the mandibular gland secretions makes them unique. Although the mandibular gland secretion of each species is different, all components have been reported from *Camponotus* previously.

The occurrence of compounds such as mellein or methyl 6-methyl salicylate in *Camponotus*, both as mandibular gland components in males and as rectal gland products in workers is unprecedented. The synthesis of the same volatile compound in two different exocrine glands in the same species poses many unanswered questions.

Comparing the chemistry of the mandibular gland components in *Camponotus* may be of limited systematic importance. While they may be very useful in separating several species, they may not contribute to differentiating among large assemblages of species. In some instances, comparing mandibular gland products may help identify some subgeneric groupings. For analysis of mandibular gland exocrine components to be of systematic value, the exocrine chemistry of

both the Dufour's gland and the rectal glands of each species may also have to be examined.

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