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# Mandibular Gland Chemistry of Two Nearctic Species of *Camponotus* (*Colobopsis*) (Hymenoptera: Formicidae)

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*Abstract.*—The chemistry of the mandibular gland secretions of three Nearctic species of carpenter ants of the subgenus *Colobopsis* was studied. No volatile compound was detected in worker mandibular secretions of *C. impressus* and *C. etiolates*. Worker secretions of *C. mississippiensis* were dominated by 2,6-dimethyl-5-heptene-1-ol and citronellol. Male head extracts of *C. impressus* and *C. mississippiensis* exhibited these two compounds and an additional volatile which was identified as mellein. Citronellol constituted 50% of the volatile components in each of these species.

*Camponotus* is a cosmopolitan genus of formicine ants. It is reported to have more than 1000 species worldwide split among several dozen subgenera (Bolton 1995a, 1995b). *Camponotus* species have diverse morphological adaptations as well as many unusual behavioral patterns. In North America north of Mexico there are approximately 50 species of *Camponotus* representing seven subgenera (Creighton 1950). Unique among the North American *Camponotus* are those that belong to the subgenus, *Colobopsis*. Major workers exhibit phragmosis: *i.e.* they insert their cylindrical heads into the opening of the nest entrance and act as living plugs. These diminutive species are arboreal, living in hollow stems and twigs.

*Colobopsis* was originally described as a genus separate from *Camponotus* (Mayr 1861). It was reclassified as a subgenus under *Camponotus* by Emery (1889) and has been more or less consistently so treated since then (Bolton 1995b). Subgenus *Colobopsis* is primarily Holarctic and mostly associated with northern hardwood forests. While numerous Southeast Asian and Melanesian species are currently placed in *Colobopsis*, they are improperly placed (R.Snelling, personal communication)

*Camponotus* mandibular gland secretions have been the focus of a number of chemical investigations. They exhibit a great diversity of chemical components. These investigations include those of Brand *et al.* (1973a, 1973b), Duffield and Blum (1975b), Lloyd et al. (1975), Duffield (1976), Jones and Fales (1983), Blum et al. (1987), Blum et al. (1988), Duffield et al. (1988), and Torres et al. (2001).

The isocoumarin, mellein, is a fungal metabolite found in *Aspergillus* species. Brand et al. (1973a, 1973b) were the first to identify mellein in ants. Since its initial identification in male mandibular gland secretions of *Camponotus*, mellein has been shown to be widely distributed in *Camponotus* (Duffield 1976). It has also been identified in the mandibular glands of *Polyrhachis doddi* Donisthorpe (Bellas and

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Holldobler 1985) (Formicinae) and in *Rhytidoponera metallica* (Smith) (Ectatomminae) (Brophy et al. 1981). Mellein is a component of the trail pheromone of *Lasius fuliginosus* (Latreille) (Formicinae) (Kern et al. 1997) and in the rectal gland secretions of several species of *Camponotus* (Ubler et al. 1995). In contrast, mellein has also been identified in the anal secretions of a thrips (Blum et al., 1992); termites (Blum et al. 1982) as well as the hair pencil secretions of the danaine butterfly, *Idea leuconoe* (Erichson) (Nishida et al. 1996). Male bumblebee and wax moth secretions are also fortified with mellein (Kunesch et al. 1987). This abbreviated summary documents the rather widespread occurrence of mellein among different orders of insects.

The three terpenoids, citronellol, citronellic acid and 2,6-dimethyl-5-heptene-1-ol are also common ant mandibular gland secretions (Wheeler and Duffield 1988). 2,6-Dimethyl-5-heptene-1-ol has been previously identified in made mandibular gland secretions of *C. clarithorax* along with citronellic acid (Lloyd et al. 1975). 2,6-Dimethyl-5-heptene-1-ol has also been identified as the major constituent in the male mandibular gland secretions of *Lasius* (as *Acanthomyops*) *clavigerus* (Roger) (Regnier and Wilson 1968) and *L. umbratus* (Nylander) (Regnier and Wilson 1969).

We report the mandibular secretions of three species of *Colobopsis*.

## MATERIALS AND METHODS

Collections of colonies of three species of *Colobopsis* were made for chemical analysis. Workers of *Camponotus etiolatus* Wheeler were collected from Live Oak and Uvalde Counties, Texas, by the senior author and the late myrmecologist, Dr. William Steel Creighton in January, 1973. *Camponotus impressus* (Roger) workers were collected from the vicinity of Paurotis Pond, Everglades National Park, Dade County, Florida (March, 1974). *Camponotus mississippiensis* Smith was collected along Whitehall Road, Clark County, Georgia and from the Oconee National Forest, Georgia during November–December, 1973. Voucher specimens were deposited in the entomology collections at the Georgia Natural History Museum, University of Georgia, Athens, Georgia, USA.

Before excising the ant heads, each colony was cooled at 4°C for several hours. Ant heads were removed with forceps and placed in spectral grade methylene chloride for 24 hours. Separate extracts were made of minor workers, major workers and male heads for *C. impressus* and *C. mississippiensis*. Only minor worker head extracts were obtained for *C. etiolatus*. Male head extracts consisted of 20–30 heads. Minor worker extracts consisted of several hundred heads and major worker head extracts consisted of approximately 200 heads, depending upon the numbers available. The solvent for each extract was drawn off and dried with sodium sulfate. Each sample was concentrated by room evaporation and analyzed by gas chromatography-mass spectroscopy.

Worker mandibular glands of *C. mississippiensis* were excised using a dissecting microscope and extracted with methylene chloride. Extracts were analyzed on a gas chromatography.

The concentrated samples were analyzed on a LKB 9000 combined gas chromatograph-mass spectrometer (GC-MS) using10% SP-1000 as the stationary phase. The column was temperature programmed at 10°C/min. to 200°C. Mass spectra and retention times of mellein, citronellol and 2,6-dimethy-5-hepten-1-ol were consistent with those of authentic standards.

## RESULTS

Compound number 1 (Table 1) showed a molecular ion at m/e 142, and ions at m/e 124, 109, 95, 82, 69, 67, 55 and 41 suggesting it was an unsaturated, terpenoid alcohol. An authentic sample of 2,6-dimethyl-5-heptene-1-ol had a retention time and mass spectrum identical to those of the unknown. The second compound

Table 1. Volatile components in the mandibular gland secretions of three species of *Camponotus* subgenus *Colobopsis*.

| Species/Volatile compounds | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| *C. etiolates* (minor workers) | – | – | – | – | – |
| *C. impressus* (minor workers) | – | – | – | – | – |
| *C. impressus* (males) | + | +[a] | + | – | + |
| *C. mississippiensis* (minor workers) | + | +[a] | – | + | – |
| *C. mississippiensis* (males) | + | + | + | – | – |

Compound 1. 2,6-dimethyl-5-heptene-1-ol; Compound 2. citronellol; Compound 3. mellein; Compound 4. citronellic acid; Compound 5 Unknown M.W. 154.
a = 50% of the volatile components.

gave a molecular ion at m/e 156 and strong ions at 41 and 69 suggesting an acyclic terpene. Ions were observed at 138, 123, 109, 95, 82, 81, 69, 67, 56, 55, and 41. An authentic sample of citronellol gave identical retention times and mass spectra as the unknown.

The third compound exhibited a molecular ion at 178 and ions at m/e 160, 149, 134, 132, 111, 106, 105, 104, 79, 77, 53, 52, 51, 43 and 41. The compound was identified as mellein. Compound 4 had a molecular ion at m/e 170 and fragment ions at m/e 41 and 69 indicating an acylic terpene. An authentic sample of citronellic acid had a retention time and mass spectrum identical to those of the unknown.

The results of the chemical analyses of the three species of *Colobopsis* are presented in Table 1. No detectable volatiles were found in the head extracts of *C. etiolatus*. This may have been due to the limited number of heads extracted. While no volatile compounds were detected in the minor worker head extracts of *C. impressus*, male head extracts contained mellein, citronellol and 2,5-dimethy-5-hepten-1-ol. It is surprising that no volatiles were detected in the worker extracts. The worker head extracts contained many more heads compared to the male head extracts.

Chemical analyses of *C. mississippiensis* minor workers and males exhibited two volatiles in common, citronellol and 2,6-dimethy-5-hepten-1-ol. Each extract also contained an additional volatile. Workers contained citronellic acid and males contained mellein.

The gas chromatogram of the excised mandibular gland extracts of *C. mississippiensis* workers exhibited two volatile compounds whose retention times matched those of authentic citronellol and 2,5-dimethy-5-hepten-1-ol. We concluded that the volatile compounds in the head extracts were mandibular gland products.

## DISCUSSION

Formicine genera of ants are unlike many other genera of ants where worker males and females exhibit the same volatile mandibular gland compounds, and in which species in the same genus often exhibit the same mandibular gland components. Several formicine genera have been shown to exhibit male-specific mandibular gland components. These include *Lasius* (Law et al. 1965) *Camponotus* (Brand et al. 1973a, b) and *Oecophylla* (Bradshaw et al. 1979), all in the subfamily Formicinae.

*Camponotus* is an ideal genus to study from a chemo-systematic standpoint. In some species males have multi-component mandibular gland secretions absent in workers and female reproductives. Other species exhibit the same components in males, female reproductives and workers (Duffield 1976). In this investigation, *C. mississippiensis* males and workers both have mandibular gland secretions that contain volatile compounds. While they

share two compounds, each has one distinctive compound.

Based on the volatile mandibular gland secretions of the two *Colobopsis* species, they form a group separate from other North American *Camponotus*. They are similar to other *Camponotus* in that they have a male mandibular gland secretion that contains mellein. The *Colobopsis* species in one sense are similar to the male mandibular gland extracts of *C. clarithorax* Creighton which are also fortified with citronellol, and 2,6-dimethyl-5-heptene-1-ol. *Camponotus clarithorax* is contrastingly different in that it exhibits a number of additional compounds and no mellein.

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