

## Methyl 6-Methyl Salicylate: Identification and Function in a Ponerine Ant (*Gnamptogenys pleurodon*)

Results obtained from the few investigations of ant species in the primitive subfamily Ponerinae indicate that these insects possess a rich natural products potential. Alkyl sulfides are known as arthropod exocrine products only because of their occurrence in the mandibular gland exudate of the ponerine *Paltothyreus tarsatus*<sup>1</sup>. More recently the first pyrazines identified in animals have been demonstrated to be mandibular gland products of several species of *Odontomachus*<sup>2</sup>. The identification of 4-methyl-3-heptanone in a glandular exudate of *Neoponera villosa*<sup>3</sup> offers further testimony to the biosynthetic versatility of members of the Ponerinae. The purpose of the present note is to report on the first aromatic compound encountered as an exocrine product of ponerine species. The mandibular gland secretion of *Gnamptogenys pleurodon*<sup>4</sup> is fortified with methyl 6-methyl salicylate which functions as a powerful releaser of alarm.

**Materials and methods.** Colonies of *G. pleurodon* were collected near Manaus, Brazil. Extracts of worker heads were prepared in spectrograde *n*-pentane. Volatile compounds were detected by gas chromatographic analyses on a Tracor MT-220 instrument using Carbowax 20 M as a stationary phase at 180°C. Additional analyses were obtained on a LKB-9000 gas chromatograph-mass spectrometer (GC-MS) utilizing 10% SP-1000 as the stationary phase at 200°C.

Behavioral tests were conducted by exposing ants in both field and laboratory colonies to either crushed worker heads or pure compounds and observing the subsequent reactions of the workers.

**Results.** Gas chromatographic analyses showed that a single compound constituted more than 95% of the observed volatiles. GC-MS analyses demonstrated that this compound exhibited a molecular ion (M) at *m/e* 166 and appeared to be aromatic (*m/e* 77 and 78). The presence of a COOCH<sub>3</sub> group (loss of CH<sub>3</sub>OH and HCOOCH<sub>3</sub> from *m/e* 166) and a phenolic hydroxyl (conversion to *O*-acetate M<sup>+</sup> 208) indicated that this compound was methyl-substituted methyl salicylate. Both the mass spectrum and retention time of methyl 6-methyl salicylate were completely congruent<sup>5</sup> with those of the *G. pleurodon* volatile whereas the retention times of methyl 3-methyl salicylate, methyl 4-methyl salicylate and methyl 5-methyl salicylate were distinctly different.

Exposure of workers to either a crushed head or methyl 6-methyl salicylate released alarm behavior. Excited workers, their mandibles spread, moved rapidly to the pheromonal emission source which they often attempted to bite or sting.

GC-MS analyses of excised mandibular glands demonstrated that these structures were the source of the pheromone.

**Discussion.** Methyl 6-methyl salicylate has been previously identified as a mandibular gland product of species in the formicine genus *Camponotus*<sup>5,6</sup>. However, this compound is only produced by males and presumably functions to coordinate the swarming behavior of the sexes<sup>7</sup>. Its occurrence and role as an alarm pheromone in *G. pleurodon* and a swarming pheromone in *Camponotus* species emphasizes that species in completely unrelated taxa can utilize the same compound for totally different functions.

At this juncture the Ponerinae must be regarded as an especially rich source of diverse pheromones. Different chemical classes of pheromones have been identified as mandibular gland products in ponerine genus which has been analyzed, and in three of these cases the compounds

are also synthesized by species in the more highly evolved subfamilies. 4-Methyl-3-heptanone, an alarm pheromone identified in *Neoponera villosa*<sup>3</sup>, is characteristic of myrmicine species in a wide range of genera<sup>8</sup>. One of the trialkylpyrazines produced by *Odontomachus* species has recently been identified in *Iridomyrmex humilis*<sup>9</sup>, a species in the subfamily Dolichoderinae. The presence of methyl 6-methyl salicylate in the mandibular gland secretions of *G. pleurodon* and species of *Camponotus* (Formicinae) offers further testimony to the capacity of ants in the more phylogenetically advanced subfamilies to synthesize the same exocrine products produced by species in the primitive subfamily Ponerinae.

In view of the key position that the Ponerinae occupy in the evolution of the Formicidae<sup>10</sup>, analyses of their natural products could provide important chemosystematic information on the phylogeny of this family. An examination of the exocrinology of additional ponerine genera may reveal that the species in this primitive taxon exhibit many of the pheromonal emphases that have already been demonstrated to be characteristic of more highly evolved ants.

**Zusammenfassung.** Das Mandibulardrüsensekret der Arbeiter der Stachelameisen *Gnamptogenys pleurodon* (Ponerinae) enthält hauptsächlich 6-Methylsalicylsäuremethylester; diese Verbindung wird als Alarmpheromon eingesetzt.

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<sup>4</sup> We thank Prof. W. L. BROWN, JR., for the identification of this ant.

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