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THE EXOCRINE SECRETIONS OF THE JUMPING ANT *HARPEGNATHOS SALTATOR*

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Abstract—1. The mandibular glands of *H. saltator* contain chiefly 4-methyl-3-heptanone, a substance found as a mandibular gland secretion in several subfamilies of ants.

2. The Dufour gland contains a complex mixture of linear hydrocarbons from C_{15} to C_{25} with (Z)-9-tricosene representing approximately half the total. The Dufour glands also contain tetradecyl propionate and traces of tetradecyl acetate and dodecyl acetate.

3. Methyl esters of the common fatty acids have been found in the postpharyngeal gland and in the cuticular wax.

INTRODUCTION

The family of the Formicidae contains an impressive variety of species, the lifestyles of which are equally remarkably diverse. One of the more extraordinary ant species is *Harpegnathos saltator* (Jerdon, 1851). This ant is confined to India, Sri Lanka and South East Asia, and because of its most peculiar appearance was chosen as the symbol of the 11th International Congress of the IUSSI at Bangalore, India, in August 1990. The workers of *H. saltator* are solitary foragers, using their astonishing jumping ability to catch a variety of arthropod prey (Soans and Soans, 1969; Maschwitz, 1981). Their large eyes and elongated mandibles, along with their slender body shape, give them a *Myrmecia*-like appearance although they are classified within the Ponerinae subfamily. With the availability of *Harpegnathos saltator* on the occasion of the Bangalore Congress, we have undertaken a chemical study of the secretions of the exocrine glands in this species as part of our comparative investigation of the glandular system in the Formicidae. We here report on the secretions of the Dufour, mandibular and postpharyngeal glands. The possibility that this species could be placed in the Myrmeciinae cannot be dismissed on chemical grounds.

MATERIALS AND METHODS

Two live colonies of *Harpegnathos saltator* were collected from Bangalore (India). The glands were dissected from the ants under water with a binocular microscope, dried lightly and sealed in soft glass capillaries (Morgan, 1990). Pieces of abdominal cuticle and antenna were prepared similarly and brought to Keele, where they were chemically analysed.

Gas chromatography–mass spectrometry (GC-MS)

The identification of the compounds present in the glands and cuticle of *H. saltator* was made by linked GC-MS, employing a Hewlett Packard 5890 gas chromatograph coupled to the mass spectrometer (Hewlett Packard 5970B mass selective detector) with control by HP59970C Chem Station. A fused silica chromatography column (12 m × 0.32 mm) coated with methylsilicone gum of 0.33 μm film thickness was used with helium as carrier gas at 10 psi column head pressure.

The samples were injected into the chromatograph using the solid-sampling technique (see Morgan and Wadhams, 1972) with the injector set at 150°C. The column oven was programmed from 30°C at 8°C/min to 260°C for Dufour and mandibular glands. For postpharyngeal glands and cuticle it was programmed from 150°C at 3°C/min to 320°C. The injections were splitless and the chromatography column was linked to the mass spectrometer by a retention gap consisting of a piece of deactivated and uncoated silica capillary (10 m × 0.2 mm). The mass spectrometer was set at 70 eV ionization energy and scanned from m/z 35 to 350 in the scan mode under Autotune conditions.

Methylthiolation—location of double bond position

Five *H. saltator* Dufour glands were ground in a small tissue grinder with hexane (100 μl) and half the solution was transferred to a Keele Microreactor (Wheaton Scientific, Millville, NJ, U.S.A.; Attygalle and Morgan, 1986), and treated with iodine and dimethyl disulphide as described by Bagnères *et al.* (1991) to obtain the methylthioethers for gas chromatography–mass spectrometry.

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Table 1. Percentage composition of the substances found in the mandibular gland of 16 samples* of heads of *Harpegnathos saltator* workers

Number in Fig. 1A	Compound	%
1	3-Methyl-1-butanol	1.4
2	4-Methyl-3-heptanone	87.3
3	4-Methyl-3-heptanol	3.7
4	6-Methyl-5-hepten-2-one	0.2
5	4-Methyl-4-hepten-3-one	1.6
6	3-Ethyl-4-methyl-1-pentanol	0.2
7	Limonene	0.4
8	2-Nonanone	0.5
9	3-Methylpropyl 3-methylbutanoate	4.7
Mean total amount = 226 ng		

*Samples were combined, with 10 heads in one capillary, four in another and one head only in each of two capillaries, so that standard deviation cannot be calculated.

Esterification—preparation of tetradecyl acetate and propionate

Tetradecanol (0.02 moles) was mixed with propionic and acetic acid (30 μl of each) in a Keele Microreactor, followed by addition of sulphuric acid (10 μl) (Attygalle *et al.*, 1987). The mixture was heated for 12 hr at 120°C, then neutralized with a minimum quantity of $NaHCO_3$ and extracted with 500 μl of hexane. The extract was directly used for GC–MS analysis.

Aldol condensation—preparation of 4-methyl-4-hepten-3-one

A mixture of 1.5 mol of pentanone (129 g) and 1.0 mol of propionaldehyde (44 g) was placed in a round bottomed flask and cooled to −5°C. The solution was saturated with dry hydrogen chloride (100 ml) and allowed to stand for 12 hr at −5°C. The crude product was washed with aqueous sodium hydroxide (2 M) and dried over sodium sulphate. Distillation through a short Vigreux column gave a mixture of 4-methyl-4-hepten-3-one and 4-methylheptan-3-ol-5-one (Faulk and Fry, 1970). A 1 μl of sample diluted in hexane was injected on to the column for GC-MS examination, to confirm the identification of the natural material.

RESULTS

Mandibular glands of *H. saltator* were analyzed by GC-MS. These glands are filled with a mixture of saturated and unsaturated alkyl ketones and their corresponding alcohols and an ester (Table 1). By far the largest component (87%) is 4-methyl-3-heptanone (Fig. 1A); next is the ester, 3-methylpropyl 3-methylbutanoate (trivial name isobutyl isovalerate). The identification of these compounds, and 4-methyl-3-heptanol and 4-methyl-4-hepten-3-one were all confirmed with synthetic specimens. It is interesting that the free alcohol, 3-methylbutanol is also present.

Analysis of Dufour gland contents of *H. saltator* showed that this gland is filled with microgram quantities of linear hydrocarbons in a range of C_{15} to C_{25}, tricosene being the major compound. A range of esters, chiefly hexadecyl acetate and hexadecyl propionate, were also found as minor components (Fig. 1B and Table 2).

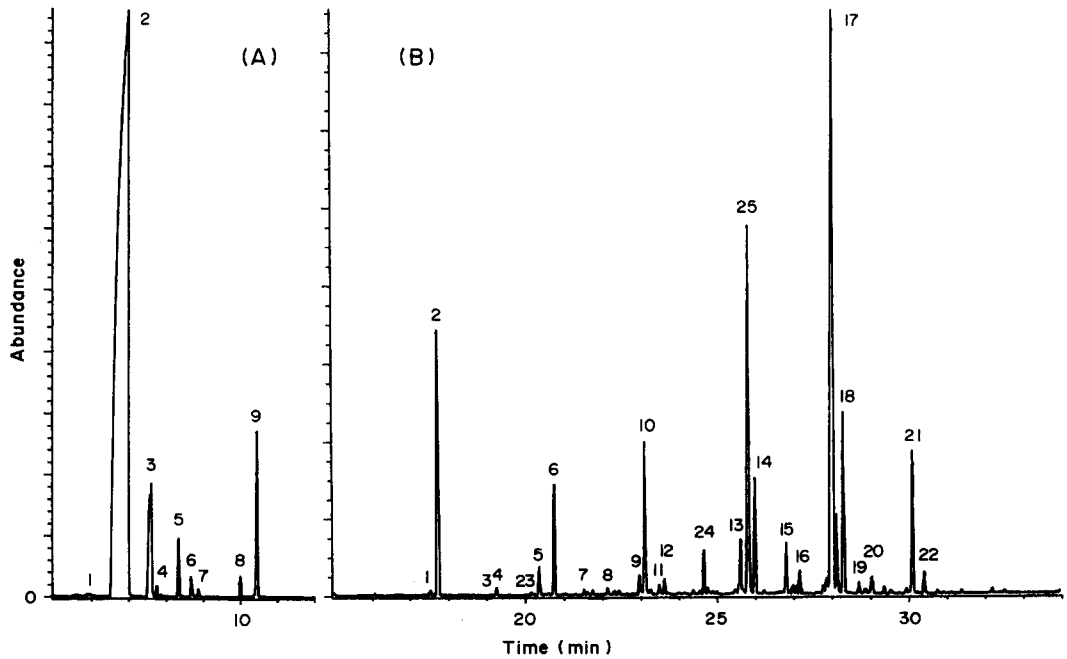

Fig. 1. Gas chromatograms of (A) the mandibular glands and (B) the Dufour gland of a single worker of *Harpegnathos saltator*. The numbering of the peaks corresponds to those in Tables 1 and 2, respectively. Chromatographic conditions are as described in Materials and Methods.

Table 2. Percentages of the substances found in the Dufour gland of *Harpegnathos saltator*

Number in Fig. 1B	Compound	% ± SD
1	Pentadecene	0.01 0.03
2	Pentadecane	7.59 6.15
3	Hexadecene	0.09 0.23
4	Hexadecane	0.13 0.21
5	Heptadecene	1.65 1.75
6	Hepadecane	2.28 2.38
7	Octadecene	0.33 1.09
8	Octadecane	0.60 1.18
9	9-Nonadecene	4.82 3.24
10	Nonadecane	3.22 3.62
11	Eicosene	1.81 3.11
12	Eicosane	0.53 1.26
13	6- and 9-Heneicosene	1.44 1.54
14	Heneicosane	4.36 0.85
15	Docosene	1.93 1.22
16	Docosane	1.95 1.86
17	9-Tricosene	44.40 9.50
18	Tricosane	5.12 2.63
19	Tetracosene	1.04 0.70
20	Tetracosane	0.78 1.20
21	9-Pentacosene	5.59 2.68
22	Pentacosane	0.47 1.02
23	Dodecyl acetate	0.01 0.01
24	Tetradecyl acetate	0.35 0.65
25	Tetradecyl propionate	9.43 6.38
Mean total amount 1.0 ± 0.99 μg		

Results are the means with sample standard deviations of 12 individuals.

To determine the double bond position of the alkene major compound, microreactions were carried out with samples containing three, five or 10 glands. The unsaturated compounds present were converted into their di(methylthio)ethers (Francis and Veland, 1981; Billen *et al.*, 1986) and submitted to GC-MS for examination. The spectrometer was set to scan ions of m/z 61, 97, 173, 227, 243, 321, 370 and 417. Searching carefully for other methylthiol derivatives, the GC-MS showed fragment ions which correspond to the adduct of 9-nonadecene (diagnostic ions at m/z 173 and 187), 6- and 9-heneicosene (m/z 131 and 257; and 173 and 215 respectively), 9-tricosene (m/z 173 and 243) and 9-pentacosene (m/z 173 and 271), that is, all compounds with the double bond at C-9, except heneicosene which was a mixture of two isomers.

Microscale esterification reactions with hexadecanol and acetic and propionic acids to give the synthetic hexadecyl acetate and propionate were carried out to confirm the identification by retention times and mass spectra of these compounds found in the glands.

Four samples of postpharyngeal glands of workers and a queen were examined and compared with the cuticular hydrocarbons from five samples of workers' antennae (Bagnères and Morgan, 1990). Both glands and cuticle contained a mixture of hydrocarbons and methyl esters. The major substance in all cases was heptacosadiene, followed by pentacosane or 4-methylpentacosane, with smaller amounts of tricosane, heptacosane and sometimes nonacosane. Of the methyl esters, methyl oleate was found in all samples with as much as 20% of the total on some antennae. Methyl stearate, methyl linoleate and in some cases methyl palmitate and methyl palmitoleate could be seen in small amounts.

DISCUSSION

There have been no previous studies of the exocrine secretions of *H. saltator* and few examples exist of such data for the subfamily Ponerinae, or the Myrmeciinae, to which this species bears notable similarities. Two species of ponerines contained only straight chain and methyl-branched hydrocarbons, while another contained unfamiliar and as yet unidentified unsaturated compounds (Morgan *et al.*, unpublished). At least one *Myrmecia* species, *M. pilosula* (Myrmeciinae)—known as the jack jumper ant in Australia—contains both hydrocarbons and esters in its Dufour glands (Jackson *et al.*, 1989) while others contain chiefly hydrocarbons (Cavill and Williams, 1967; Jackson *et al.*, 1989). Some myrmeciine species contain alkylpyrazines in their mandibular glands (Brophy and Nelson, 1985), and so do some ponerine species (e.g. *Odontomachus hastatus* and *O. brunneus*; Wheeler and Blum, 1973) while at least one, *Pachycondyla* (*Neoponera*) *villosa* contains 4-methyl-3-heptanone and 4-methyl-3-heptanol (Duffield and Blum, 1973). But 4-methyl-3-heptanone and 4-methyl-3-heptanol are also familiar mandibular gland pheromones in a number of myrmicine species (Blum and Hermann, 1978).

The relative simplicity of the chemicals found here, and the diversity of substances found in the few ponerine species studied, make it difficult to come to any chemical conclusion that would help to place *H. saltator* in either of these subfamilies.

Postpharyngeal gland and cuticle

We have recently shown that in several species of ant, the postpharyngeal gland contains a mixture of hydrocarbons, closely similar to the waxy coating of the insect cuticle (Bagnères and Morgan, 1991). The full significance of this discovery is not yet known. We have here made a brief examination of the cuticle and of postpharyngeal gland of *H. saltator*. We find the same general principles applied, that the gland contents and cuticle wax have related composition, but with a few special points worth noting. First, saturated straight chain and unsaturated and methyl branched alkanes are present in both gland and cuticle. This appears to be a general phenomenon (Bagnères and Morgan, 1991). Secondly, variable amounts of methyl esters of the common fatty acids are present in both. We are not aware of any earlier reports of them in insect cuticle but we have since found them in other ant species (unpublished work). The antennal cuticle wax of workers contains from 18 to over 50% methyl stearate, oleate and linoleate but the exact composition of both wax and postpharyngeal gland contents varies widely between individuals.

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REFERENCES

Attygalle A. B. and Morgan E. D. (1986) A versatile microreactor and extractor. *Analyt. Chem.* **58,** 3054–3058.

Attygalle A. B., Vostrovsky O., Bestmann H. J. and Morgan E. D. (1987) New chemicals from the Dufour gland of the Formicine ant *Lasius niger* (Hymenoptera: Formicidae). *Insect Biochem.*, **17,** 219–225.

Bagnères A. G. and Morgan E. D. (1990) A simple method for analysis of insect cuticular hydrocarbons. *J. Chem. Ecol.* **16,** 3263–3276.

Bagnères A. G. and Morgan E. D. (1991) The post-pharyngeal glands and the cuticle of *Formicidae* contain the same characteristic hydrocarbons. *Experientia* **47,** 106–111.

Bagnères A. G., Billen, J. and Morgan E. D. (1991) The volatile secretion of the Dufour gland of workers of an army ant, *Dorylus* (*Anomma*) *molestus*. *J. Chem. Ecol.* **17,** 1633–1639.

Billen J. P. J., Evershed R. P., Attygalle A. B., Morgan E. D. and Ollett D. G. (1986) The contents of the Dufour glands of workers of three species of *Tetramorium*. *J. Chem. Ecol.* **12,** 669–685.

Blum M. S. and Hermann H. R. (1978) Venoms and venom apparatuses of the Formicidae. In *Arthropod Venoms* (Edited by Bettini S.), p. 801. Handbook of Experimental Pharmacology, New Series, Springer, Berlin.

Brophy J. J. and Nelson D. (1985) 2,5-Dimethyl-3-n-propylpyrazine from the head of the bull ant *Myrmecia gulosa* (Fabr.) *Insect Biochem.* **15,** 363–365.

Cavill G. W. and Williams P. J. (1967) Constituents of Dufour's gland in *Myrmecia gulosa*. *J. Insect Physiol.* **13,** 1097–1103.

Duffield R. M. and Blum M. S. (1973) 4-Methyl-3-heptanone: identification and function in *Neoponera villosa* (Hymenoptera: Formicidae). *Ann. Ent. Soc. Am.* **66,** 1357.

Faulk D. D. and Fry A. (1970) Spectral correlations for α,β-unsaturated ketones. *J. Org. Chem.* **35,** 364–369.

Francis G. W. and Veland K. (1981) Alkylthiolation for the determination of double-bond positions in linear alkenes. *J. Chromatogr.* **219,** 379–384.

Jackson B. D., Billen J. P. J. and Morgan E. D. (1989) Dufour gland content of three species of *Myrmecia* (Hymenoptera: Formicidae), primitive ants of Australia. *J. Chem. Ecol.* **15,** 2191–2205.

Jerdon T. C. (1851) A catalogue of the species of ants found in southern India. *Madras J. Lit. Sci.* **17,** 103–127.

Maschwitz U. (1981) Predatory behaviour and its correlation to recruitment behaviour morphology and nesting habits in three species of ponerine ants. In *Neurobiology and Strategies of Adaptation* pp. 52–59. Joint symposium of the Hebrew University of Jerusalem, Université de Lyon and J. W. Goethe Universität Frankfurt a. M.

Morgan E. D. (1990) Preparation of small samples for chromatography of insect pheromones. *Analyt. Chim. Acta.* **236,** 227–235.

Morgan E. D. and Wadhams L. J. (1972) Gas chromatography of volatile compounds in small samples of biological materials. *Chromatogr. Sci.* **10,** 528–529.

Soans A. B. and Soans J. S. (1969) Some observations of the habits of the ant, *Harpegnathos saltator* Forel (Hymenoptera: Formicidae). *J. Bombay Nat. Hist. Soc.* **69,** 668–669

Wheeler W. M. and Blum M. S. (1973) Alkylpyrazine alarm pheromones in ponerine ants. *Science* **182,** 501–503.