

The contents of the pygidial gland of the primitive ant *Nothomyrmecia macrops* (Hymenoptera:Formicidae)

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Summary. The principal constituent of the pygidial gland of *Nothomyrmecia macrops* is 3,7-dimethyloct-6-en-2-one, a substance not previously identified in insects. Also identified were 2,6-dimethylhept-5-enal, 2-nonanone, indole, γ -dodecalactone, and the hydrocarbons pentadecane, heptadecane, heptadecene and heptadecadiene, all in low nanogram quantities.

Key words. Ant; pygidial gland; *Nothomyrmecia*; dimethyloctenone; γ -dodecalactone.

We have recently collected specimens of the extremely elusive and primitive ant *Nothomyrmecia macrops* Clark at Poochera, South Australia. This is considered the most primitive living ant, and because of some peculiar anatomical features, has been placed by itself in the subfamily Nothomyrmeciinae¹. It has therefore been the subject of several recent studies of its anatomy and phylogenetic position², its behaviour³, genetics⁴, and sting morphology⁵. We have now undertaken a combined study of the ultrastructure⁶ and chemical contents of its exocrine glands. We have

already described the large number of substances identified in its Dufour gland⁷.

We describe here the contents of the pygidial gland and our attempt to study the mandibular gland. The pygidial gland (fig. 1), is associated with the intersegmental membrane between the 6th and 7th abdominal tergites of ants. Large pygidial glands are found in most species of the subfamily Dolichoderinae (where they were erroneously called 'anal glands') and in a number of species scattered throughout other subfamilies⁸. Excepting the dolichoderines, they have

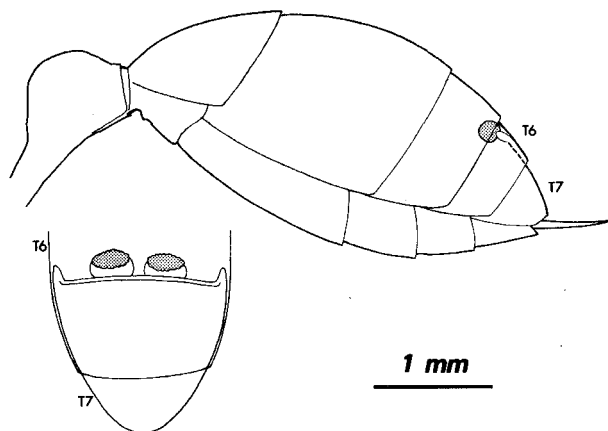


Figure 1. Longitudinal section of the abdomen and dorsal section of the abdominal tip of *N. macrops* showing the 6th and 7th tergites (T6, T7) and the location of the pygidial glands.

been little studied. The pygidial glands occur laterally in pairs and are relatively large in *N. macrops*. Pairs of glands were dissected out and analysed chemically and found to contain nanogram quantities of volatile substances.

Material and methods. Workers of *Nothomyrmecia macrops*, identified by Dr R. W. Taylor, were collected in South Australia, on 24 and 25 February 1987 and taken immediately to Canberra (CSIRO) and there anaesthetized and killed by placing for 3 min in a Biofreezer at -50°C . The ants were then dissected under water. Pygidial glands were removed with some cuticle and sealed immediately in soft-glass capillaries ($2\text{ mm} \times 2\text{ cm}$) in pairs and one group of 4 pairs. Samples of cuticle from the 2nd abdominal tergite, pairs of mandibular glands, and whole heads were similarly sealed. The samples were analysed by GC-MS on a Hewlett Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation. A fused silica capillary column ($12\text{ m} \times 0.2\text{ mm}$) coated with HP-1 (cross linked methyl silicone gum \cong OV-1) of $0.33\text{ }\mu\text{m}$ film thickness was used. The carrier gas was helium at 10 psi column head pressure.

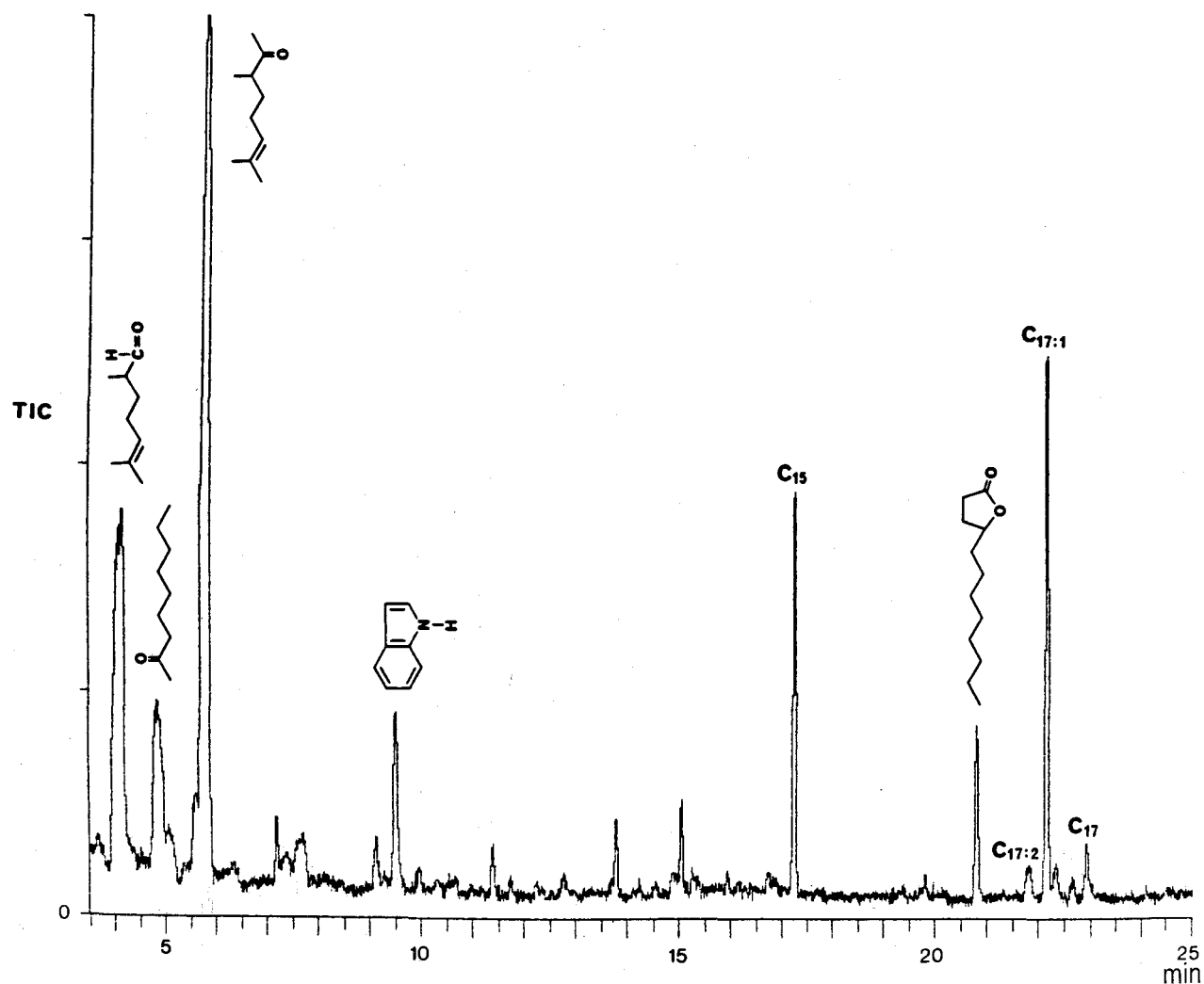


Figure 2. Typical total ion chromatogram of the contents of the pygidial glands of a single worker of *N. macrops*. The peak at 11.4 min is methyl

decanoate. This, and other unlabelled peaks did not appear in other samples.

Mean amount of substances found in the pygidial glands of a single worker of *N. macrops*, together with the sample standard deviation (σ_{n-1} , $n = 8$)

Substance	Mean composition by weight		Mass spectral data m/z (%)
	ng/ant	SD	
2,6-Dimethylhept-5-enal	7	17	140 (M ⁺ , 2), 82 (73), 69 (30), 67 (63), 55 (31), 41 (100), 39 (35)
2-Nonanone	6	5	142 (M ⁺ , 2), 71 (14), 59 (17), 58 (72), 57 (21), 43 (100), 41 (31)
3,7-Dimethyloct-6-en-2-one	33	29	154 (M ⁺ , 5), 83 (27), 82 (61), 72 (37), 67 (57), 43 (100), 41 (73)
Indole	2	4	118 (8), 117 (M ⁺ , 100), 116 (3), 90 (53), 89 (38), 63 (19), 62 (11), 58 (12), 39 (11)
Pentadecane	16	12	212 (M ⁺ , 1), 113 (3), 99 (6), 85 (31), 71 (52), 57 (100), 43 (89)
γ -Dodecalactone	3	2	†128 (6), 114 (3), 85 (100), 69 (9), 55 (19), 43 (17), 41 (27)
Heptadecadiene	2	2	236 (M ⁺ , 3), 109 (19), 95 (42), 81 (63), 67 (100), 55 (55), 41 (83)
Heptadecene	38	32	238 (M ⁺ , 4), 111 (20), 97 (42), 83 (55), 69 (74), 55 (95), 43 (83), 41 (100)
Heptadecane	4	3	240 (M ⁺ , 1), 113 (6), 99 (9), 85 (32), 71 (56), 57 (100), 43 (90)
Total	111	69	

† 198 (M⁺, 0.03) synthetic compound only.

The capillary tubes containing the samples were kept in the solid injector⁹ in the injection port at 200 °C for 2–3 min before crushing. A splitless injection was performed with the injection port purge turned on after 0.5 min. The oven temperature was initially 30 °C for 2 min then increased at a rate of 4 °C min⁻¹ to 250 °C. The mass selective detector was set to monitor m/z 35–350 in the scan mode ($\cong 1.5$ scans s⁻¹) under "Autotune" conditions using 70 eV ionization. The quantity of each component was determined by external standards.

Results and discussion. Insects were transported live to Canberra, and there the pairs of glands from each of four workers were dissected and sealed in glass capillaries and brought to Keele. Glands from another four workers were sealed together in a single capillary. Whole worker heads and mandibular glands were prepared similarly.

These samples were analysed by combined gas chromatography-mass spectrometry using our solid sampling method⁹. Because a certain amount of cuticle has to be included with the dissected pygidial glands, comparable samples, free of glandular tissue were taken from the 2nd abdominal tergite to serve as controls.

Nine substances were recognized in the pygidial gland. A typical gas chromatogram of a single worker's gland is given in figure 2. The major substance was 3,7-dimethyloct-6-en-2-one, a compound which has not previously been identified in insects. Also present in smaller amounts were 2,6-dimethylhept-5-enal, 2-nonanone, indole and γ -dodecalactone. None of these substances has been identified before in pygidial glands of ants, though all have been found in other glands of ants or other insects¹⁰. 2,6-Dimethylhept-5-enal has been found in the mandibular glands of the ants *Acanthomyops claviger*, *Lasius alienus* and *L. carnolicus*¹⁰. 2-Nonanone has been identified in the pygidial ('anal') gland of *Azteca* spp. and in the mandibular glands of *Trigona* bees¹⁰. Indole is the major constituent of the paired intersegmental sternal glands of the trichopteran *Pycnopsyche scabripennis*¹⁰ and γ -dodecalactone is the major substance of the pygidial glands of the staphylinid beetles *Bledius mandibularis* and *B. spectabilis*¹⁰. Also found were the four hydrocarbons pentadecene, heptadecane, heptadecene and heptadecadiene. These have already been encountered in *N. macrops* Dufour glands⁷. Indeed, they were present in roughly the same proportions as in the Dufour gland, though in less than 1% to 0.1% of the amount found there. The hydrocarbons, but not the other substances, were also found in the samples of tergal tissue. It is possible that they arise as contaminants during dissection or they are distributed over the cuticle by discharge of the Dufour gland. Still smaller amounts were found in the heads (see below). The mean quantities of all these substances found in the glands are given in the table.

The hydrocarbons are already familiar from many other species examined. For the other substances, synthetic specimens

were obtained to confirm their identification from mass spectra and retention times. 2,6-Dimethylhept-5-enal, indole and 2-nonanone were available commercially. γ -Dodecalactone was prepared in high yield, accompanied by a little δ -lactone via a Knoevenagel condensation of decanal and malonic acid followed by acid-catalysed ring closure¹¹. 3,7-Dimethyloct-6-en-2-one was prepared from 6-methylhept-5-en-2-one and ethyl 2-chloropropionate via a Darzens glycidic ester condensation followed by hydrolysis and decarboxylative rearrangement¹².

The large pygidial glands of dolichoderine ants, which contain monoterpene iridoids, have been extensively studied first by Pavan and co-workers and later by Cavill and co-workers (reviewed by Blum and Hermann¹³ and ourselves¹⁴). The iridoids are used as defensive secretions. Apart from the work on dolichoderines, there is only one other chemical examination reported, that on the pygidial gland of the ponerine ant *Rhytidoponera metallica*¹⁵, where 3-hydroxybenzaldehyde, isogeraniol, heptadecane and heptadecene were found.

Hölldobler and Taylor carried out simple tests on the behaviour of *N. macrops* workers towards their glandular secretions, and found the pygidial gland secretion caused some response, weaker than their reaction to their mandibular glands and Dufour gland, but the pygidial gland secretion was the only one that caused a positive repellent response in three species of *Camponotus*³. They concluded that the pygidial gland secretion has an alarm-defense function, perhaps in encounters with other ants. γ -Dodecalactone may contribute to this effect, since γ -decalactone, a component of the anal exudate of the thrips *Bagnalliella yuccae* (Thysanoptera), repels small predators such as ants (*Monomorium minimum*, *Iridomyrmex humilis*)¹⁶.

Mandibular glands are probably present in all ants, and in many, but by no means all species, the glands contain volatile chemicals. The type of substance varies with the sub-family, but generally they have a defensive or pheromonal function. We have analysed individual heads, dissected glands, with and without mandibles attached, of workers of *N. macrops* and one sample consisting of six heads, and another of eight, and found no volatile material in any of them, other than still smaller traces of Dufour gland hydrocarbons. This was surprising since Hölldobler and Taylor found the greatest response of all the glandular secretions from the mandibular glands in simple behavioural tests on *N. macrops* workers. We must consider the possibility that the ants had discharged these glands in the disturbance of travelling.

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