

Gas chromatography without solvents: pheromone studies: the Dufour gland of the ant *Pogonomyrmex occidentalis*

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Résumé L'analyse d'échantillons biologiques comme les sécrétions ou glandes de phéromones d'insectes, peut être réalisée à distance dans le temps et l'espace à partir de la collection ou de la dissection au moyen du système simple consistant à enfermer le tissu dans des tubes capillaires en verre et à injecter ces derniers dans une colonne chromatographique en phase gazeuse sans l'intervention d'un solvant. On a découvert que la glande Dufour du *P. occidentalis* contient un mélange carburant de C₁₂ à C₁₉ comprenant des alcanes en chaînes méthyliques ramifiée et droite et un nouvel hydrocarbure bishomosesquiterpénoïde C₁₇H₂₈, le *n*-pentadécane étant l'élément principal.

Zusammenfassung Die Analyse von biologischen Mustern, wie zum Beispiel Insektenpheromondrüsen oder Sekretionen kann vom Probeziehen und von der Sezierung zeitlich und räumlich entfernt ausgeführt werden, indem man einfach das Gewebe in Glaskapillaren einschmelzt und diese dann direkt, ohne die Verwendung eines Lösungsmittels, in eine Gaschromatographiesäule einsetzt.

Es wurde entdeckt, dass die Dufour Drüse des *P. occidentalis* eine Mischung von C₁₂ bis C₁₉ Kohlenwasserstoffen enthält, die aus Methyl-verzweigten und geradkettigen Alkanen und einem neuen bishomosesquiterpenoid Kohlenwasserstoff C₁₇H₂₈ mit *n*-pentadekan als Hauptkomponent besteht.

INTRODUCTION

Samples for analysis of chemically or biologically unstable material may frequently have to be collected far from the laboratory and transported long distances. The possibility of deterioration in such samples is a serious drawback to their usefulness or reliability. A good example of this is the collection of animal or insect glands or secretions in the field where little or no scientific equipment is available. We have been using for some time a simple system for collection of these types of samples which requires only a supply of glass capillary tubing and a small flame for heat-sealing.

This system has the additional advantage of requiring no solvent, either at the time of collection or in the laboratory. Analysis is performed directly on the sealed capillary by gas chromatography, with or without coupled mass spectrometry¹. This technique is illustrated with an examination of the Dufour glands of harvester ants from the USA, which were found to be difficult to transport live to the laboratory.

The ant genus *Pogonomyrmex*, or harvester ants of North America, form large colonies

which collect seeds from a defined territory around the nest². This territory may cover many square metres and has permanent, branching trails which are patrolled by the workers, who defend it against incursion from other colonies or species.

The alarm pheromone from the mandibular glands has been identified in several species as 4-methyl-3-heptanone. It is present in *P. badius*, *P. barbatus*, *P. desertorum*, *P. californicus*, *P. occidentalis* and *P. rugosus*³. The mandibular glands of *P. badius* workers contain on average 16 µg of this ketone, with smaller amounts of 4-methyl-3-heptanol³. Hölldobler asserts that the concentration of the alarm pheromone determines the response of workers of *P. badius*⁴.

The Dufour glands and their secretion in *Pogonomyrmex* have not yet received much attention. Hölldobler and Wilson⁵ report that Dufour gland secretion serves as an additional orientation clue in recruitment trails, and that Dufour gland trails are longer lasting than poison gland trails. After 25 to 30 min significantly more ants followed the Dufour gland trail. Hölldobler also says that the Dufour gland

secretion of *Pogonomyrmex* acts as a homing signal⁶. Regnier *et al.* examined the secretion of *P. barbatus* and *P. rugosus*⁷. They found homologous families of straight chain alkanes from C₁₂ to C₁₅ and three types of methyl-branched alkanes, and two dimethyl-alkanes. The major substance in *P. barbatus* was *n*-dodecane with 6-methyldodecane next. In *P. rugosus* the major peak obtained in gas chromatography was an apparently unresolved mixture of 6-methylundecane and 5-methylundecane.

The Dufour gland is a small bulbous reservoir attached to the poison apparatus in the posterior tip of the abdomen of all aculeate Hymenoptera. Its original or main function remains unknown, though in many ants, as here, a pheromone function has been demonstrated. From numerous studies of ants, the Dufour gland has been shown to contain species-specific mixtures of oily substances, chiefly hydrocarbons⁸. A colony of *Pogonomyrmex occidentalis* (Cresson) was collected at Denver, Colorado in August 1982, and a preliminary examination of the Dufour glands made. A further sample collected

at Denver in 1985 was used to repeat and complete the study. The species-specific nature of the contents of the Dufour gland of *P. occidentalis* and the existence of a new sesquiterpenoid compound discovered in the secretion are also described here.

MATERIALS AND METHODS

Source and preparation of insect material

Workers of *P. occidentalis* collected at Denver, Colorado, August 1982, were transported live to Belgium, where they were dissected to remove

the poison apparatus which was then sealed in soft glass capillaries. Further material was obtained at Denver in 1985, and sent to Athens, Georgia, where again the poison apparatus was sealed in soft glass capillaries. These were transported to Keele, where they were kept in a refrigerator until analysed.

Gas chromatography (GC)

Gas chromatography was carried out on a Carlo Erba Fractovap 4160 series GC equipped with a flame ionization detector (FID) and a Pye

Unicam Spectra Physics DP101 computing integrator. A 25 m fused silica OV1 (0.4 mm) capillary column was used for the analysis. Helium was used as the carrier gas at a flow rate of 2 ml min⁻¹.

The dissected glands, which had been sealed in glass capillaries (1 to 2 cm × 1.2 mm OD) were placed in the injection port of the gas chromatograph using the solid sampling method¹, and heated there to 170° for 2-3 min before depressing the plunger, to crush the glass and release the volatiles from the tissue.

Gas chromatography-mass spectrometry (GC-MS)

Mass spectrometry was performed on a Finnigan 3200E quadrupole spectrometer with a Data System 6000. A fused silica column (CP-19 38 m × 0.22 mm, 0.2 μm film) was directly coupled to the mass spectrometer. Helium was used as the carrier gas at a flow rate of 1 ml min⁻¹. 70 eV EI spectra were recorded at a rate of 2 s scan⁻¹. The dissected glands were solid sampled onto the GC via a splitless injector with the split vent opened after one minute. The GC was then left at room temperature for 4 min and then programmed from 70 to 280°C at 6°C min⁻¹.

Quantification

A standard solution of hydrocarbons was made up in hexane. Three injections containing 200 ng μl⁻¹ each of C₃, C₁₅, C₁₇ and 113 ng of C₁₉ were made and the results used in the quantification of the glandular components.

RESULTS

Gas chromatography-mass spectrometry has revealed a number of *n*-alkanes, recognised by their simple mass spectra and branched alkanes, by their molecular masses and by their fragmentation patterns. In many cases the identification could be confirmed from the tables of characteristic ions used in the identification of the branched alkanes in *P. barbatus* and *P. rugosus* by Regnier *et al.*⁷.

For quantification of the components of the second sample, six replicates of individual workers were examined. A typical gas chromatogram is shown in Figure 1. The letters designating the peaks correspond to the numbers and identification in Table 1, where the mean amounts of each substance, and its percent of the total are recorded.

The major component present in the gland was found to be *n*-pentadecane (M⁺ 212, C₁₅H₃₂). This comprised just over 30% (20 ng) of the total glandular contents. The gland is relatively small for the size of the ant, and in the second sample it contained on average only 70 ng of hydrocarbons. In the first sample the mean amount was 527 ± 115 ng. However, in the first sample, *n*-pentadecane still represented 29.0 ± 1.6%. The only other component over 10% of the total was tridecane (M⁺ 184, C₁₃H₂₈, 7.6 ng, 11.6%; 14.6% in the first sample). The other

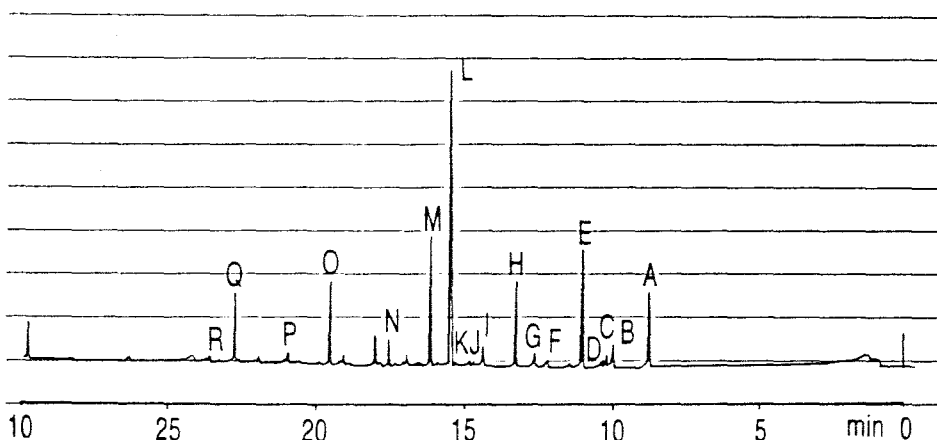


Figure 1. Gas chromatogram of a single Dufour gland of the ant *Pogonomyrmex occidentalis* on an OV-1 capillary column (25 m × 0.44 mm) programmed from 70 to 280°C at 6°C min⁻¹ and using helium carrier gas at 2 ml min⁻¹. The sample was introduced without solvent by the sampling method of Morgan and Wadhams¹. Peak identifications are given in Table 1.

Peak ^a	Identification	Mean composition by weight ^b ng per ant ± SD		Mean % by weight ± SD	
A	<i>n</i> -Dodecane	5.5	1.3	8.4	2.0
B	6-Methyldodecane	2.4	1.5	3.7	2.3
C	4-Methyldodecane	0.8	0.4	1.2	0.6
D	3-Methyldodecane	0.7	0.4	1.1	0.6
E	<i>n</i> -Tridecane	7.6	1.8	11.6	2.7
F	7-Methyltridecane 5-Methyltridecane	1.1	0.9	1.7	1.4
G	3-Methyltridecane	1.0	0.6	1.5	0.8
H	<i>n</i> -Tetradecane	5.6	0.9	8.6	1.4
I	3,5-Dimethyltridecane	1.7	1.0	2.6	1.5
J	4-Methyltetradecane	0.4	0.2	0.6	0.3
K	3-Methyltetradecane	0.4	0.1	0.6	0.3
L	<i>n</i> -Pentadecane	20.6	3.3	31.5	5.0
M	Bishomofarnesene-3	5.3	2.8	8.1	4.3
N	<i>n</i> -Hexadecane	1.7	0.5	2.6	0.8
O	<i>n</i> -Heptadecane	5.6	1.3	8.6	2.0
P	Octadecene	4.6	1.1	7.0	1.8
Q	Nonadecene	4.8	2.2	7.3	3.4
R	<i>n</i> -Nonadecane	0.2		0.3	
	Total	70.0			

Table 1. Composition of the secretion of the Dufour gland of workers of the ant *Pogonomyrmex occidentalis*. a. See Figure 1 b. Mean of 6 determinations

more important components were all straight chain hydrocarbons from *n*-dodecane to *n*-nonadecane, but including two *n*-alkenes.

A large number of methyl-branched and dibranched hydrocarbons were present in very small quantities, from a few ng to less than 1 ng.

Most interesting was the discovery of the sesquiterpenoid compound described as bishomofarnesene (peak M in Figure 1, M^+232 , $C_{17}H_{28}$). This is not the same substance that has been found in *Myrmica* ants^{8,9,10} since its retention time and the relative intensities of the peaks in its mass spectrum are different. Nor is it the same as the bishomofarnesene recently found in the Dufour glands of *Monomorium pharaonis* and *Solenopsis geminata*¹¹.

DISCUSSION

Using samples of pheromones taken from ants in the laboratory, sealed, and then analysed at different periods up to one year we have found no deterioration in the substances, including relatively air sensitive sesquiterpenes. Where it is not possible to seal the tissue inside the capillary, or where any fear or pyrolysis products from the tissue must be eliminated, the secretion or glandular contents can be drawn into a fine glass capillary which is then sealed in a larger capillary.

Attempts to transport a colony of *P. occidentalis* ants live to the laboratory, as has been done for many other species, failed in two attempts, therefore the poison apparatus of workers, consisting of Dufour gland and poison gland, were dissected and sealed separately in glass capillaries, which were then transported by airmail to the laboratory, where they were stored for use. One gland at a time was then introduced directly into the gas chromatograph, where it was heated, the glass capillary crushed and the contents of the gland immediately volatilised and chromatographed. Because the poison gland contains an aqueous protein solution, which gives no volatile products under these conditions, the tedious separation of the poison reservoir from the Dufour gland is not necessary. The finding of

air-sensitive substances, such as farnesenes, in glands which have been dissected and sealed in this way for months and the constant composition of glands sampled at different times attests to the lack of deterioration on storage using this technique.

Insufficient material was obtained in the first sample for both quantification and identification, therefore the second sample was obtained to complete the work. The mean total amount in the glands varied between the two samples (527 ng in the first, 70 ng in the second) but in other species we have found the amount in a gland may vary widely between individual workers, though the percentage composition tends to remain constant within certain limits. The percentage composition was therefore calculated for each individual worker and the means of these percentage values calculated and listed in Table 1. Thus, the proportions of the two principal constituents, pentadecane and tridecane were very similar in both samples.

The composition of hydrocarbons in *P. barbatus*, *P. rugosus* and *P. occidentalis* (the only species of *Pogonomyrmex* yet studied in detail) are similar in type but differ in detail. Regnier *et al.* record substances only up to C_{15} in *P. rugosus* and *P. barbatus*, but this may be because they did not take the chromatography column to sufficiently high temperatures. We have extended the chromatogram into the C_{25} region (not shown in Figure 1) where some linear hydrocarbons, probably from the cuticle of the sting lance were observed, but find no significant peaks above nonadecane (Figure 1).

The new bishomofarnesene (peak M in Figure 1) of *P. occidentalis* represents a significant fraction of the contents. To distinguish it from the *Myrmica* bishomofarnesene⁹ and the bishomofarnesene-2 of *Solenopsis* and *Monomorium*¹¹ this is referred to as bishomofarnesene-3 until its full structure is known. This compound may well be present in the other two *Pogonomyrmex* species, the unidentified peak 16 in *P. rugosus* and peak 12 in

P. barbatus, since these peaks appear at the expected retention times on non-polar columns (shortly after *n*-pentadecane)⁷. The mass spectrum of bishomofarnesene-3 is reproduced in Figure 2. The mass spectra of sesquiterpenes are notoriously difficult to interpret, because changes in structure lead to minor changes in ion intensities and that is complicated here by the addition of two extra CH_2 groups. In spite of four double-bond equivalents in the molecular formula, the prominent ions at m/z 161 (loss of C_5H_{11}), and at 57 (C_5H_9) suggest at least one saturated isopropene unit in the molecule. Further study using microchemical methods¹² will be necessary to elucidate the structure.

It is noteworthy that linear hydrocarbons from hexadecane upwards are solids at room temperature. It is unusual to find *n*-alkanes of C_{17} upwards in Dufour glands. Large proportions of these hydrocarbons would cause the oily mixture to solidify, however, the relatively small proportions of *n*-heptadecane and *n*-nonadecane in the gland of *P. occidentalis* would remain in solution in the lower *n*-alkane and branched alkane mixture.

It is conceivable that the alkanes act as a solvent and preservative for the air-sensitive sesquiterpenes, such as the bishomofarnesene-3 encountered here. Pure farnesenes react rapidly in air with oxygen by a free radical process to give involatile rubbery polymers. Dissolved in hydrocarbons, these substances would be much more stable to aerial oxidation and could volatilise slowly to provide a communication odour.

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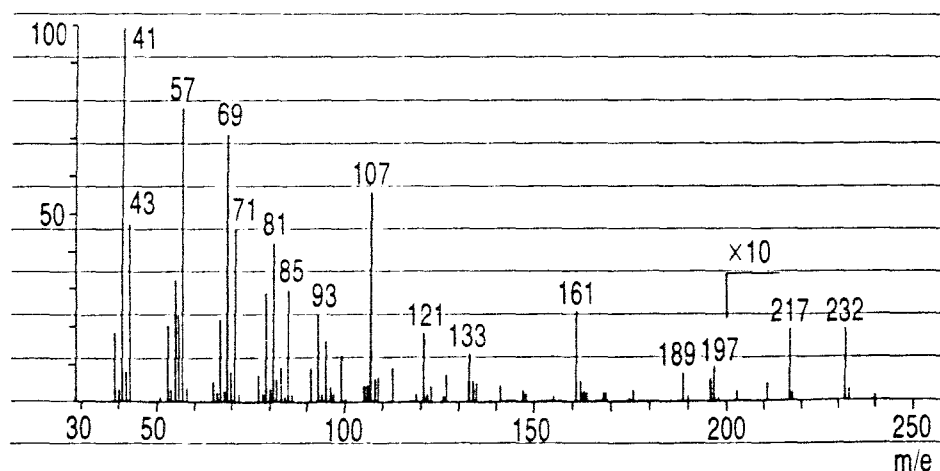


Figure 2. Mass spectrum of bishomofarnesene-3, peak M in Figure 1, obtained as described under GC-MS in the materials and methods section.