

# The Contents of the Dufour Gland of the Ant *Harpagoxenus sublaevis* Nyl. (Hymenoptera: Formicidae)

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The volatile components of the Dufour gland secretion of workers of the ant *Harpagoxenus sublaevis* have been analysed by gas chromatography and mass spectrometry. Over 20 components have been identified, consisting of linear and terpenoid hydrocarbons. Each gland contains approximately 6  $\mu$ g of hydrocarbons with *n*-heptadecene (40%) and *n*-heptadecadiene (30%) as the major components. Two terpenoid hydrocarbons, (*E*)- $\beta$ -farnesene and a homofarnesene isomer were also identified.

## Introduction

*Harpagoxenus sublaevis* Nyl. is a myrmicine ant unusual in that it does not forage for itself, but exists by dulosis, that is, an extreme form of slavery by which it captures the brood of its relative, *Leptothorax acervorum* and the latter perform all the labour of food gathering, nest maintenance and nursing within the *Harpagoxenus* nest [1].

A slave raid of *H. sublaevis* usually begins with a single "scout" seeking out a nest of *L. acervorum*, then single nest mates are led by tandem running to the slave nest until there are sufficient of them to start the attack. The raiding party enter the nest, capturing brood and return with them to their own nest [2, 3]. The *H. sublaevis* workers smear the captured brood with Dufour gland secretion which makes the brood unattractive to the *Leptothorax* defenders [4, 5]. *H. canadensis* (closely related to *sublaevis*) behaves similarly. *H. zaisanicus* from Mongolia may be identical with *H. sublaevis*. The rather different *H. americanus* (Emery) [6], raids *L. curvispinosus* and *L. longispinosus* [7]. Here the scout leads the raiding party all the way to the slave nest. The *H. americanus* raiders rarely attack workers of the raided colony, rather they induce panic using

"propaganda substances", probably disseminated by the sting [8]. The propaganda substances and the repellent spread on the brood may be the same substance or mixture of substances.

The Dufour gland is a rich source of chemicals [9, 10] found in all ants and other aculeate Hymenoptera, and yet its function is little understood. In *Myrmica* it certainly contains a home range marking pheromone [11], in *Monomorium pharaonis* and *Solenopsis* species it also contains the trail pheromone [12, 13] and the sex pheromone in *Monomorium* [14]. It was of interest to us to study the Dufour gland secretion of *H. sublaevis* to see if it differed greatly from that of many other myrmicine species we have examined and to see if it contained any unusual compounds, that might be used in slave raiding.

We find that *H. sublaevis* Dufour glands contain hydrocarbons like other myrmicines, but additionally contain two unusual sesquiterpenoid compounds.

## Materials and Methods

### Source and preparation of insect material

Colonies of ants collected in Germany were maintained in the laboratory in artificial nests of glass and moistened plaster of Paris, and fed on sugar and dipteran larvae. The ants were killed by chilling them momentarily in liquid nitrogen, and the Dufour

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glands were removed by dissection in water under a binocular microscope. The individual glands were immediately sealed in a soft glass capillary [15].

#### Gas chromatography (GC)

Gas chromatography was carried out on a Pye 104 instrument equipped with a flame ionization detector (FID) and a Pye Unicam: Spectra Physics DP101 computing integrator for qualitative analysis. A 10% PEG 20M column (2.75 m × 0.4 mm) was used for the analysis. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min.

The dissected glands were injected onto the chromatography column without solvent using the method of Morgan and Wadhams [16]. The sample was kept in the injection port (200 °C) for 2–3 minutes before crushing. A temperature programme from 130–210 °C at 3 °C/min was used to elute all of the compounds.

#### 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) was directly coupled to the mass spectrometer. Helium was used as the carrier gas at a flow rate of 1 ml/min. 70 eV EI spectra were recorded at a rate of 2 sec/scan.

#### Dehydration of nerolidol

A mixture of (E)- and (Z)-nerolidol were heated with *p*-toluene sulphonic acid (20 mg) in refluxing toluene (50 ml) in a Dean and Stark apparatus until no more water was collected (~30 mins). The toluene was evaporated off and the concentrate was purified by eluting with petroleum ether (B.p. 40–60 °C, 50 mls) through a florisil column (2.5 × 25 cm). The eluant was again concentrated by rotary evaporation. The products were then dissolved in hexane and were analysed by GC.

For comparison of the natural and synthetic isomers a Carlo Erba 4160 Fracto-Vap series GC was used with a flame ionization detector (FID). A 25 m × 0.32 mm fused silica column coated with OV1 stationary phase (0.4 µm thickness) was used for analysis. Helium was used as the carrier gas with a flow rate of 2 ml/min and the oven was programmed from 120–300 °C at 3 °C/min.

#### Quantification of components

The absolute quantity of each component was determined by measuring peak areas and comparing with a standard solution of pentadecane in hexane (800 ng/µl) as an external standard.

Ten samples of individual glands of *H. sublaevis* were chromatographed on the PEG 20M column, and the mean amount of each substance and its percentage of the total, plus the mean percentage were calculated.

#### Results

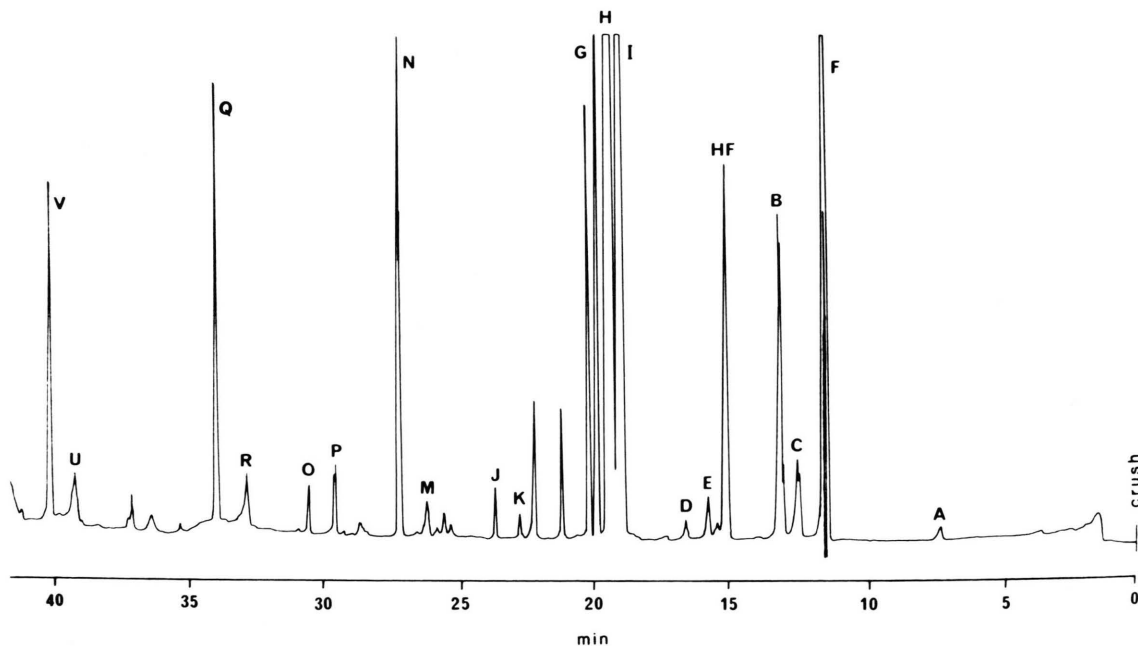
The investigation of the volatile constituents of the Dufour gland of *Harpagoxenus sublaevis* showed the presence of more than 20 components all of which proved to be hydrocarbons. Initially, analyses were carried out on packed columns, and no suitable column was available that separated all the components. Quantification was carried out on a polyethylene glycol (PEG 20 M) packed column that did not resolve the higher sesquiterpenoid homologue from heptadecene. This is reflected in the quantities in Table I. Later a capillary column became available on which all the components were well resolved (Fig. 1) and good mass spectra could be obtained for both the farnesene and homofarnesene. The letters given to the peaks in the chromatogram refer to Table I where the identification of the peaks are listed and the relative abundance of the compounds are shown. The mean values presented in the table are the results of ten replicated determinations.

The major components present in the gland are found to be *n*-heptadecene ( $M^+$  238  $C_{17}H_{34}$ ) which co-elutes with a homofarnesene isomer ( $M^+$  218  $C_{16}H_{26}$ ) on the PEG 20M column and heptadecadiene ( $M^+$  236  $C_{17}H_{32}$ ). Together these components total 70% of the total glandular components. The heptadecene and homofarnesene total 2390 ng, however the homofarnesene which is well resolved on the capillary column (Fig. 1) only represents a small fraction of this total. The heptadecadiene totals 1800 ng (see Table I), the entire glandular contents totalling almost 6 µg. The next largest peak is octadecane and an unidentified component which together total 502 ng and form 8.4% of the total contents, however a clear mass spectrum of the minor component could not be obtained so the structure remains unknown.

Table I. Chemical composition of the Dufour gland contents of workers of *Harpagoxenus sublaevis* from gas chromatography and mass spectrometry, mean of ten samples.

Peak*	Compound	Mean composition by weight ng/ant $\pm$ S.D.	Mean % by weight $\pm$ S.D.
A	<i>n</i> -tridecane	trace	—
B	<i>n</i> -pentadecane	128 $\pm$ 36.6	2.1 $\pm$ 0.6
C	pentadecene	trace	—
D	<i>n</i> -hexadecane	trace	—
E	hexadecene	33.5 $\pm$ 10.5	0.6 $\pm$ 0.2
F	farnesene	146 $\pm$ 176	2.4 $\pm$ 2.9
G	<i>n</i> -heptadecane	145 $\pm$ 53.8	2.4 $\pm$ 0.9
H + HF	heptadecene + homofarnesene	2390 $\pm$ 755	40 $\pm$ 12.6
I	heptadecadiene	1800 $\pm$ 652	30 $\pm$ 10.9
J	<i>n</i> -octadecane	502 $\pm$ 377	8.4 $\pm$ 6.3
K	octadecene	trace	—
M	unknown	trace	—
N	<i>n</i> -nonadecane	191 $\pm$ 52	3.2 $\pm$ 0.9
O	<i>n</i> -eicosane	trace	—
P	eicosene	trace	—
Q	<i>n</i> -heneicosane	162 $\pm$ 79	2.7 $\pm$ 1.3
R	heneicosene	trace	—
S	unknown	49.6 $\pm$ 55.9	0.8 $\pm$ 0.9
T	unknown	76.8 $\pm$ 74.4	1.3 $\pm$ 1.2
U	<i>n</i> -tricosane	162 $\pm$ 104	2.7 $\pm$ 1.7
V	tricosene	34.7 $\pm$ 28	0.6 $\pm$ 0.5
Total		5630 ng	

\* Letters correspond to those on the gas chromatograph in Fig. 1.

Fig. 1. Gas chromatograph trace obtained from the solid injection of one Dufour gland of a *Harpagoxenus sublaevis* worker on the OV1 capillary column, temperature programmed from 120 °C to 300 °C at 3 °C/min. The identification of peaks corresponds to that of Table I.

Of the other hydrocarbons, compounds with an odd number of carbon atoms were found as the major components (peaks A, B, G, N, Q, U in Fig. 1). The alkene of the same carbon number was also usually present but in a much lower quantity (peaks C, R, V in Fig. 1).

Even numbered hydrocarbons were also present but generally in lower concentrations (less than 0.5%, peaks D, J, O in Fig. 1) than the odd numbered homologues. Again the alkene of the same carbon number was present, in similar quantities to the alkane (peaks E, K, P).

A farnesene isomer (peak F in Fig. 1,  $C_{15}H_{24}$ ) was also found in the gland (145 ng, 2.4%).

Other substances identified by mass spectrometry but not shown in Fig. 1 and 2 were nonadecene, nonadecadiene, eicosadiene and two branched chain hydrocarbons, 7-methylnonadecane and 7-methylheneicosane.

The two sesquiterpenoid compounds (peaks F and HF, Fig. 1) were also identified from their mass spectra. The first was an isomer of farnesene (Fig. 2) and the second a homologue of it with one more carbon atom ( $C_{16}H_{34}$ , Fig. 3). The farnesene isomer was tentatively identified as (*E*)- $\beta$ -farnesene, but because the mass spectra of the six farnesene isomers differ only slightly [17], a mixture of the six farnesenes was prepared from (*E*)- and (*Z*)-nerolidol

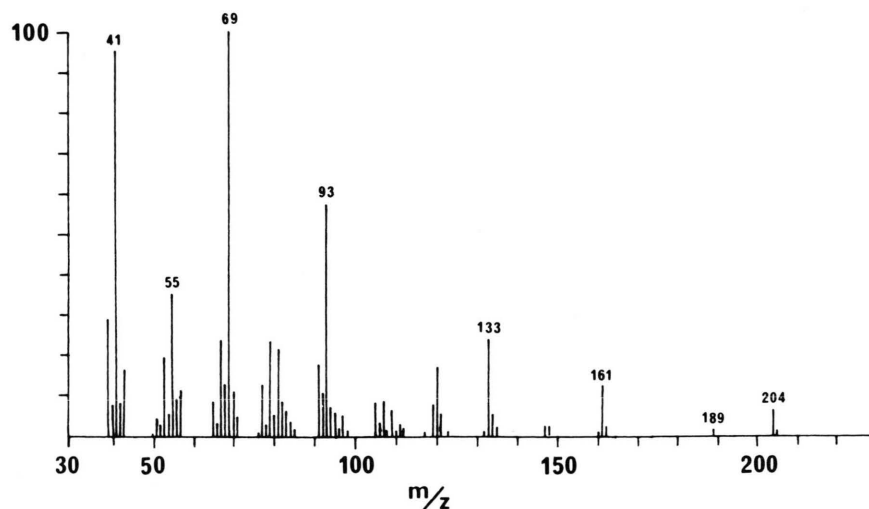


Fig. 2. Mass spectrum of (*E*)- $\beta$ -farnesene obtained from *H. sublaevis* workers.

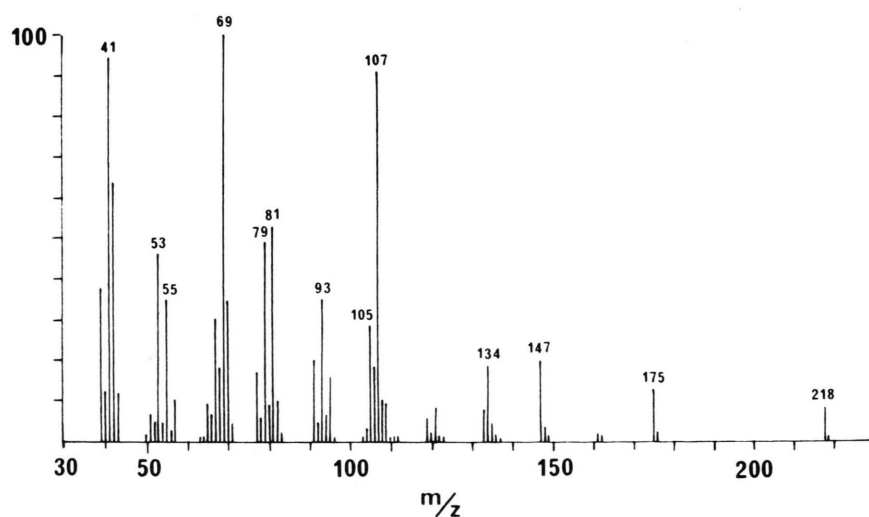


Fig. 3. Mass spectrum of the homofarnesene isomer found in *H. sublaevis* workers.

[17] and chromatographed on a capillary column. From the known order of elution of these farnesenes the (*E*)- $\beta$ -farnesene was recognised and shown to have a retention time identical with that of the isomer from *H. sublaevis*. The higher homologue is a new substance. Nor further compounds corresponding to bishomofarnesene [18] or trishomofarnesene [19] were detected.

## Discussion

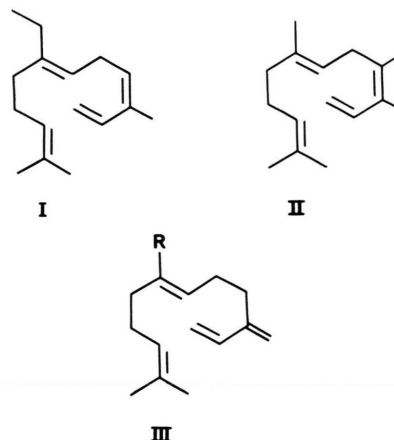
The Dufour gland of *H. sublaevis* contains only hydrocarbon substances. The chief constituents are linear alkanes and alkenes from C<sub>15</sub> to C<sub>23</sub> with the odd numbered carbon chains predominating as has been frequently found in other species. The spread in chain length is unusual here, typically most species of *Myrmica* have a spread from C<sub>15</sub> to C<sub>19</sub> [11], *Atta* species have been found to have rather longer chains from C<sub>19</sub> to C<sub>23</sub> [20, 21]. Two branched chain hydrocarbons were identified from their retention times and mass spectra as 7-methylnonadecane (M<sup>+</sup> 282, prominent ion at *m/z* 197, 6% intensity) and 7-methylheneicosane (M<sup>+</sup> 310, prominent ion at *m/z* 225, 4%).

One diene, heptadecadiene was present in large amounts. The position of the double bond in this and the other alkenes was not determined for lack of sufficient insects.

Only two compounds did not belong to the aliphatic hydrocarbons, these were identified as (*E*)- $\beta$ -farnesene and a homologue of it with one more carbon atom.

Farnesenes have been frequently found in ants, but where the isomer has been determined this has either been (*Z,E*)- $\alpha$ -farnesene [19] or occasionally (*E,E*)- $\alpha$ -farnesene [13]. This is the first time (*E*)- $\beta$ -farnesene has been identified. It is found widely in plants, chiefly in essential oils [22], and is well known as an alarm pheromone in aphids [23, 24]. Release of the substance from the cornicles causes aphids to drop from the plant on which they are feeding or disperse. (*E*)- $\beta$ -farnesene has also been found in six species of *Andrena* bees [25]. There would seem to be no interaction between *Harpagoxenus* and aphids. *H. sublaevis* does not collect food and never visits aphids (Buschinger, personal communication). All foraging is done by its slaves, *L. acervorum*, but they only collect honey dew droplets from the forest floor and very rarely go near aphids [26].

The homologue of farnesene is an interesting substance because it does not have the structure of homofarnesene (I) found in *Myrmica* ants [18, 27] nor that of the homofarnesene (II) found in *Solenopsis* [13]. It would appear to be related to (*E*)- $\beta$ -farnesene (III, R = CH<sub>3</sub>) and may well have the structure (III, R = CH<sub>2</sub>CH<sub>3</sub>).



The Dufour gland of *H. sublaevis* is extremely large for the size of the individuals (a mean of 6  $\mu$ g of contents per worker in the sample analysed). The workers of *L. acervorum* are about the same size but have much smaller Dufour glands (mean value of 0.14  $\mu$ g per ant) and do not contain (*E*)- $\beta$ -farnesene (Ali and Morgan, unpublished). Normally we find only liquid hydrocarbons (linear alkanes up to C<sub>17</sub>) in Dufour glands, higher linear alkenes would be expected to solidify. Unusually we find small percentages of C<sub>21</sub> and C<sub>23</sub> alkanes in *H. sublaevis*, but these small amounts would remain dissolved in the liquid mixture of lower alkanes, alkenes and branched alkanes, but they should have a small effect to reduce the volatility of the total mixture.

We hope later to collect some of the farnesenes for biological test to see whether they provide the repellent which *H. sublaevis* apply to the brood or the "propaganda substance". When pure, these highly unsaturated substances have limited stability in air, due to reaction with oxygen, but dissolved in the larger quantities of alkanes and alkenes they may have lifetimes appropriate to the needs of the raiding *H. sublaevis* within the raided nest, but without caus-

ing upset to their *Leptothorax* slaves by the time they return with their captives to their own nest.

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