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Hexadecanol and hexadecyl formate in the venom gland of formicine ants

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SUMMARY

The venom apparatus of workers of five representative species of formicine ant, *Anoplolepis custodiens*, *Camponotus vagus*, *Formica polyctena*, *Lasius niger* and *Polyrhachis gagates*, have all been shown to contain a mixture of hexadecanol, its formate and acetate, unaccompanied by homologous compounds. These compounds are not found in venom or in the venom reservoir but in the convoluted gland. It is suggested that the gland is lined with hexadecanol and its esters to protect the tissue from the corrosive venom.

1. INTRODUCTION

Wray (1670) first described the venom of formicine ants when he distilled wood ants and obtained a new acid which he called formic. Today it is recognized that all formicine ants have a venom consisting of a strong aqueous solution of formic acid, of concentration up to 60% (Stumper 1951, 1952; Osman & Brander 1961). Cavill & Clark (1971) reported that formic acid is the only volatile compound present in formicine venom, but the presence of peptides and free amino acids have also been reported in the venoms of *Formica polyctena* (Osman & Brander 1961) and *Camponotus pennsylvanicus* (Hermann & Blum 1968). Acetic acid is a minor component in at least some cases, as A. B. Attygalle (personal communication) has identified it in the venom of *Camponotus floridanus* and we have identified it in the venom of *Anoplolepis custodiens* (unpublished results). In the present work we have sometimes identified both formic and acetic acids.

Formic acid is also used as a defensive secretion of some other arthropods, including millipeds (Polydesmidae), Coleoptera and certain moth larvae (Blum & Hermann 1978). In the case of a carabid beetle producing a mixture of formic acid and alkyl esters as a defensive secretion, Attygalle *et al.* (1992) have again shown that 1% of the secretion is acetic acid.

The venom apparatus of ants consists essentially of three parts, the tubular filaments, attached at one end to the convoluted gland and otherwise floating in the haemolymph, the convoluted gland, which Billen (1990) has shown for *Solenopsis*, has the same cellular structure as the filaments but is coiled into a compact ball and attached to the third part, the reservoir, in which the venom is stored. Hefetz & Blum (1978) have shown that the formic acid of formicine ants is biosynthesized in the venom gland from a C_1 source with the aid of folic acid. It is presumed that synthesis occurs in the filaments and convoluted part of the gland. Hefetz & Blum (1978) refer to intact and homogenized glands but do not say if the reservoir was included or not.

In the course of examination of some formicine ants we found that either extracts of venom glands or whole venom apparatuses contained a small amount of hexadecyl formate accompanied by hexadecanol and hexadecyl acetate. Closer examination has shown that these compounds are not in the venom or its reservoir but are located in the convoluted gland. We have demonstrated their presence in five representative genera of the subfamily Formicinae.

2. MATERIALS AND METHODS

Insects were obtained from colonies of *Anoplolepis custodiens* and *Polyrhachis gagates* collected in South Africa and sent live to Keele. *Camponotus vagus* and *Lasius niger* collected in southern France and *Formica polyctena* from Belgium were sent live to Keele where they are maintained in artificial nests until dissection.

Individual venom glands and parts of glands were dissected under water with the aid of a binocular dissecting microscope and venom collected from the reservoir in glass capillaries as described by Morgan (1990). The dissected materials were freed of moisture and either solvent extracted in a small tissue grinder with hexane (100 µl) or sealed in glass capillaries for gas chromatography, using the solvent-less solid sampling method of Morgan & Wadhams (1972).

Gas chromatography–mass spectrometry was done with a Hewlett Packard 5890 gas chromatograph coupled to a 5970B Mass Selective Detector, which was controlled with a HP 59970C Chemstation. Chromatography was done on a fused silica capillary

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Table 1. *Average amounts of hexadecanol, its formate and acetate and their average proportions, in the venom glands of some formicine species*

(Results are averages of five determinations.)

	amount µg			proportion %		
species	$C_{16}OH$	C_{16} formate	C_{16} acetate	$C_{16}OH$	C_{16} formate	C_{16} acetate
Anaplolepis custodiens	0.12	0.90	0.1	11	82	9
Camponotus vagus	1.8	0.27	0.2	79	12	9
Formica polyctena	0.08	1.84	—	4	96	—
Lasius niger	0.7	1.1	0.03	38	60	2
Polyrhachis gagates	1.1	2.2	trace	33	67	—

column (12 m × 0.22 mm i.d.) coated with immobilized polydimethylsiloxane. The injection port temperature was 250°C. The sample capillary was held in the injection port for 3 min before crushing it. The column was initially at 30°C for 2 min after crushing, then raised to 250°C at 3°C min^{-1}.

The identification of compounds was confirmed by comparison of their mass spectra and retention times with those of commercial or synthesized materials.

Hexadecyl formate and hexadecyl acetate were prepared by heating hexadecanol (5 mg), formic acid (2 µl) and acetic acid (2 µl) together with a drop of concentrated sulphuric acid in a Keele Microreactor (Attygalle & Morgan 1986) for 12 h at 100°C. The reaction mixture was neutralized with $NaHCO_3$, extracted with hexane (200 µl) and a portion (1 µl) was injected (splitless) onto the chromatography column.

3. RESULTS

Three compounds identified as hexadecanol, hexadecyl formate and hexadecyl acetate were first observed in solvent extracts of *A. custodiens* and *P. gagates*. Three other species of formicine ant were then selected at random to represent other genera, i.e. *C. vagus*, *F. polyctena* and *L. niger*, and in each the same substances were identified. Hexadecanol is not an unusual substance in hymenopteran exocrine glands (Wheeler & Duffield 1988; Blum 1981), nor are various of its simple esters, but these are normally accompanied by homologous compounds (tetradecanol, octadecanol, etc.), but in these venom glands we found no evidence of homologues at all.

The proportions of the three substances varied with sample and species. Some values are given in table 1, together with the amounts of these substances per individual gland. The amounts did not vary greatly; the largest amount was 4.7 µg of hexadecyl formate in a sample of *F. polyctena*. The smallest were two samples of *A. custodiens* which contained 0.4 µg of hexadecyl formate, the major compound. The mean proportions hide a wide variation in the actual proportions found. For example, three out of four samples of *C. vagus* contained no hexadecyl formate. Hexadecyl acetate was sometimes absent, frequently an unquantifiable trace component, but in one sample of *A. custodiens* and one of *C. vagus* it was over 20%.

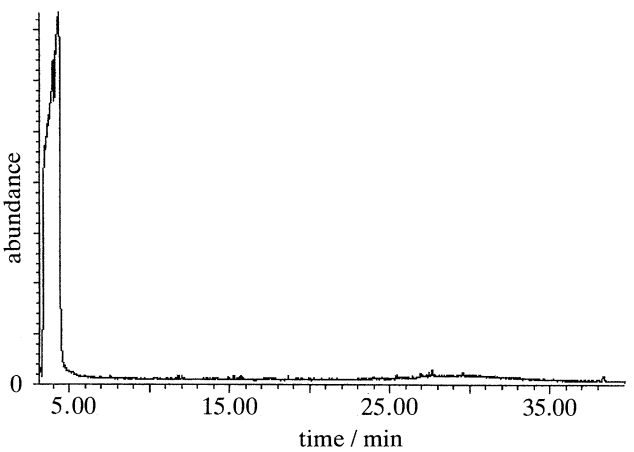

Figure 1. Total ion chromatogram of the secretion from a single venom reservoir of a worker of *Formica polyctena*. The only substance visible is formic acid (large peak at 4 min).

Frequently very small amounts of undecane and other hydrocarbons were present. These from their pattern were easily recognized as Dufour gland contaminants and indicate contamination with a very small proportion of the Dufour gland contents.

To investigate the location of the hexadecyl formate further, the venom was removed from the reservoir of

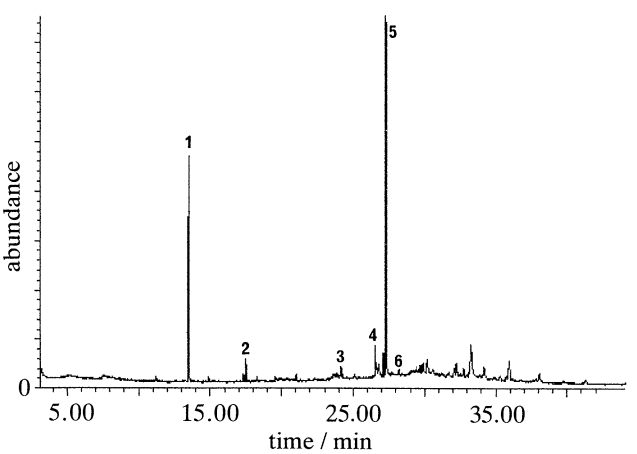

Figure 2. Total ion chromatogram of the empty venom apparatus of a single worker of *Formica polyctena* after the removal of the venom. Numbered peaks are: 1, undecane; 2, tridecane; 3, heptadecane (all contaminants from the Dufour gland secretion); 4, 1-hexadecanol; 5, hexadecyl formate; 6, hexadecyl acetate. Other peaks are produced by silicone products from the stationary phase.

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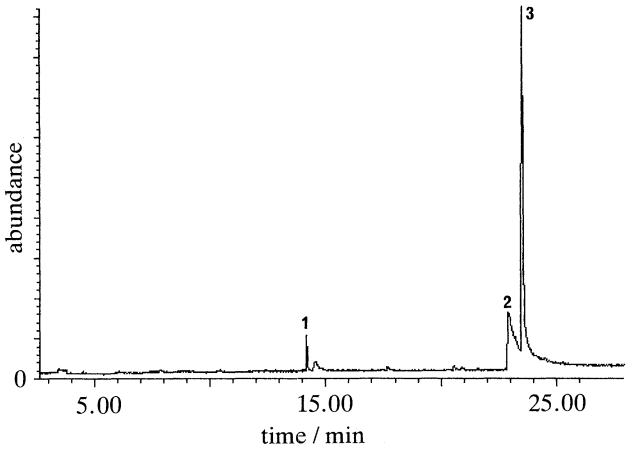

Figure 3. Total ion chromatogram from a single convoluted gland portion of the venom apparatus of a worker of *P. gagates*. Numbered peaks are: 1, tridecane; 2, hexadecanol; 3, hexadecyl formate.

some glands of *F. polyctena* and *P. gagates*, and the venom and the empty glands were chromatographed separately. The venom surprisingly did not contain the alcohol and esters (figure 1), but they were in the gland itself (figure 2). Further dissection of the gland located the compounds in the convoluted gland portion (figure 3), although it was absent from the reservoir and the filaments.

4. DISCUSSION

The venom glands of a random selection of five species of formicine ants have all been shown to contain microgram quantities of hexadecanol, hexadecyl formate and hexadecyl acetate in variable proportions. One of us (J.M.B.) has also identified hexadecyl formate in two further species, *Camponotus arminius* and *Polyrhachis schistacea*. These compounds are not located in the venom itself but are found in the convoluted gland. We did not find them in the filament part of the gland, but the filaments are much smaller than the convoluted section, and that makes a firm statement about the filaments more difficult. It was also surprising to find the hexadecanol and its esters unaccompanied by C_{14} or C_{18} homologues, as is usually found in nature for long-chain alcohols or carboxylic acids.

From our experiments, no explanation for the presence of these compounds was forthcoming. Water-insoluble long-chain compounds with an apolar chain and a polar end group, such as hexadecanol, form ordered monolayers on aqueous solutions. These monolayers of close-packed oriented molecules are known as Langmuir-Blodgett films. The films have unusual properties, for example, they inhibit evaporation and act as insulators. Although the venom reservoir has a thick cuticular lining, it would appear from Billen (1990) that the convoluted gland has no such protection. It is tempting to suggest (but difficult to envisage a technique for testing) that hexadecanol forms a monolayer film on the secretory ducts to protect cells from the corrosive venom. Contact of hexadecanol with the venom would convert some of it to formate and acetate. In some cases both these acids could be observed in the venom by their mass spectra. Langmuir-Blodgett films are best prepared from compounds with homogeneous chain length. This would be consistent with finding only C_{16} chains. The polar hydroxyl groups would be directed towards the venom. If these became esterified with polar formate and acetate groups they would alter the monolayer very little.

We have examined the venom glands of a large number of other ants, chiefly myrmicines, both those with alkaloidal and protein venoms and those such as attines with only vestigal stings, but have not encountered the present compounds in them. Hexadecanol and its formate and acetate in the venom gland have no correlation with the presence of esters or alcohols in Dufour gland secretions. Only hydrocarbons have been identified in the Dufour glands of *A. custodiens* (Schreuder & Brand 1972) and *F. polyctena* (Francke *et al.* 1980). Those of *C. vagus* (B. D. Jackson, unpublished results) and *L. niger* (Attygalle *et al.* 1987) contain hexadecanol and its acetate, but there, as in some other formicines, e.g. *Formica rufa* (Francke *et al.* 1985), a whole range of homologous alcohols and esters are found. *P. gagates* and *P. schistacea* Dufour glands contain primarily a series of hydrocarbons (L. C. Lopez, E. D. Morgan & J. M. Brand, unpublished results).

The mass spectra of alcohols, alkenes and formates are all very similar (Bagnères *et al.* 1991); the formates are best recognized by their retention times in gas chromatography and the very weak but characteristic mass spectral ions at m/z 47, corresponding to protonated formic acid.

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