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# THE ALARM-DEFENCE SYSTEM OF THE ANT *ACANTHOMYOPS CLAVIGER*

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**Abstract**—All of the principal and some of the minor volatile products of *Acanthomyops claviger* were identified chemically, their glandular source determined, and their functions analysed. Undecane was shown to be an efficient spreading agent for formic acid. All of the more abundant substances in the $C_{10}$–$C_{13}$ range, including undecane, were shown to be alarm pheromones, with behavioral threshold concentrations varying in order of magnitude from $10^{10}$ to $10^{12}$ and yielding potential signal distances in the centimetre range. Among the substances tested behaviorally, olfactory efficiency varies with molecular weight rather than structure. The $C_{10}$–$C_{13}$ substances are optimal for alarm signalling because, apparently on the basis of molecular weight alone, they combine moderate olfactory efficiency with sufficiently high vapour pressure to broadcast in the centimetre range when present in microgram quantities or less.

## INTRODUCTION

WE BEGAN this study with several goals in mind that could best be approached sequentially: (1) To examine in greater depth than previously attempted the chemistry and function of glandular secretions in a group of ant species; (2) to reconstruct the evolution of the chemical systems at the species level; and (3) to disentangle the relations of the communicative and defensive functions of the volatile secretory substances. The members of the ant subfamily Formicinae are well suited for this task. They store relatively large quantities of secretions in easily dissected reservoirs; abundant colonies of many phyletic stocks are available in the northern United States; and the phylogeny of the group, in particular the genus *Lasius* (WILSON, 1955), is comparatively well understood on morphological and zoogeographic grounds.

Moreover, a considerable amount of promising ground has already been broken in the chemistry and behaviour of the formicines. Carthy (1951) provided indirect evidence of the presence of a trail substance in the worker hind gut of *Lasius fuliginosus*; later HANGARTNER and BERNSTEIN (1964) proved it directly by the artificial trail test. BLUM and WILSON (1964) demonstrated by artificial trail tests that trail substances originate in the hind gut in various genera scattered widely through the Formicinae. QUILICO *et al.* (1956) discovered the furan

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dendrolasin in the mandibular gland secretion of *L. fuliginosus* workers; and the same group of researchers (BERNARDI *et al.*, 1967) later identified a variety of other volatile substances in the mandibular gland and hind gut of the same species, including *n*-tridecane, *n*-pentadecane, 2-tridecanone, 2-pentadecanone, and a previously unknown short-chain homologue of dendrolasin referred to as perillen. QUILICO *et al.* (1957) and PIOZZI *et al.* (1959) reported the presence of 2-tridecanone in workers of *L. umbratus* and *L. bicornis*. CHADHA *et al.* (1962) identified citronellal and citral as major components of the mandibular gland secretion of *Acanthomyops claviger*, and HAPP and MEINWALD (1965) demonstrated the biosynthesis of citronellal from labelled acetates and mevalonates fed to workers of the same species. GHENT (1961) showed that citronellal can serve as an alarm pheromone for *A. claviger* workers. MASCHWITZ (1964) demonstrated the existence of chemically unidentified alarm pheromones in the mandibular glands, Dufour's glands, and poison glands of workers of *Plagiolepis*, *Lasius*, and *Formica*. LAW *et al.* (1965) identified a variety of terpenoid alcohols in the hypertrophied mandibular glands of males of *Lasius* and *Acanthomyops* and showed that these substances are expelled during the nuptial flights. HÖLLDOBLER and MASCHWITZ (1965) reported that the mandibular gland secretions of male *Camponotus*, not yet chemically identified, function to help initiate the nuptial flights.

In this first report of a series we present the results of an intensive study of a single formicine species, *Acanthomyops claviger*, in which an attempt has been made for the first time to combine quantitative chemical and functional analyses.

## PROCEDURES IN CHEMICAL ANALYSIS

### *Sources of synthetic materials*

Citronellal, 2,6-dimethyl-5-hepten-1-al, citral, and undecane were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Tridecane and 2-tridecanone were obtained from Eastman Organic Chemicals, Rochester, New York. K and K Laboratories, Plainview, New York, were the suppliers of 2-pentadecanone and samples of the alkane series. All gas chromatography packing material and the OV-17 grease were obtained from Applied Science Laboratories, State College, Pennsylvania. Precoated thin-layer chromatography plates were purchased from Brinkmann Instruments Inc., Westbury, New York. The 2,6-dimethyl-5-hepten-1-ol was supplied by Dr. John H. Law.

### *Extraction of exocrine gland substituents*

Exocrine gland substances were extracted from *A. claviger* workers by crushing whole ants and separately dissected glands in carbon disulphide. The low response of carbon disulphide in the hydrogen flame detector makes it a particularly favourable solvent for the analysis of small amounts of volatile substances by gas chromatography. Because disturbed living workers discharge variable amounts of the substances, the ants were first chilled at 4°C for several days, during which time the glandular contents were observed to regenerate.

*Gas chromatographic analysis*

Qualitative analyses were performed on 7 ft × 0·25 in. glass columns packed with (a) 3 per cent OV-17 coated on 100/120 mesh Gas Chrom Q and (b) 15 per cent Apiezon L coated on 80/100 mesh Gas Chrom Q. An F & M Model 402 gas chromatograph equipped with hydrogen flame detectors was used in all gas chromatographic analyses.

Quantitation of exocrine gland components was achieved by adding dodecane to the total ant extracts as an internal standard at a level of 0·75 $\mu$g/mg of worker body weight. Relative peak areas for each compound were determined after corrections for the relative molar response of the hydrogen flame detector.

*Combination mass spectrometric–gas chromatographic analyses*

Mass spectrometric–gas chromatographic (MS–GC) analyses were performed on a Model 9000 LKB combination mass spectrometer–gas chromatograph fitted with an 8 ft × 0·25 in. glass column packed with 3 per cent OV-17 on 100/120 mesh Gas Chrom Q. The mass spectrometer was operated at an electron energy of 70 eV, accelerator voltage of 3·5 kV, and an ion source temperature of 250°C. The molecular separators and injection port were maintained at 150°C.

*Thin-layer chromatography*

Brinkmann precoated silica gel-G analytical thin-layer plates were used for all thin-layer separations. Dufour's gland substances were resolved by developing the plates in a hexane–acetone–ethanol (40 : 10 : 4) solvent system. Components of the mixture were detected by sulphuric acid spray or exposing to iodine vapour. Individual fractions were scraped from the plates before visualization and the compounds eluted with diethyl ether for gas chromatographic analysis. Formic acid was identified by developing the plates with a mixture of isopropanol, ammonium hydroxide, and water (100 : 5 : 15) and spraying with ammoniacal silver nitrate. The presence of formic acid was indicated by a black spot after the plates were heated in a 110°C oven for 15 min. The ammoniacal silver nitrate spray was 0·05 N silver nitrate in 2·5 N ammonium hydroxide.

*Trapping volatile odorants*

Volatile odorants were trapped from air streams by passing them through a 0·25 × 2 in. glass tube packed with 3 per cent OV-17 on Gas Chrom Q. The packing was retained in the traps by plugging both ends with glass wool. After the trap had been loaded with odorant, the trap packing material was transferred to the top of a regular gas chromatograph and purged with helium at room temperature. After igniting the flame, the trap contents were analysed in a normal temperature-programmed run.

## RESULTS OF CHEMICAL ANALYSIS

Gas chromatographic analysis of carbon disulphide extracts of *A. claviger* workers indicated the presence of at least fourteen volatile organic compounds

(Fig. 1). These compounds, which constituted some 0·43 per cent (9·6 $\mu$g) of the body weight of the whole ant, were located in the mandibular and Dufour's glands. Identification of these compounds was achieved by comparing their gas chromatographic and mass spectral properties with those of authentic standards. All compounds indicated in Fig. 1 were identified with the exception of compounds 1, 2, 5, and 9.

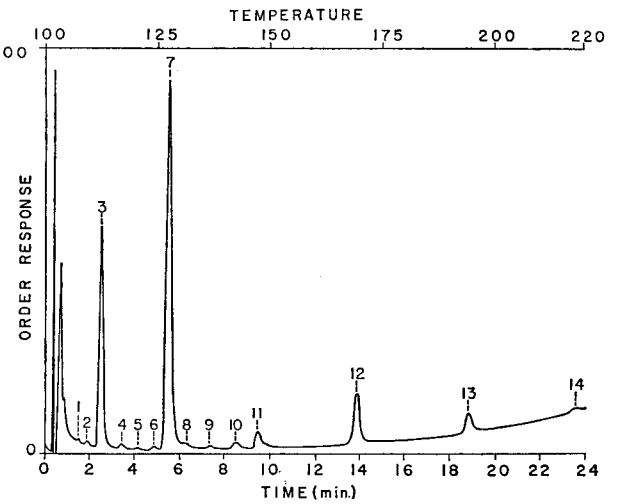


FIG. 1. Gas chromatogram of the total extract of *A. claviger* workers.

*Identification of the mandibular gland substances*

Excised mandibular glands were found to contain compounds 4, 6, 7, 9, 10, and 11 of Fig. 1. The total amount of organic compounds/gland of an average worker is 0·21 per cent (4·7 $\mu$g) of its body weight. All quantitative measurements were made by using the internal standard technique described in the experimental section.

Compounds 7, 10, and 11, which CHADHA *et al.* (1962) have identified as citronellal and the two citral isomers, constitute 98 per cent of the mandibular gland contents. An average worker's mandibular gland contains 4·3 $\mu$g of citronellal and 0·3 $\mu$g of the citral isomers. Compounds 4 and 6 are present at concentrations of 22 ng and 54 ng/gland respectively.

The mass spectra of compound 4, presented in Fig. 2, and of compound 6 indicate the presence of intense fragment ions at m/e = 69, 67 and 41. These ions have been associated by VON SYDOW (1963, 1964) with the terminal 1,1-dimethyl-$\Delta^1$-propenyl moiety of acyclic terpenes, thus suggesting a terpene skeleton for these compounds. The presence of fragments at M-18 suggested the elimination of water and therefore the presence of oxygen in both compounds. With molecular ions of m/e = 140 and 142 for compounds 4 and 6, empirical formulas of $C_9H_{16}O$ and $C_9H_{18}O$ were proposed.

The empirical formula and general mass spectral features of compound 6 appeared to be the same as the 2,6-dimethyl-5-hepten-1-ol identified by LAW *et al.* (1965) from the mandibular gland of *A. claviger* males. Instrumental analysis of an authentic sample of 2,6-dimethyl-5-hepten-1-ol showed it to be identical to compound 6.

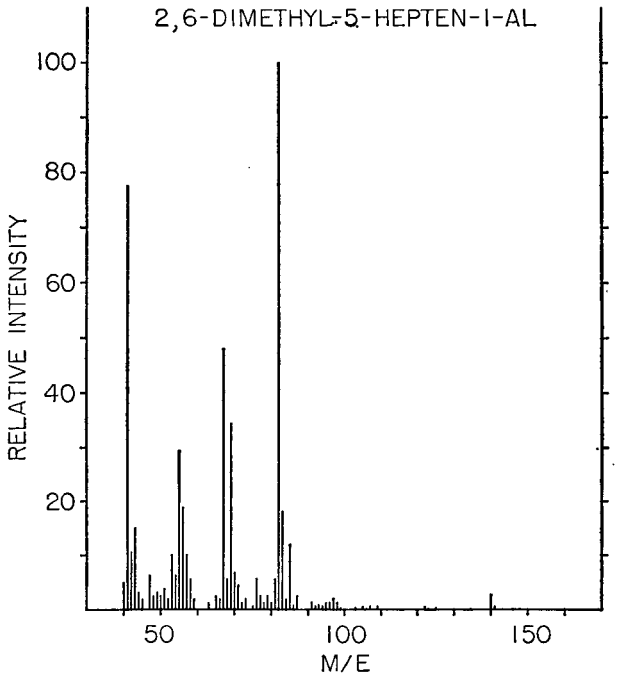


FIG. 2. Mass spectrum of 2,6-dimethyl-5-hepten-1-al.

The similarity of the spectra of compounds 4 and 6 made it seem probable that compound 4 was the aldehyde of compound 6. The base peak at m/e = 82 in the spectrum of compound 4 (Fig. 2) is atypical in the acyclic terpenes and is therefore of considerable value in structure assignment. This ion is probably the result of a MCLAFFERTY (1959) type rearrangement with stabilization and charge retention on the olefin fragment. The structural requirements for this transition strongly indicated that compound 4 was 2,6-dimethyl-5-hepten-1-al. Identical mass spectral and gas chromatographic behaviour of the unknown and the synthetic standard confirmed this structural assignment.

*Identification of Dufour's gland substances*

Excision and analysis of the contents of Dufour's glands from several *A. claviger* workers localized compounds 1, 2, 3, 5, 8, 12, 13, and 14 in Fig. 1 in this gland. The concentration of these compounds in the Dufour's gland of an average worker was 4·8 $\mu$g or 0·21 per cent of the body weight of the worker. The high volatility of compounds 1 and 2 prevented identification of these compounds.

Preliminary analysis of Dufour's gland substances was done by thin-layer chromatography on silica gel-G plates. Two spots were located which were scraped from the plate and eluted with diethyl ether. Gas chromatographic analysis of the fraction at $R_f$ 0·93 indicated the presence of compounds 3 and 8

TABLE 1—A TABULATION OF THE INTENSE IONS IN THE SPECTRA OF THE HYDROCARBONS IN DUFOUR'S GLAND

| | Relative intensity | |
|---|---|---|
| m/e | Undecane | Tridecane |
| M* | 10·8 | 7·9 |
| M–29 | 3·3 | 1·3 |
| M–43 | 5·9 | 5·3 |
| 99 | 7·5 | 7·9 |
| 85 | 26·5 | 33 |
| 71 | 45 | 55 |
| 57 | 100 | 100 |
| 55 | 12·5 | 13·2 |
| 43 | 88 | 84 |
| 41 | 26 | 27 |

* M Indicates the molecular ion.

TABLE 2—A TABULATION OF THE INTENSE IONS IN THE SPECTRA OF THE METHYL KETONES IN DUFOUR'S GLAND

| | Relative intensity | | |
|---|---|---|---|
| m/e | 2-Tridecanone | 2-Pentadecanone | Compound 14 |
| M* | 2·5 | 2·1 | 1·8 |
| M–15 | 1·0 | 0·8 | 0·7 |
| M–18 | 0·5 | 0·5 | 0·5 |
| M–43 | 0·5 | 0·4 | 0·3 |
| M–58 | 3·0 | 2·0 | 1·4 |
| 71 | 34·5 | 36·2 | 37·6 |
| 58 | 100 | 100 | 100 |
| 43 | 88 | 90 | 82·5 |
| 41 | 27·1 | 28·8 | 28·6 |

* M Indicates the molecular ion.

in Fig. 1. This behaviour on thin-layer chromatography suggested that these compounds were hydrocarbons. A tabulation of the intense ions in the mass spectra of these compounds will be seen in Table 1. These spectra are distinctly those of normal aliphatic hydrocarbons with molecular weights of 156 and 184; suggesting *n*-undecane and *n*-tridecane respectively. Identical mass spectral and

gas chromatographic properties for the unknowns and synthetic *n*-undecane and *n*-tridecane confirmed the structures of compounds 3 and 8.

Dufour's gland substances 12, 13, and 14 of Fig. 1 were located gas chromatographically in the $R_f$ 0·73 thin-layer fraction. These compounds on mass spectral analysis gave intense ions at m/e = 43 and 58 with molecular ions at m/e = 198, 226, and 252 as is indicated in Table 2. The similarity of these spectra and the difference of 28 mass units in molecular weights suggested a homologous series with a $C_2H_4$ increment. The additional peaks at M-15, M-43, and M-58 indicated that these compounds were methyl ketones. Likely candidates were 2-tridecanone, 2-pentadecanone, and 2-heptadecanone. Authentic samples of 2-tridecanone and 2-pentadecanone were identical to compounds 10 and 11 on MS–GC analysis. Since 2-heptadecanone was not available for analysis the structure of compound 14 is still tentative.

TABLE 3—RELATIVE CONCENTRATION OF DUFOUR'S GLAND SUBSTANCES IN A SINGLE GLAND

| Compound | Amount (μg) | Total (%) | Body weight (%) |
|---|---|---|---|
| Undecane | 2·48 | 50·4 | 0·11 |
| Tridecane | 0·02 | 0·4 | 0·0009 |
| 2-Tridecanone | 1·88 | 38·2 | 0·084 |
| 2-Pentadecanone | 0·45 | 9·1 | 0·020 |
| Compound 12 | 0·09 | 1·8 | 0·004 |

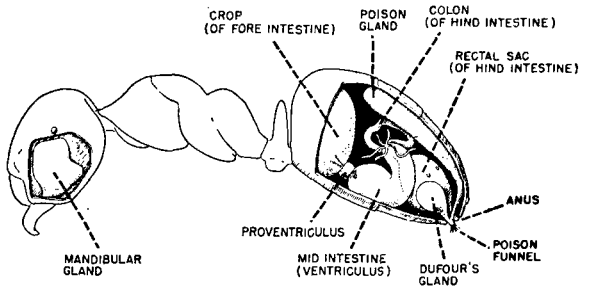


FIG. 3. Location of the exocrine glands of the *A. claviger* worker included in the present study.

Concentrations of the Dufour's gland substances are found in Table 3. Undecane is the principal constituent of this gland. The other saturated hydrocarbon identified is present in only a trace. Tridecanone is the primary methyl ketone while pentadecanone and compound 14 are present in smaller concentrations.

The location of the glands and a summary of the substances identified in them are given in Figs. 3 and 4. The existence of a trail substance in the hind

gut vesicle was established by artificial trail tests by the method of WILSON (1959), but the chemistry of the pheromone has not yet been pursued.

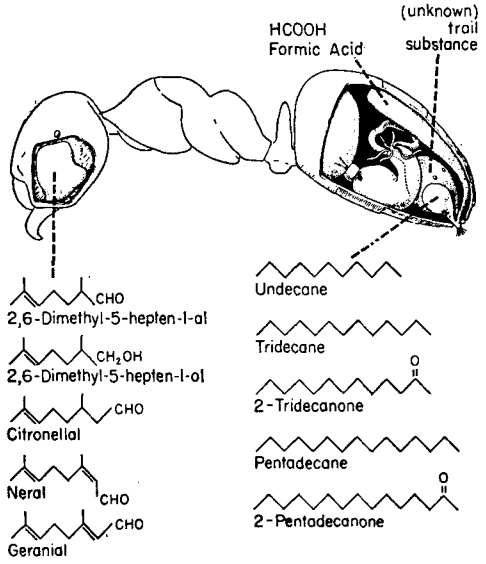


FIG. 4. Structural formulas of volatile substances found in various of the exocrine glands.

*Venom composition*

The work of STUMPER (1952) has indicated that most of the Formicinae produce formic acid which is released in fine droplets from the venom gland as a defence substance when the ant is attacked. It was suggested by STUMPER (1959) that the Dufour's gland contents were also released during the act of stinging. This hypothesis was tested by collecting and analysing the venom for formic acid and the various organic constituents of Dufour's gland.

Venom was collected by inducing the ants (with the aid of tweezers) to eject onto thin-layer chromatography plates and glass slides coated with OV-17. The thin-layer plates were developed with isopropanol–ammonium hydroxide–water and sprayed with ammoniacal silver nitrate. Since formic acid reduces this spray to metallic silver, the presence of this acid was indicated by a black spot at $R_f$ 0·31. This test is specific for reducing acids and therefore easily differentiates formic acid from other aliphatic acids.

Elution and gas chromatographic analysis of the venom components trapped in the OV-17 grease on glass slides showed the presence of all of the Dufour's gland substances. Thus it may be concluded that the venom gland and Dufour's gland contents are discharged simultaneously during the act of spraying.

## ANALYSIS OF DEFENSIVE FUNCTION

The presence of undecane and tridecanone in large quantities in the Dufour's gland, an organ associated intimately with the poison gland, suggested to us that

these compounds might serve as defensive substances in conjunction with formic acid. Since the venom gland and Dufour's gland contents are rapidly forced out of a common orifice, the venom probably emerges as a dispersion of the aqueous formic acid in the predominantly hydrocarbon mixture from Dufour's gland. When ants were induced to attack a clean glass surface or the cuticle of *Periplaneta americana*, the venom was seen to spread rapidly over a large surface area. The undecane in venom deposited on a glass surface evaporates in several minutes leaving tiny droplets of formic acid on the surface. Quantitation of the physical behaviour of venom on surfaces was achieved using venom reconstituted from synthetic compounds, since large quantities of natural venom were difficult to obtain. Reconstituted venom consisted of Dufour's gland substances in the ratios described in Table 3 and 50 per cent formic acid mixed and emulsified shortly before using. Synthetic emulsions of the Dufour's gland constituents and 50 per cent aqueous formic acid (1 : 10) behaved in roughly the same manner as native venom. The synthetic venom was observed to spread over ten times greater surface area on *P. americana* cuticle than an equal volume of 50 per cent formic acid alone. The low surface tension of undecane appears to be the primary reason for the enhancement of spreading. Droplets of formic acid move easily over surfaces when released with undecane alone.

## ANALYSIS OF COMMUNICATIVE FUNCTION

The rôle of citronellal as an alarm pheromone in *A. claviger* has already been established (GHENT, 1961), and the occurrence of unidentified alarm pheromones in the poison and Dufour's glands has been indicated by the experiments of MASCHWITZ (1964). In our studies we undertook to determine the precise nature of the behavioural response to citronellal, citral, and each of the newly identified substances, and, of more importance, the behavioural threshold concentrations of the substances in molecules/cm$^3$ of air. Although the latter measurement had not been attempted by previous workers, it is nevertheless essential to the understanding of function, because only when the threshold concentration is known can the effectiveness of the substance and its transmission characteristics be determined.

### *The alarm response*

The tests were conducted on groups of resting workers in artificial nests. The response to the various glandular products diffusing through air is basically the same for all but varies in intensity as molecular concentration is increased. The variation can be expressed as a gradient of ever stronger responses arbitrarily divided into four ascending degrees, as follows. (1) The funiculi of the antennae are raised and both scape and antennae extended forward and moved in an exploratory fashion through the air. (2) The antennae are moved as just described, and the mandibles are opened. (3) The antennae and mandibles are moved as described, and the ant begins to walk; if it walks more than a few steps, it usually turns in the direction of the odour source, evidently using the antennae for osmotropotactic orientation in the still air; after walking part-way to the source it

turns back and its excitement dies down. (4) The behaviour is as just described in (3) but the ant walks or runs all the way to the odour source. We arbitrarily refer to a given response exhibiting some intensity from (1) to (3) as a *weak response* and a response of intensity (4) as a *strong response.*

Communication of alarm by pheromones was demonstrated in laboratory colonies by seizing workers by the appendages with a pair of clean forceps and holding them slightly off the nest floor just within the entrance. A strong alarm response spread through resting workers nearby to a maximum distance of about 10 cm within 90 sec. The rate of spread was at least roughly commensurate with that predicted by the gas diffusion model (see below) for quantities of the known substances held within the reservoirs of single ants. When workers were crushed, the spread was much faster and the maximum distance greater, also as predicted by the model. When quietly anaesthetized workers were presented in the same fashion, the response was elicited only over much shorter distances.

Finally, it remained to be demonstrated that alarmed workers discharged both the mandibular and Dufour's gland substances when they were alarmed. This was accomplished by aspirating a stream of air over 100 workers in a $1 \times 30$ cm glass tube and trapping the organic compounds entrained in the air. The construction of traps and the analysis of their contents are described in the section on procedures in chemical analyses. Anemonic agitation from the air stream was sufficient to cause the workers to go into typical alarm behaviour. Gas chromatographic analysis of the trap contents indicated the presence of both mandibular and Dufour's gland components in approximately the same relative concentrations as in Fig. 1. These results indicate (1) that the animal does not have to be under attack to release Dufour's gland substances and (2) that both the mandibular and Dufour's glands are discharged when a worker is alarmed.

*Measurements of threshold concentrations*

*Method.* The model for continuous emission of an odorant in still air developed by BOSSERT and WILSON (1963, p. 445, equation 2.3) was modified for use in the present study. Because the new method is both rather involved and of potential wide applicability in olfaction and behaviour studies, a full account is being presented in a later paper. It includes the following essential steps: (a) a droplet of the pure pheromone is placed in a capillary applicator tube near one end, and the opposite end is sealed; (b) after 24 hr tests are conducted by inserting the open end of the applicator tube into artificial nests and halting it within 4 cm of groups of resting workers; then the distance from the opening of the tube and time to onset of the response are recorded; (c) during the period of testing the rate of evaporation of the droplet is measured volumetrically. The behavioural threshold concentration $K$, in molecules/cm$^3$ is then estimated by the formula,

$$K = \frac{Q}{2D\pi r} \operatorname{erfc}\left(\frac{r}{\sqrt{4Dt}}\right),$$

where $Q$ is the evaporation rate from the tube in molecules/sec, $D$ is the diffusion

TABLE 4—DATA FROM BEHAVIOURAL EXPERIMENTS AND ESTIMATED $K$ OF *A. claviger* SECRETORY PRODUCTS (INDICATED BY ASTERISK) AND ADDITIONAL SELECTED COMPOUNDS

| Substance | Nest temp. (°C) | $D$: diffusion coefficient | $Q$: evaporation (Mol/sec) | $r$: distance to ant (cm) | $t$: time to response (sec) | Response intensity | Estimated $K$: threshold concentration (Mol/cm$^3$) |
|---|---|---|---|---|---|---|---|
| Formic acid* | 27 | 0·18 | $>10^{5}$ | 1·5 | — | No response in 60 sec | — |
| | 27 | 0·18 | $>10^{15}$ | 2·0 | — | No response in 60 sec | — |
| | 27 | 0·18 | $>10^{15}$ | 1·0 | 35·0 | Weak | $1·93\times10^{15}$ |
| 2,6-Dimethyl-5-hepten-1-ol* | 27 | 0·06 | $8·46\times10^{12}$ | 1·5 | 3·5 | Strong | $3·09\times10^{11}$ |
| | 27 | 0·06 | $8·46\times10^{12}$ | 1·0 | 3 | Strong | $2·15\times10^{12}$ |
| Citronellal* | 27 | 0·06 | $5·65\times10^{11}$ | 1·6 | 9·5 | Strong | $1·26\times10^{11}$ |
| | 27 | 0·06 | $5·65\times10^{11}$ | 1·0 | 9 | Strong | $5·03\times10^{11}$ |
| | 27 | 0·06 | $5·65\times10^{11}$ | 3·0 | 19·0 | Strong | $2·35\times10^{10}$ |
| | 27 | 0·06 | $5·65\times10^{11}$ | 1·5 | 7 | Strong | $1·02\times10^{11}$ |
| | 27 | 0·06 | $5·65\times10^{11}$ | 2·0 | 11 | Strong | $6·12\times10^{10}$ |
| Citronellol (male substance only)* | 27 | 0·06 | $4·85\times10^{11}$ | 1·5 | 3 | Strong | $1·07\times10^{10}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 1·5 | 4 | Strong | $2·61\times10^{10}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 2 | 4 | Strong | $2·51\times10^{9}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 1·2 | 2 | Strong | $1·54\times10^{10}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 1·3 | 3 | Strong | $3·00\times10^{10}$ |
| Citral* | 27 | 0·06 | $4·85\times10^{11}$ | 1·5 | 7 | Strong | $1·23\times10^{11}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 1·3 | 3·5 | Strong | $6·54\times10^{10}$ |
| | 27 | 0·06 | $4·85\times10^{11}$ | 1·2 | 2 | Strong | $2·26\times10^{10}$ |
| 2-Tridecanone* | 27 | 0·05 | $2·21\times10^{11}$ | 0·8 | 1·5 | Strong | $3·42\times10^{10}$ |
| | 27 | 0·05 | $2·21\times10^{11}$ | 1·5 | 5 | Strong | $1·59\times10^{10}$ |
| | 27 | 0·05 | $2·21\times10^{11}$ | 1·6 | 8 | Strong | $3·24\times10^{10}$ |
| Undecane* | 26 | 0·06 | $5·02\times10^{12}$ | 1·0 | 1·8 | Strong | $4·19\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·8 | 8·8 | Strong | $5·19\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·2 | 2·4 | Strong | $2·82\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·0 | 4·0 | Strong | $1·98\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·2 | 6·0 | Weak | $1·75\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 0·6 | 2·0 | Strong | $4·90\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·2 | 4·0 | Strong | $9·24\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 2·8 | 8·8 | Strong | $3·07\times10^{10}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·5 | 6·2 | Strong | $7·28\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·8 | 5·1 | Strong | $1·58\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·3 | 2·4 | Weak | $1·58\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·0 | 3·5 | Strong | $1·64\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·5 | 8·0 | Strong | $1·12\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·0 | 10·2 | Strong | $4·87\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·8 | 4·6 | Strong | $1·14\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·2 | 1·6 | Strong | $6·86\times10^{10}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·0 | 3·8 | Strong | $1·85\times10^{12}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·3 | 4 | Strong | $6·21\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·8 | 7 | Strong | $3·27\times10^{11}$ |
| | 26 | 0·06 | $5·02\times10^{12}$ | 1·5 | 2·5 | Strong | $5·49\times10^{10}$ |
| Butyl ether | 26 | 0·07 | $1·01\times10^{10}$ | 1·4 | 24 | Strong | $7·30\times10^{13}$ |
| | 26 | 0·07 | $1·01\times10^{10}$ | 0·6 | 8 | Strong | $2·18\times10^{14}$ |
| | 26 | 0·07 | $1·01\times10^{10}$ | 0·8 | 10·4 | Weak | $1·46\times10^{14}$ |
| | 26 | 0·07 | $1·01\times10^{10}$ | 1·2 | 60·6 | Strong | $1·30\times10^{14}$ |
| Methyl decanoate | 26 | 0·05 | $5·29\times10^{12}$ | 0·6 | 2·4 | Strong | $6·19\times10^{12}$ |
| | 26 | 0·05 | $5·29\times10^{12}$ | 1·0 | 4 | Strong | $1·92\times10^{12}$ |
| | 26 | 0·05 | $5·29\times10^{12}$ | 1·2 | 7·4 | Strong | $2·29\times10^{12}$ |
| | 26 | 0·05 | $5·29\times10^{12}$ | 1·2 | 27 | Strong | $6·53\times10^{12}$ |

Each row represents a single trial.

coefficient of the substance in air, in $cm^3/sec$, $r$ is the distance in cm from the tip of the tube to the antennae of the first reacting ant, $t$ is the time in seconds to the onset of reaction of the first ant, and erfc($x$) is the complementary error

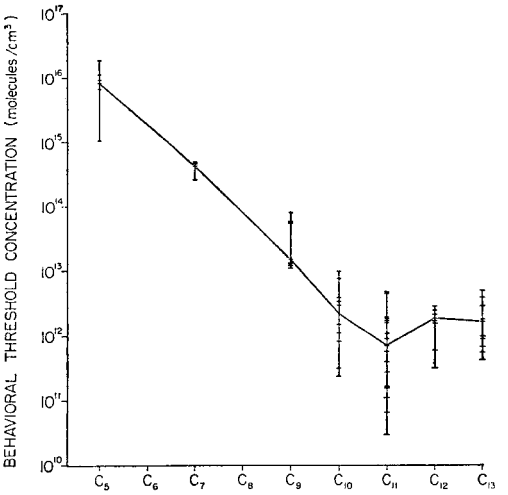


FIG. 5. Estimates of the behavioural threshold concentrations ($K$) of members of the alkane series, from pentane ($C_5H_{12}$) to tridecane ($C_{13}H_{28}$). Undecane ($C_{11}H_{24}$) and tridecane are the hydrocarbons that occur naturally in the Dufour's gland of *A. claviger*. The evaporation rates used in the estimates are given in Table 5.

TABLE 5—RESPONSES OF *A. claviger* WORKER GROUPS TO MEMBERS OF THE ALKANE SERIES, WHERE THE SUBSTANCES WERE ALLOWED TO EVAPORATE FROM DROPLETS INSIDE CAPILLARY TUBES 1 mm i.d., AT 26–27°C

| | No. of strong responses | No. of weak responses | No. not responding | Total No. of trials | Artificial $Q$ (Mol/sec) | Trials giving responses (%) |
|---|---|---|---|---|---|---|
| $C_5H_{12}$ | 0 | 6 | 4 | 10 | $8{\cdot}28 \times 10^{15}$ | 60 |
| $C_7H_{16}$ | 1 | 3 | 6 | 10 | $6{\cdot}36 \times 10^{14}$ | 40 |
| $C_9H_{20}$ | 6 | 2 | 2 | 10 | $3{\cdot}64 \times 10^{13}$ | 80 |
| $C_{10}H_{22}$ | 8 | 2 | 0 | 10 | $1{\cdot}40 \times 10^{13}$ | 100 |
| $C_{11}H_{24}$ | 9 | 1 | 0 | 10 | $5{\cdot}02 \times 10^{12}$ | 100 |
| $C_{12}H_{26}$ | 0 | 8 | 2 | 10 | $1{\cdot}30 \times 10^{12}$ | 80 |
| $C_{13}H_{28}$ | 2 | 1 | 7 | 10 | $\sim 3{\cdot}50 \times 10^{11}$ | 30 |
| $C_{15}H_{32}$ | 0 | 0 | 10 | 10 | $\sim 1{\cdot}00 \times 10^{11}$ | 0 |
| $C_{17}H_{36}$ | 0 | 0 | 10 | 10 | $\sim 9{\cdot}00 \times 10^{10}$ | 0 |

The symbol $\sim$ indicates that $Q$ was estimated by extrapolation of the curve from the lower numbers.

function (values of this function are given in CARSLAW and JAEGER, 1947, Table 8).

All measurements in the present study were made on queenless groups of several hundred workers, collected from a large colony in woodland at Lexington, Massachusetts, and housed in 12 in. wide artificial Plexiglas nests of the kind described by WILSON (1962).

*Results.* The estimates of the behavioural threshold concentrations ($K$) and the measurements on which they were based are given in Table 4 and Fig. 5. Percentages of responses to members of the alkane series allowed to evaporate from 1 mm wide capillary tubes are given in Table 5.

Because the discovery that undecane is an efficient odorant came as a surprise to us, additional precautions were taken. Synthetic material was further purified both by hydrogenation, to remove double bonds that might have existed in a small fraction of the molecules, and by chromatographic refinement. The $K$ values obtained with the treated undecane sample were consistent with those obtained with the untreated samples.

## DISCUSSION

It is now apparent that the venom of the formicine ant *A. claviger* is composed of a series of aliphatic hydrocarbons and ketones in addition to formic acid. The studies of OTTO (1960) and OSMAN and KLOFT (1961) have indicated that the principal venom toxicant in the Formicinae is formic acid. However, it is also possible that undecane provides some toxicity. GILBY and COX (1963) have shown that small amounts of aliphatic hydrocarbon placed on the cuticle of cockroaches causes a loss of co-ordinated movement and in some cases death. Aside from the toxic effects of the Dufour's gland substances, these studies have indicated that they are also spreading agents for the formic acid in the venom.

Since the molecular weight of the organic venom constituents does not exceed 256, most of the venom is volatile. Undecane and formic acid are particularly volatile. When venom is deposited on a surface, these components will either penetrate the surface or evaporate into the surrounding air. The formic acid which penetrates the cuticle kills the underlying tissue while the Dufour's gland substances may disrupt the cuticular lipids and block the tracheae. Formic acid evaporating from the surface will form a repellent shield while the undecane emanating from the source broadcasts a continuous alarm. Emission from this source will remain constant until it is depleted.

Spreading the venom over the largest possible area has several advantages for the ant. When under attack, *A. claviger* attempts to protect itself by biting and spraying its enemy. This biting results in numerous abrasions on the cuticle of the attacker. Increasing the area over which the venom is spread increases the possibility that the toxic components will come in contact with tracheae or one of the cuticular abrasions. It also increases the rate of evaporation of volatile venom constituents. This enables an ant under attack to establish a defensive shield more quickly and to communicate alarm over greater distances.

There can be little doubt that undecane actually serves as both a defensive substance and as an alarm pheromone. If the entire contents of the Dufour's gland, containing 2·48 $\mu$g of undecane, were discharged as a puff from the poison funnel, the diffusion model of BOSSERT and WILSON (1963, p. 446) predicts that the pheromone signal would reach a maximum of about 20 cm in still air. If, on the other hand, only 0·1 per cent were discharged, the signal would still reach a maximum of 2 cm. Experiments with living ants show that the signals, generated by all the volatile substances combined, actually reach a maximum of 10 cm or more.

Similarly, 2-tridecanone, citronellal, and citral must contribute significantly to the alarm signal by virtue of both their moderately low $K$ values and relatively high concentrations in the Dufour's gland and mandibular gland reservoirs. Also, the Dufour's gland and two mandibular glands are roughly comparable in their signalling capacity. In other words, about as effective a signal is generated from the head as from the abdomen. When agitated, *A. claviger* discharges copiously from both ends. The mandibular glands produce citronellal and citral in total quantity several times greater than the combined quantity of undecane and 2-tridecanone from the Dufour's gland, yet the difference is offset by the greater olfactory efficiency of 2-tridecanone.

Formic acid, on the other hand, is not an effective pheromone. Although it is discharged in large quantities from the abdomen, it is exceedingly ineffective as an odorant. Tridecane, 2-pentadecanone, 2-heptadecanone, 2,6-dimethyl-5-hepten-1-ol, and 2,6-dimethyl-5-hepten-1-al are probably ineffective for the opposite reason: they are efficient odorants but produced in too small a quantity. Moreover, tridecane, 2-pentadecanone, and 2-heptadecanone have too low vapour pressures to permit them to signal over centimetre distances, although they might conceivably serve as residual signals operating at very close range after most of the more volatile substances have dispersed.

The apparent positive correlation, among the natural *A. claviger* volatile substances, between molecular weight and olfactory efficiency led us to examine the efficiency of other selected substances. The tests with the alkane series produced results consistent with this simple hypothesis. Note that the relationship is not altogether an elementary one. The efficiency, measured by $K$, levels off in the $C_{10}$–$C_{13}$ range rather than continuing to increase in a straight line (Fig. 5). Our inability to obtain many responses in the $C_{15}$–$C_{19}$ range (Table 5) is undoubtedly due to the low evaporation rate of the substances from the 1 mm tubes rather than to lower olfactory efficiency. The molecular weight hypothesis was also supported by the $K$ values obtained for butyl ether and methyl decanoate (Table 4). The $K$ values are consistent with the molecular weight of the two substances but not with their structures, which are radically different from those of the natural secretory products.

It would seem that substances in the $C_{10}$–$C_{13}$ range are optimally efficient with respect to the alarm function. First, the relation between molecular weight and olfactory efficiency is such that the $C_{10}$–$C_{13}$ substances are vastly more

effective, molecule for molecule, than substances of lesser molecular weight. At the same time, their vapour pressures are still high enough to generate active spaces of centimetre distances from discharge of microgram quantities or less, without the aid of wind, and during a period of only a few seconds. The latter feature does not occur in slightly heavier substances, for example alkanes in the range above $C_{13}$.

Finally, we conclude that at least in the case of undecane, the defensive and communicative functions are combined. It is strictly correct to refer to undecane as both a defensive substance and a pheromone. This conclusion nevertheless leaves open for future analysis two important questions: Which function appeared first in evolution, and to what degree have the secretory substances been modified in evolution to serve either or both of the functions?

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