CHEMICAL INVESTIGATIONS OF EXOCRINE GLANDS IN SOME *MYRMICA* AND ATTINE ANTS.

BY

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A thesis submitted to the University of Keele in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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May 1982

Unless otherwise stated, all the work reported in this thesis was carried out by the author under the supervision of Dr. E.D. Morgan.

R.P. Evershed

ACKNOWLEDGEMENTS

In presenting this thesis I would like to thank:-

Dr. E.D. Morgan for his patience and guidance.

Professor I.T. Millar and the University of Keele for providing the laboratory facilities.

The S.R.C. for providing the financial support.

Dr. M.C. Cammaerts of the University of Brussels for her collaboration and for performing many of the bioassays.

Dr. A. Mudd of Rothampstead Experimental Station, Harpenden, Hertfordshire, for his collaboration.

Dr. M. Cherrett of University College of North Wales, Mr. C.A. Collingwood of Leeds Museum, Mrs. J. Wardlaw of Furzbrook Experimental Station, Dr. Nielsen of Jutland, Denmark and Rothampstead Experimental Station for providing the ant colonies.

Mr. J. Billen of the University of Limberg, Belgium for preparing the thin sections.

All the technical staff of the Departments of Chemistry and Biology at the University of Keele whose assistance was invaluable; special thanks to Messrs. J. Clews, C. Cork, P. Holbrook, D. Mountford, T. Alston and T. Bolin.

My wife Sue for her understanding and assistance in typing this thesis.

TO MY WIFE

AND PARENTS

ABSTRACT

By employing a combination of microanalytical techniques including solid sampling, reaction gas chromatography, gas chromatography, bioassay and linked gas chromatography-mass spectrometry, the volatile constituents of the venom, Dufour and mandibular glands of workers of eleven species of myrmicine ant (including two sub-species) have been examined.

Trail following behaviour in the ant *M. rubra* is evoked by a single substance, 3-ethyl-2,5-dimethylpyrazine, identified from the venom of this species. Seven other *Myrmica* species which follow trails made with each other's venom also produce this substance in similar quantities. Analysis of ten single venom glands by a solid sampling technique showed *M. rubra* workers to contain on average 5.8± 1.7ng of the trail substance.

The same solid sampling technique has been used to quantify the major trail pheromone components in *A. octospinosus*, *A. cephalotes*, *A. s. sexdens* and *A. s. rubropilosa*.

The Dufour glands of the above Attines and those of *M. ruginodis*, *M. sabuleti* and *M. lobicornis* have been shown to contain species specific blends of either predominently linear or terpenoid hydrocarbons in the C_{12} to C_{23} chain length range. In common with *M. rubra* the major glandular component of *M. ruginodis* is heptadecene, while *M. sabuleti* and *M. lobicornis*, like *M. scabrinodis*, produce predominently farnesene and homologous terpenoid hydrocarbons. Of the Attines, *A. octospinosus* also produces terpenoid material, homofarnesene is its major glandular component. *A. cephalotes* on the

other hand contains mainly n-heptadecane. There are quantitative differences in the hydrocarbon compositions of the two A. sexdens sub-species. The major glandular component of A. s. sexdens is (Z)-9-tricosene, while in A. s. rubropilosa the most abundant component is (Z)-9-nonadecene. From a chemical taxonomic viewpoint A. s. rubropilosa and A. s. sexdens could be regarded as separate species.

All the above species produce microgram amounts of hydrocarbon material in their Dufour glands with the exceptions of A. octospinosus and A. cephalotes which produce nanogram quantities. In the Attines there is a linear relationship between body size and hydrocarbon content.

The mandibular glands of M. schencki, M. rugulosa, M. sulcinodis, M. ruginodis, M. sabuleti and M. lobicornis have been shown to contain microgram amounts of similar mixtures of compounds in species specific proportions. The mixtures are composed of the same 3-alkanones and 3-alkanols in the C_5 to C_{10} chain length range that are found in M. rubra and M. scabrinodis. The highly volatile portion of this secretion in these latter two species consists mainly of ethanal, propanone and methylpropanal. The identification of methylpropanal has required a correction be made to previous work. Nanogram quantities of these three compounds are found in similar proportions in the mandibular glands of other Myrmica species.

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INTRODUCTION

INTRODUCTION

It is essential for most animals to communicate with their environment or members of their own species. Three distinct modes of communication have been observed in the animal kingdom:

1. Chemical (olfactory or gustatory)
2. Mechanical (tactile or sonic)
3. Radiational (light perception or visual)

The chemical mode is by far the most widespread and primitive of the three modes (HALDANE, 1955; WILSON, 1970), and although it is often dominant, a blend of two or even all three of the communicative modes may be employed.

In chemical communication a message is transmitted between individuals by the release of one or more chemicals by an individual into the atmosphere, water or onto the substratum. The substances involved in transmitting the chemical messages are collectively termed Semiochemicals (REGNIER, 1971). Semiochemicals that deliver messages between individuals of different species are called Allelochemics (WHITTAKER and FEENY, 1970). These are subdivided into Allomones, which give the emitter an adaptive advantage e.g. repellent odours and venoms; Kairomones, giving the receiver an adaptive advantage e.g. food attraction stimuli from a prey species that attract predators; Synomones, acting to the advantage of both the emitter and receiver; and Apneurones, which are attractive substances arising from non-living material.

Pheromones

Semiochemicals that are employed in intraspecific communication are termed pheromones (KARLSON and BUTENANDT, 1959; KARLSON and LUSCHER, 1959) (derived from the Greek 'pherein' meaning 'to transfer' and 'hormon' meaning 'to excite'). The accepted definition of the term pheromone was proposed by KALMUS (1965); a pheromone is a chemical that is released to the exterior by an organism and that causes one or more specific reactions in a receiving organism of the same species.

Pheromones, in turn, have been conveniently divided into two categories (WILSON, 1963):

1. Releaser pheromones trigger an almost immediate behavioural response in the receiving animal e.g. the alarm behaviour exhibited by worker ants in response to the mandibular gland secretion of a fellow worker, emitted during a defensive action.

2. Primer pheromones produce a delayed response and often result in hormonally controlled physiological changes occuring in the receiver. The effects of primer pheromones generally become evident some time after their initial reception, e.g. the inhibition of queen rearing and ovarian development in worker bees in response to 'queen substance' (BUTLER, et al., 1961).

In insects, pheromones are generally produced and stored in exocrine glands (as opposed to endocrine glands which produce hormones, resembling pheromones in some respects). Pheromones are usually active in minute quantities. The exocrine gland secretions containing pheromones are often complex mixtures of substances, and although it is frequently possible to assign the pheromonal activity

of a secretion to a single component, the other substances may act synergistically to enhance the activity of the principle active constituent, have a moderating action or confer species specifity to the particular secretion.

A pheromone may be a single substance. However if two or more substances are released simultaneously and all are necessary to elicit the specific behavioural reaction then the chemical mixture must be termed the pheromone, and the individual active constituents are termed the pheromone components.

Naturalists began to realise that insects communicated through pheromones as early as the 18th Century (DE REAUMUR, 1742). However as pheromones are often produced at the nanogram level per individual, their analysis by traditional degradative and chemical techniques would require vast numbers of insects. Even as recently as 1969, TUMLINSON *et al.* required 4.5 million boll weevils (*Anthonomis grandis*) to obtain enough material to identify the four components of its aggregation and sex pheromone. However, progress in the fields of gas chromatography (GC), mass-spectrometry (MS) and more recently in nuclear magnetic resonance (NMR), infrared (IR) and ultraviolet (UV) spectroscopies, now means that identifications of multicomponent pheromones can be made on a few micrograms of material or less. Consequently, more thorough investigations can now be performed on significantly fewer insects. The rapid progress in this area of research has led to the chemical identification of hundreds of insect pheromone components.

In the ethological and chemical investigation of pheromones, bioassays are essential in testing for the presence of biological

activity in insect extracts, synthetic samples or controls. Live insects are the detectors of activity and their responses are usually recorded as alterations in their behaviour or physiology. As the effects of primer pheromones usually manifest themselves a considerable time after the initial reception , as physiological changes within the animal under test, the bioassay of insect primers can be very lengthy and their effects difficult to detect. It would appear that these two factors are, in part, responsible for so few insect primer pheromones being chemically identified. From this point of view, releaser pheromones are rather easier to study as an immediate behavioural response is usually observed which can be readily quantified. As a result releaser pheromones have been much more extensively investigated.

Attraction is the most widespread form of releaser behaviour in insects. The best examples of this form of communication are sex pheromones which are secreted by one sex to attract the other as an initial part of the mating process. Sex pheromones have been most extensively investigated in the Lepidoptera and Coleoptera; the structures of pheromone components have been unambiguously established in scores of cases. Some Coleoptera species also produce aggregation or recruitment pheromones. These differ from the sex pheromones in that they are produced by both sexes and serve more than one function. (For review of insect pheromones see BRAND et al., 1979).

Much of the early interest in these attractant pheromones has stemmed from the desire to break away from the total reliance on the broad spectrum chemical pesticides for the control of insect pests,

particularly in cases where these so called 'hard' chemical pesticides have been found to be uneconomical and very damaging biologically (BIRCH, 1974).

Pheromones of the above type have been employed in pest control programmes in two distinct ways. The synthetic pheromone is either incorporated into a trap which is used to monitor the adult population as an aid to assessing the optimum time for spraying with chemical insecticide, or the synthetic pheromone is used more directly, either in mass trapping to reduce the adult population, or, in the case of sex pheromones, a pheromone is employed to interfere with the adult insect's navigation in order to decrease the likelihood of the two sexes coming into contact, so reducing the incidence of copulation (BIRCH, 1974).

Pheromones in social insects

Interest in pheromones has led to the communication systems of the social Hymenoptera and Isoptera being extensively investigated. Communication is particularly highly developed in the eusocial insects and it is now known that the interaction of the individuals through pheromones is the predominant communicative mode. Pheromones are, in fact, implicated in every aspect of their social behiaviour (WILSON, 1971). Table 1 summarises the categories of eusocial behaviour in which pheromones have been observed to play a part (BLUM, 1974a).

Although all the eusocial groups of insects have been found to employ primer pheromones (Table 1), they have been chemically characterised in very few cases. Probably the best known example of

TABLE 1

Catagories of eusocial behaviour in which pheromones of exocrine origin have been demonstrated to play a part.*

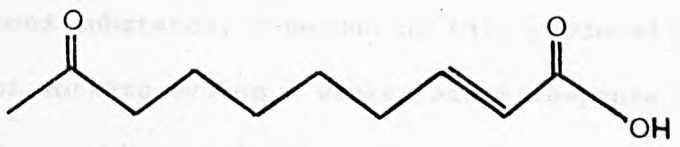
Reaction	Type of Chemical Stimulus	Group
Primers		
Caste determination	Reproductive-caste inhibitory pheromone	Bees, wasps, termites
Ovarian inhibition	Queen substance	Ants, bees, termites
Inhibition or stimulation of queen-cell construction	Queen substance	Ants, bees, termites
Releasers		
Regurgitation and solid food exchange	Solicitation pheromone	Ants, bees, wasps, termites
Alarm behaviour	Alarm pheromone; trail pheromone	Ants, bees, wasps, termites
Recruitment and emigration	Trail pheromone; alarm pheromone; specialized attractant pheromones	Ants, bees, termites
Oral grooming	Surface pheromone	Ants, bees, wasps, termites
Aggregative (= clustering) behaviour	Aggregation pheromone	Ants, bees, termites
Attraction of alate reproductives	Territorial pheromone; sex attractant	Ants, bees, wasps, termites
Queen identification by workers	Queen-recognition pheromone; queen substance	Ants, bees, wasps, termites
Flight induction of sexual alates	Flight induction pheromone	Ants
Repellency	Alarm (?) pheromone	Ants, bees
Abdominal pumping by adults	Thermoregulatory pheromone	Wasps

* Colony odor has not been demonstrated to be of exocrine origin and is not included in this list.

(From BLUM, 1974a)

a primer pheromone is (E)-9-oxo-2-decenoic acid (1) which constitutes the 'queen substance' of the honeybee (Apis mellifera). The stucture of this compound, which is produced in the mandibular glands of queens, was determined on the milligram scale by means of elemental analysis, IR spectrometry, microscale reactions, GC and synthesis. Bioassay of both the natural and synthetic material showed that (1) inhibits queen rearing and ovarian development in worker bees. It also possesses releaser properties serving as an attractant for workers in colony cohesion and swarming and as a sex attractant or mating stimulant for drones (BUTLER et al., 1961; BUTLER and SIMPSON, 1967; GARY, 1970). The mandibular glands of queen honeybees are the source of a second primer pheromone, (E)-9-hydroxy-2-decenoic acid (2). This substance acts synergistically with the 9-oxo acid to inhibit queen cell construction by workers (BUTLER and CALLOW, 1968). A further example of a social insect primer is δ-n-hexadecalactone (3) which was isolated from the heads of queens of the oriental hornet wasp, (Vespa orientalis). The structural determination was made from IR and mass spectra of material isolated by solvent extraction and thin layer chromatography (TLC). The synthetic and natural materials were found to stimulate workers to construct queen cells (IKAN et al., 1969).

In contrast to primers, releaser pheromones have been extensively studied, particularly those which elicit alarm and foraging behaviour. One of the earliest descriptions of releaser behaviour was by CHARLES BUTLER, (1609), in 'The Feminine Monarchie', where he describes the mass attraction of honeybee workers resulting from the emission of alarm chemicals following a single stinging.



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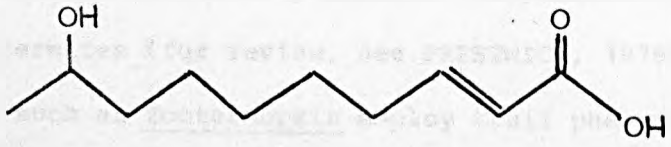

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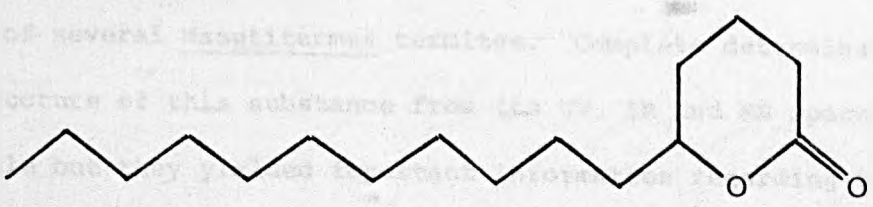

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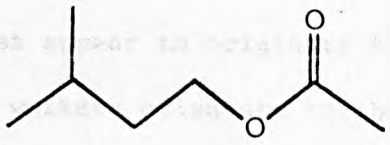
The principle alarm stimulant is isopentyl acetate (**4**), which is produced by the glandular tissue of the sting shaft (BOCH et al., 1962, 1970). A second substance, 2-heptanone (**5**), produced by the mandibular glands of workers evokes a weaker alarm response (SHEARER and BOCH, 1965). In a number of Isoptera genera the cephalic glands of soldiers have been found to be the source of various monocyclic terpenoid hydrocarbons which are capable of eliciting alarm behaviour in the form of a short-lived biting and snapping frenzy (MOORE, 1968; ERNST, 1959). More recent investigations have resulted in the identification of a number of novel polycyclic terpenoid compounds from the heads of termites (for review, see PRESTWICH, 1979). Some primitive termites such as Zootermopsis employ trail pheromones as a means of recruitment in defensive actions. The substance largely responsible for this activity in Z. nevadensis is n-hexanoic (caproic) acid which originates from the sternal gland found at the base of the fifth abdominal sternite (HUMMEL and KARLSON, 1968). The diterpene hydrocarbon neocembrene-A (12-isopropyl-1,5,9-trimethyl-cyclotetradeca-1,5,9-triene) (**6**) has been identified as the trail pheromone of several Nasutitermes termites. Complete determination of the structure of this substance from its UV, IR and MS spectra was not possible but they yielded important information regarding the molecular weight and the presence of $C{=}CH_2$ (MOORE, 1966). Subsequent hydrogenation and PMR studies indicated the number of double bonds and the nature of the protons attached to them. However, the assignment of the formula (**6**) rested on the results of a quantitative micro-ozonolysis investigation (BIRCH et al., 1972). It is believed that this substance is derived directly from the food the termites



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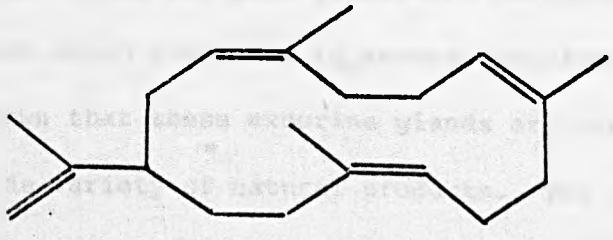

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eat.

Trail pheromones are also known in bees and wasps. BUTLER et al. (1969) have demonstrated that honeybees and wasps lay trails with footprints, the pheromones appear to originate from the dermal glands and are believed to help workers orientate to the nest entrance.

Aerial trails are formed from liquid droplets originating from the mandibular glands in stingless bees in the tropical rain forests (LINDAUER and KERR, 1958). Such trails are employed to aid the bees in locating the flora of the forest canopy.

Pheromones and exocrine glands of ants

Although no primer pheromone has been characterised from the Formicidae, releaser pheromones and their associated exocrine components have been extensively studied.

The pheromones of ants are produced and stored in the glands and vesicles shown in Figure 1. The supra-anal glands and Pavan's gland were thought to be restricted to the Dolichoderinae. However HOLLDOBLER and ENGEL (1978) have recently reviewed tergal and sternal glands, and they believe that the anal glands are analagous to the tergal pygidial glands which are found in several sub-families.

Research has shown that these exocrine glands are capable of biosynthesising a wide variety of natural products. The glandular secretions are usually complex mixtures of substances, many of which appear to have no significant behavioural activity in bioassay. Closely related species are often found to produce similar substances in a particular gland, however the compositions are usually species specific. Therefore morphologically similar species are often

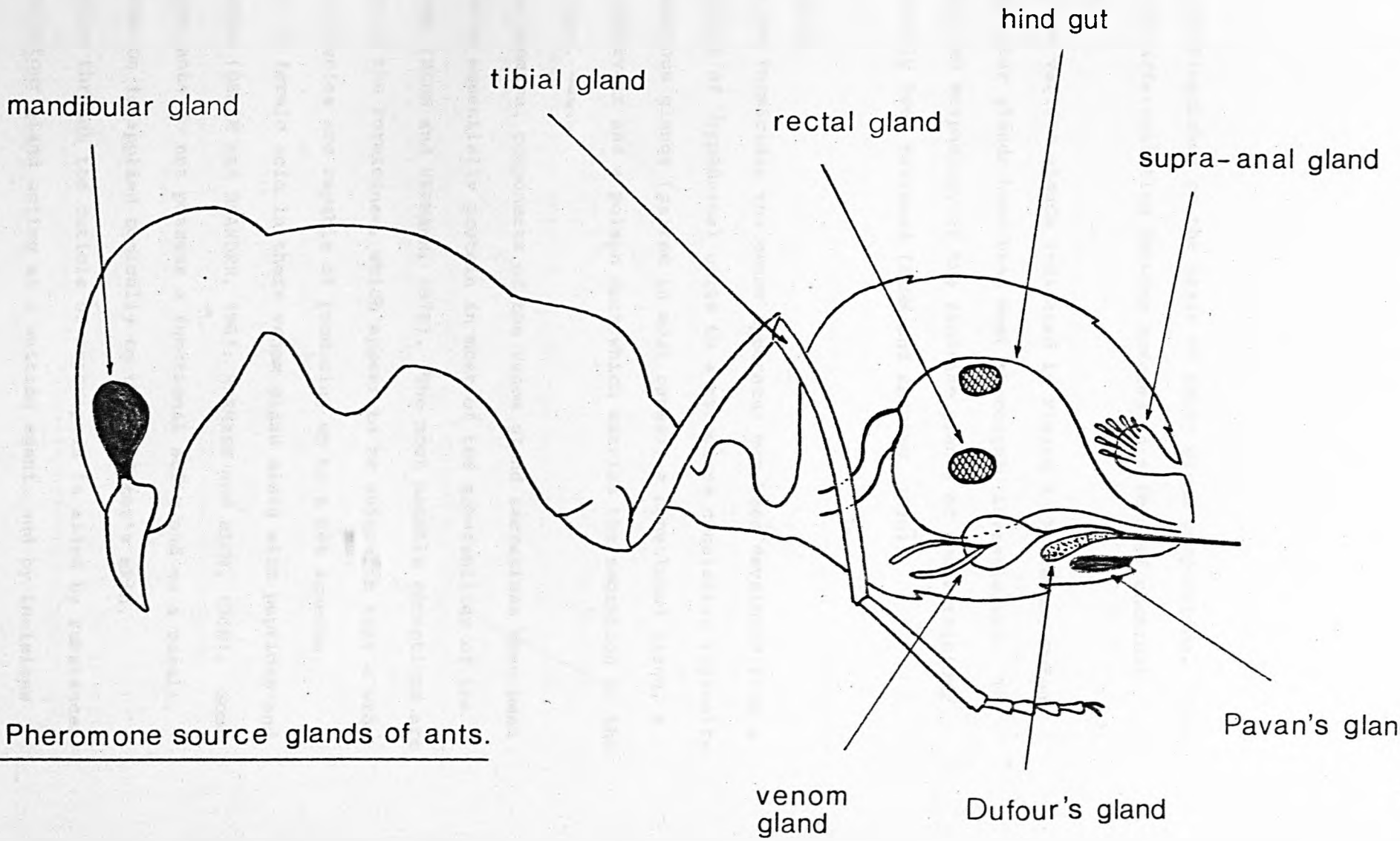


FIGURE 1 Pheromone source glands of ants.

readily distinguished on the basis of their gland composition. This method of differentiating between species is a form of chemical taxonomy.

Of the various glands indicated in Figure 1 the venom, Dufour and mandibular glands have been most thoroughly investigated. The chemistry and morphology of the exocrine glands of the Formicidae have recently been reviewed (BLUM and HERMANN, 1978).

Venom Gland

In the Formicidae the venom apparatus has been developed from a small pocket of hypodermal cells to a structure consisting typically of filamentous glands (paired in most cases), a convoluted gland, a poison reservoir and a poison duct which carries the secretion to the sting lance.

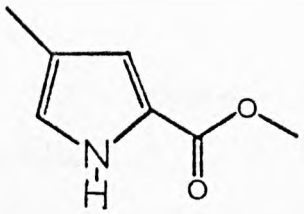
The chemical components of the venom gland secretions have been found to be essentially protein in most of the sub-families of the Formicidae (BLUM and HERMANN, 1978). The most notable exceptions are the ants of the Formicinae, which appear to be unique in that a wide range of species are capable of producing up to a 60% aqueous solution of formic acid in their venom gland along with peptides and amino acids (OSMAN and BRANDER, 1961; HERMANN and BLUM, 1968). Some Formicinae ants do not possess a functional sting and as a result, this solution is applied topically to the predator's skin. Penetration through the cuticle of arthropods is aided by substances from the Dufour gland acting as a wetting agent, and by incisions from the ant's mandibles.

In contrast to the Formicinae, protein constituents appear to be

characteristic of the myrmicine venom gland. However, studies on a number of genera have shown that it has the ability to produce a wide variety of other natural products, some of which have been adapted to a communicative role. At least twelve myrmicine genera produce volatile trail substances in their venom glands and employ them in recruiting during foraging and defensive actions (BLUM, 1974b).

The first trail substance to be identified was methyl 4-methyl pyrrol-2-carboxylate (**7**) (TUMLINSON et al., 1971, 1972). This compound was isolated from the tropical myrmicine Atta texana. The same compound was subsequently identified as the trail substance of two other Attines; Atta cephalotes (RILEY et al., 1974a) and Acromyrmex octospinosus (CROSS et al., 1979). It is also found in the venom gland of Atta sexdens rubropilosa but elicits no behavioural response in this species at the glandular concentration. Instead a second nitrogen heterocycle (**8**), 3-ethyl-2,5-dimethyl pyrazine, was shown to be the major component of the trail pheromone of this species (CROSS et al., 1979).

Another tropical myrmicine species, Monomorium pharaonis has been found to produce three nitrogen containing compounds in its venom gland, namely: all-(Z)-5-methyl-3-butyloctahydroindolizine (Monomorine 1) (**9**), 2-butyl-5-pentylpyrrolidine (Monomorine 2) (**10**), and (E)-2-pentyl-5-(5'-hexenyl)pyrrolidine (Monomorine 3) (**11**) (RITTER et al., 1973). Components (**9**) and (**10**) elicited a weak trail following response in the worker ants. A fourth substance, faranal, a minor component of the Dufour gland of this species, was later found to be much more active as a pheromone (RITTER et al.,



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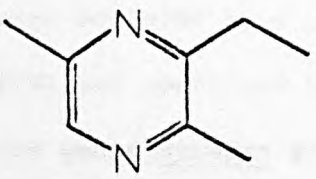

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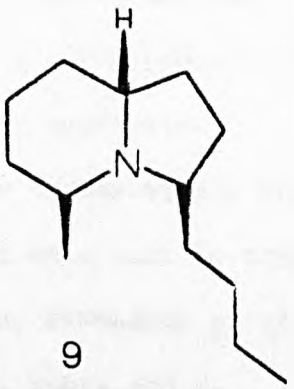

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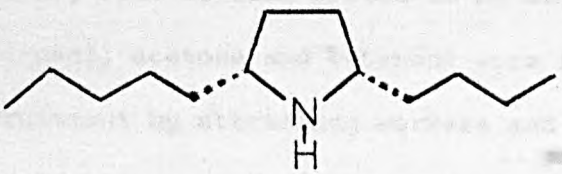

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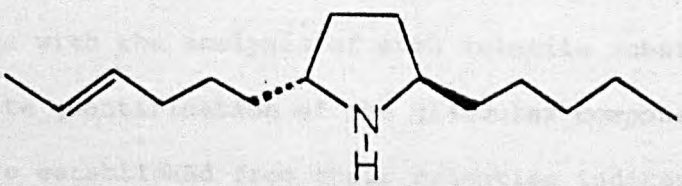

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1977, 1980). Faranal will be described in more detail in the discussion of Dufour glands. Very similar analytical procedures were employed to determine the identities of all the above trail substances. In each investigation the volatile material was initially extracted from many thousands of workers and repeatedly fractionated by preparative GC. The fractions were bioassayed to check for the presence of active components. The structural elucidations were achieved by a combination of spectroscopic methods (IR, MS, and PMR) and confirmed by unambiguous synthesis.

Ants of the genus Myrmica are also known to lay trails from their venom glands. Although at the start of this work no trail substance had been identified from these ants, CAMMAERTS et al. (1978), had shown that the venom glands of M. rubra and M. scabrinodis contained nanogram amounts of a number of oxygenated organic compounds. Of the sixteen substances that were found in each species, (see Table 2), four of them proved to be ethologically active; ethanal, ethanol, acetone and butanone were found to participate in recruitment by attracting workers and increasing their speed and sinuosity of walking. In contrast to the investigations of the Attine and Monomorium trail substances, this study used a solid sampling technique (MORGAN and WADHAMS, 1972a) to analyse the contents of single glands and capillary extracts (MORGAN and TYLER, 1977) by GC. This approach avoided the use of extracting solvents which interfere with the analysis of such volatile substances, and allowed accurate quantification of the glandular components. Their identities were established from their retention indices (by comparison with those of standard compounds). The funtional groups

TABLE 2

Volatile compounds observed in the venom of *M. rubra* and *M. scabrinodis*.

Compound	Species		Ethologically active
	M. rubra	*M. scabrinodis*	
methane	+	+	
ethane	+	+	
propane	+	+	
methanol	+	+	
ethanal	+	+	+
butane	+	+	
ethanol	+	+	+
propanal	+	+	
propanone	+	+	+
methylpropanal	+	+	
butanone	+	+	+
methylbutanal	+	+	
+ 4 unidentified components	+	+	

present in each component were confirmed by reaction GC involving a combination of pre-column reactions and post-column reaction loops.

Ants of the genus Solenopsis differ from other myrmicines as their venoms consist mainly of alkaloidal constituents. (Reviewed by BLUM and HERMANN, 1978). The venoms of the Solenopsis fire ants consist mostly of 2,6-dialkylpiperidines (MACCONNELL et al., 1971, 1974; BRAND et al., 1972; BLUM et al., 1973) while those of the Solenopsis thief ants are composed of 2,5-di-n-alkylpyrrolidines (BLUM and HERMANN, 1978 ; JONES et al. , 1979). Unlike the previously described nitrogen heterocycles of the Attines and M. pharaonis, these compounds have no pheromonal activity, and instead are employed defensively. The diversity of myrmicine venoms is further emphasised in Myrmicaria natalensis which produces predominantly d- and l-limonene (GRUNANGER et al., 1960) in addition to other monoterpene hydrocarbons (BRAND et al., 1974). These terpenes serve as an alarm pheromone in this species (QUILICO et al., 1960).

Dufour gland

Dufour (1841) first described the gland in bees and ants that now bears his name. It is found at the posterior end of the abdomen, closely associated with the venom gland and it has now been shown to be present in the female and worker castes of all the Hymenoptera sub-families (BORDAS, 1894, 1897). In ants, including non-venomous species, the Dufour and venom glands have a common exit through the sting. In common with the venom gland, the Dufour gland has evolved from a pocket of hypodermal cells which later developed into a sac-

like structure. Little change occured except in the Camponotus and Formica genera where the sac became bilobed (BLUM and HERMANN, 1978).

Chemical investigations have been made on the Dufour gland of relatively few species of ant, but where this has been done simple hydrocarbons or oxygenated compounds have been found. Most work has been performed on the Myrmicinae, Formicinae and the Dolichoderinae. The reports up to 1974 have been well reviewed by BLUM and HERMANN (1978). There is no recognised function for the Dufour gland that embraces all ant species. It has been suggested that the Dufour gland has evolved primarily to provide a lubricant for the sting or eggs during oviposition (WHEELER, 1910; ROBERTSON, 1968). In some species the Dufour gland has evolved a secondary function, and now produces substances which act in communicative or defensive roles.

In some formicine species the contents of the Dufour and venom glands are released together as a defensive secretion. In a recent investigation of the chemistry and behaviour of the African weaver ant, Oecophylla longinoda, BRADSHAW et al. (1979) demonstrated how n-undecane from the Dufour gland and formic acid from the venom gland act synergistically to elicit a 'mass attack' reaction in major workers. This behaviour is part of a complex alarm and defence system which also involves the mandibular gland secretion. The alarm -defence role of the Dufour and venom glands secretions is common amongst the Formicidae (for a review, see PARRY and MORGAN, 1979).

In slave keeping formicines such as Formica subinterga, Formica pergandei and Formica sanguinea the Dufour glands are hypertrophied and produce large amounts of C_{10}-C_{14} acetates. During slave raids these substances are sprayed to excite and attract the slave maker

ants but panic and disperse the slave species (REGNIER and WILSON, 1971; BERGSTROM and LOFQUIST, 1968).

The Dufour gland of Myrmica ants contains microgram amounts of hydrocarbons in the C_{13}-C_{19} chain length range. In M. scabrinodis they consist mainly of α-farnesene (**12**) and three homologous terpenoid hydrocarbons (**13,14,15**) (MORGAN et al., 1979).

M. rubra, however, produces predominantly straight chain alkanes and alkenes with (Z)-8-heptadecene (**16**) as the major component (MORGAN and WADHAMS, 1972b). Also present in the Dufour gland of each of the species are nanogram quantities of low molecular weight oxygenated compounds similar to those found in the venom gland (see Table 3). It has been shown that the less volatile fraction is used as a species specific territorial marking pheromone, while the more volatile fraction serves as a short acting non-species specific attractant (CAMMAERTS et al., 1978).

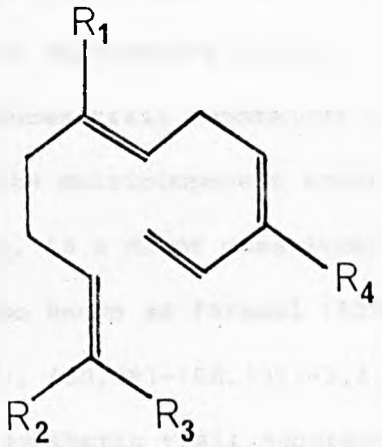
As so many of the compounds identified from the Dufour glands of ants are straight chain hydrocarbons and simple oxygenated compounds, their mass spectra are often sufficient to characterise them. Graphical plots of log of GC retention time (t_r) against the number of carbon atoms in the hydrocarbon chain are useful for picking out homologous series of saturated and unsaturated compounds from GC profiles obtained from isothermal analyses. MORGAN and WADHAMS (1972a) used a pre-column bromination in conjunction with a GC solid sampling technique to distinguish between saturated and unsaturated hydrocarbons in the ant M. rubra. The position of the double bonds in unsaturated compounds is readily determined by micro-ozonolysis of material trapped from the GC. The configuration of the double bonds

TABLE 1

Compounds identified in the Dufour gland of M. rubra and M. scabrinodis (WADHAMS, 1972; TYLER, 1977; PARRY, 1978).

	Compound	Species		
		M. rubra	M. scabrinodis	Ethologically active
oxygenated volatiles	methanol	+	+	
	ethanal	+	+	+
	ethanol	+	+	+
	propanal	+	+	
	propanone	+	+	+
	methylpropanal	+	+	
	butenone	+	+	
	butanone	+	+	+
	1-butanol	+	+	
linear hydrocarbons	tridecane	+	-	
	tetradecane	+	-	
	pentadecane	+	+	
	pentadecene	+	+	
	hexadecane	+	-	
	hexadecene	+	-	
	heptadecane	+	+	
	heptadecene	+	-	
	heptadecadiene	+	-	
	octadecane	+	-	
	octadecene	+	-	
	nonadecane	+	+	
	nonadecene	+	-	
terpenoid hydrocarbons	α-farnesene	+	+	
	homofarnesene	+	+	
	bishomofarnesene	+	+	
	trishomofarnesene	-	+	

The ethological activity of the higher hydrocarbons has not been studied with synthetic samples.

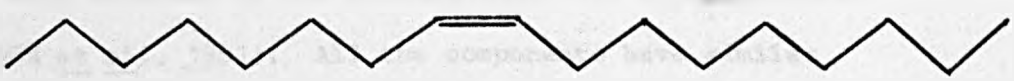


12 = $R_1 R_2 R_3 R_4 = H$

13 = $R_1 = Me$; $R_2 R_3 R_4 = H$

14 = $R_1 R_2 = Me$; $R_3 R_4 = H$ or $R_2 = H$; $R_3 = Me$

15 = $R_1 R_2 R_4 = Me$; $R_3 = H$ or $R_2 = Me$; $R_3 = H$



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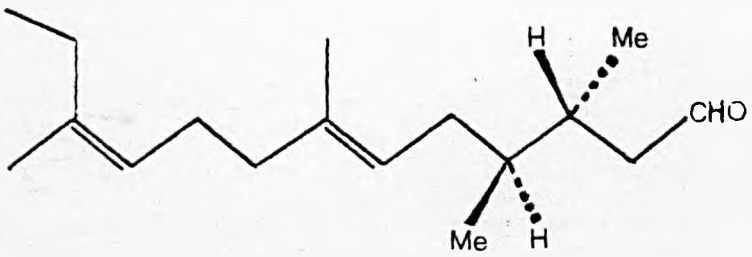
can be established by TLC of the natural material against synthetic standards on silver nitrate impregnated plates.

The Dufour gland produces trail substances in some ants. The most active component of the multicomponent trail pheromone of Pharaohs ant, M. pharaonis, is a minor constituent of its Dufour gland. The substance, also known as faranal (RITTER et al., 1977, 1980), is a terpenoid (**17**), (3S,4R)-(6E,10Z)-3,4,7,11-tetramethyl-6,10-tridecadienal. The synthetic trail substance has been found to produce a pronounced trail following reaction at less than 1pg/cm. The structure was elucidated using 70µg of material isolated from 100,000 worker ants, by micro-ozonolysis, GC-MS, IR, PMR and synthesis.

Solenopsis invicta also produces a trail pheromone in its Dufour gland. Preliminary MS analysis had shown the molecular mass of the major trail pheromone component to be 204 (empirical formula $C_{15}H_{24}$;BARLIN et al., 1976). Subsequent analysis by capillary GC, GC-MS, hydrogenation, PMR and UV of material isolated from nearly a kilogram of whole ants resulted in the identification of four compounds (VANDER MEER et al., 1981). All the components have similar structures to the farnesenes of M. scabrinodis (MORGAN et al., 1979). However only two of the substances (Z,E) and (E,E)-α-farnesene showed a positive trail bioassay. The most active of the two was the (Z,E) isomer which showed positive trail following activity at 0.1pg/cm.

Mandibular glands

The mandibular glands are probably present in all of the Formicidae. They consist of a reservoir and associated glandular



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mass. The reservoir contents are released through ducts which open on the inner edge of the mandibles. It has been suggested that the contents of the reservoir are released automatically when the mandibles are opened (KRATKY, 1931), although the exact mechanism regarding its release is the subject of some controversy (HERMANN et al., 1971).

The mandibular gland secretions have been shown to elicit an alarm response in a large number of species of Formicidae. The chemical compositions of the mandibular gland secretions have been more thoroughly analysed than any other exocrine exudate. These have been well reviewed up to 1974 by BLUM and HERMANN (1978).

The mandibular gland secretions are generally composed of oxygenated compounds such as alcohols, ketones and aldehydes; many of them terpenoid. Notable exceptions include certain ponerine and dolichoderine species that produce a variety of alkylpyrazines (**18**) which are employed in alarm and defensive roles (WHEELER and BLUM, 1973; CAVILL and HOUGHTON, 1974a,b; LONGHURST et al., 1978). Another ponerine species, Paltothyreus tarsatus has been shown to contain dimethyldisulphide (**19**) and dimethyltrisulphide (**20**) (CASNATI et al., 1967). These are the only known natural alkylpolysulphides and they function as defensive compounds and releasers of digging behaviour.

The mandibular gland secretions of the Myrmicinae and Formicinae have been more extensively studied than those of any other sub-families of the Formicidae. Myrmicine ants have been shown to produce an abundance of 3-alkanols and their corresponding 3-alkanones (BLUM and HERMANN, 1978). Terpenoid aldehydes and alcohols including neral, geranial, citronellol and geraniol are produced by a

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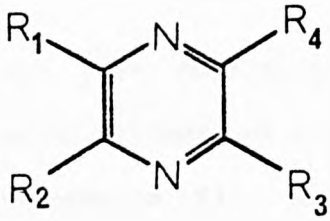
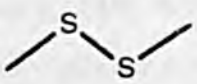


R_1	R_2	R_3	R_4	Species
H	Me	Me	Et	Odontomachus troglodytes, Odontomachus brunneus
H	Me	Me	n-Butyl	O. troglodytes, O. brunneus, Anochetus sedilloti, Brachyponera sennaarensis
H	Me	Me	n-Pentyl	O. trolodytes, O. brunneus, B. sennaarensis
H	Me	Me	n-Hexyl	O. troglodytes
Me	H	Me	iso-Butyl	A. sedilloti
Me	H	Me	sec-Butyl	A. sedilloti
H	Me	Me	iso-Butyl	A. sedilloti
H	Me	Me	sec-Butyl	A. sedilloti
Me	H	Me	n-Pentyl	A. sedilloti
Me	H	Me	iso-Pentyl	Odontoachus hastatus, Iridomyrmex humilis
H	Me	Me	n-Propyl	O. brunneus
Me	H	Me	n-Propyl	I. humilis
Me	H	Me	(E)-Styryl	I. humilis



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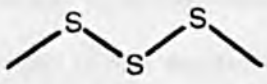

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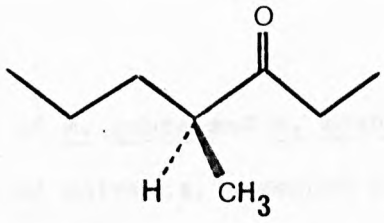
few Atta species (BUTENANDT et al., 1959; BLUM et al., 1968). A principle alarm component of several Attines and a number of other myrmicine species is 4-methyl-3-heptanone (**21**). RILEY et al. (1974b) synthesised both optical isomers of this compound and showed through bioassay experiments, that A. texana and A. cephalotes produce only the S(+) form. The S(+) form was found to be about 400 times more active in releasing alarm behaviour than the R(-) form and that the racemic mixture of the two forms was found to be inactive. This emphasises the importance of stereochemistry in pheromone perception. In this particular case the antennal receptors are able to distinguish between the spatial arrangement of the two optical forms. In general, more attention is being paid today than in the early days of pheromone isolation, to the chiral nature of pheromones.

In a large number of formicine species the mandibular gland secretion acts in conjunction with substances from the poison apparatus, as part of a complex alarm-defence system (for a review see PARRY and MORGAN, 1979).

The alarm-defence behaviour of Myrmica ants, like that of many formicines, is released by secretions of more then one of their exocrine glands. The aggressive behaviour of M. rubra workers is produced by the combined effects of the mandibular gland and the venom gland secretions.

The mandibular gland secretions of a number of Myrmica species were first investigated by CREWE and BLUM (1970). They characterised 3-octanone (**22**), 3-octanol (**23**) and in some cases 6-methyl-3-octanone (**24**), 6-methyl-3-octanol (**25**) and 3-nonanone (**26**) from solvent extracts by means of GC-MS. Further investigations (MORGAN et al.,



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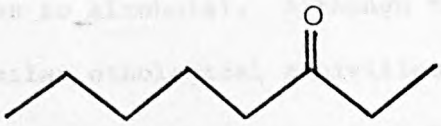

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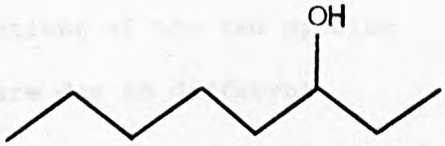

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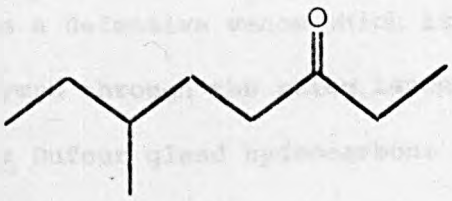

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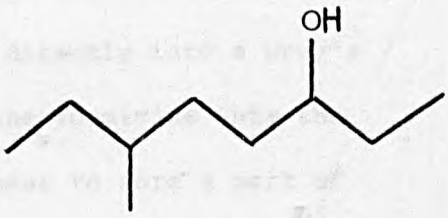

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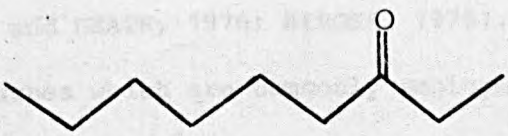

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1978; CAMMAERTS et al., 1978) of M. rubra and M. scabrinodis by solid sampling-GC, avoiding the use of solvents, revealed further important constituents (see Table 4). Characterisation of the individual components was carried out by means of GC-MS, also a comparison of their GC retention times with those of standards and GC pre-column reactions with sodium borohydride (which reduces ketones and aldehydes to alcohols). Although the secretions of the two species have similar ethological activities, they are due to different compounds in the two species (see Table 5); the greater proportion of higher molecular weight components in M. scabrinodis ensures that the species can recognise their own secretion. The volatiles from the venom gland are employed in conjunction with the mandibular gland volatiles to aid in recruitment. The non-volatile portion of the venom gland secretion, consisting mainly of protein constituents, acts as a defensive venom which is injected directly into a prey's heamolymph through the sting lance. Unlike the formicine ants the Myrmica Dufour gland hydrocarbons do not appear to form a part of their alarm-defence system.

A wide variety of microanalytical techniques are used in the structural elucidation of pheromone components and other exocrine products (for reviews see INSCOE and BEROZA, 1976; YOUNG and SILVERSTEIN, 1975; TUMLINSON and HEATH, 1976; BEROZA, 1975). However there are two distinct approaches which are commonly employed in the analysis of pheromones in ants. These two alternative analytical schemes are shown in Figure 2.

The methods of Scheme 1 are concerned purely with identifying pheromone components, and set out to achieve this by first extracting

TABLE 4

Volatile components of *M. rubra* and *M. scabrinodis* mandibular glands (CAMMAERTS *et al.*, 1978).

Compound	Species	
	M. rubra	*M. scabrinodis*
ethanal	+	+
methylpropanal	+	+
propanone	+	+
3-hexanone	+	+
3-hexanol	+	+
3-heptanone	+	+
3-heptanol	+	+
3-octanone	+	+
3-octanol	+	+
6-methyl-3-octanone	+	+
6-methyl-3-octanol	-	+
3-nonanone	+	+
3-nonanol	-	+
3-decanone	+	+
3-decanol	-	+
3-undecanone	+	+

TABLE 5

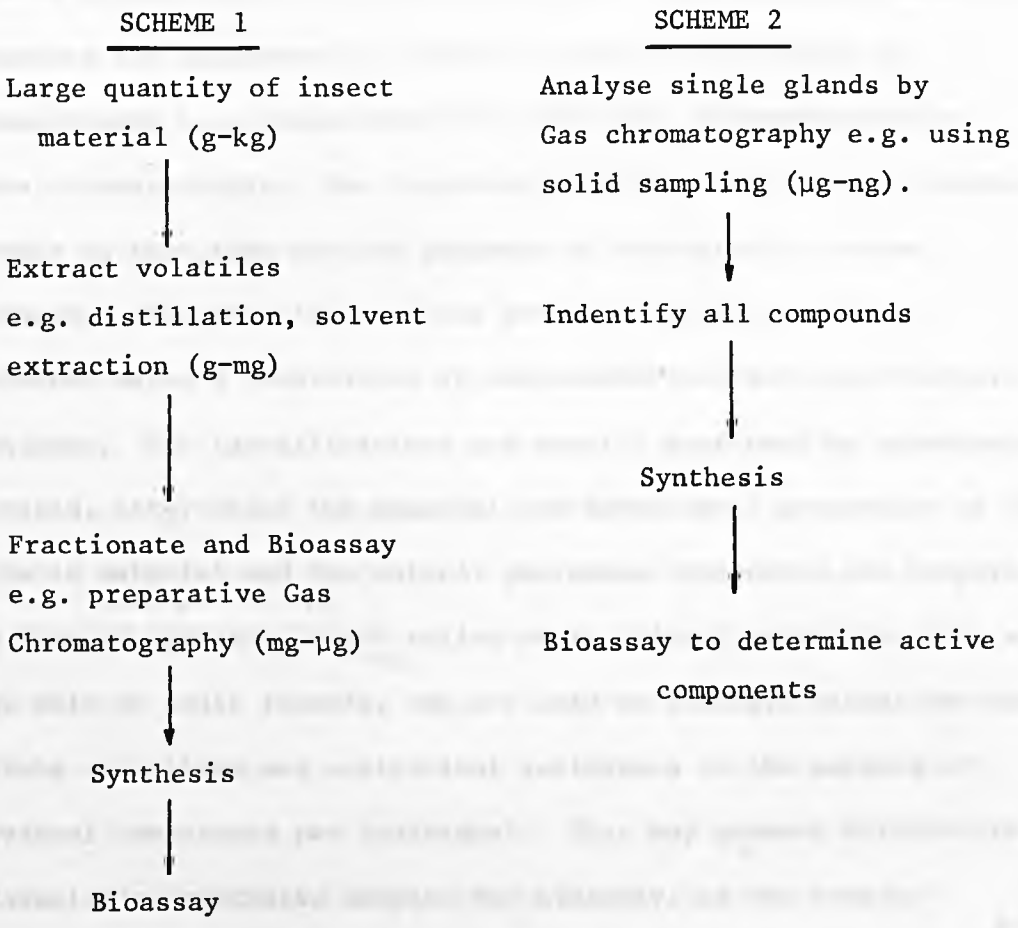
Ethological activities of substances produced by M. rubra and M. scabrinodis workers' mandibular glands (CAMMAERTS et al., 1978)

Compound tested	Specific ethological activities: attractive power	Increasing linear speed	Increasing sinuosity
M. rubra workers			
3-octanone	+	+	+
3-octanol	- x	-	-
3-nonanone	-	-	-
6-methyl-3-octanone	-	-	-
M. scabrinodis workers			
3-hexanone	-	+	-
3-heptanone	-	-	-
3-octanone	- x	+ x	-
3-octanol	+	-	-
3-nonanone	- x	+	-
3-decanone	- x	- x	-

+ : ethological activity statistically revealed
- : no ethological activity statistically evident
x : substance acts synergistically to augment activity.

FIGURE 2

Approaches to Pheromone Analysis



all the volatiles from a large mass of whole or part insect bodies by means of distillation or solvent extraction. The volatile material containing the pheromone of interest is then fractionated by chromatography e.g. preparative GC, thin layer chromatography or column chromatography. The fractions are then subjected to bioassay in order to test them for the presence of biologically active components. The identities of the active components are then determined using a combination of microanalytical and spectroscopic techniques. The identifications are usually confirmed by unambiguous synthesis, after which the chemical and behavioural properties of the synthetic material and the natural pheromone components are compared. This type of approach, which relies on an initial extraction from a large bulk of adult insects, can not lead to accurate values for the absolute quantities and statistical variations in the amounts of individual components per individual. This may present difficulties in formulating synthetic samples for bioassay, as the insects' behavioural responses often depend upon the pheromone concentration: attractants can repel if presented at too high a concentration. Furthermore Scheme 1 neglects those substances that have no significant biological activity, consequently synergists and novel natural products can be overlooked.

Analysis by a Scheme 2 type of approach are usually performed on small samples e.g. the volatile material from either single glands or small tissue samples may be analysed by gas chromatography (GC) in conjunction with a solid sampling technique (MORGAN and WADHAMS, 1972a; STALLBERG-STENHAGEN, 1972).

Attempts are then made to identify all the components

originating from the sample. Once the necessary determinations have been performed, syntheses are carried out where necessary, and the synthetic compounds tested for biological activity at the glandular concentration. Assay of the components singly and in combination often reveals the presence of synergistic constituents.

The major advantage of this latter type of approach is that solid sampling methods enable glandular volatiles to be analysed in the absence of extracting solvents which may obscure a number of behaviourally active volatile constituents. In addition these techniques enable very accurate determinations of glandular composition to be performed.

Furthermore, a Scheme 2 approach often results in the characterisation of numerous behaviourally inactive compounds which may well be of importance in future taxonomic, biochemical and biosystematic studies.

The only major drawback of a Scheme 2 type approach is encountered when the compound or compounds are new substances. Determinations by this type of procedure rely heavily on data obtained through linked GC-MS analysis and often this is insufficient for making full stuctural determinations of previously unknown compounds. In such a case more material must be accumulated in order to obtain further structural information e.g. by PMR.

Active components can be missed in a Scheme 2 type analysis if they are below the level of detection. This rarely happens, however as the limit of detection of the gas chromatograph flame ionisation detector (FID) is of the order of 1.0-0.1ng, and there are very few reports of ant pheromones present below this level.

The major difference between the two approaches lies in the amount of insect material required: Scheme 1 often employs many thousands of individual workers to identify a few active components while Scheme 2 frequently results in the characterisation of many components and requires only a few hundred individual insects or less for complete analysis.

The determinations made during the course of this work rely heavily on the solid sampling techniques employed by WADHAMS (1972) and TYLER (1977).

A Scheme 2 approach has been adopted to investigate, principally by GC, the exocrine gland secretions of three tropical Attine ants (including two sub-species) and eight non-tropical Myrmica ants.

Colonies of the Myrmica species are often observed living in close proximity. Therefore to determine whether or not workers of each species are able to distinguish between their own pheromones and those of neighbouring species, a systematic analysis of the chemistry and ethology (by M. C. Cammaerts) has been undertaken.

The chemical compositions and ethological activities of the mandibular and Dufour glands of M. rubra and M. scabrinodis have already been thoroughly examined (CAMMAERTS et al., 1978). The Dufour glands of each species were found to be completely filled with hydrocarbons. In M. rubra these were predominantly simple saturated and unsaturated linear hydrocarbons while in M. scabrinodis, the secretion was composed mainly of sesquiterpenoid hydrocarbons.

In order to determine whether other Myrmica species produce such characteristic mixtures of substances, the Dufour glands of three further species have been examined: M. lobicornis, M. sabuleti and M.

ruginodis. In addition, further quantitative analyses have been carried out on the Dufour gland contents of M. rubra and M. scabrinodis.

The mandibular gland secretion of Myrmica ants has been found to release alarm behaviour in the workers. Chemical examination of this secretion in M. rubra and M. scabrinodis has shown that these two species produce similar mixtures of 3-alkanols and 3-alkanones. M. scabrinodis however, produces a significantly larger proportion of the higher molecular weight components, which enable the workers of the two species to distinguish between one anothers mandibular gland secretions.

This study of Myrmica mandibular gland secretions has been extended to include six further species, namely: M. lobicornis, M. sabuleti, M. ruginodis, M . sulcinodis, M. schencki and M. rugulosa. Reported here are the results of the chemical investigations of their mandibular gland secretions. These results are intended to serve as a basis for further ethological examinations, aimed at determining the biologically active constituents and establishing whether the species specificity observed in the M. rubra and M. scabrinodis secretions is also apparent in the secretions of these other closely related species.

All eight of the above species readily follow trails formed from one anothers venom glands (CAMMAERTS et al., 1978, 1981 and unpublished) Previous investigations of the venom of M. rubra and M. scabrinodis have demonstrated the presence of nanogram amounts of a number of oxygenated compounds which aid in recruitment. None of them were found to elicit trail following. As no trail pheromone had been

chemically characterised from a temperate ant species a thorough investigation of the trail pheromone of M. rubra has been performed to establish its identity. A method was adopted which involved the systematic fractionation of gland extracts by TLC and micro-preparative GC in conjunction with a circular trail bioassay and GC-MS. Once characterised, the investigations were continued in order to determine whether all eight Myrmica species produce the same trail pheromone.

Although the trail and alarm pheromones of a number of Attini have been thoroughly investigated (for review see PARRY and MORGAN, 1979), the chemical compositions and ethological significance of the Attine Dufour glands appears to have been completely neglected. Reported here are the results of some preliminary investigations into the chemical compositions of the Dufour gland contents of A. cephalotes, A. octospinosus, A. s. rubropilosa and A. s. sexdens. The techniques employed to investigate the Myrmica trail pheromone have also been used to determine the mean amounts of the major trail pheromone components in the venom glands of the above Attines.

As the work performed during the course of this project has concentrated on the Dufour glands, mandibular glands and the venom glands of Myrmica and Attine ants, the results of the investigations and their complementary discussions are presented under the following headings:

1. Investigations of trail pheromones.
2. Chemical investigation of Dufour gland substances.
3. Chemical investigation of mandibular gland substances.

DISCUSSION

The Investigation of Trail Pheromones

The odour trail system is the most elaborate of all the forms of communication in ants (WILSON, 1971). In ants it is believed to have evolved from the recruitment technique of tandem running which was first described in the Indian formicine ant Camponotus sericeus (HINGSTON, 1928). Evolution to the typical trail following behaviour enables workers to navigate by the odour trail for considerable distances in the absence of the trail layer.

With the exception of Crematogaster ashemeadi and C. peringueyi which have been shown to produce a trail substance from the tibial gland in the metatibia (LEUTHOLD, 1968; FLETCHER and BRAND, 1968; PASTEELS et al., 1970), all the other ants studied to date secrete their trails from exocrine glands located in the gaster, e.g. Dufour gland, Pavan's gland, hindgut and venom gland, (reviewed by PARRY and MORGAN, 1979). Although a large number of ant species have been found to employ this form of communication during food gathering or defensive actions the constituents of the odour trails have been identified in very few ant species.

Research into ant trail pheromones has concentrated on determining those of pest species such as leaf-cutting ants with the aim of using the synthetic trail pheromones as part of control programmes. To date the chemical components of the trails have been identified only in five species, all tropical inhabitants.

Leaf-cutting ants employ trail pheromones in order to exploit effectively sources of vegetation on which to cultivate the fungus that they eat. Attine ants produce trail pheromones in their venom glands (BLUM and et al., 1964) and in 1971, TUMLINSON et al.

identified the first trail substances methyl 4-methylpyrrol-2-carboxylate (7), from the crushed abdomens of A. texana. The same compound has since been identified as the trail substance of A. cephalotes (RILEY et al., 1974a) and A. octospinosus (CROSS et al., 1979). ROBINSON et al. (1974) found that all the above species follow trails formed from one another's venom gland contents in addition to that of A. s. sexdens. However A. s. sexdens, whilst following trails formed from the venom gland contents of A. texana and A. cephalotes, would not follow the trails of A. octospinosus. From these results it appeared that A. s. sexdens was responding to a component of the A. cephalotes and A. texana venom that was absent from that of A. octospinosus. Subsequently, CROSS et al. (1979) showed that the major component of the trail pheromone of A. sexdens rubropilosa was 3-ethyl-2,5-dimethylpyrazine (8); methyl 4-methyl pyrrole-2-carboxylate was also identified from an extract of whole abdomens and was probably a minor component. These findings were in agreement with the results of ROBINSON et al. (1974) and it seemed likely at this stage that while A. cephalotes, A. texana and A. octospinosus were responding to the pyrrole in A. s. sexdens, this latter species was probably responding to another component, probably 3-ethyl-2,5-dimethylpyrazine present in A. texana and A. cephalotes but absent in A. octospinosus.

This project began as an investigation into the trail pheromone of the common British red ant M. rubra. The aim was to use this readily available non-pest species to develop analytical techniques which would then be applied to the determination of trail pheromones in pest species.

EIDMAN (1927) first showed that M. rubra used odour trails as a means of recruitment to food, although the trails of Myrmica species are not so apparent as those of more highly evolved Myrmicinae. When changing nest sites, M. rubra make trails upto 6m long (PASTEELS and VERHAEGHE, 1974), and CAMMAERTS-TRICOT (1974a) has shown that trails are laid by workers returning to the nest from a food source or when they discover an enemy close to the nest. Related species, M. ruginodis, M. scabrinodis and M. sabuleti adopt a very similar kind of behaviour, although M. scabrinodis and M. sabuleti also deposit a secretion as they return to a food source from the nest (CAMMAERTS and CAMMAERTS, 1980).

Among the Myrmicinae, the trail pheromone has been located in the poison gland, Dufour gland and tibial glands. CAMMAERTS-TRICOT (1974) demonstrated that in M. rubra the trail following behaviour arose from the secretion of the venom gland, and BLUM (1974b) also showed that for M. rubra and a number of related species of Myrmica and Manica the venom was the source of the trail pheromone.

TYLER (1977) analysed the volatile components of the venom gland of M. rubra and M. scabrinodis by GC and found only nanogram amounts of very low molecular weight substances in the C_1 to C_5 range (Table 2) which had short acting attractive properties, and which increased explorative action (CAMMAERTS et al., 1978).

It was presumed that the trail pheromone was present in quantities below the level of detection by GC. Consequently a less direct approach has been adopted to investigate this trail pheromone, which involved isolating the pheromone by means of systematic TLC and GC fractionation in conjunction with a bioassay (PASTEELS and

VERHAEGHE, 1974). Subsequent characterisation of the trail substance was achieved by GC-MS and synthesis. Described here are the results of these investigations into the Myrmica trail pheromone together with those of some further investigations into Attine venom gland volatiles.

Investigation of the Myrmica trail pheromone

A preliminary investigation of the Myrmica trail pheromone was performed by TLC. The contents of five M. rubra venom glands were chromatographed on silica gel TLC plates. Cutting the silica into R_f bands, extraction of the silica and trail bioassay showed the pheromonal activity to be concentrated in the R_f 0.4-0.6 band (eluent, 7:3 hexane-acetone) or R_f 0.2-0.4 band range (eluent, 6:4 toluene-diethyl ether). These R_f properties were similar to those of simple alcohols.

Chemical treatment of the poison gland extracts, followed by TLC and bioassay of the R_f bands showed that the pheromone was readily destroyed by oxidation, inert to bromine (no simple double bonds), sodium borohydride in methanol (absence of aldehyde or ketone) and acetylating conditions (absence of primary or secondary alcohols or amines, Table 6).

Since the FID detector of the gas chromatograph was apparently not sensitive enough for its detection, the worker ants themselves were used as detectors through the bioassay.

The effluent from the gas chromatograph, after the injection of five venom glands, was trapped, first as a very broad band, and then as successively narrower bands. One gland equivalent of the trapping

TABLE 6

Effect of chemical reagents on trail pheromone, as measured by bioassay of R_f bands after TLC. In three cases one R_f band was as active as before the chemical treatment. With chromic acid no bands remained active. P gives the probability of statistical difference from solvent controls, using the non-parametric χ^2 test.

Chemical treatment	Median no. of arcs.	P	Inference
Br_2/CCl_4	4.9	0.001	no significant reaction not alkene.
$NaBH_4$/MeOH	6.3	0.001	no significant reaction not aldehyde or ketone
AC_2O/pyridine	6.2	0.001	no significant reaction not 1^o or 2^o alcohol or amine.
Chromic acid	0.2	N.S.	significant reaction oxidizable.

solvent (acetone) was used for bioassay. The results are given in Table 7. The first experiment showed that the pheromone could be trapped and recovered from the column. The second, that the trail pheromone activity was in the Kovats Index (KI) range 0-1200, the third trapping from the OV-101 column showed it in the KI range 1000-1100 with some overlap beyond this. This indicated that the molecular mass of the trail pheromone was approximately 150. The trail activity was recovered from the KI range 1300-1400 after switching to a polar DEGS column, indicating the pheromone to be of relatively low polarity.

Subsequently, GC analysis of single poison glands and capillary extracts of glands were performed on both OV-101 and DEGS columns with the amplifier set at maximum practical sensivity (attenuation x10), and in both cases, a single peak at the appropriate t_r could be clearly seen (Figure 3). This peak, and the activity could be completely removed by attaching a post column loop containing boric acid to the GC column.

The definitive characterisation of the trail pheromone was achieved by linked GC-MS using the Pye 104-AEI MS12 system, on a sample consisting of capillary extracts from poison reservoirs of fifty workers, sealed in a 5cm long solid sample vial. The mass spectrum obtained after manual background subtraction is shown in Figure 4a; it is consistent with that of 3-ethyl-2,5-dimethylpyrazine **(8)** (STENHAGEN *et al.*, 1974). Although there are three possible arrangements of the alkyl substituent around the pyrazine nucleus **(8,27,28)**, they are, however, readily distinguishable from the relative intensities of their fragment ions (GOLDMAN *et al.*, 1967;

TABLE 7

Results of trapping experiments and bioassay after the gas chromatography of five venom glands of M. rubra. The probability, P, of statistical difference from control values is shown, using the non-parametric χ^2 test.

Expt.	GC liquid phase	fraction (KI range)	Median no. of arcs.	P
(i)	OV - 101	0 - 2000	5.00	0.001
(ii)	OV - 101	0 - 1200	9.00	0.001
		1200 - 1400	0.75	0.05
		1400 - 1600	0.78	N.S.
		1600 - 1800	1.11	N.S.
		1800 - 2000	0.50	N.S.
(iii)	OV - 101	0 - 800	0.14	N.S.
		800 - 900	0.14	N.S.
		900 - 1000	0.10	N.S.
		1000 - 1100	5.42	0.001
		1100 - 1200	0.71	0.01
		1200 - 1300	0.21	N.S.
(iv)	DEGS	0 - 1300	0.46	0.02
		1300 - 1400	7.13	0.001
		1400 - 1500	0.33	N.S.

FIGURE 3.

A. GC of a single M. rubra venom gland on OV-101

B. GC-bioassay of five M. rubra venom glands on OV-101

C. GC of a single M. rubra venom gland on DEGS

D. GC-bioassay of five M. rubra venom glands on DEGS

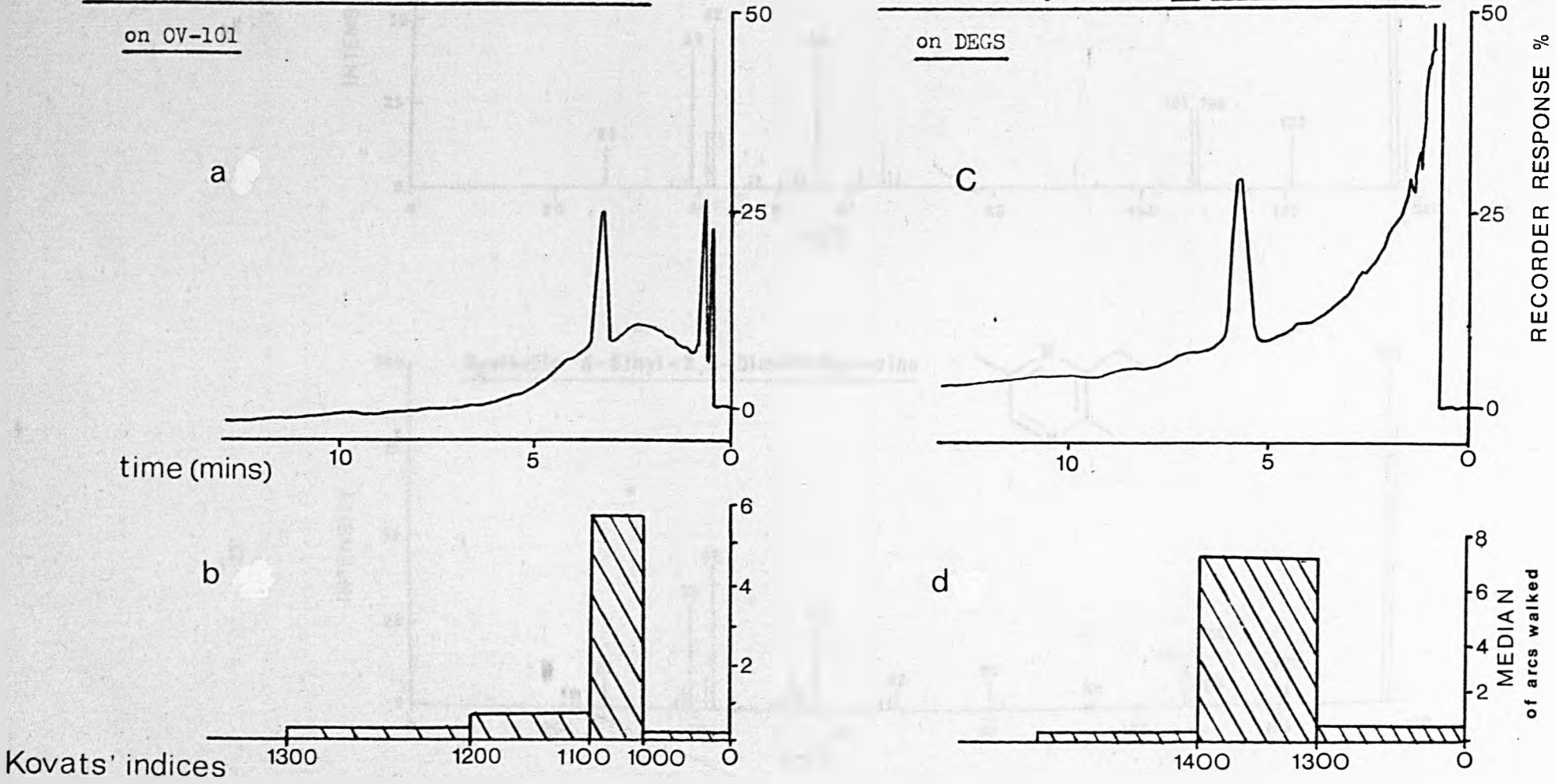
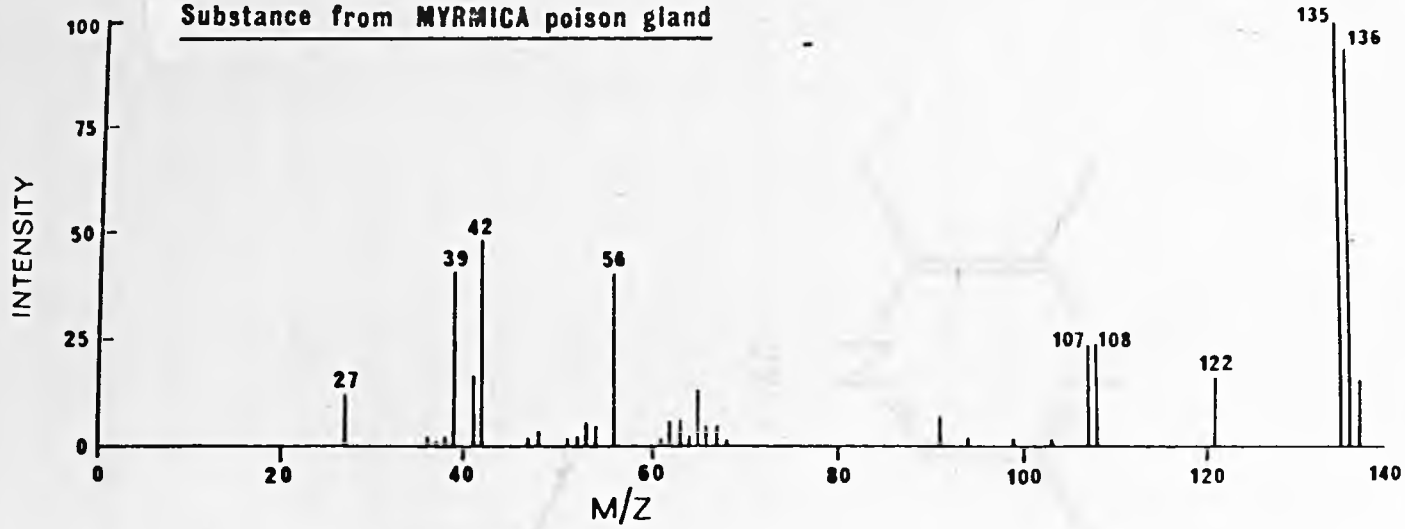
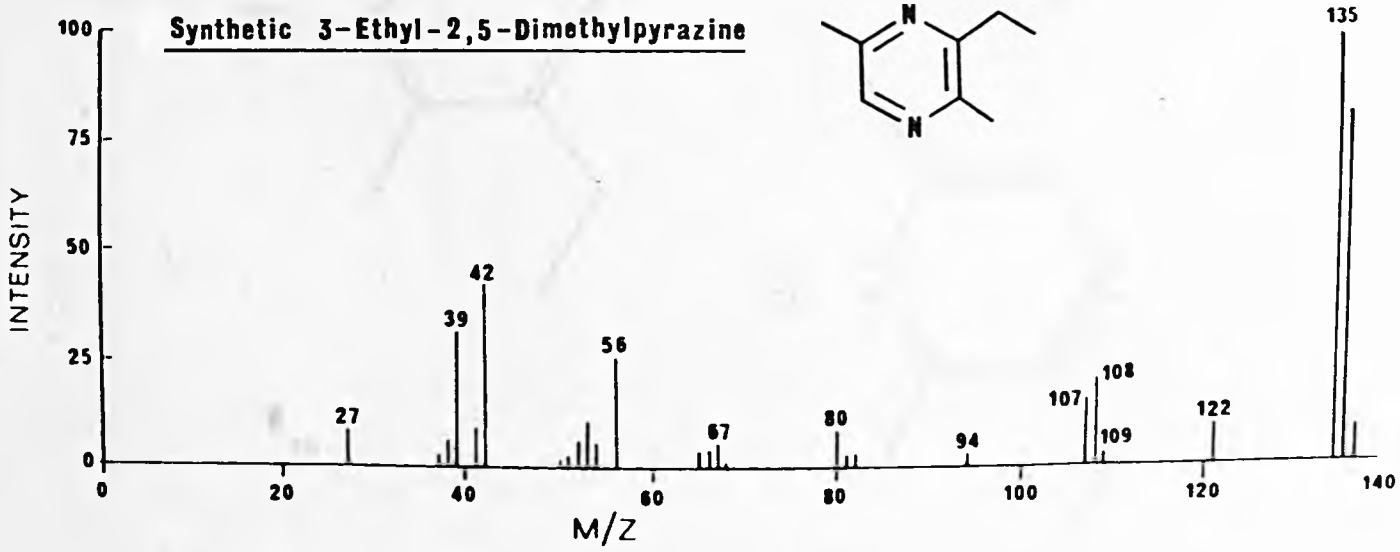


FIGURE 4

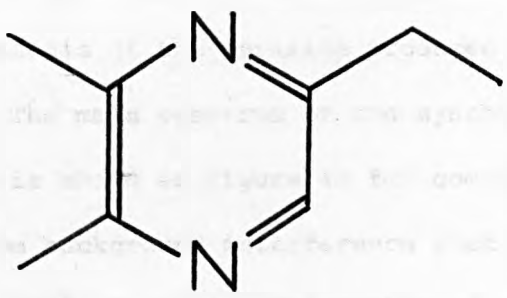
a


Substance from MYRMICA poison gland
INTENSITY
M/Z
100
75
50
25
0
0
20
40
60
80
100
120
140
27
39
42
56
107
108
122
135
136

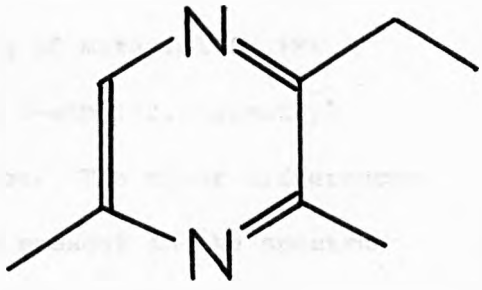
b


Synthetic 3-Ethyl-2,5-Dimethylpyrazine
INTENSITY
M/Z
100
75
50
25
0
0
20
40
60
80
100
120
140
N
N
27
39
42
56
67
80
94
107
108
109
122
135

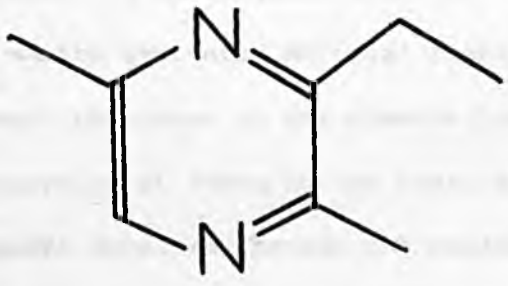
Structural isomers of ethyldimethylpyrazine



27



28



8

FRIEDEL et al., 1971). The fragments observed in the spectrum are typical of those arising through the cleavage of a substituted alkylpyrazine (BUDZIKIEWICZ et al., 1964). Figure 5 shows how the fragments of 3-ethyl-2,5-dimethylpyrazine arise.

Synthesis of the pyrazine produced 1.2g of material (a 19% yield). The mass spectrum of the synthetic 3-ethyl-2,5-dimethyl pyrazine is shown in Figure 4b for comparison. The minor differences arise from background interference that was present in the spectrum of the insect material. The synthetic material had the same retention time on three columns (G,K and M) as the natural material. Furthermore, its R_f when subjected to TLC in the eluents corresponded exactly to the region in which the ethological activity was observed (Table 8). The behaviour of the synthetic material exactly reproduces that of the insect pheromone in all chemical tests including the complete absorption of 100ng by the boric acid loop.

By comparing the GC peaks obtained through the analyses of ten M. rubra workers' venom apparatuses to those of the synthetic standard, it has been found that the workers contain an average of 5.8±1.7ng of 3-ethyl-2,5-dimethylpyrazine. Table 9 lists the absolute amounts of the trail substance in the ten workers analysed.

Table 10 summarises the reactions of the worker ants to 5.8ng of 3-ethyl-2,5-dimethylpyrazine (the mean amount per worker ant) presented in a bioassay experiment compared to their responses to hexane extracts of single poison apparatuses. Statistically, there is no significant difference between the trail following responses of the workers to the synthetic material at the glandular level and an extract of their poison apparatuses at a 95% level of significance

FIGURE 5

MS fragmentation of 3-ethyl-2,5-dimethylpyrazine

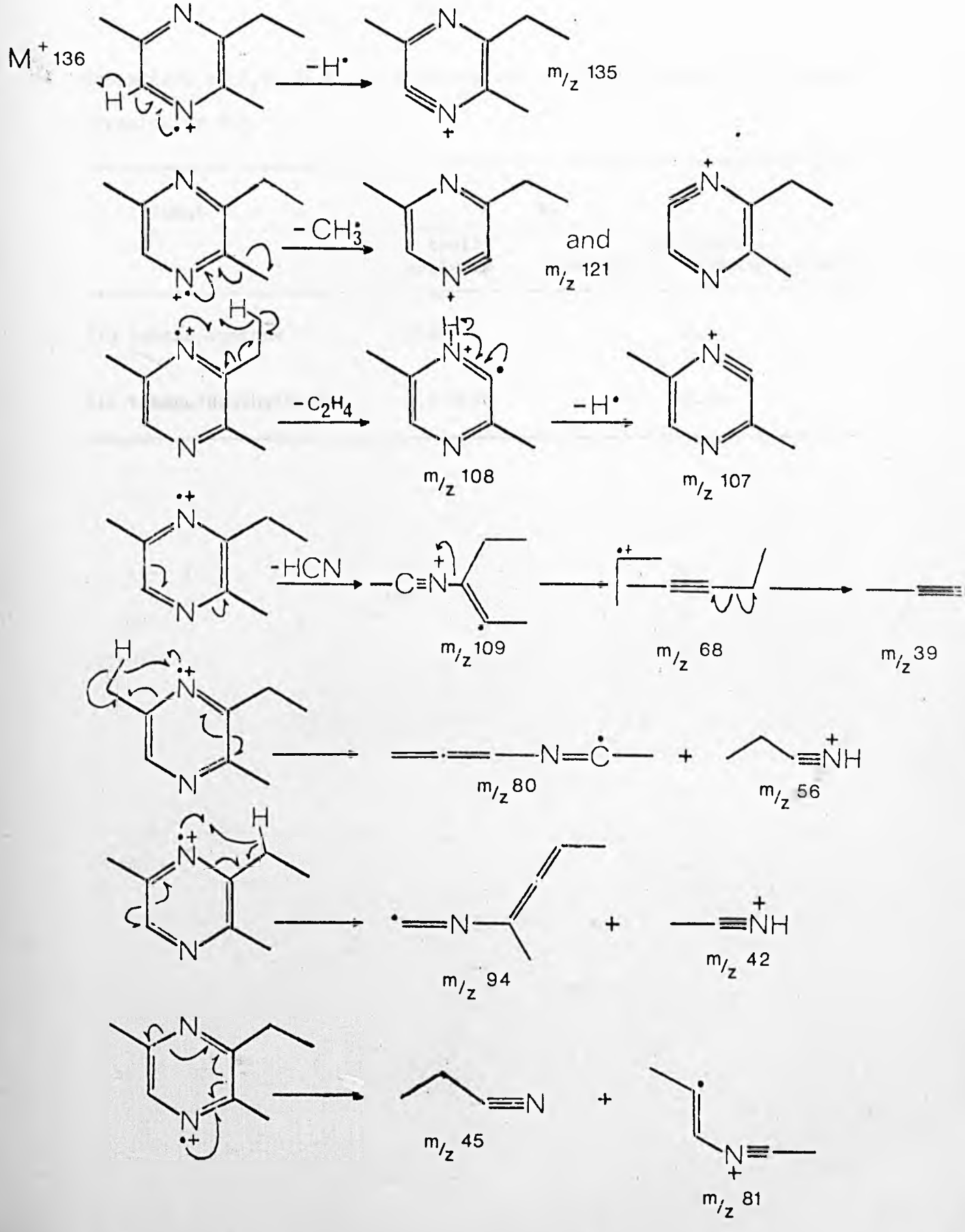


TABLE 8

Comparison of R_fs of trail activity and synthetic 3-ethyl-2,5-dimethylpyrazine by TLC.

Eluent	R_f	
	trail activity	synthetic 3-ethyl-2,5-dimethylpyrazine
7:3 hexane/acetone	0.4-0.6	0.44
6:4 toluene/diethylether	0.2-0.4	0.34

TABLE 9

Absolute amounts of 3-ethyl-2,5-dimethylpyrazine in ten M. rubra workers.

Replicates		Absolute amount (ng)
1		5.4
2		3.3
3		8.1
4		5.6
5		8.0
6		4.1
7		3.1
8		6.8
9		6.1
10		7.6
	Mean	5.8
	SD	1.7

TABLE 10

Comparison of trail following activity of 1 gland-equivalent of *M. rubra* poison reservoir contents (duplicate results) with 5.8ng (the average amount calculated to be present) of synthetic 3-ethyl-2, 5-dimethylpyrazine. The probability of difference from solvent controls, P, was found using the non-parametric χ^2 test. The Mann-Whitney U test was used to look for difference between natural and synthetic substances.

Sample/31.4 cm trail	Median no. of arcs	P	Mann-Whitney U test
1 gland eqv.	3.90	0.001	no sign. diff. (95 %)
1 gland eqv.	3.14	0.001	no sign. diff. (95 %)
5.8 ng	3.33	0.001	-

(Mann-Whitney U test). This implies that the 3-ethyl-2,5-dimethyl pyrazine is the only substance present in the venom of M. rubra which elicits a trail following response from the workers.

The upper and lower trail following thresholds of the workers to 3-ethyl-2,5-dimethylpyrazine were determined by presenting a range of concentrations to them as a circular trail (Table 11). The 3-ethyl-2,5-dimethyl pyrazine elicited trail following in the ants at 10^{-2}-10^{2}ng/trail i.e. 3.19 x 10^{-4} to 3.19ng/cm range. The response was found to be strongest at 1ng per trail i.e. 31.9pg/cm. Figure 6 summarises the results of these bioassays of the synthetic 3-ethyl-2,5-dimethylpyrazine.

It has been demonstrated that the eight species of Myrmica: M. rubra, M. scabrinodis , M. ruginodis, M. sabuleti, M. sulcinodis, M. rugulosa, M. lobicornis and M. schencki show no species specificity in following trails made from the contents of each other's poison reservoirs (CAMMAERTS et al., 1978, 1981, and unpublished).

The presence of the 3-ethyl-2,5-dimethylpyrazine in the venom of all the above species has been confirmed by GC of single glands on polar and non-polar phases. Although all the species produce similar amounts (nanograms) of the pyrazine, the large number of determinations necessary for a statistical analysis has only been carried out for M. rubra.

Analysis of the Attine venom glands

Previous investigations of the pheromones of leaf cutting ants have involved extractions of volatiles from very large masses (kgs) of whole or parts of insects bodies. In this investigation single

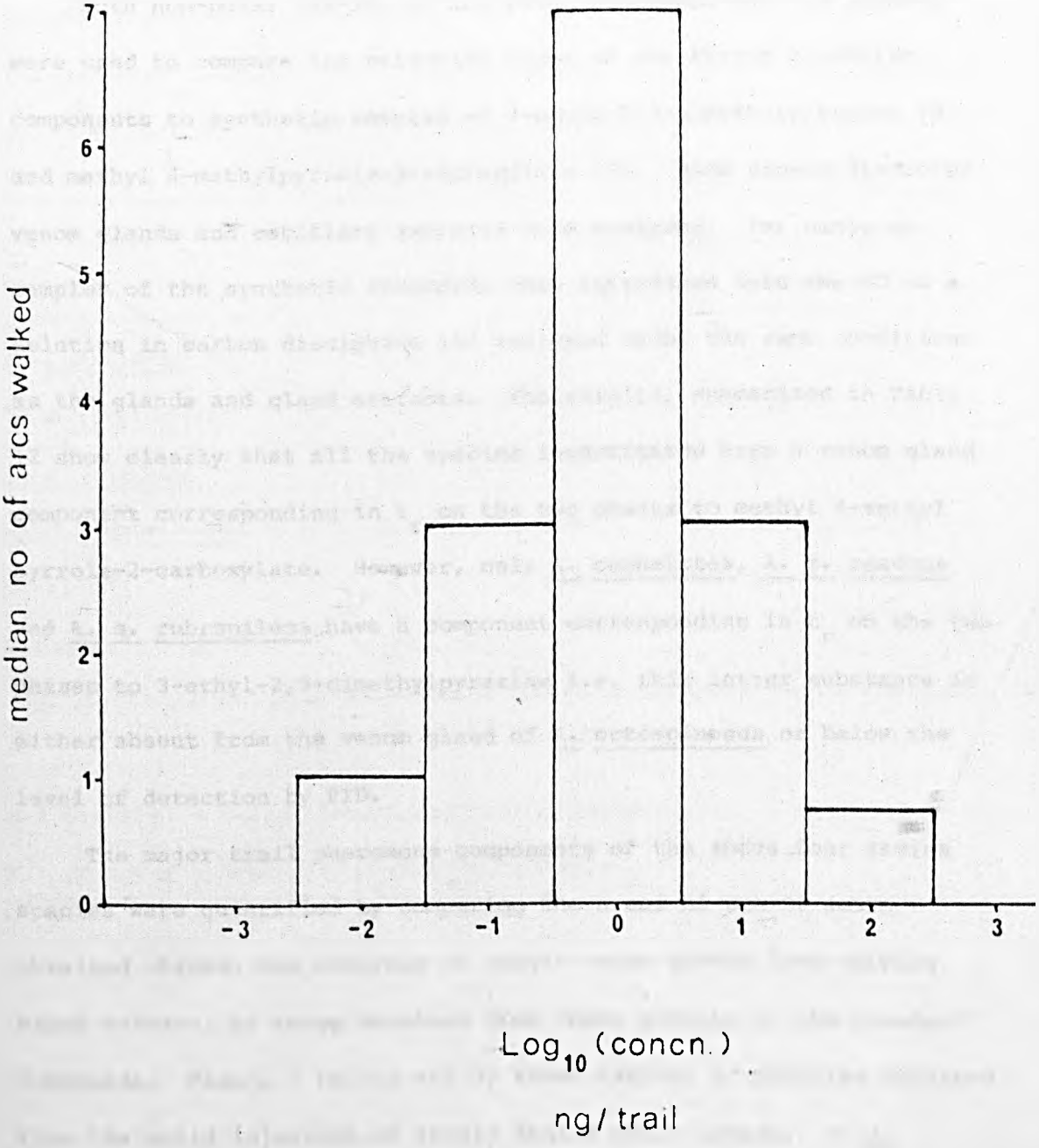
TABLE 11

Activity of pure 3-ethyl-2,5-dimethylpyrazine over a range of concentrations in the trail-following bioassay. The probability, P, of difference from solvent controls and the median number of arcs walked by each worker of M. rubra is given in each case.

Concentration/trail (ng/31.4 cm)	ng/cm	Median no. of arcs.	P
10^{-3}	3.19×10^{-3}	0.30	N.S.
10^{-2}	3.19×10^{-4}	1.38	0.001
10^{-1}	3.19×10^{-3}	3.00	0.001
1.0	3.19×10^{-2}	7.00	0.001
10	3.19×10^{-1}	3.00	0.001
10^{2}	3.19	0.68	0.001
10^{3}	31.9	0.26	N.S.

FIGURE 6

Bioassay of synthetic 3-ethyl-2,5-dimethylpyrazine over a range of concentrations



dissected glands and capillary extracts of single glands were analysed by GC using a solid sampling technique.

Both non-polar (SE-30, M) and polar (CARBOWAX 20M, K) columns were used to compare the retention times of the Attine glandular components to synthetic samples of 3-ethyl-2,5-dimethylpyrazine (**8**) and methyl 4-methylpyrrole-2-carboxylate (**7**). Both single dissected venom glands and capillary extracts were analysed. Ten nanogram samples of the synthetic standards were introduced into the GC as a solution in carbon disulphide and analysed under the same conditions as the glands and gland extracts. The results, summarised in Table 12 show clearly that all the species investigated have a venom gland component corresponding in t_r on the two phases to methyl 4-methyl pyrrole-2-carboxylate. However, only A. cephalotes, A. s. sexdens and A. s. rubropilosa have a component corresponding in t_r on the two phases to 3-ethyl-2,5-dimethylpyrazine i.e. this latter substance is either absent from the venom gland of A. octospinosus or below the level of detection by FID.

The major trail pheromone components of the above four Attine species were quantified by comparing the areas of the GC peaks obtained through the analysis of single venom glands from varying sized workers, to those obtained from known amounts of the standard compounds. Figure 7 (a,b,c and d) shows typical GC profiles obtained from the solid injection of single Attine venom glands. In A. octospinosus there is not such a wide variation in the size of workers as in the Atta species, and Figure 8a shows that there is a linear relationship between the amount of methyl 4-methylpyrrole-2-carboxylate and the live body weights of the worker ants. For the

TABLE 12

Comparison of GC retention times of Attine venom gland components with standard compounds on non-polar (SE-30) and polar (Carbowax 20M) GC phases. For the analysis of the pyrrole and pyrazine on the SE-30 phase the GC column temperature was maintained at 119 °C and 87 °C respectively. On the Carbowax 20M phase the temperatures for the pyrrole and pyrazine analyses were 134 °C and 75 °C respectively.

Species	Retention times (mins)			
	SE-30		Carbowax 20M	
A. cephalotes	2.4	2.8	2.2	3.2
A. octospinosus	-	2.8	-	3.2
A. s. sexdens	2.4	2.8	2.2	3.2
A. s. rubropilosa	2.4	2.8	2.2	3.2
pyrrole[a]	-	2.8	-	3.2
pyrazine[b]	2.4	-	2.2	-

a : pyrrole = methyl 4-methylpyrrole-2-carboxylate

b : pyrazine = 3-ethyl-2,5-dimethylpyrazine

FIGURE 7

Gas chromatography of the major trail pheromone components of leaf-cutting ants

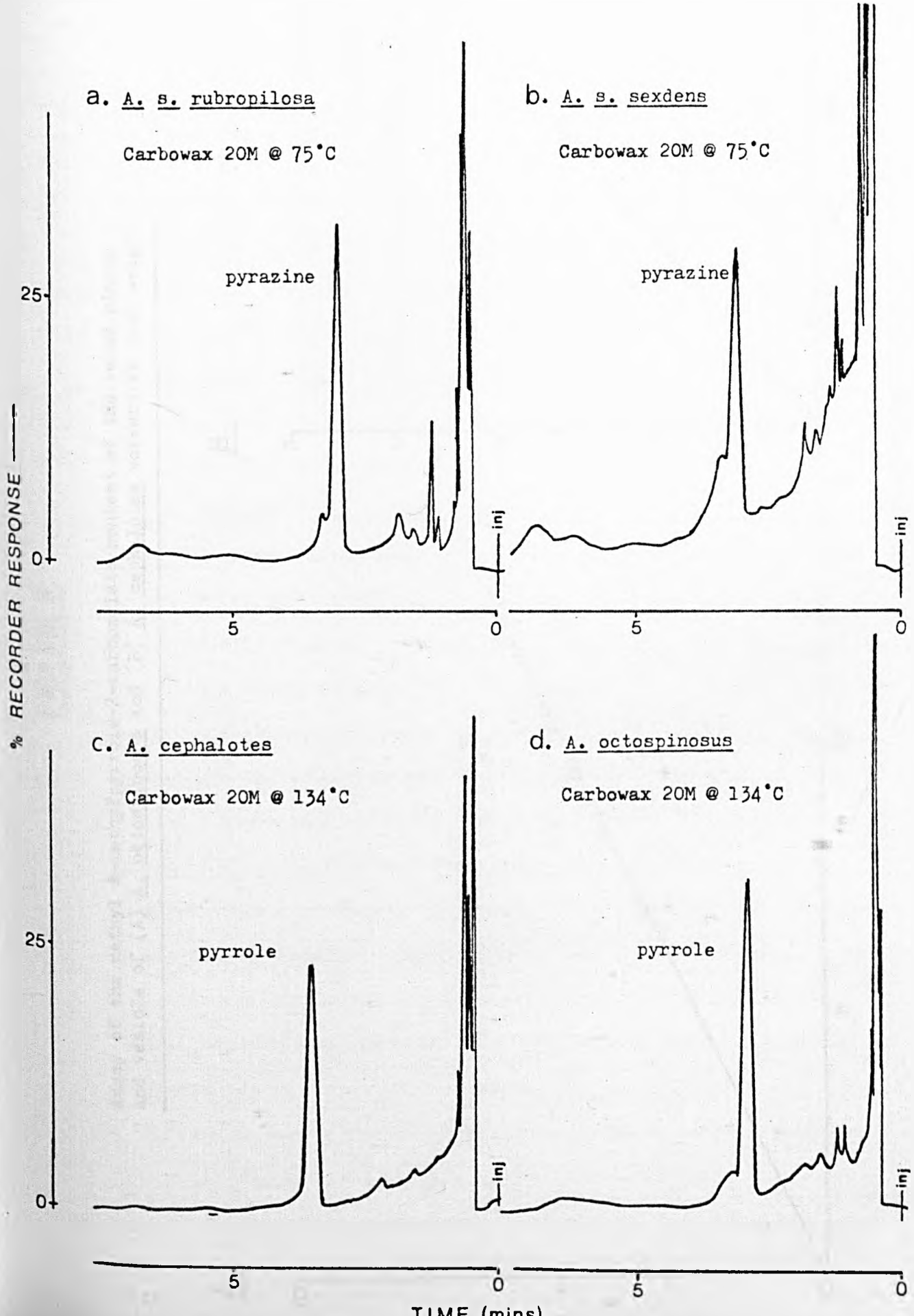
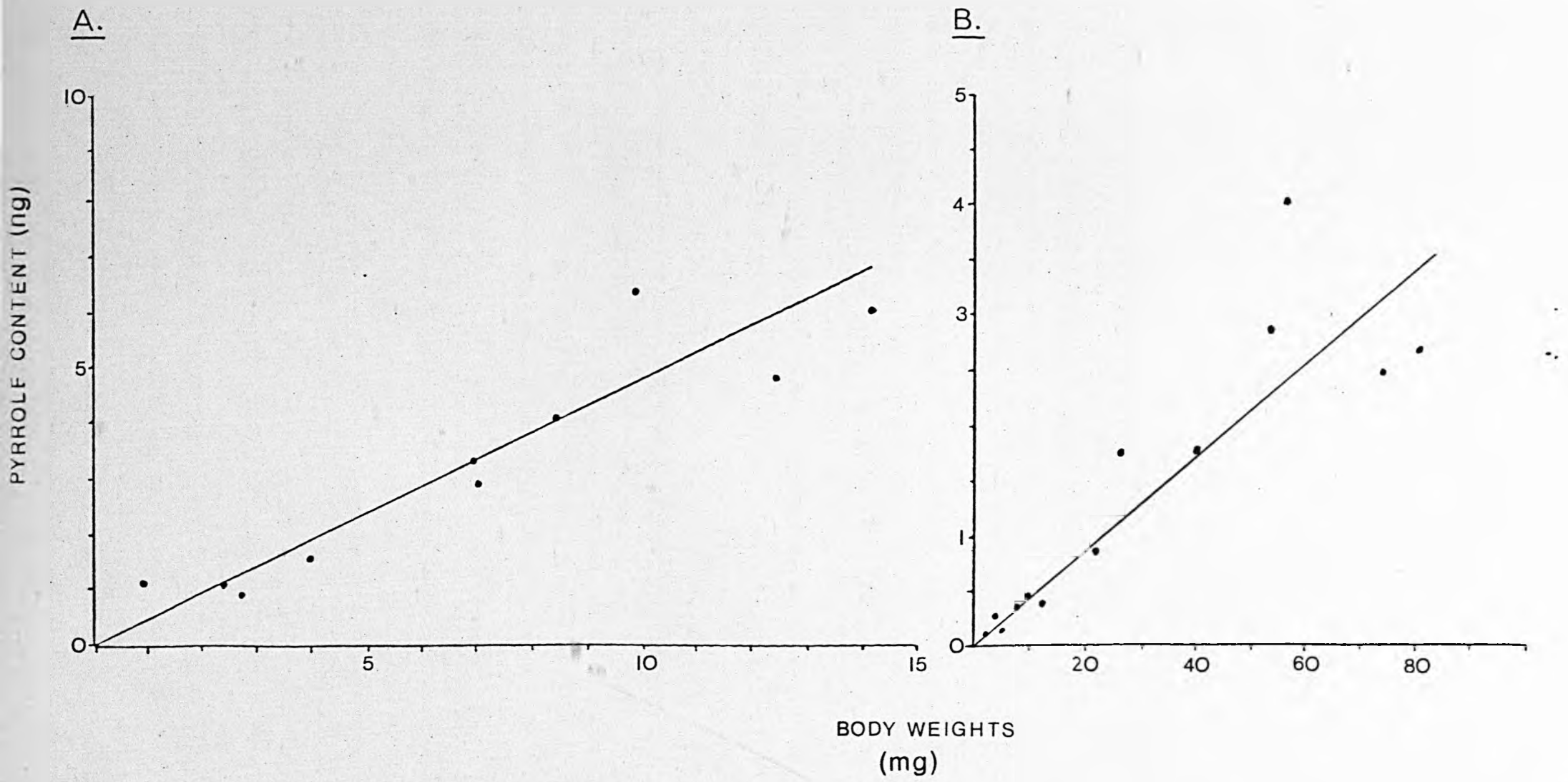


FIGURE 8

Assay of the methyl 4-methylpyrrole-2-carboxylate content of the venom glands and vesicle of (A) A. octospinosus and (B) A. cephalotes workers vs body weight



ten workers that were analysed having body weights 1.1mg-14.2mg an average of 3.3ng (range 0.9-6.5ng) of trail substance was found.

The major component of the trail pheromone of A. cephalotes is also methyl 4-methylpyrrole-2-carboxylate; the analyses showed that this species produces on average only 1.1ng (range 0.2-2.9ng) per worker. Furthermore although the quantity of trail pheromone is directly proportional to body weight in the workers, this linearity is not extrapolated to the soldiers. Figure 8b shows that the relationship is less well-defined in the soldier caste.

In contrast to A. octospinosus and A. cephalotes, the two A. sexdens subspecies produce 3-ethyl-2,5-dimethylpyrazine as the major component of their trail pheromone. As in A. cephalotes, a soldier caste is also present, and similarly the quantity of trail substance observes a linear relationship to body weight in the worker caste which is less obvious in the soldiers (Figures 9a and 9b). The A. s. sexdens and A. s. rubropilosa workers produce an average of 4.3ng (range 0.2ng-9.1ng) and 3.9ng (range 0.1ng-8.8ng) of the pyrazine respectively. Similar sized workers of each subspecies produce similar amounts of 3-ethyl-2,5-dimethylpyrazine. Table 13 summarises the mean and range of amounts of the Attine trail substances.

The raw quantitative data obtained from the analyses of the trail pheromone components of these leaf cutting ants is given in Appendix 1.

In this work 3-ethyl-2,5-dimethylpyrazine has been shown to be the trail pheromone of M. rubra, it has also been found in the venom gland of seven other Myrmica species namely: M. ruginodis, M. rugulosa, M. scabrinodis, M. sabuleti, M. sulcinodis, and M.

FIGURE 9

Assay of the 3-ethyl-2,5-dimethylpyrazine content of the venom glands and vesicle of (A) A. s. sexdens and (B) A. s. rubropilosa workers vs body weight

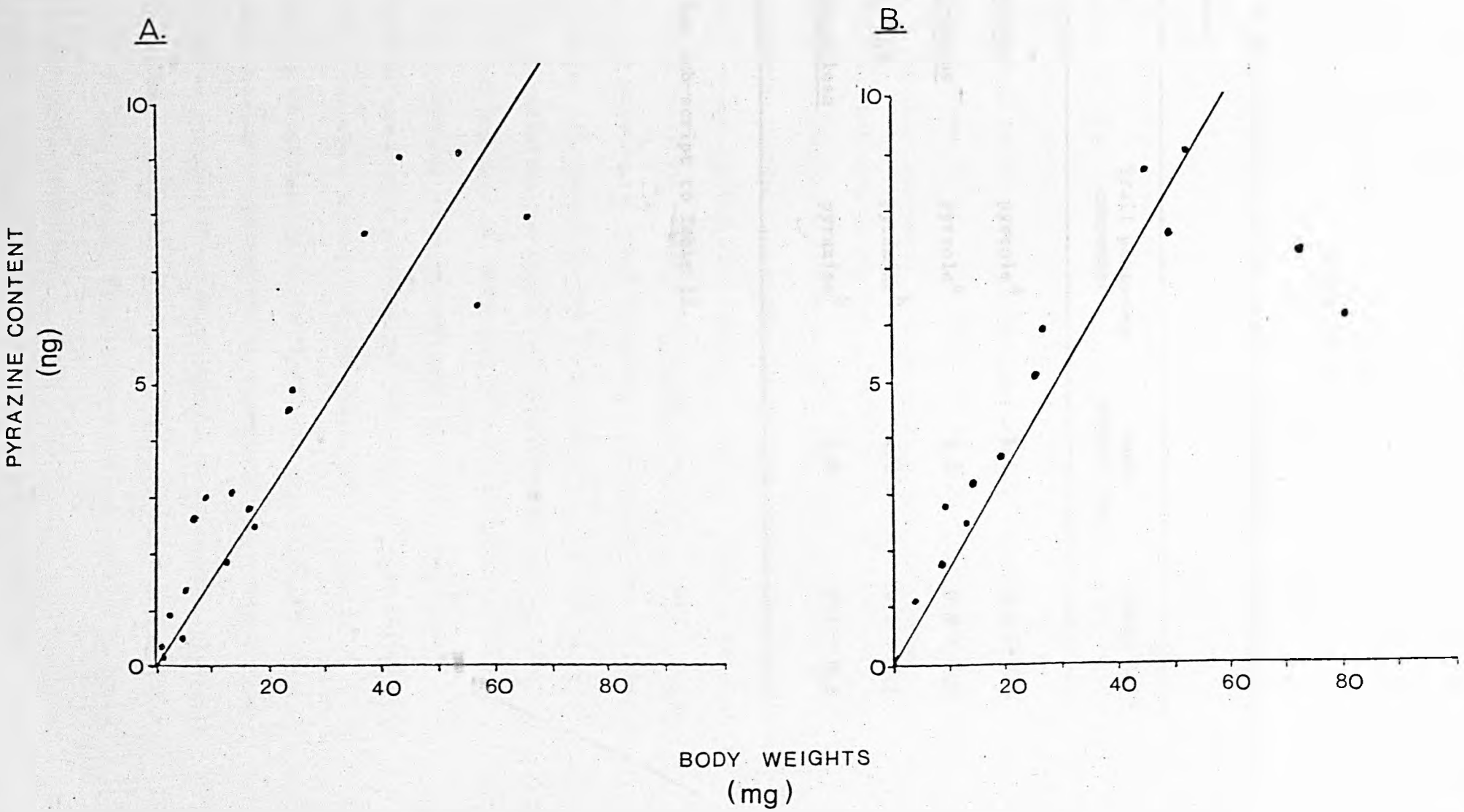


TABLE 13

Summary of quantification of the major trail pheromone components of Attine ants.

Species	Trail pheromone component	mean amount (ng)	Range (ng)
A. cephalotes	pyrrole[a]	1.1	0.2 - 2.9
A. octospinosus	pyrrole[a]	3.3	0.9 - 6.5
A. s. sexdens	pyrazine[b]	4.3	0.2 - 9.1
A. s. rubropilosa	pyrazine[b]	3.9	0.1 - 8.8

a + b : see sub-script to Table 12.

lobicornis. All of these ants follow trails formed from one another's venom. Only five of the above eight species have so far been tested with the synthetic pheromone: M. rubra, M. sabuleti, M. scabrinodis, M. ruginodis and M. rugulosa, all of which show a strong trail following response at 1ng per 10cm diameter circular trail (see Table 14). This is the first identification of a trail pheromone in a temperate species of ant. All the above species store nanogram amounts of the trail substance in their venom glands. The amount of trail pheromone has been determined accurately for the first time by employing a solid sampling technique and performing determinations on single dissected glands. For workers of M. rubra this figure is 5.8± 1.7ng (mean of ten replicate analyses). A range of concentrations of the synthetic trail was presented to M. rubra workers. They showed statistically significant trail following responses at the 10^{-2}-10^{2}ng circular trail. At concentrations lower than 10^{-2} the workers have difficulty in detecting the trail. At concentrations higher than 100ng per trail the ants are unable to follow a discrete trail as a result of the critical odour passage becoming too broad through evaporation of the active material and the ants, although strongly attracted to the area, become confused and wander aimlessly.

There is no statistically significant difference in the trail following responses of the workers to artificial trails formed from either 5.8ng of the synthetic trail compound or to extracts of single venom apparatuses. This indicates that the 3-ethyl-2,5-dimethyl pyrazine is the only compound present in the venom gland of M. rubra which elicits trail following.

The simple trail following test of PASTEELS and VERHAEGHE (1974)

TABLE 14

Responses of Myrmica species to synthetic 3-ethyl-2,5-pyrazine in circular trail bioassay (1 ng/31.4 cm trail). Tabulated figures correspond to the number of workers following the trail for a given number of arcs.

Species	No. of arcs walked ##							
	0	1	2/3	4/5	6/8	9/12	13/20	21<
M. rubra†	19	6	8	10	9	5	4	9
M. sabuleti*	0	2	8	7	10	5	18	8
M. rugulosa*	0	0	1	9	12	16	12	10
M. scabrinodis†	6	4	5	7	5	8	14	7
M. ruginodis†	6	4	7	5	8	8	10	8
Control	47	12	11	2	2	-	-	-

† : experiments performed by R.P.E. at the University of Keele.

* : experiments performed by M.C. CAMMAERTS at the Université de Bruxelles.

: all trail following responses significantly different from the hexane control at a level of probability of 0.001

was used throughout this work. The degree of trail following observed in a particular experiment is usually expressed as the median number of arcs walked on the circular trail. It was found that the degree of trail following was directly related to the density of workers in the region of the test trail. This is related to its positioning relative to the nest entrance. It was found that by placing the test trail 1m away from the nest entrance on a clean area temporarily attached to the nest tray a much higher level of trail following was observed than when the synthetic trail was positioned in the nest tray 30cm from the nest entrance (Table 15). These variations arise as a result of differences in the numbers of ants in the region of the trail in the two cases. When the trail is positioned close to the nest the density of ants on the trail is high and encounters between workers often lead to trail following ants being diverted from the trail. As a result the median is reduced relative to that obtained when the trail is positioned further away or when less ants are foraging. Consequently, care must be taken to ensure that, as near as possible, reproducible conditions are employed, if the results of two or more tests are to be compared.

It has been demonstrated that the poison and Dufour glands secretions are used in food gathering and recruitment (CAMMAERTS et al., 1978; CAMMAERTS and CAMMAERTS, 1980) but trails made with the poison gland and the Dufour gland are less attractive than the poison gland alone (Table 16). BLUM (1974b) has also shown that M. americana, M. brevinodis, M. emeryana, M. fracticornis and M. rubra all follow each others trails. It may be inferred that these species also employ 3-ethyl-2,5-dimethylpyrazine in their trail pheromone, although the

TABLE 15

Trail following bioassay of 1 gland-equivalent of contents of poison reservoir of workers of M. rubra, tested against M. rubra workers. The variability of the number of ants reaching the test paper (N) and the median number of arcs run (Md) is given, with the paper at different distances (D) from the nest entrances. The numbers in each column are the number of individuals running continuously that number of arcs of the circle.

				number of arcs run								
	Experiment	N	Md+	0	1	2/3	4/5	6/8	9/12	13/20	21/30	31/60	60<
A	Control	74	0.29	47	12	11	2	2	-	-	-	-	-
	D = 30 cm	70	3.9	19	6	8	10	9	5	4	4	1	4
B	Control	8	0.3	5	1	2	-	-	-	-	-	-	-
	D = 100 cm	10	20	-	-	-	-	-	1	4	2	3	-

+ : the higher the value of the median (Md) the greater is the degree of trail following

TABLE 16

Comparison of trail following by M. rubra workers to trails formed from the venom alone and from the venom presented together with the Dufour gland contents.

Test trail	No. of arcs walked								Md (Median no. of arcs)+
	0	1	2/3	4/5	6/8	9/12	13/20	21<	
venom	0	2	9	5	8	11	4	1	6.5
venom/Dufour	0	20	11	6	1	2	-	-	1.0

+ : the higher the value of the median (Md) the greater is the degree of trail following

possibility of additional compounds is not ruled out. Surprisingly, BLUM found that M. monticola does not share trail following behaviour with the other species. Therefore it must be concluded for the time being that the pyrazine is widely but not exclusively used as a trail pheromone in the genus Myrmica. The same pyrazine has recently been shown to be present in the trail pheromone of A. s. rubropilosa (CROSS et al., 1979). A. s. sexdens also responded to the synthetic pyrazine in a trail bioassay, and the GC analyses reported here have confirmed its presence in the venom gland of this species. BLUM (1974b) also demonstrated cross activity between the venom gland secretions of the Myrmica species he tested and Manica bradleyi, M. hunteri, M. mutica, Pogonomyrmex badius and P. barbatus (but not between P. badius and P. barbatus themselves). The substance 3-ethyl -2,5-dimethylpyrazine would appear to be used with great economy throughout a number of myrmicine genera as a trail substance.

It may be noted that methyl 4-methylpyrrole-2-carboxylate, the trail pheromone of A. texana, A. cephalotes and A. octospinosus has also been found in A. s. rubropilosa but it has no activity in the latter species at the pheromone level (CROSS et al., 1979). This investigation has also confirmed the presence of the pyrrole in A. s. sexdens. This discovery together with the finding of 3-ethyl-2,5-dimethylpyrazine in A. cephalotes (approximately 0.5ng/worker) but not in A. octospinosus are in complete agreement with the interspecific trail following reactions observed by ROBINSON et al. (1974) and it would appear that these findings offer a complete explanation of the cross-reactions noted between A. cephalotes, A. s. sexdens and A. octospinosus. However there may well be other

substances present which contribute to the species specificity of these trail pheromones. Although methyl 4-methylpyrrole-2-carboxylate appeared to be widely distributed in the Attine ants none could be detected in the venom of M. rubra. For the present, methyl 4-methylpyrrole-2-carboxylate would seem to be restricted to the Attine species while 3-ethyl-2,5-dimethylpyrazine is more widespread in the Myrmicinae.

Heterocyclic nitrogen compounds occur frequently in the glands of myrmicine ants. In addition to those mentioned above, and the octahydroindolizine, Monomorine I and the pyrrolidines, Monomorines II and III, which contribute to the trail following in Monomorium pharaonis (RITTER et al., 1980), other species possess similar compounds but these have not been shown to be ethologically active. Solenopsis richteri, S. xyloni, S. invicta. S. geminata and S, aurea produce species and caste specific mixtures of a number of 2,6-dialkylpiperidines (MACCONNELL et al., 1971, 1974; BRAND et al., 1972; BLUM et al., 1973) which possess insecticidal, hemolytic, antibacterial and necrotic activity. S. punctaticeps, S. molesta and S. texanas produce 2,5-dialkylpyrrolines and pyrrolidines (BLUM and HERMANN,1978; JONES et al., 1979) while Pheidole fallax produces 3-methylindole (skatole) in its venom gland (LAW et al., 1965). A number of trisubstituted pyrazines are produced as alarm pheromones in the mandibular glands of some Ponerine and Dolichoderine species (WHEELER and BLUM, 1973; BLUM and HERMANN, 1978 ; CAVILL and HOUGHTON, 1974a,b; LONGHURST et al., 1978).

Although the techniques employed here are not new, the use of solid sampling in the investigation of trail pheromones is somewhat

innovative. The overall method has the advantage of being highly economical with respect to the amount of insect material used, a feature borne out by the fact that only fifty venom gland extracts were required for the GC-MS determination. With the most up to date mass spectrometers, the number required would have been even less. Furthermore the chemical analyses, bioassays and GC trapping experiments required only five glands each, while the quantification by GC required only a single gland for each determination, in contrast to previous investigations of trail pheromones, all of which have employed many thousands of ants. For example, the identification of 3-ethyl-2,5-dimethylpyrazine from A. s. rubropilosa required 9kg of ants i.e. 500,000 workers.

Chemical Investigation of Dufour Gland Substances.

The leaf-cutting ants are members of the myrmicine tribe Attini, which shares the habit of cultivating and eating fungus with certain termites and wood-boring beetles. Their distribution is limited to the New World, occuring in Mexico, Central and South America between the latitudes 40°N and 40°S.

The leaf-cutting ants are particularly advanced social insects which have a polymorphic caste system characterised by both a gradation of size and division of labour (WEBER, 1966). There are three distinct castes; the sexuals (Queens and males), soldiers (occuring only in Atta species) which protect the nest and workers (mediums and minims). The mediums do all the foraging while the minims are concerned with tending the Queen and brood, and preparation of the leaves brought in by the mediums. The leaves provide a substrate for the symbiotic fungus on which the ants feed.

The leaf-cutting ants constitute serious agricultural pests in certain areas as a result of their defoliating behaviour. Investigations have been performed into the pheromones of several Attine species with a view to incorporating them into pest control programmes. While the trail pheromones of these ants originate from the venom gland, and alarm pheromones from their mandibular glands, the biological significance of the Dufour gland (found in close association with the venom glands and vesicle) is unknown.

Ants of the genus Myrmica occur in the temperate zones of Eurasia and North America. Unlike their Attine relations they do not cultivate fungus but are instead largely carnivorous, feeding mainly

on small insect prey. Previous investigations have shown that their mandibular and venom gland secretions are employed for the purpose of alarm, defence and recruitment. The chemistry and ethology of the Dufour gland secretion is particularly well documented for M. rubra and M. scabrinodis (MORGAN and WADHAMS, 1972b; CAMMAERTS et al., 1978; MORGAN et al., 1979). Their Dufour gland secretions consist mainly of hydrocarbons with smaller amounts of oxygenated compounds. In both species the secretion is employed in territorial marking, in addition to having short lived attractive properties. The hydrocarbons produced by the above two Myrmica species are of two distinct types; workers of M. rubra for example produce predominantly linear hydrocarbons in their Dufour gland while those of M. scabrinodis produce mainly sesquiterpenoid hydrocarbons.

This study has been undertaken in order to investigate, principally, the chemical components of the Dufour gland in workers of several pest species of the Attine tribe. The results are intended to serve as the basis for future ethological and biosynthetic studies.

The results of investigations into the Dufour glands of three further species of Myrmica are also reported. The aims of these latter studies were to identify substances present in the glands of workers in order to discover whether these three species also produce linear or terpenoid hydrocarbons, or perhaps some other class of compounds. These results are also intended to serve as a basis for future ethological and biosynthetic studies. They may also have some taxonomic significance.

Investigation of Attine Dufour glands

A. cephalotes

The Dufour glands of A. cephalotes workers appear as opaque pear shaped sacs (Figure 10) having slightly yellowish colouration.

Initially single dissected workers Dufour glands were introduced into the GC via a solid sampler and the volatile constituents analysed on a non-polar OV-101 column (A). These analyses revealed nanogram amounts of fourteen components (Figure 11a).

Gas chromatography of a mixture of standard n-alkanes showed components 1,2,3,5,7,9,11 and 14 to have the same trs on the non-polar OV-101 and polar DEGS phases to n-alkanes in the C_{12} to C_{19} chain length range. The major component of the gland, component 9, was found to have the same tr as n-heptadecane.

Microbromination of the material from a single gland resulted in the elimination of peaks 4,6,8,10,12 and 13 from the GC profile (Figure 11b), indicating that the substances are unsaturated. Components 1,2,3,5,7,9,11 and 14 were unaffected by the bromine. This is consistent with them being n-alkanes.

Components 8 and 13 have the same tr on polar and non-polar GC phases as synthetic heptadecene and nonadecene respectively. Plotting log tr against the number of carbon atoms in the chain suggested that components 4,8,10 and 13 were part of a homologous series of mono-unsaturated alkenes (Figure 12). On this basis components 4 and 10 would be pentadecene and octadecene respectively.

Linked GC-MS was performed on a Pye 104 GC-Hitachi-Perkin Elmer RMU 6E system. Ten Dufour glands were introduced via a solid

FIGURE 10

Sketch of the Dufour gland and poison gland complex of *Atta cephalotes*

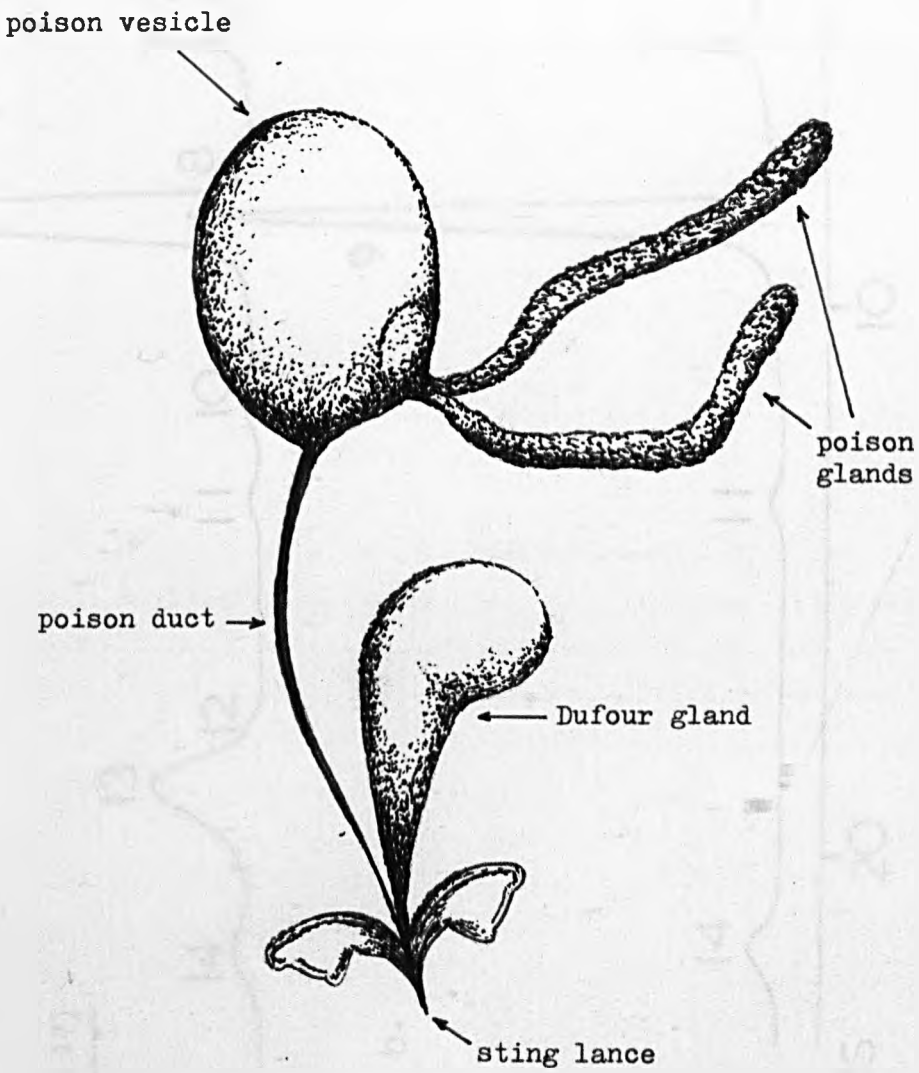


FIGURE 11

GC profiles of the Dufour gland hydrocarbons of *A. cephalotes* (a) Before (b) After treatment with bromine. Analyses performed on 5% OV-101 (Nos. of peaks refer to TABLE 17)

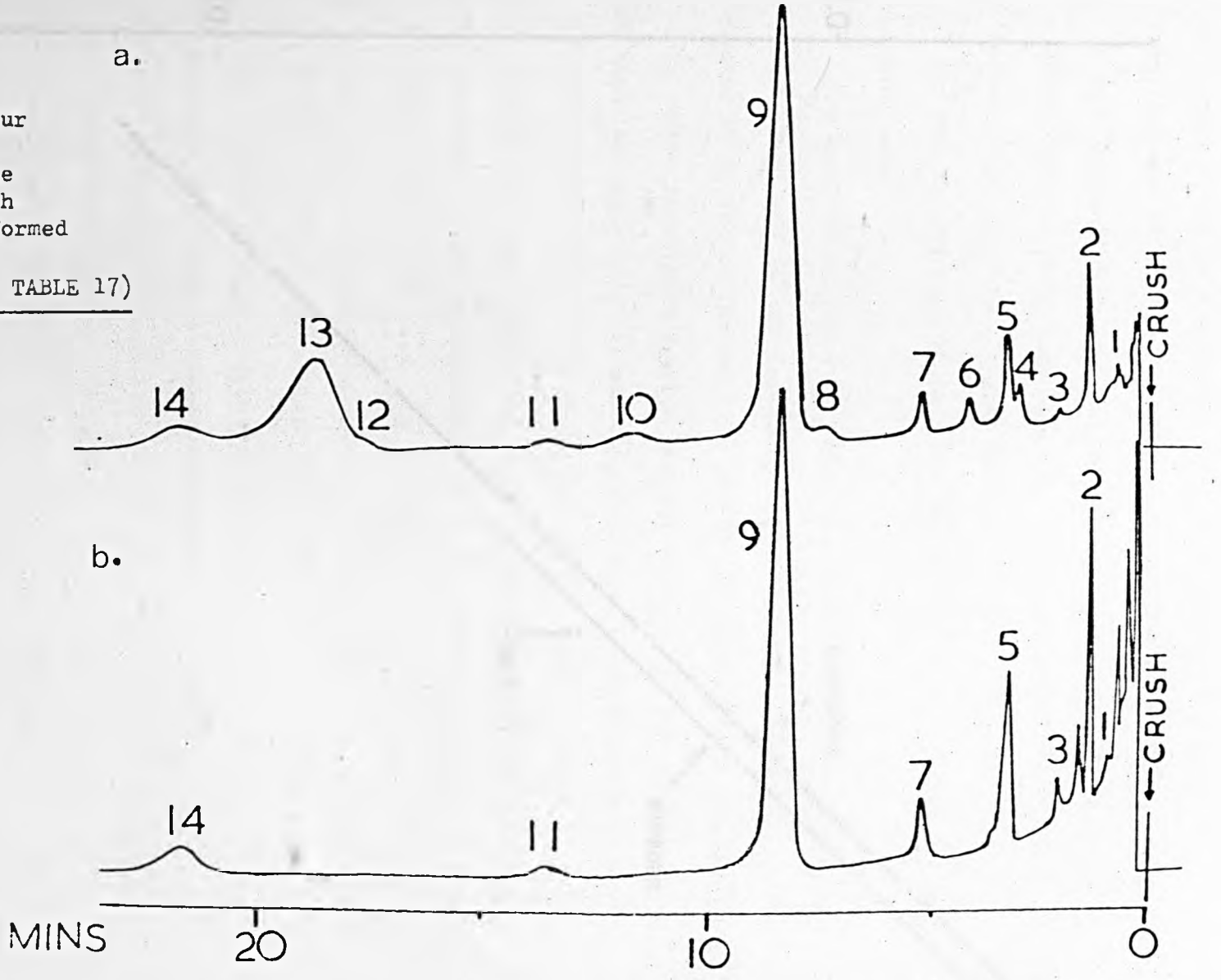
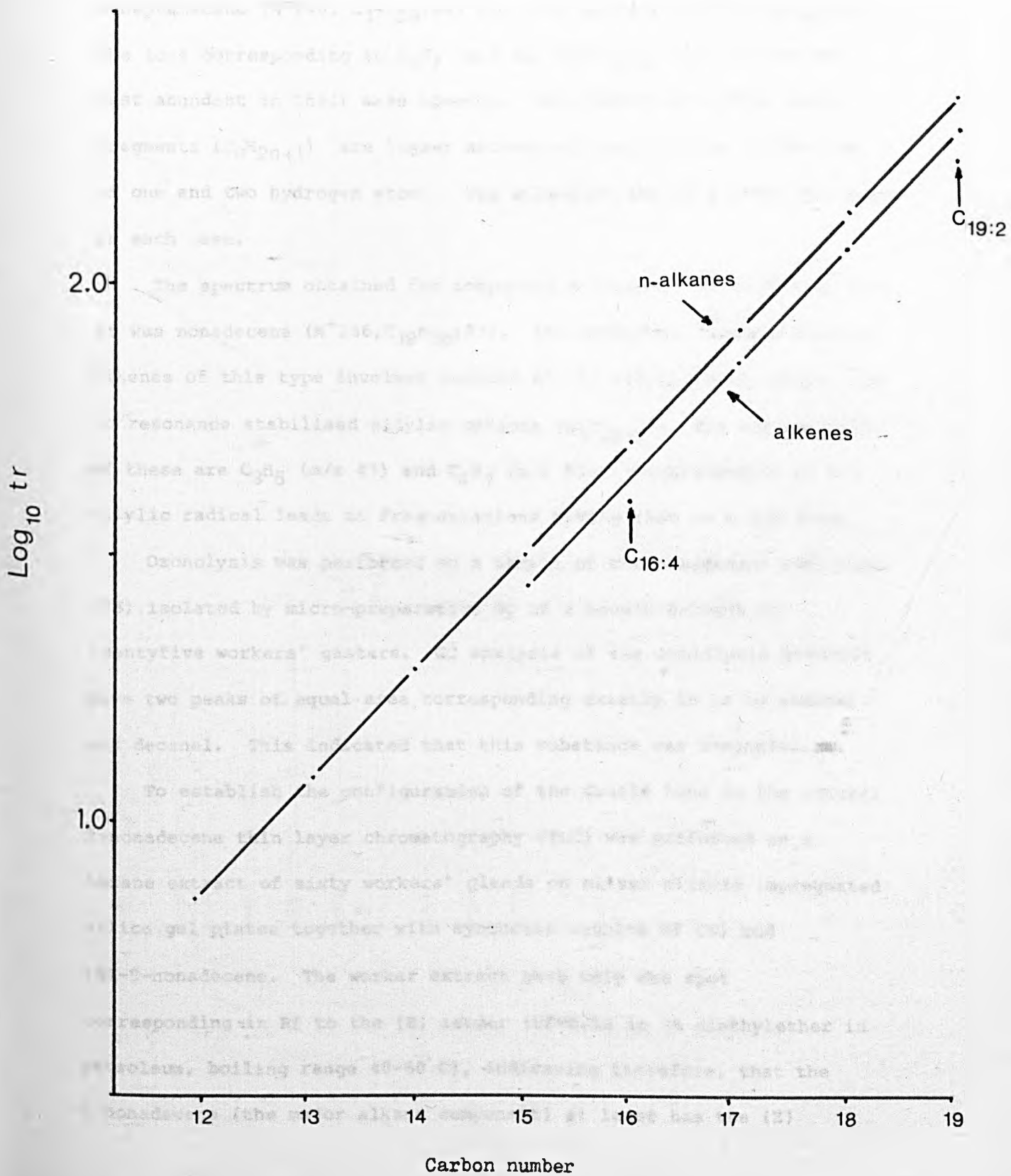


FIGURE 12

Plot of Log_{10} tr vs the number of carbon atoms in the n-alkanes and alkenes of Dufour gland of *A. cephalotes*



sampler. The mass spectra (Figures 13a and b) obtained from components 9 and 14 confirmed that they were the linear alkanes n-heptadecane (M^+240, $C_{17}H_{36}$;29) and n-nonadecane (M^+268,$C_{19}H_{40}$;30). The ions corresponding to C_3H_7 (m/z 43) and C_4H_9 (m/z 57) are the most abundant in their mass spectra. Associated with these alkyl fragments (C_nH_{2n+1}) are lesser amounts of ions arising by the loss of one and two hydrogen atoms. The molecular ion is present but weak in each case.

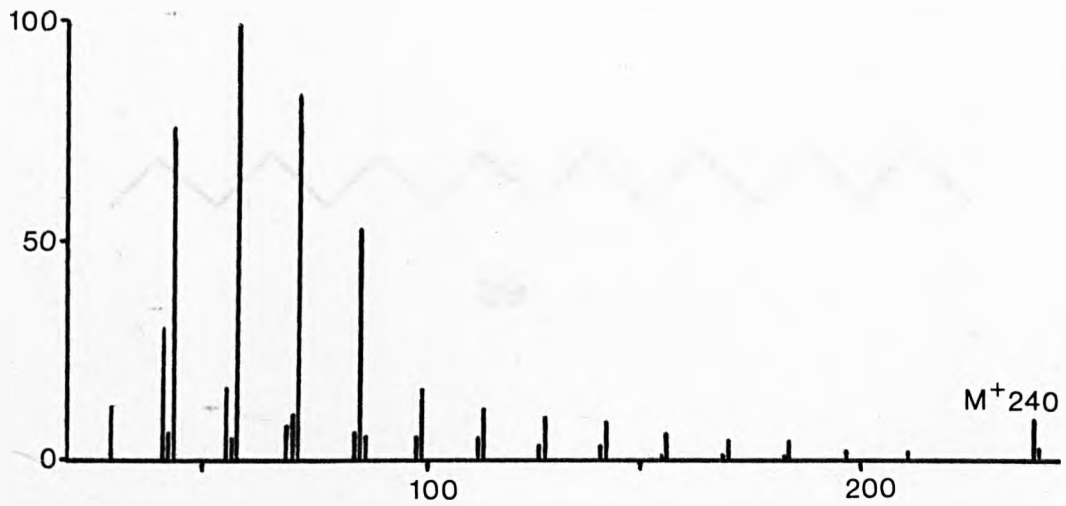
The spectrum obtained for component 6 (Figure 14) confirmed that it was nonadecene (M^+266,$C_{19}H_{38}$;31). The commonest fragmentation in alkenes of this type involves rupture of the allylic bond giving rise to resonance stabilised allylic cations (C_nH_{2n-1}). The most abundent of these are C_3H_5 (m/z 41) and C_4H_7 (m/z 55). Stabilisation of the allylic radical leads to fragmentations giving rise to alkyl ions.

Ozonolysis was performed on a sample of the nonadecene component (13) isolated by micro-preparative GC of a hexane extract of twentyfive workers' gasters. GC analysis of the ozonolysis products gave two peaks of equal area corresponding exactly in tr to nonanal and decanal. This indicated that this substance was 9-nonadecene.

To establish the configuration of the double bond in the natural 9-nonadecene thin layer chromatography (TLC) was performed on a hexane extract of sixty workers' glands on silver nitrate impregnated silica gel plates together with synthetic samples of (Z) and (E)-9-nonadecene. The worker extract gave only one spot corresponding in Rf to the (Z) isomer (Rf=0.52 in 1% diethylether in petroleum, boiling range 40-60°C), indicating therefore, that the 9-nonadecene (the major alkene component) at least has the (Z)

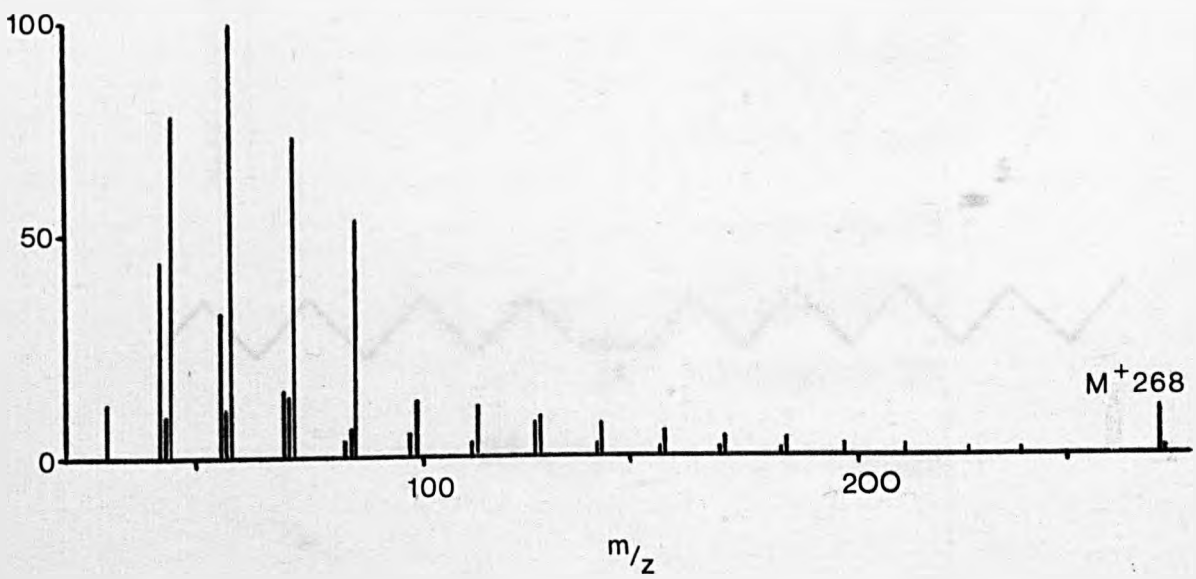
FIGURE 13

a. Mass spectrum of n-heptadecane

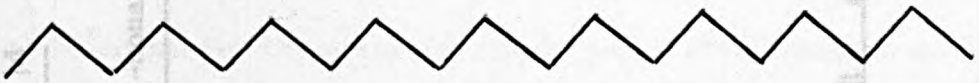


INTENSITY

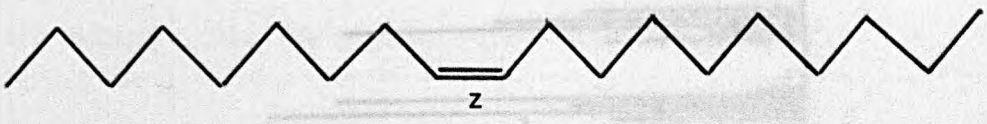
b. Mass spectrum of n-nonadecane



29



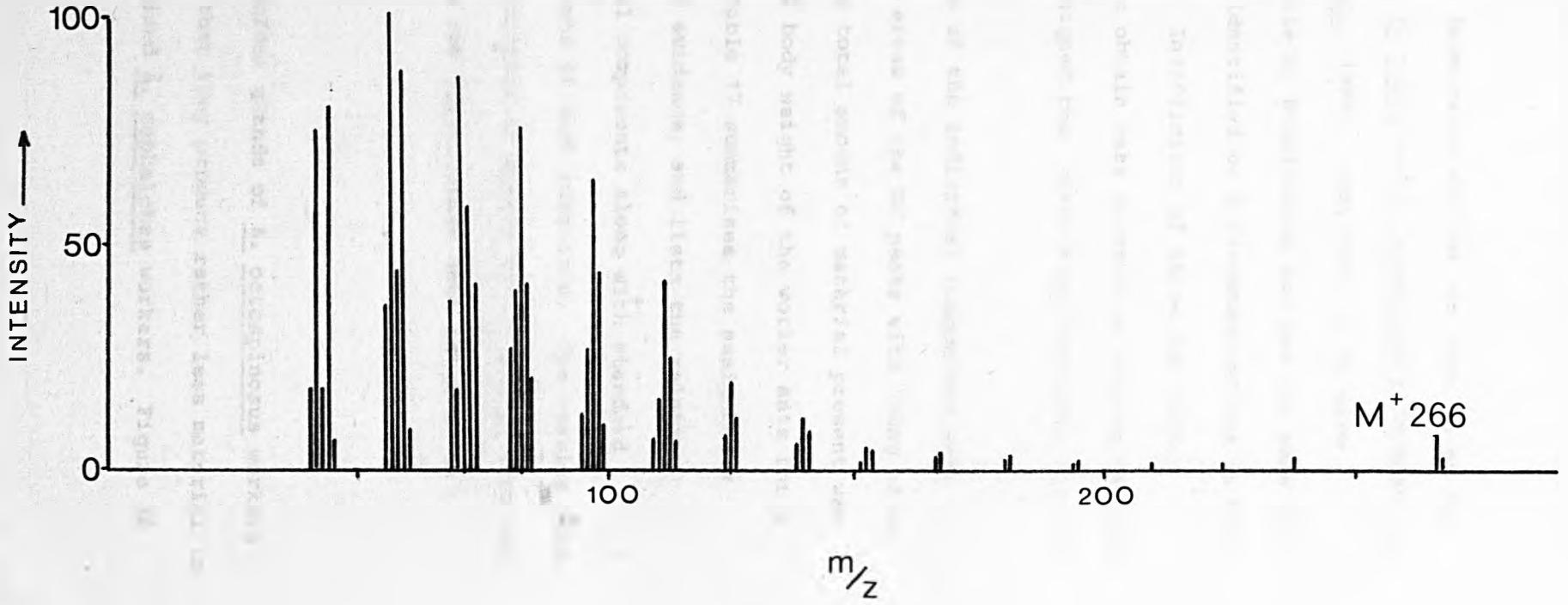
30



31

FIGURE 14

Mass spectrum of Z-(9)-nonadecene



configuration. The Rf of the (E)-9-nonadecene was 0.72 in the above eluent.

Component 6 is readily brominated and has the same tr as the homofarnesene identified in *M. rubra* and *M. scabrinodis* (MORGAN and WADHAMS, 1972b; MORGAN *et al.*, 1979). Component 13 is also eliminated from the GC profile by bromination and has the same tr as the component subsequently identified as 8,11-nonadecadiene in the two *A. sexdens* sub-species. Insufficient of these two latter components were available to obtain mass spectra so components 6 and 13 have been tentatively assigned the identities homofarnesene and nonadecadiene.

The absolute quantities of the individual components were determined by comparing the areas of the GC peaks with 100ng of an n-heptadecane standard. The total amount of material present was directly proportional to the body weight of the worker ants for a given colony (Figure 15). Table 17 summarises the assignments together with the analytical evidence, and lists the relative proportions of the individual components along with standard deviations and range of amounts of each substance. The results were obtained through replicate analyses of single Dufour glands from ten workers of each colony. The raw quantitative data is given in Appendix 2.

A. octospinosus

GC analysis of single Dufour glands of *A. octospinosus* workers using solid sampling showed that they produce rather less material in this gland than do similar sized *A. cephalotes* workers. Figure 16

FIGURE 15

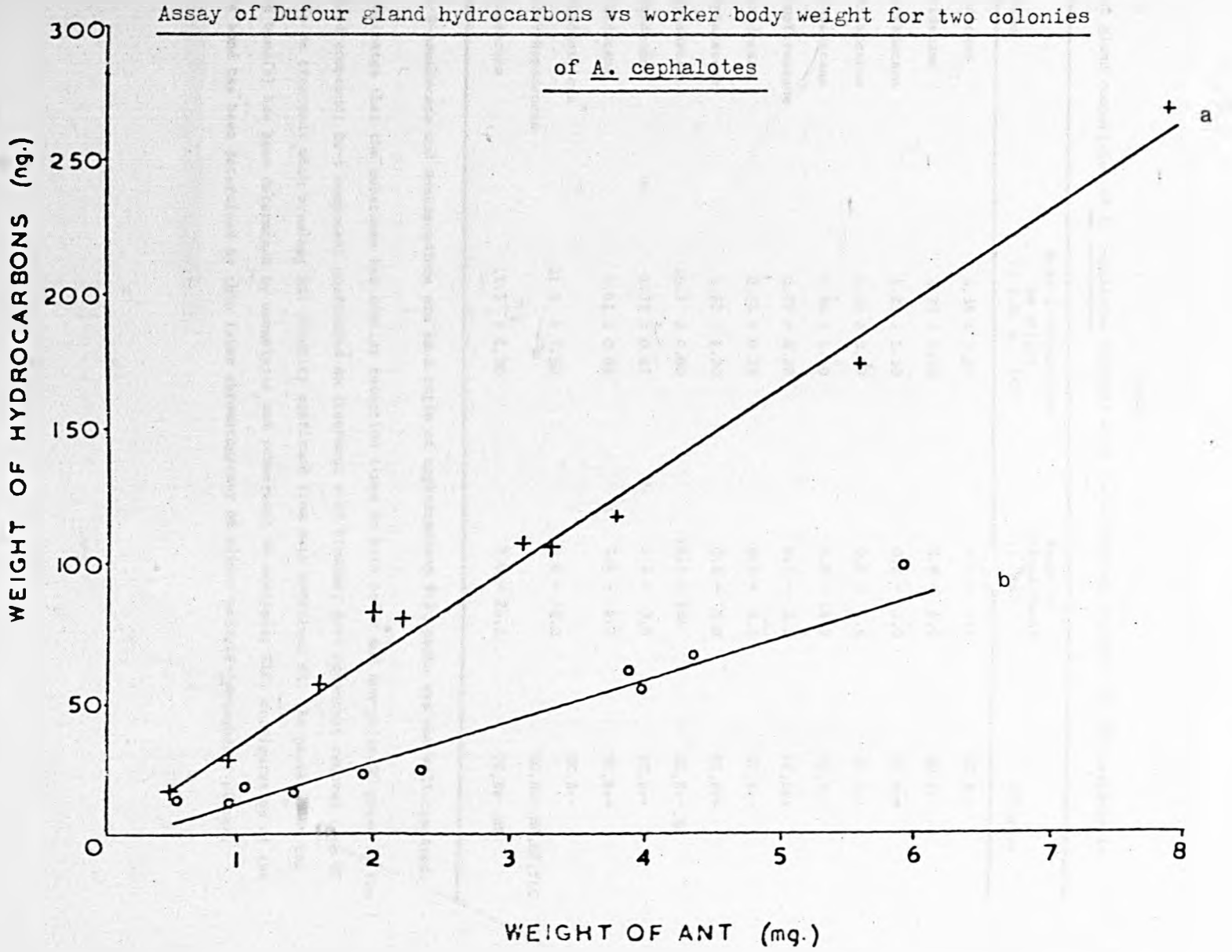

Assay of Dufour gland hydrocarbons vs worker body weight for two colonies of A. cephalotes
WEIGHT OF HYDROCARBONS (ng.)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
WEIGHT OF ANT (mg.)
a
b

TABLE 17

Dufour gland composition of A. cephalotes together with the analytical evidence for the assignments.

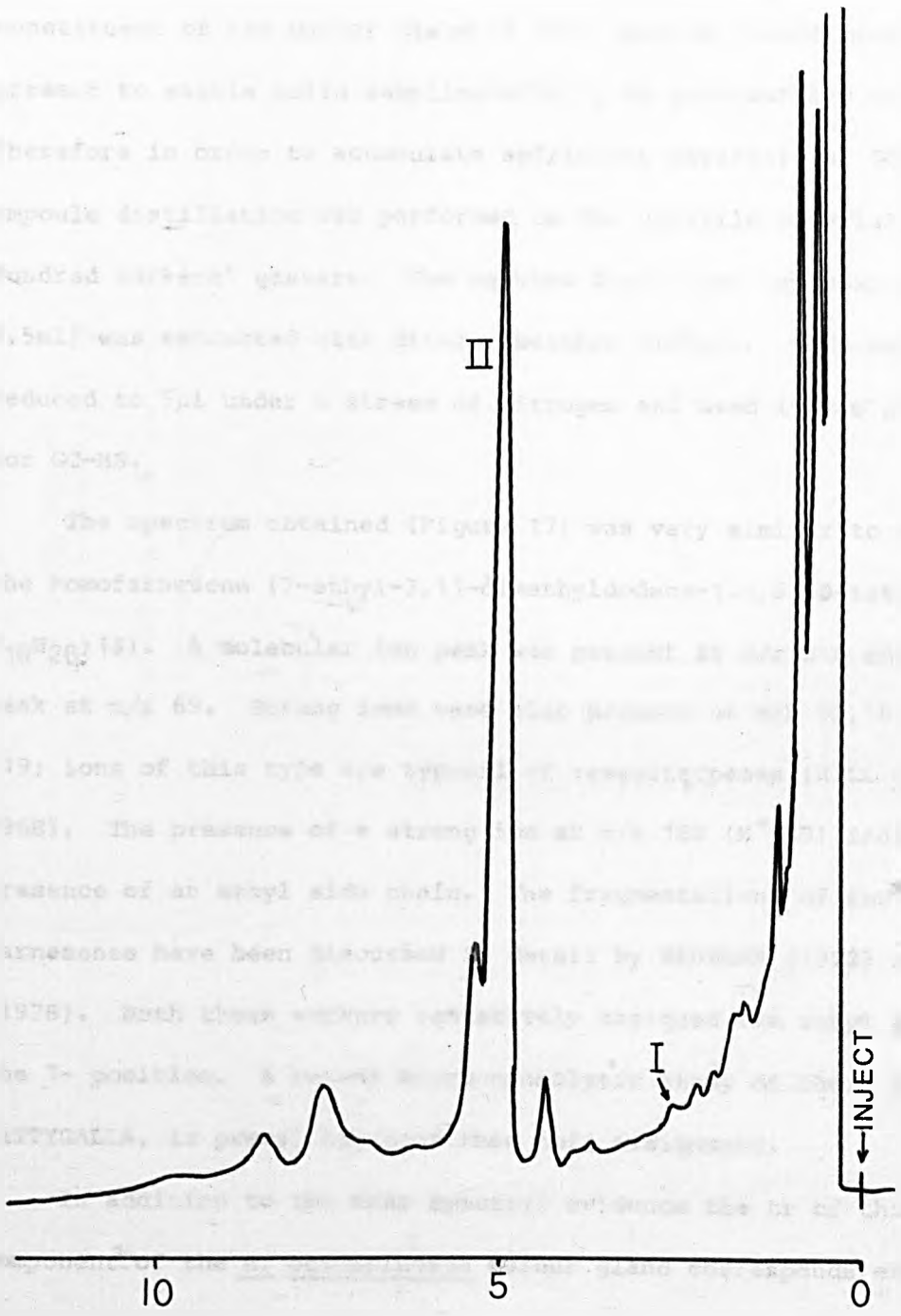
Compound	Mean % composition by weight (± S.D. n = 10)	Range of values found (ng)	Evidence
1 Dodecane	0.38 ± 0.25	0.1 - 1.1	GC,Br-
2 Tridecane	3.23 ± 1.20	0.8 - 6.0	GC,Br-
3 Tetradecane	1.23 ± 1.20	0.2 - 1.0	GC,Br-
4 Pentadecene	1.11 ± 0.37	0.2 - 4.6	GC,Br+
5 Pentadecane	4.86 ± 1.60	0.8 - 14.2	GC,Br-
6 Homofarnesene	0.77 ± 0.38	0.1 - 2.2	GC,Br+
7 Hexadecane	2.64 ± 0.75	0.5 - 5.4	GC,Br-
8 Heptadecene	1.82 ± 1.20	0.2 - 4.0	GC,Br+
9 Heptadecane	46.7 ± 4.60	12.1 - 104	GC,Br-,MS
10 Octadecene	1.77 ± 0.61	0.3 - 3.6	GC,Br+
11 Octadecane	2.61 ± 0.85	0.6 - 4.2	GC,Br-
12 Nonadecadiene*	21.3 ± 7.50	1.8 - 78.2	GC,Br-
13 (Z)-9-Nonadecene			GC,Br+,MS,OZ,TLC
14 Nonadecane	11.1 ± 1.30	2.0 - 25.2	GC,Br-,MS

* (Z)-9-nonadecene and nonadecadiene are in a ratio of approximately 9:1, peaks are not well resolved.

GC: indicates that the substance has similar retention times on both polar and non-polar GC phase to the assigned compound; Br-: component unaffected on treatment with bromine; Br+: component removed from GC profile on treatment with bromine; MS: identity confirmed from mass spectrum; OZ: the position of the double bond(s) has been determined by ozonolysis and subsequent GC analysis; TLC: configuration of the double bond has been determined by thin layer chromatography on silver nitrate-impregnated plates.

FIGURE 16

Typical GC profile of the Dufour gland volatiles of *A. octospinosus* analysed on 3% OV-101



shows a typical GC trace obtained through the analysis of a single Dufour gland from an A. octospinosus worker weighing 12mg on a 3% OV-101 column. Only one of the peaks (I) corresponds in tr to an n-alkane i.e. n-tetradecane.

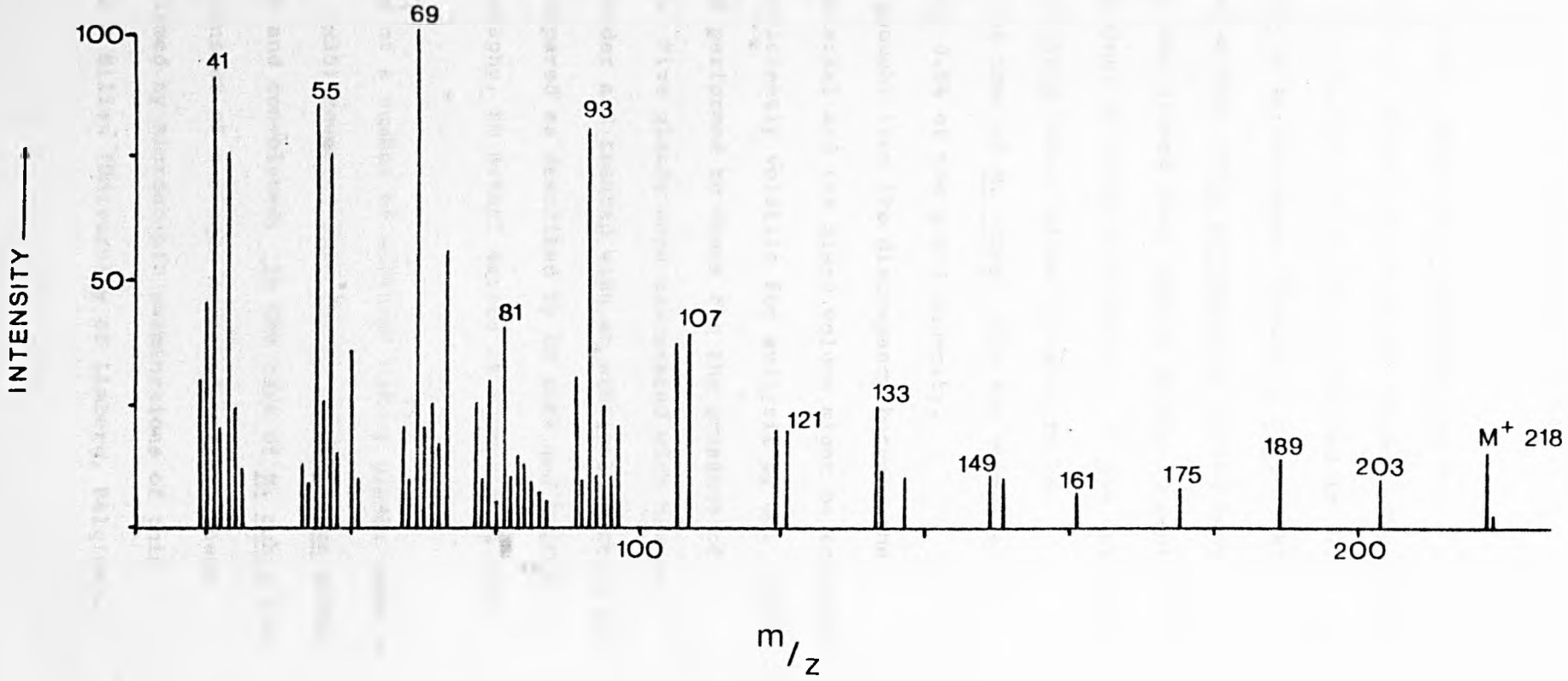
Component (II) was completely removed from the GC profile on treatment with bromine. Although it was the predominant volatile constituent of the Dufour gland of this species insufficient was present to enable solid sampling-GCMS to be successfully carried out. Therefore in order to accumulate sufficient material for GC-MS an ampoule distillation was performed on the volatile material from two hundred workers' gasters. The aqueous distillate (approximately 0.5ml) was extracted with dichloromethane (100µl). This extract was reduced to 5µl under a stream of nitrogen and used in two portions for GC-MS.

The spectrum obtained (Figure 17) was very similar to that of the homofarnesene (7-ethyl-3,11-dimethyldodeca-1,3,6,10-tetrene, $C_{16}H_{26}$; 13). A molecular ion peak was present at m/z 218 and base peak at m/z 69. Strong ions were also present at m/z 93,105,107 and 119; ions of this type are typical of sesquiterpenes (HILL et al., 1968). The presence of a strong ion at m/z 189 (M^+-29) indicated the presence of an ethyl side chain. The fragmentations of ant farnesenes have been discussed in detail by WADHAMS (1972) and PARRY (1978). Both these workers tentatively assigned the ethyl group to the 7- position. A recent micro-ozonolysis study of these faresenes (ATTYGALLA, in press) has confirmed this assignment.

In addition to the mass spectral evidence the tr of this component of the A. octospinosus Dufour gland corresponds exactly to

FIGURE 17

Mass spectrum of Homofarnesene



that of the homofarnesene from M. scabrinodis (MORGAN et al., 1979; Table 18).

The Dufour glands of M. rubra and A. octospinosus have approximately the same outer dimensions (Figures 18a,b). The volume of the former has been fairly accurately determined and is 7.5nl and contains on average, 5µg of hydrocarbons (MORGAN et al., 1978). Assays performed on ten workers of A. octospinosus having body weights in the range 8-14mg showed their Dufour glands to contain on average 44ng (range 28-92ng) of volatile material. If the internal volume of the A. octospinosus Dufour gland is taken to be approximately the same as that of M. rubra, then the volatile material represents only 0.5% of the gland capacity.

Initially it was thought that the discrepancy between the quantity of volatile material and the gland volume might be accounted for by substances insufficiently volatile for analysis by GC. With this in mind a test was performed to check for the presence of involatile fatty acids. Five glands were mascerated with hexane (100µl) in a tissue grinder and treated with an ethereal solution of diazomethane (100µl, prepared as described by DE BOER and BACKER (1963). On rechromatography, no methyl esters of fatty acids were observed.

Careful inspection of a number of workers' Dufour glands under a light microscope (mag. x35) revealed that the A. octospinosus gland wall may be quite thick and convoluted. In the case of M. rubra the gland wall appears to consist of a single layer of cells. These observations were confirmed by microscopic examinations of thin sections prepared by Mr J Billen (University of Limberg, Belgium).

TABLE 18

Comparison of retention time (tr) of the homofarnesene of the M. scabrinodis Dufour gland, with that of the major volatile Dufour gland component of A. octospinosus. Analyses were performed on the DEGS (G) and OV-101 (A) GC phases at oven temperatures of 129° and 161 °C respectively.

	Retention times (mins)	
Species	OV-101	DEGS
A. octospinosus	4.4	3.8
M. scabrinodis	4.4	3.8

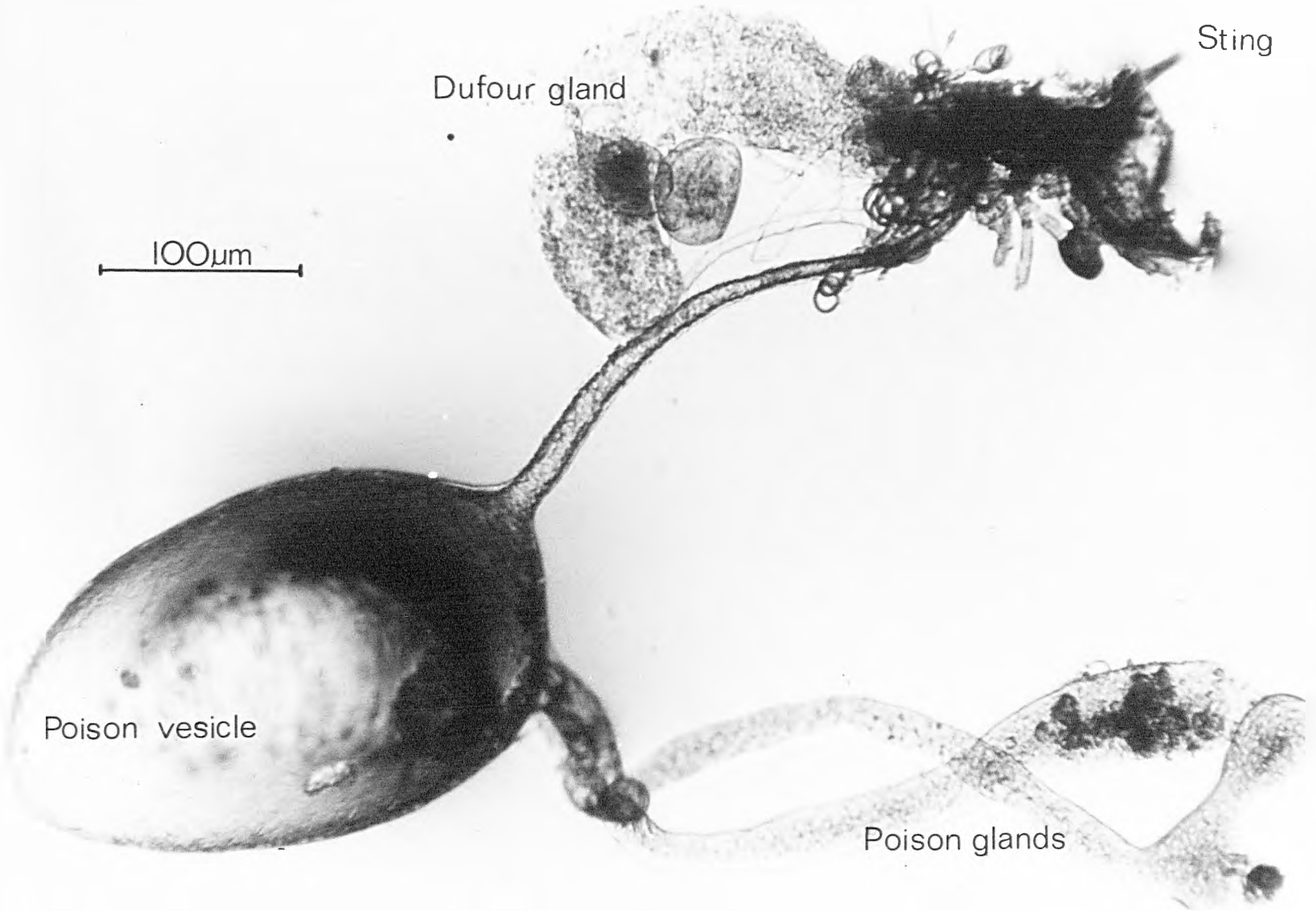


FIGURE 18a Poison apparatus of A. octospinosus

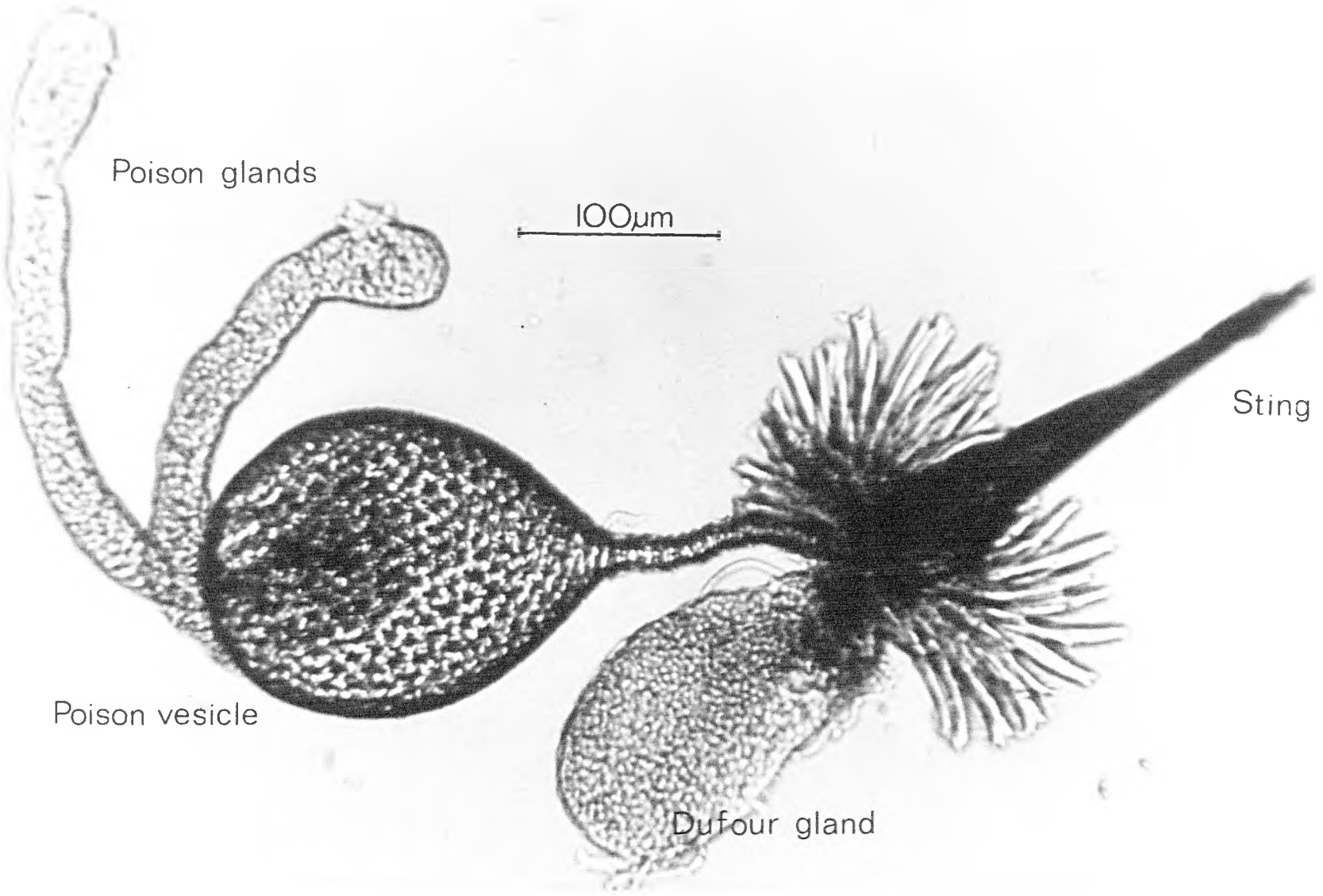


FIGURE 18b Poison apparatus of M. rubra

The preparations showed clearly that the gland wall in A. octospinosus was indeed thick with numerous apical invaginations. As a result of the thick epithilium the volume of the lumen is very much less than had been predicted from the outer dimensions. Furthermore transverse sections showed that the Dufour gland of A. octospinosus was flattened, unlike that of M. rubra which was found to have approximately circular cross-section. Figure 19 shows sketches of the logitudinal thin sections prepared from the Dufour glands of A. octospinosus and M. rubra. The structural differences between the Dufour glands of the two species appear to account for the differences in the amounts of volatiles observed by GC.

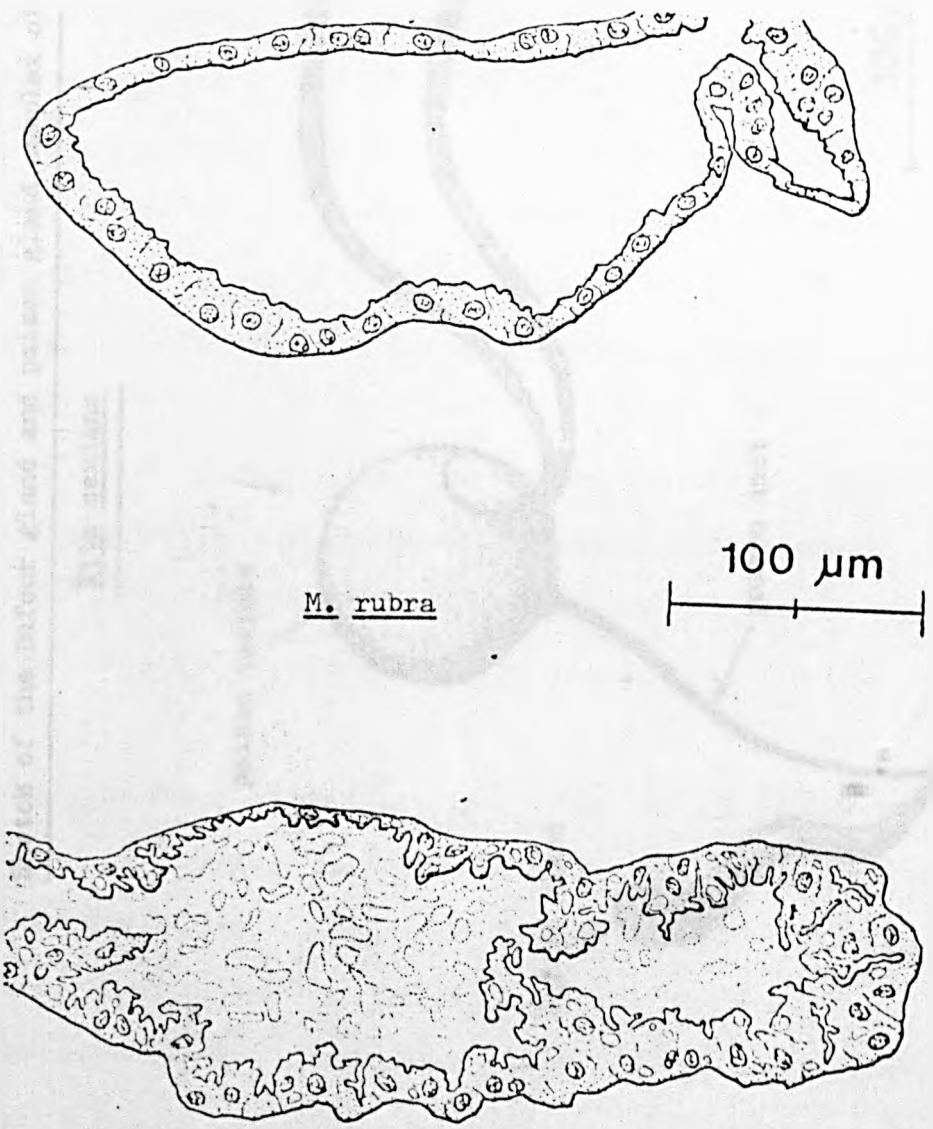
Similarly, in A. cephalotes workers the amount of volatile material observed by GC only accounts for a tiny fraction of the gland volume (<5%, predicted from the outer dimensions of the gland). Although no structural investigation has been performed on the Dufour gland of this species it is very likely that the discrepency between the observed and expected amounts of volatile material maybe explained in a similar manner to that outlined above.

A. s. sexdens and A. s. rubropilosa

The Dufour glands of both these sub-species are very similar in appearance, however they are rather more elongated (Figure 20) than those of M. rubra and M. scabrinodis and the two Attines described above. The yellowish colouration of the A. sexdens gland is somewhat similar to that of the A. cephalotes gland, however it does appear more translucent and distended than those of A. cephalotes and A. octospinosus.

FIGURE 19.

Sketches of Dufour gland thin sections (10μm).

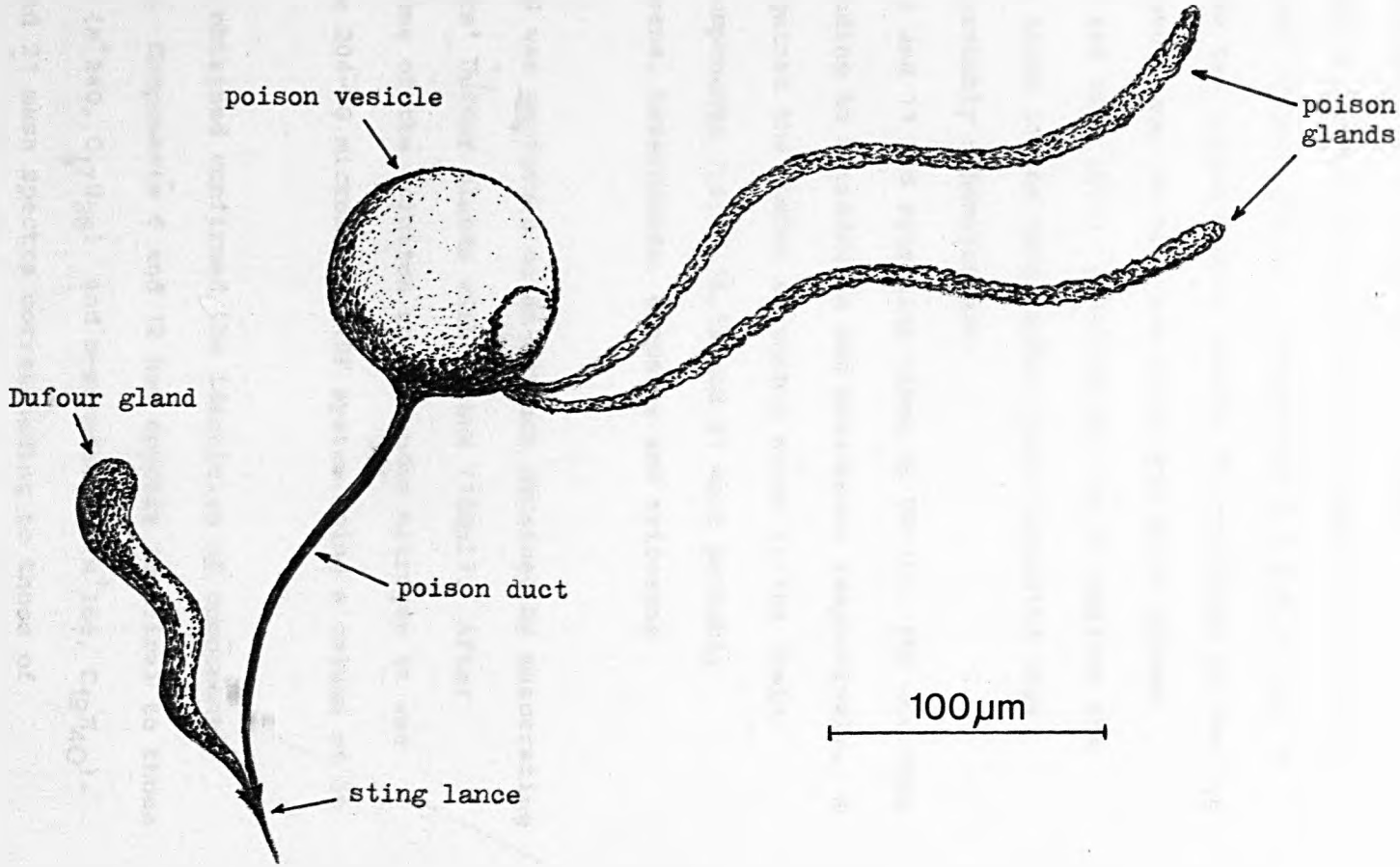


M. rubra

A. octospinosus

FIGURE 20

Sketch of the Dufour gland and poison gland complex of *Atta sexdens*



Gas chromatography of a single gland from each of the sub-species on the non-polar OV-101 column (B) revealed twentyone volatile components (Figure 21a,b). Components 2,4,6,9,12 and 15 were found to have trs corresponding exactly to n-alkanes in the C_{15} to C_{20} chain length range, on both non-polar and polar phases (OV-101, PEG (F) and PEGA (D)). Plotting the log tr against the number of carbon atoms in the hydrocarbon chain suggested that component 17 is probably n-heneicosane.

Components 5 and 11 had retention times on OV-101, PEG and PEGA columns corresponding to heptadecene and nonadecene respectively. A plot of log tr against the number of carbon atoms in the chain indicated that components 1,8,14,16,19 and 21 were probably octadecene, eicosene, heneicosene, docosene and tricosene respectively.

Linked GC-MS was performed on an extract obtained by mascerating twentyfive workers' Dufour glands with hexane (100µl). After reducing the volume of the solution to 5µl under nitrogen it was analysed on a Pye 204-VG micromass 7070F system using a column of 5% OV-101.

The spectra obtained confirmed the identities of components 6,8,11,12 and 21. Components 6 and 12 had spectra identical to those of n-heptadecane (M^+240, $C_{17}H_{36}$) and n-nonadecane (M^+268, $C_{19}H_{40}$). Components 8,11 and 21 mass spectra corresponding to those of mono-unsaturated alkenes, octadecene (M^+252, $C_{18}H_{36}$, Figure 22a), nonadecene (M^+266, $C_{19}H_{38}$, Figure 14) and tricosene (M^+322, $C_{23}H_{46}$, Figure 22b) respectively.

Microbromination of the glandular components resulted in the

FIGURE 21

Typical GC profiles of the Dufour gland contents of (a) A. s. sexdens and (b) A. s. rubropilosa analysed on 5% OV-101 (Nos. refer to Tables 21 and 22)

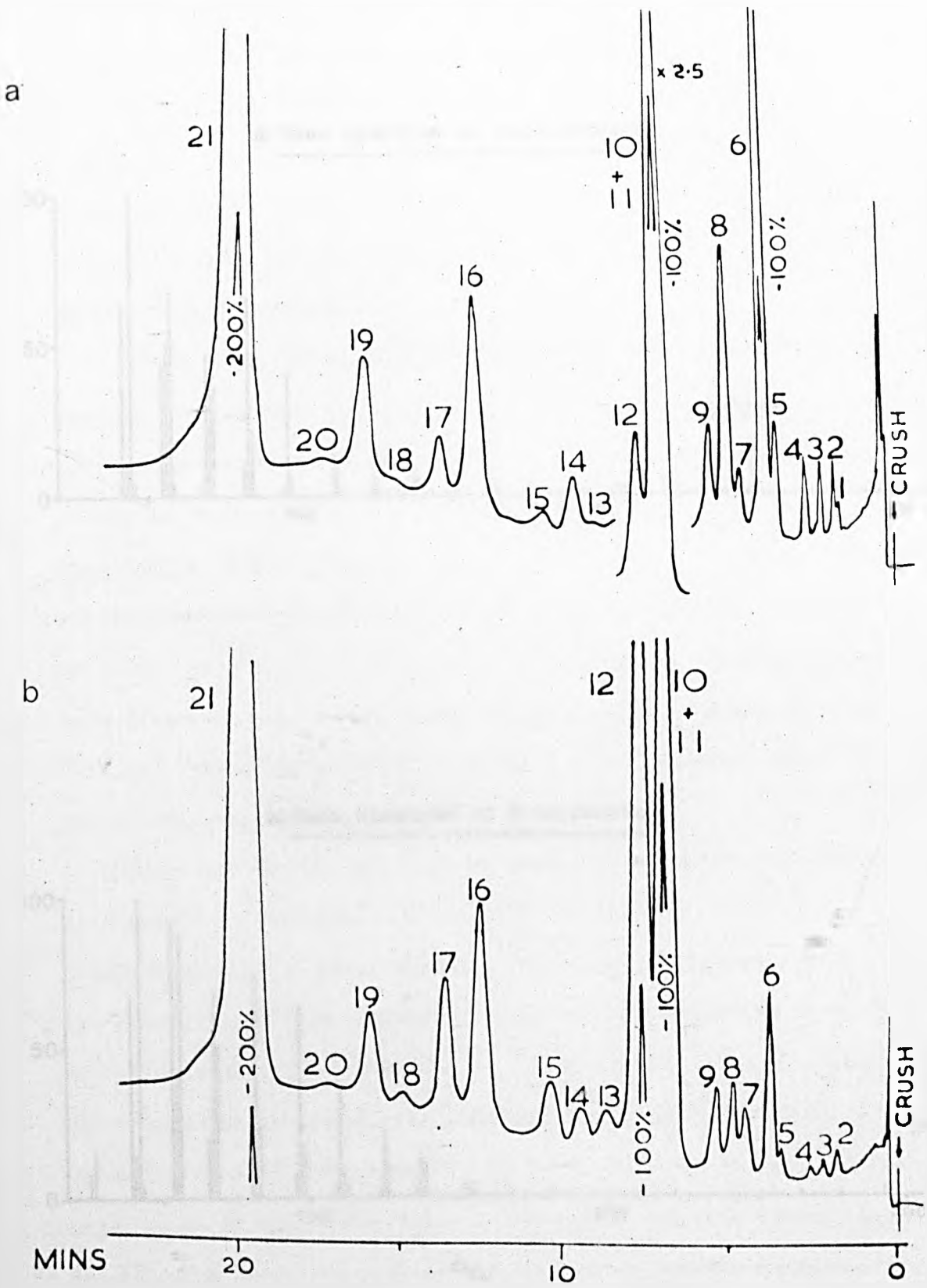
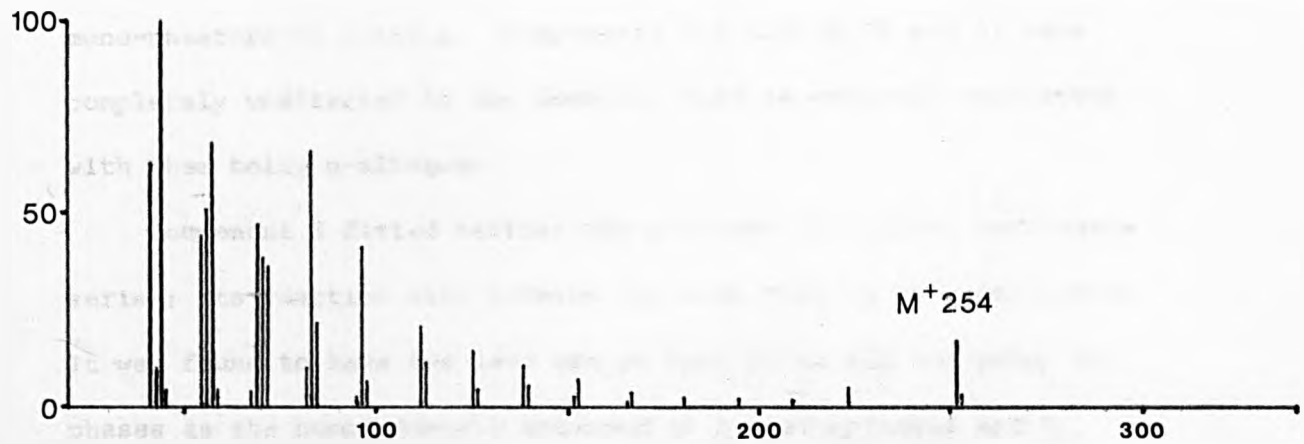


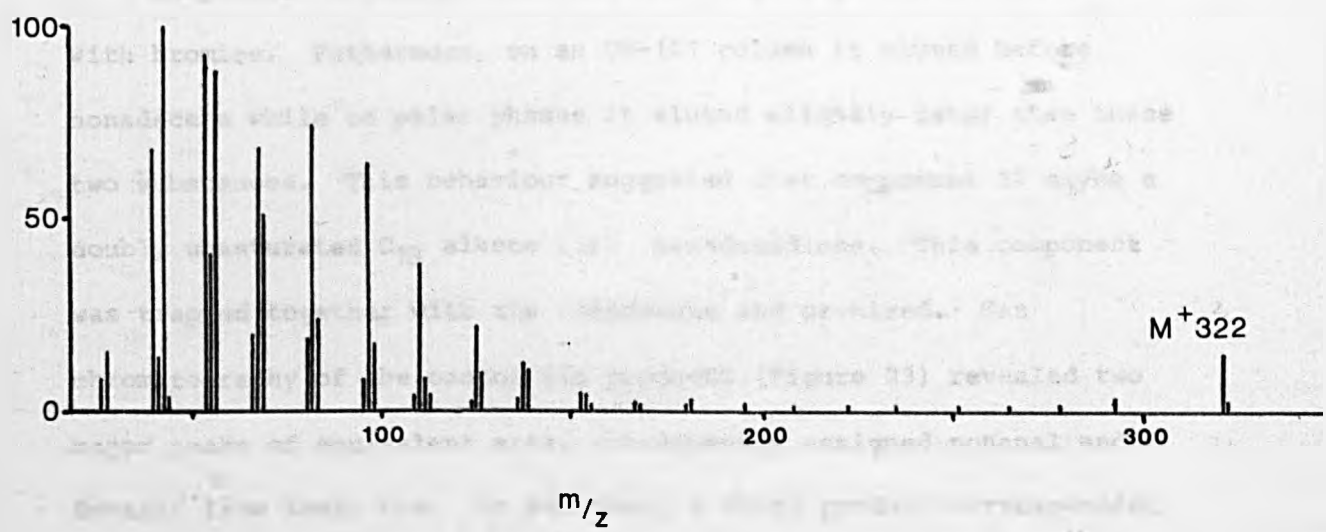
FIGURE 22

a. Mass spectrum of 9-octadecene



INTENSITY

b. Mass spectrum of 9-tricosene



elimination of peaks 1,3,5,8,10,11,14,19 and 21 from the GC profile, showing that they are unsaturated compounds. The log plots and mass spectrometry had previously shown that with the exception of components 3 and 10, all these substances correspond to mono-unsaturated alkenes. Components 2,4,6,9,12,15 and 17 were completely unaffected by the bromine, this is entirely consistent with them being n-alkanes.

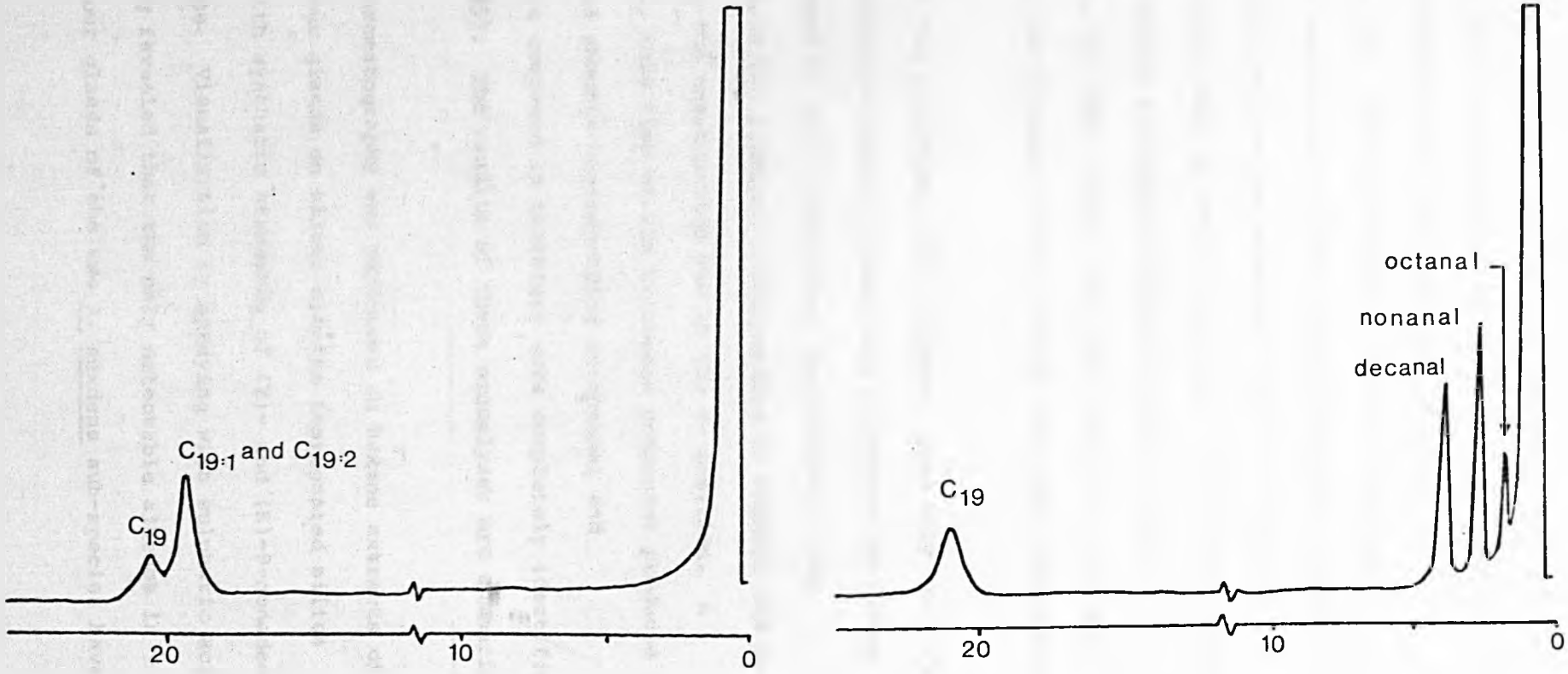
Component 3 fitted neither the n-alkane nor alkene homologous series; its reaction with bromine confirms that it is unsaturated. It was found to have the same trs on both polar and non-polar GC phases as the homofarnesene observed in A. octospinosus and M. scabrinodis (MORGAN et al., 1979). There was too little of this substance present to obtain its complete mass spectrum. However, in spite of the absence of an M^+ peak and several of the expected higher mass fragments the remainder of the spectrum was similar to that obtained from homofarnesene. Component 3 has therefore been tentatively assigned as homofarnesene.

Component 10 was also removed from the GC profile when treated with bromine. Futhermore, on an OV-101 column it eluted before nonadecene while on polar phases it eluted slightly later than these two substances. This behaviour suggested that component 10 maybe a doubly unsaturated C_{19} alkene i.e. nonadecadiene. This component was trapped together with the nonadecene and ozonised. Gas chromatography of the ozonolysis products (Figure 23) revealed two major peaks of equivalent area, subsequently assigned nonanal and decanal from their trs. In addition, a third product corresponding to octanal was observed. The two major components have undoubtedly

FIGURE 23.

GC profile of the C_{19} hydrocarbons trapped from A. sexdens

Products of the ozonolysis of the C_{19} unsaturated hydrocarbons



arisen from component 11, which can be more completely assigned 9-nonadecene. The minor ozonolysis product, octanal, is believed to represent two C_8 fragments from the symetrical diene, 8,11-nonadecadiene (**32**). The C_3 fragment resulting from the ozonolysis of this substances would be malondialdehyde; originally it was thought that owing to its low molecular weight it would be obscured by the solvent peak in the GC analysis. However BEROZA and BIERL (1967) were unable to observe malondialdehyde to any appreciable extent, and they suggest that this was due to its poor stability and very low response factor towards the flame ionisation detector (FID).

Ozonolysis of the octadecene (**33**) component gave only one major product, corresponding to nonanal, indicating therefore that this alkene is unsaturated in the 9- position. Ozonolysis of the heneicosene (**34**) gave two products corresponding to nonanal and dodecanal indicating the unsaturation was at the 9- position. A further ozonolysis, this time of the tricosene component produced two components in equal amounts corresponding to nonanal and tetradecanal. This compound is therefore more completely identified as 9-tricosene (**35**). The results of these ozonolyses are summarised in Table 19.

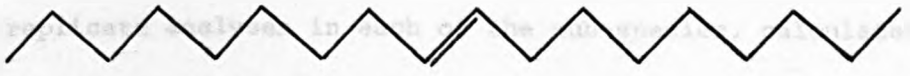
Thin layer chromatography was performed on hexane extracts of five workers' Dufour glands on silver nitrate impregnated silica plates together with synthetic standards of (Z)- and (E)-9-nonadecene and (Z)-9-tricosene. Visualisation by spraying with sulphuric acid followed by baking revealed that the only detectable alkenes in either of the Dufour glands of the two A. sexdens sub-species have

TABLE 19

Compounds arising through the ozonolysis of A. sexdens Dufour gland alkenes.

Component number (in Table 21 and 22)	Ozonolysis products	Assignment
8	Nonanal	9-Octadecene
10	Octanal (malondialdehyde, not observed)	8,11-Nonadecadiene
11	Nonanal + decanal	9-Nonadecene
16	Nonanal + dodecanal	9-Heneicosene
21	Nonanal + tetradecanal	9-Tricosene

32



33

34

35

the (Z)- configuration. No component was observed corresponding to an (E)- isomer (Table 20). As the most abundant alkenes in the two sub-species are 9-nonadecene (**31**) and 9-tricosene (**35**) these have both been assigned the (Z)- configuration. The remaining alkenes are present at too low a concentration to allow a confident assignment to be made.

The identities of the various components in the Dufour glands of the sub-species are summarised in Tables 21 and 22. Also listed are the percentage compositions, ranges of values found and the analytical evidence for the assignments. The values obtained were from ten replicate analyses in each of the sub-species, calculated by comparing the areas of the GC peaks from the analysis of a single gland with a known amount of hydrocarbon standard. The raw quantitative data is given in Appendices 3 and 4.

The results show clearly that although the workers of the two sub-species produce similar substances in their Dufour glands, there are significant differences in the relative amounts of the individual components. The composition of the secretion is however constant in workers of each sub-species, especially with respect to the major components.

In the Dufour gland of A. s. sexdens (Z)-9-tricosene is the most abundant component at 43.7%, while (Z)-9-nonadecene is the second most abundant component, constituting on average 17.7% of the total hydrocarbons present. In A. s. rubropilosa the reverse is the case; (Z)-9-nonadecene is the major component (38.9%) and (Z)-9-tricosene the second most abundant component (25.2%).

Calculation of the glandular volume from eye graticule

TABLE 20

Comparison of R_f values of natural and synthetic hydrocarbons on silica gel thin layers impregnated with 10% silver nitrate using an eluent of 1% diethylether in petroleum (boiling range 40 - 60 ^{o}C).

Sample	R_f
(Z)-9-nonadecene	0.52
(E)-9-nonadecene	0.76
(Z)-9-tricosene	0.54
A. sexdens Dufour gland extract	0.53

TABLE 21

Dufour gland composition of A. s. rubropilosa together with the analytical evidence for the assignments.

Compound	Mean % composition by weight (± S.D. n = 10)	Range of values found (ng)	Evidence
1 Pentadecene	0.06 ± 0.04	0 - 6	GC,Br+
2 Pentadecane	0.32 ± 0.16	1 - 24	GC,Br-
3 Homofarnesene	0.32 ± 0.08	3 - 24	GC,Br+,MS
4 Hexadecane	0.39 ± 0.16	5 - 41	GC,Br-
5 Heptadecene	0.88 ± 0.21	6 - 41	GC,Br+
6 Heptadecane	8.35 ± 2.80	65 - 374	GC,Br-,MS
7 Unknown	0.24 ± 0.10	2 - 89	
8 9-Octadecene	3.27 ± 1.03	10 - 205	GC,Br+,MS,OZ
9 Octadecane	1.15 ± 0.36	10 - 68	GC,Br-
10 8,11-Nonadecadiene	5.81 ± 0.52	57 - 403	GC,Br+,OZ
11 (Z)-9-Nonadecene	38.9 ± 3.50	380 -2697	GC,Br+,MS,OZ,TLC
12 Nonadecane	7.17 ± 1.70	65 - 532	GC,Br-,MS
13 Unknown	0.01 ± 0.02	0 - 3	
14 Eicosene	0.84 ± 0.45	6 - 139	GC,Br+
15 Eicosane	0.38 ± 0.20	3 - 39	GC,Br-
16 9-Heneicosene	4.03 ± 0.93	16 - 354	GC,Br+,OZ
17 Heneicosane	0.92 ± 0.37	8 - 71	GC,Br-
18 Unknown	0.01 ± 0.02	0 - 3	
19 Docosene	1.72 ± 0.55	10 - 205	GC,Br+
20 Unknown	0.01 ± 0.02	0 - 8	
21 (Z)-9-Tricosene	25.24 ± 6.50	106 -2598	GC,Br+,MS,OZ,TLC

See sub-script to Table 17 for the definitions of the abbreviations in the Evidence column.

TABLE 22

Dufour gland composition of A. s. sexdens together with the analytical evidence for the assignments.

Compound	Mean % composition by weight (± S.D. n = 10)	Ranger of values found (ng)	Evidence
1 Pentadecene			GC,Br+
2 Pentadecane	0.13 ± 0.06	2 - 16	GC,Br-
3 Homofarnesene	0.09 ± 0.05	1 - 12	GC,Br+,MS
4 Hexadecane	0.10 ± 0.06	1 - 9	GC,Br-
5 Heptadecene	0.21 ± 0.11	2 - 26	GC,Br+
6 Heptadecane	1.80 ± 0.40	28 - 142	GC,Br-,MS
7	0.55 ± 0.27	4 - 74	
8 9-Octadecene	1.22 ± 0.26	14 - 84	GC,Br+,MS,OZ
9 Octadecane	1.21 ± 0.28	16 - 95	GC,Br-
10 8,11-Nonadecadiene	3.91 ± 0.70	46 - 216	GC,Br+,OZ
11 (Z)-9-Nonadecene	17.7 ± 3.10	308 - 984	GC,Br+,MS,OZ,TLC
12 Nonadecane	16.0 ± 2.10	145 - 999	GC,Br-,MS
13	0.14 ± 0.17	2 - 22	
14 Eicosene	0.61 ± 0.26	6 - 63	GC,Br+
15 Eicosane	0.97 ± 0.20	11 - 63	GC,Br-
16 9-Heneicosene	5.57 ± 0.89	61 - 425	GC,Br+,OZ
17 Heneicosane	3.44 ± 0.71	36 - 271	GC,Br-
18	0.01 ± 0.15	0 - 22	
19 Docosene	2.55 ± 0.43	28 - 193	GC,Br+
20	0.01 ± 0.01	0 - 12	
21 (Z)-9-Tricosene	43.8 ± 4.70	485 - 2944	GC,Br+,MS,OZ,TLC

See subscript to Table 17 for the definitions of the abbreviations in the Evidence column.

measurements followed by GC determinations of the hydrocarbon contents showed the glands to be completely filled with hydrocarbons (Table 23).

Analysis of the hydrocarbon content of workers of varying sizes showed that for both species the amount of volatile material present in the glands is directly proportional to the live body weight of the worker ants (Figure 24a,b). The graphs also show that similar sized workers of each sub-species produce similar amounts of volatile hydrocarbons.

Investigation of Myrmica Dufour glands

Viewing Myrmica Dufour glands under a light microscope (mag. x35) showed them to be colourless distended pear-shaped sacs. In M. rubra and M. scabrinodis workers this gland has been shown to be completely filled with microgram amounts of hydrocarbons and nanogram quantities of volatile oxygenated compounds (WADHAMS, 1972; TYLER, 1977; PARRY, 1978).

M. sabuleti

GC analysis of a single Dufour gland of an M. sabuleti worker produced a GC profile bearing a remarkable resemblance to that obtained from the analysis of the Dufour gland of an M. scabrinodis worker under the same conditions (Figure 25a,b). Several components were observed at short tr in M. rubra and M. scabrinodis which corresponded to oxygenated compounds (TYLER, 1977; Figure 25a,b). Several of the peaks in M. sabuleti at very short tr corresponded to these volatile oxygenated compounds previously identified in M. rubra and M. scabrinodis (TYLER, 1977). At longer

TABLE 23

Comparison of glandular volume (calculated from eye graticule measurements) and hydrocarbon content (determined from GC analysis).

Species	Glandular volume (nl)	Volume of hydrocarbon (nl)	Hydrocarbon as % of total gland volume
A. s. sexdens	7.95	7.86	98.3
A. s. rubropilosa	9.72	9.65	99.3

FIGURE 24

Assay of Dufour gland hydrocarbons vs worker body weight for
(a) A. s. rubropilosa
(b) A. s. sexdens

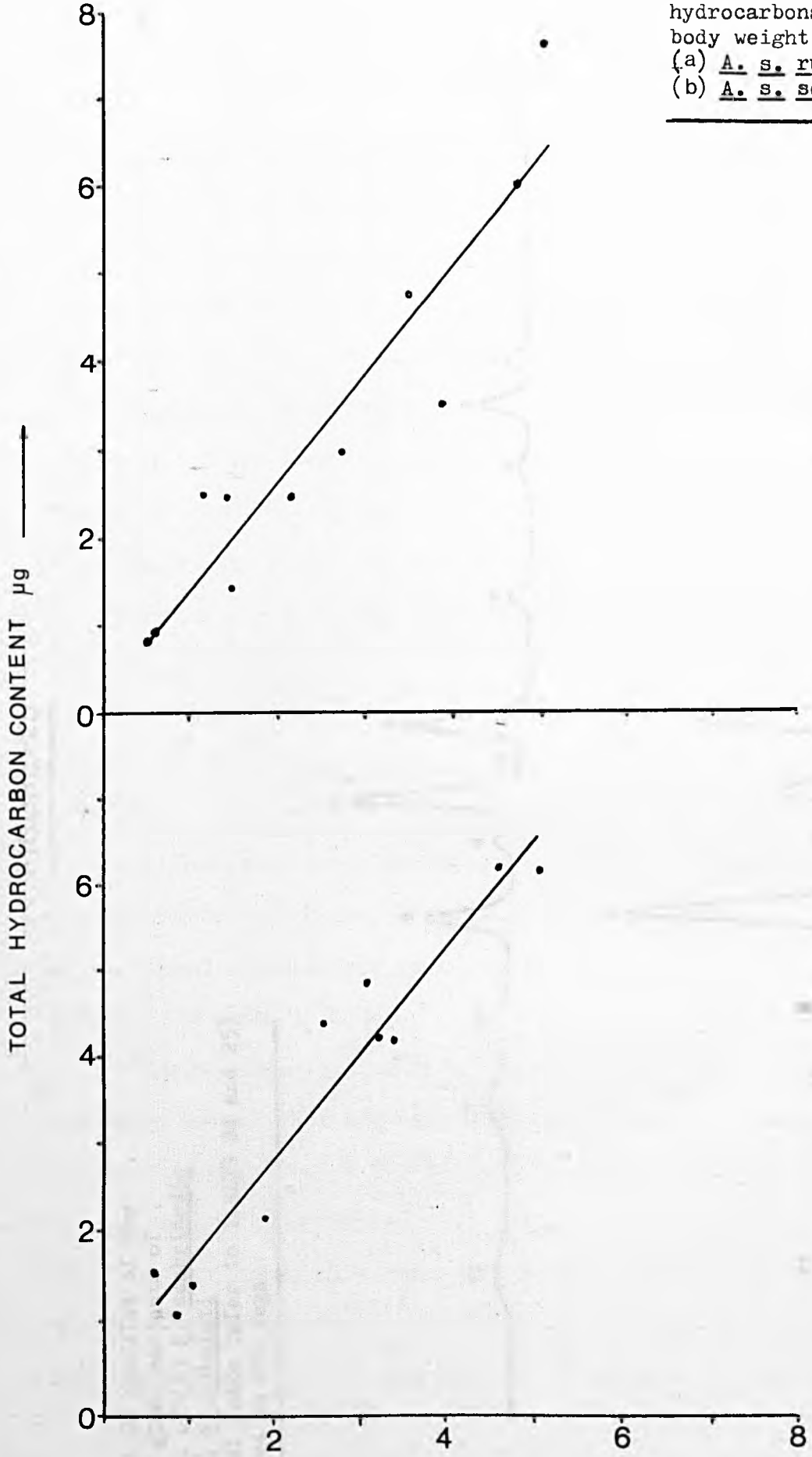
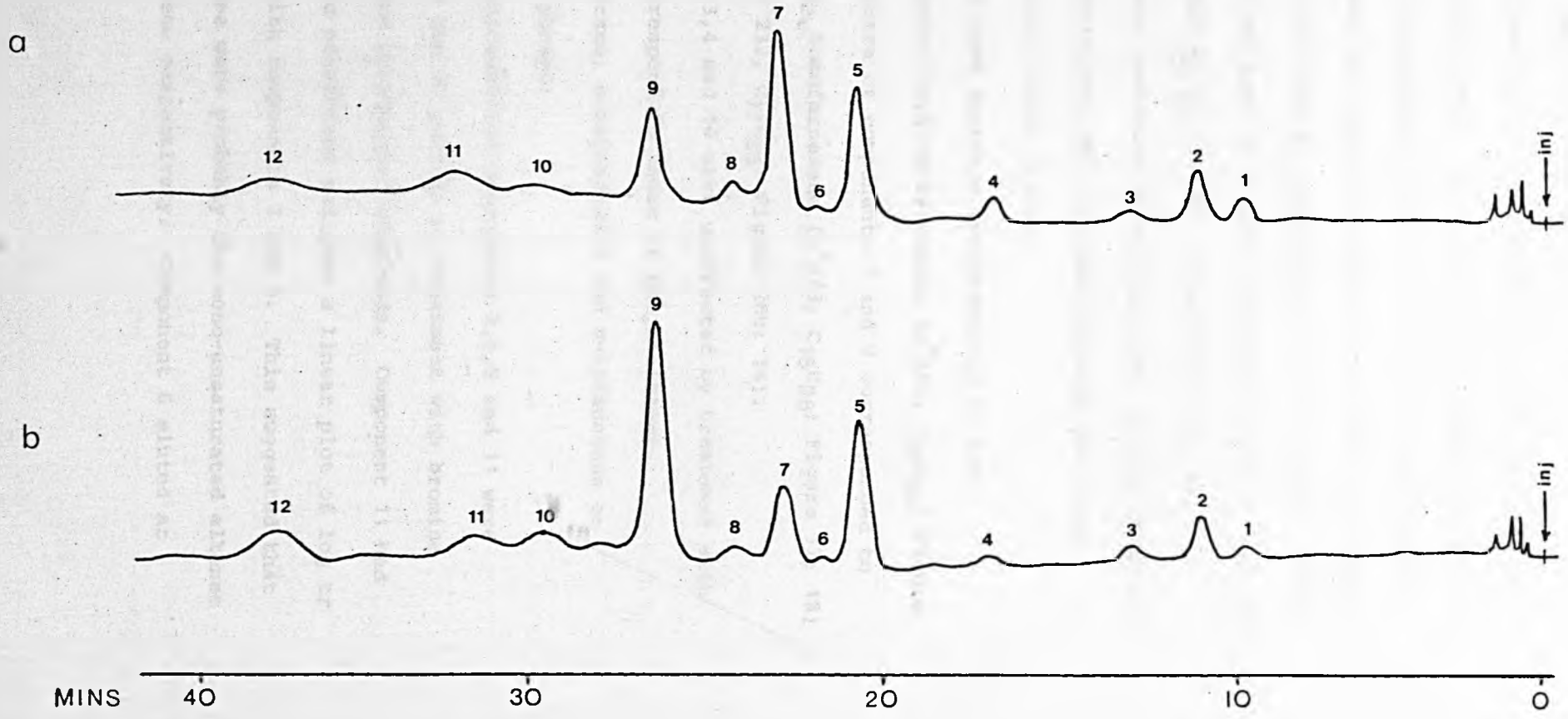


FIGURE 25

Typical GC profiles of the Dufour gland contents of workers of (a) M. scabrinodis and (b) M. sabuleti
(Nos. of peaks refer to TABLES 24 and 25)
Analysed on 20% Pega



tr (five minutes) 12 components were observed.

The three major components 5,7 and 9 (constituting over 80% of the total gland volatiles) had trs on non-polar and polar (OV-101(B) and PEGA (C)) phases corresponding exactly to those of the (Z,E)-α -farnesene, homofarnesene and bishomofarnesene observed in M. rubra (MORGAN and WADHAMS, 1972b) and M. scabrinodis (MORGAN et al., 1979) while component 12 had the same tr as the trishomonfarnesene observed in M. scabrinodis (MORGAN et al., 1979). The identities of components 5,7 and 9 were confirmed from their mass spectra obtained by GC-MS on the Pye 104-Hitachi RMU 6E system through the solid injection of five workers' Dufour glands.

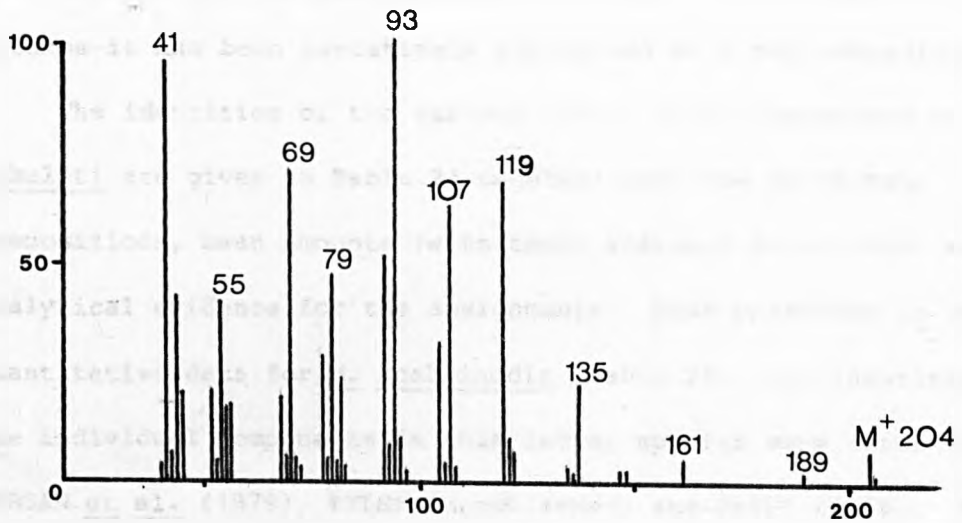
Component 5 gave a mass spectrum corresponding to the sesquiterpenoid hydrocarbon (Z,E)-α-farnesene (M^+204, $C_{15}H_{24}$; Figure 26a; 12), while the spectra of components 7 and 9 corresponded to those of its homologues, homofarnesene (M^+218, $C_{16}H_{26}$; Figure 17; **13**) and bishomofarnesene (M^+232, $C_{17}H_{28}$; Figure 26b; **14**).

Only components 1,3,4 and 10 were unaffected by treatment with bromine. Their trs correspond to those of the n-alkanes, n-pentadecane, n-hexadecane, n-heptadecane and n-nonadecane on non-polar and polar GC phases.

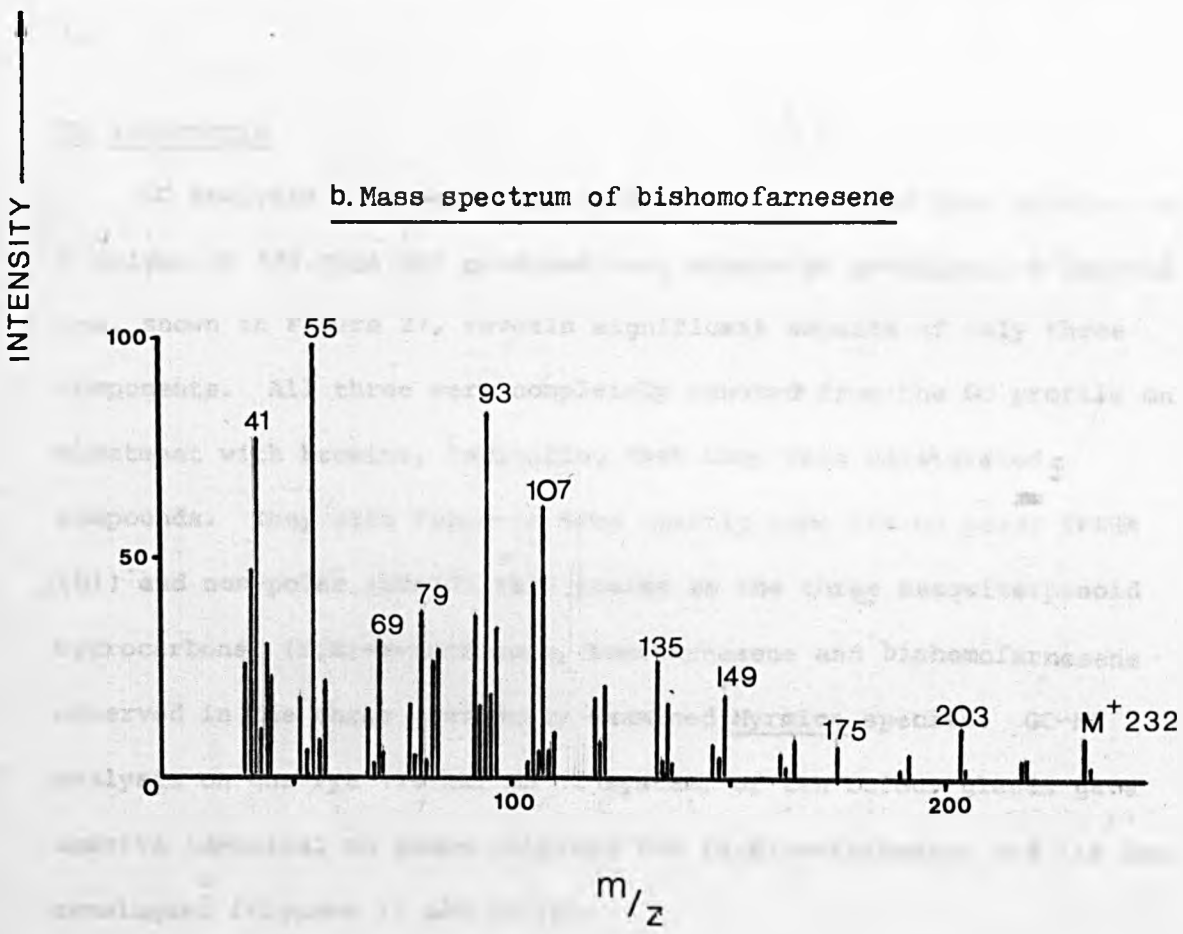
Of the remaining unidentified components 2,6,8 and 11 were completely removed from the GC profile on treatment with bromine indicating that they were unsaturated compounds. Component 11 had the same tr as synthetic nonadecene and gave a linear plot of log tr against carbon number with components 2 and 8. This suggested that the latter two substances were probably the mono-unsaturated alkenes pentadecene and octadecene respectively. Component 6 eluted at

FIGURE 26

a. Mass spectrum of (Z,E)-α-farnesene



b. Mass spectrum of bishomofarnesene



slightly longer tr than would be expected for a mono-unsaturated hydrocarbon; unfortunately insufficient of it was present to confirm its identity by mass spectrometry. However, as it reacts with bromine it has been tentatively identified as a heptadecadiene.

The identities of the various Dufour gland components of M. sabuleti are given in Table 24 together with the percentage compositions, mean amounts (with their standard deviations) and the analytical evidence for the assignments. Also presented is the quantitative data for M. scabrinodis (Table 25), the identities of the individual components in this latter species were determined by MORGAN et al. (1979), TYLER (unpublished) and PARRY (1978). The raw quantitative data for both species is presented in Appendices 5 and 6.

M. lobicornis

GC analysis of single dissected Dufour glands of this species on a column of 10% PEGA (D) produced very simple GC profiles. A typical one, shown in Figure 27, reveals significant amounts of only three components. All three were completely removed from the GC profile on treatment with bromine, indicating that they were unsaturated compounds. They were found to have exactly same trs on polar (PEGA (D)) and non-polar (OV-101 (B)) phases as the three sesquiterpenoid hydrocarbons, (Z,E)-α-farnesene, homofarnesene and bishomofarnesene observed in the three previously examined Myrmica species. GC-MS analysis on the Pye 104-AEI MS 12 system, of ten Dufour glands gave spectra identical to those obtained for (Z,E)-α-farnesene and its two homologues (Figures 17 and 26a,b).

TABLE 24

Dufour gland composition of M. sabuleti together with the analytical evidence for the assignments.

Compound	Mean % composition by weight (± S.D. n = 10)	mean amount (ng ± S.D. n = 10)	Evidence
1 Pentadecane	0.71 ± 0.40	7 ± 4	GC,Br-
2 Pentadecene	1.65 ± 0.58	18 ± 7	GC,Br+
3 Hexadecane	1.57 ± 0.73	17 ± 7	GC,Br-
4 Heptadecene	0.37 ± 0.22	4 ± 2	GC,Br-
5 α-Farnesene	28.92 ± 4.61	312 ± 106	GC,Br+,MS
6 Heptadecadiene	0.43 ± 0.28	5 ± 5	GC,Br+
7 Homofarnesene	17.84 ± 2.55	200 ± 80	GC,Br+,MS
8 Octadecene	2.68 ± 0.73	30 ± 14	GC,Br+
9 Bishomofarnesene	37.21 ± 6.75	425 ± 194	GC,Br+,MS
10 Nonadecane	2.77 ± 2.74	29 ± 32	GC,Br-
11 Nonadecene	2.06 ± 1.80	20 ± 19	GC,Br+
12 Trishomofarnesene	3.79 ± 3.43	39 ± 41	GC,Br+

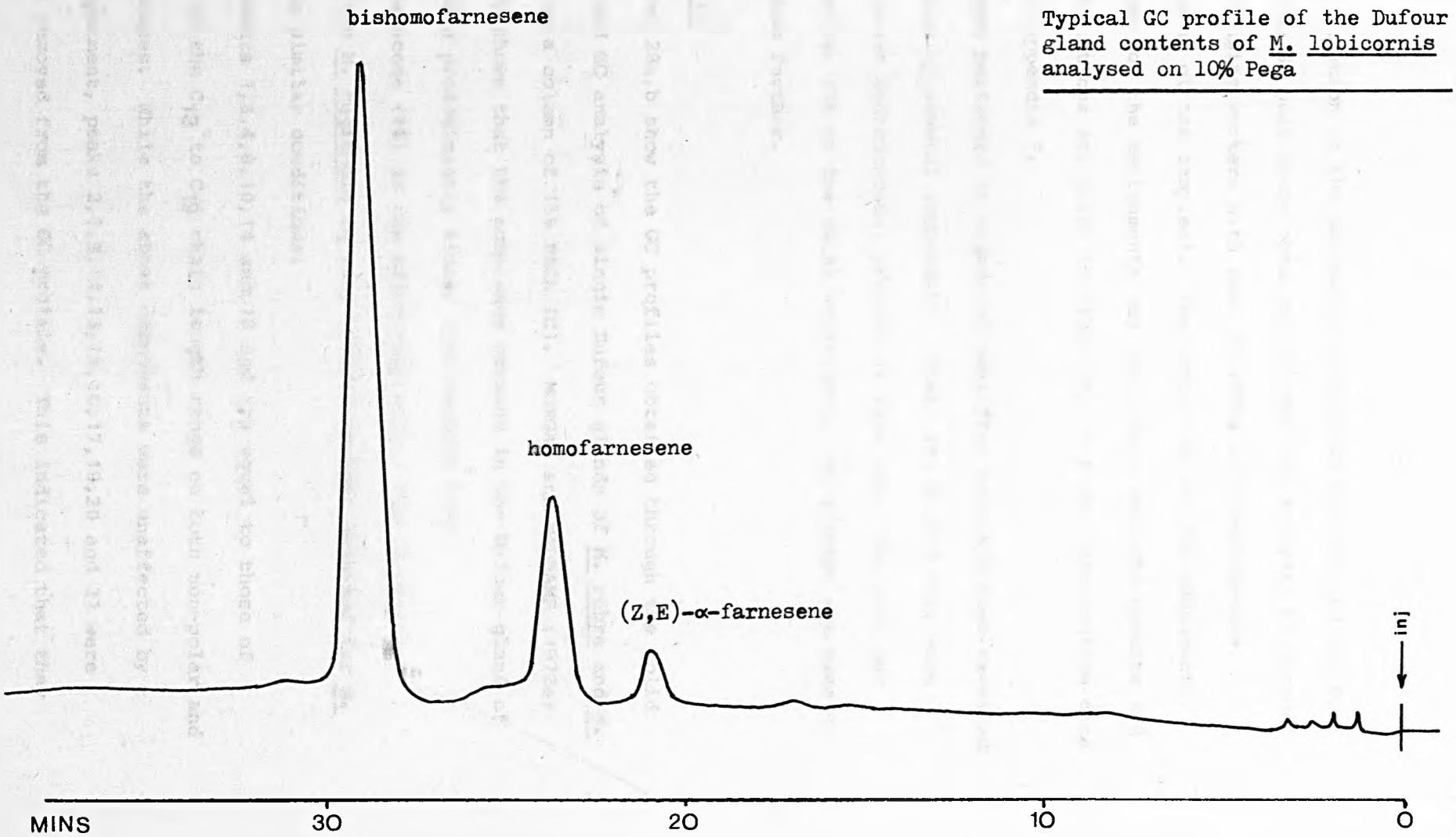
An explanation of the abbreviations in the 'Evidence' column is given in the sub-script to Table 17.

TABLE 25

Dufour gland composition of M. scabrinodis assignments according to PARRY (1978) and TYLER (unpublished).

Compound	Mean % composition by weight (± S.D. n = 8)	mean amount (ng ± S.D. n = 8)
1 Pentadecane	2.01 ± 0.69	20 ± 16
2 Pentadecene	2.82 ± 0.96	28 ± 22
3 Hexadecane	1.35 ± 0.35	12 ± 7
4 Heptadecane	1.43 ± 0.56	13 ± 7
5 α-Farnesene	14.36 ± 5.00	138 ± 86
6 Heptadecadiene	1.33 ± 0.62	12 ± 8
7 Homofarnesene	42.22 ± 6.59	379 ± 179
8 Octadecene	1.80 ± 0.94	19 ± 18
9 Bishomofarnesene	26.80 ± 7.86	228 ± 104
10 Nonadecane	1.59 ± 1.10	15 ± 11
11 Nonadecene	2.57 ± 1.86	28 ± 30
12 Trishomofarnesene	1.71 ± 1.23	19 ± 19

FIGURE 27



Typical GC profile of the Dufour gland contents of M. lobicornis analysed on 10% Pega

Quantification of the glandular components was carried out by comparing the GC peak areas obtained through the analysis of glands from ten foraging workers with that of 500ng of n-heptadecane standard (mean of ten samples). The identities of the components, the evidence for the assignments and their mean absolute amounts and standard deviations are given in Table 26. The raw quantitative data is given in Appendix 7.

Analyses performed at higher GC amplifier sensitivities revealed trace amounts of several components. Their trs showed they were probably linear hydrocarbons, present at less than 10ng each per gland (together <1% of the total volatiles). No attempt was made to identify them further.

M. ruginodis

Figures 28a,b show the GC profiles obtained through the solid injection and GC analysis of single Dufour glands of M. rubra and M. ruginodis on a column of 15% PEGA (C). MORGAN and WADHAMS (1972a) had already shown that the compounds present in the Dufour gland of M. rubra were predominantly linear hydrocarbons with (Z)-8-heptadecene (16) as the major component. The GC profile obtained from M. ruginodis is very similar to that obtained for M. rubra under similar conditions.

Components 1,3,4,8,10,14 and 18 had trs equal to those of n-alkanes in the C_{13} to C_{19} chain length range on both non-polar and polar GC phases. While the above components were unaffected by bromine treatment, peaks 2,5,9,11,13,15,16,17,19,20 and 21 were completely removed from the GC profile. This indicated that they

TABLE 26

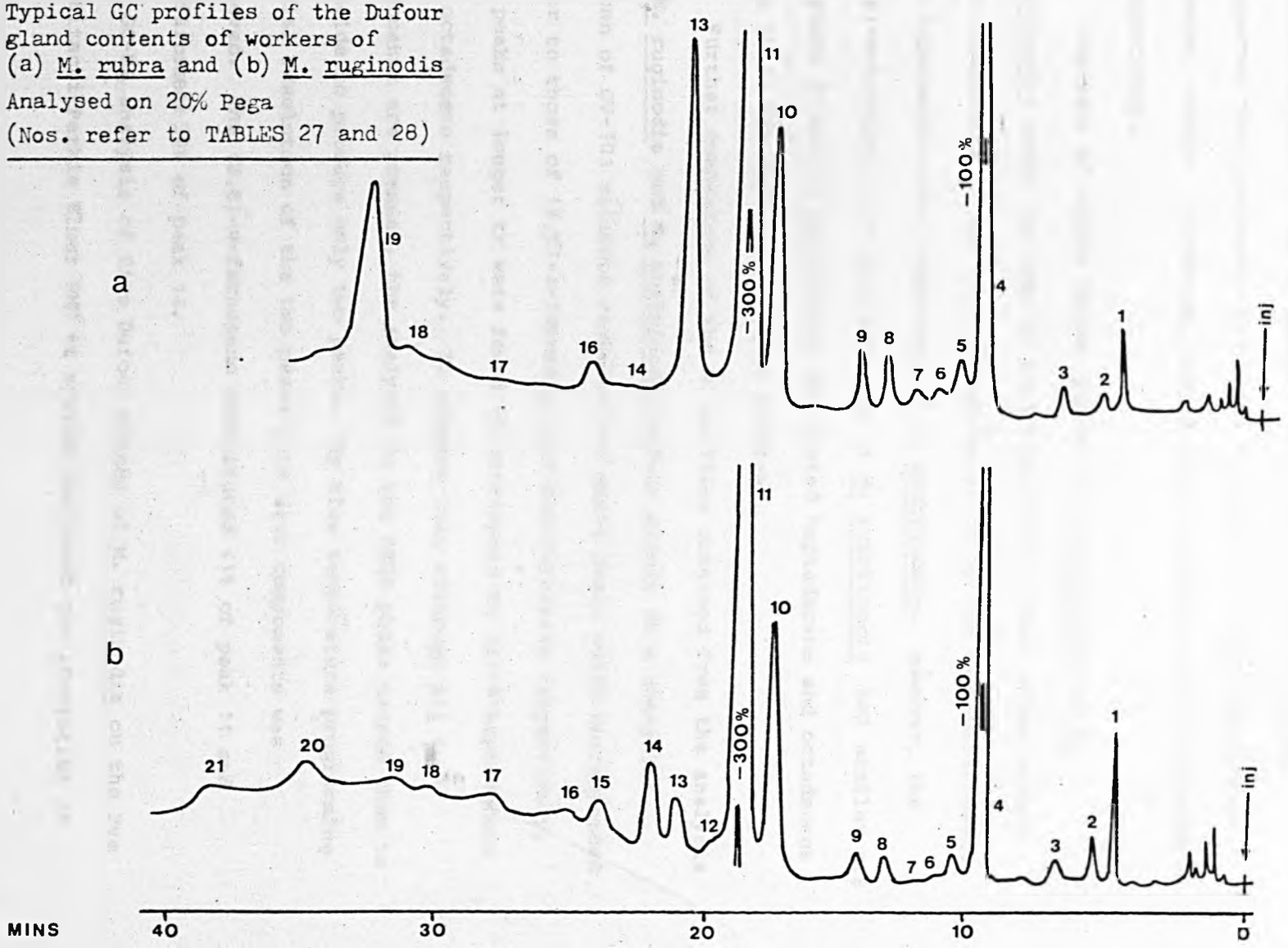
Dufour gland composition of M. lobicornis together with the analytical evidence for the assignments (mean of ten replicates).

Compound	Mean % composition by weight (± S.D. n = 10)	mean amount (ng ± S.D. n = 10)	Evidence
1 α-Farnesene	5.51 ± 2.03	192 ± 41	GC,Br+,MS
2 Homofarnesene	26.75 ± 6.15	979 ± 312	GC,Br+,MS
3 Bishomofarnesene	67.76 ± 7.35	2516 ± 644	GC,Br+,MS

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 17.

FIGURE 28

Typical GC profiles of the Dufour gland contents of workers of (a) M. rubra and (b) M. ruginodis
Analysed on 20% Pega
(Nos. refer to TABLES 27 and 28)



were unsaturated components. Components 11 and 19 had trs corresponding to synthetic heptadecene and nonadecene (two of the major components of the M. rubra Dufour gland) on OV-101 and PEGA phases. A plot of log tr against the number of chain carbons suggested that components 2,5,9 and 15 were also mono-unsaturated alkenes, namely: tridecene, pentadecene, hexadecene and octadecene respectively.

Analysis of single Dufour glands of M. ruginodis and M. scabrinodis under the same GC conditions on the PEGA column showed that components 17 and 21 corresponded in tr to the bishomofarnesene and trishomofanesene components of M. scabrinodis. However, the (Z,E)-α-farnesene and homofarnesene of M. scabrinodis had similar trs to peaks 11 and 14 (previously designated heptadecene and octadecene from their trs and reaction with bromine).

Further comparison of the GC profiles obtained from the analysis of M. ruginodis and M. scabrinodis Dufour glands on a non-polar column of OV-101 silicone revealed two small peaks which corresponded in tr to those of (Z,E)-α-farnesene and homofarnesene respectively. Two peaks at longer tr were found to correspond to (Z)-9-heptadecene and octadecene respectively. It appears that although all four components are present, the analysis on the PEGA phase caused them to coincide to produce only two peaks. By slow temperature programming partial resolution of the two peaks into four components was achieved. The (Z,E)-α-farnesene constituted <1% of peak 11 and homofarnesene 30% of peak 14.

GC-MS analysis of five Dufour glands of M. ruginodis on the Pye 104-Hitachi-Perkin Elmer RMU 6E system confirmed the identities of

the major components: n-pentadecane (M^+212, $C_{15}H_{32}$, Figure 29a), n-heptadecane (M^+238, $C_{17}H_{36}$) and heptadecene (M^+236, $C_{17}H_{34}$, Figure 29b).

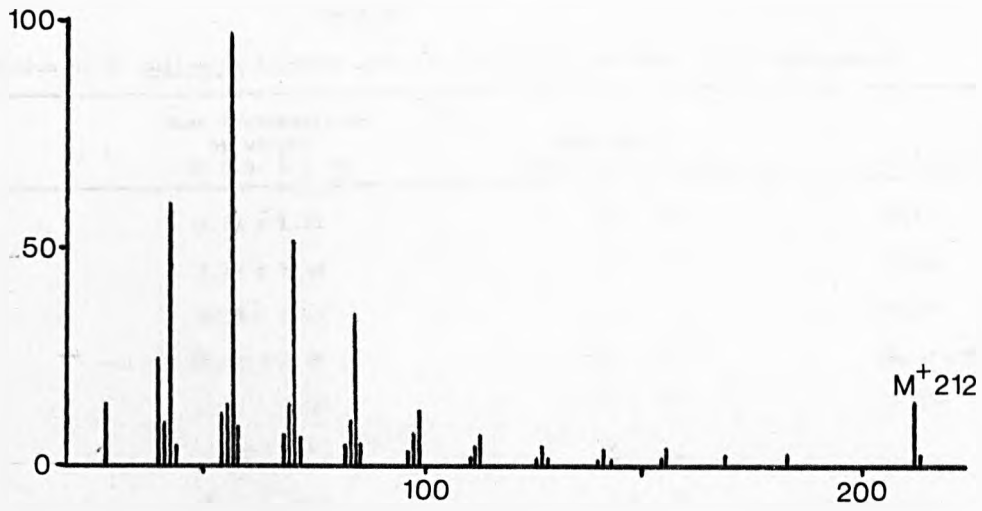
Peaks 13 and 20 fell into neither the terpenoid, n-alkane nor alkene series. Component 13 corresponded in tr to the heptadecadiene component of M. rubra, while component 20 was tentatively assigned as nonadecadiene through its tr and reaction with bromine.

The identities of the components of the Dufour gland of M. ruginodis are listed in Table 27 together with a summary of the analytical evidence for the assignments and the quantitative data. Also presented is the complementary quantitive data for the Dufour gland secretion of M. rubra based on the assignments made previously by WADHAMS (1972; Table 28). The raw quantitative data for both species Dufour gland secretion is given in Appendicies 8 and 9.

Relatively little study of the Dufour gland contents of myrmicine ants has been made, other than these and previous investigations performed at the University of Keele (WADHAMS,1972; TYLER, 1977; PARRY, 1978). Other studies have been confined to Aphaenogaster longiceps, Novomessor cockerelli, Pogonomyrmex rugosus, P. barbatus, Solenopsis invicta, S. richteri, S. geminata and Monomorium pharaonis (see table on page 809 in BLUM and HERMANN, 1978; RITTER et al., 1977,1980; VANDER MEER et al., 1981). The Dufour glands of these species have been shown to contain mainly straight chain alkanes, alkenes, methyl branched alkanes (in Pogonomyrmex) and sesquiterpenoid hydrocarbons in the C_{12} to C_{19} chain length range. The myrmicines studied during the course of

FIGURE 29

a. Mass spectrum of n-pentadecane



b. Mass spectrum of heptadecene

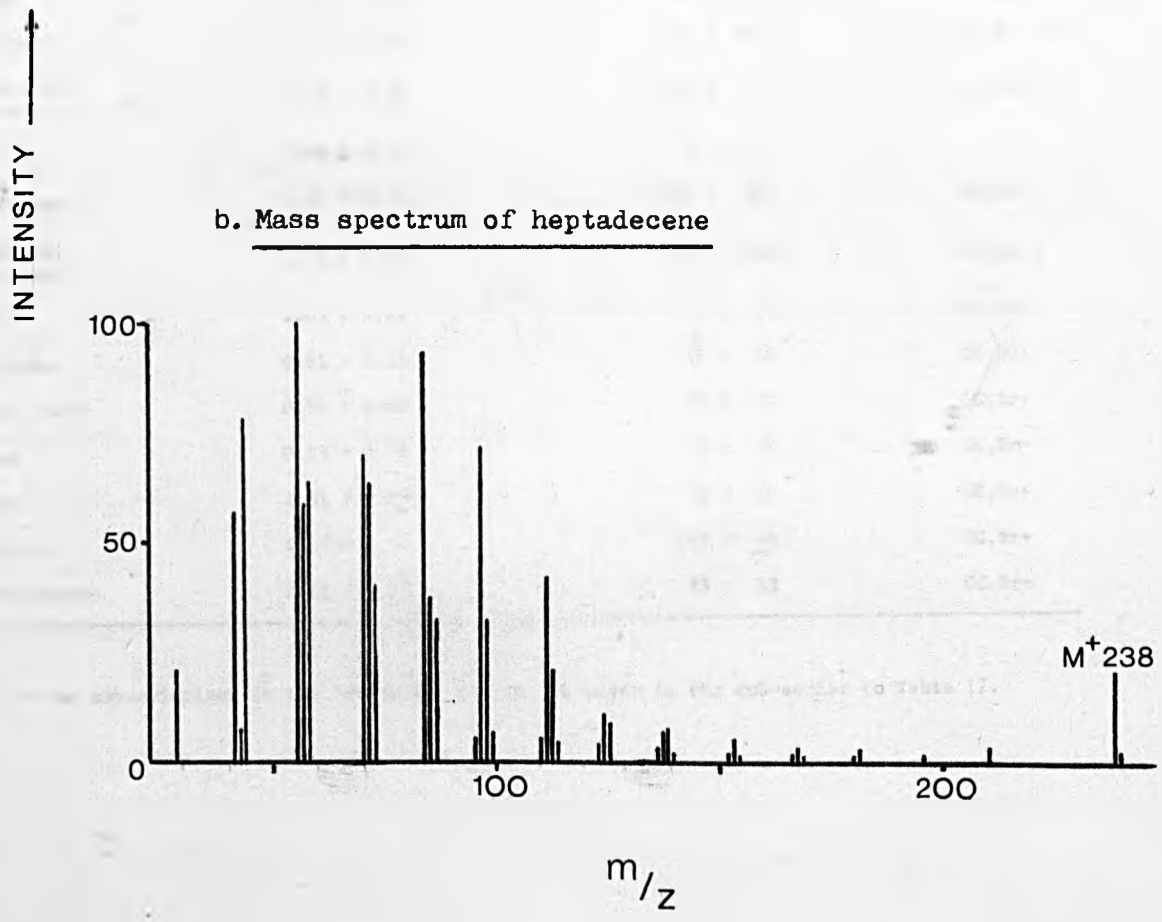


TABLE 27

Dufour gland composition of M. ruginodis together with the analytical evidence for the assignments.

Compound	Mean % composition by weight (± S.D. n = 10)	mean amount (ng ± S.D. n = 10)	Evidence
1 Tridecane	2.52 ± 1.22	76 ± 27	GC,Br-
2 Tridecene	2.19 ± 2.30	61 ± 51	GC,Br+
3 Tetradecane	0.38 ± 0.19	13 ± 5	GC,Br-
4 Pentadecane	18.33 ± 2.08	638 ± 225	GC,Br-,MS
5 Pentadecene	1.73 ± 1.66	39 ± 20	GC,Br+
6 Unknown 1	0.23 ± 0.21	7 ± 5	
7 Unknown 2	0.33 ± 0.13	7 ± 4	
8 Hexadecane	1.07 ± 0.58	39 ± 20	GC,Br-
9 Hexadecene	0.89 ± 0.21	30 ± 11	GC,Br+
10 Heptadecane	7.42 ± 1.43	256 ± 86	GC,Br-,MS
11 Heptadecene and α-Farnesene	43.45 ± 6.56	1557 ± 708	GC,Br+,MS
12 Unknown 3	0.22 ± 0.17	7 ± 5	
13 Heptadecadiene	3.67 ± 1.61	125 ± 58	GC,Br+
14 Octadecane and Homofarnesene	5.36 ± 5.28	173 ± 202	GC,Br-
15 Octadecene	2.63 ± 1.86	90 ± 68	GC,Br+
16 Octadecadiene	0.61 ± 0.29	20 ± 10	GC,Br+
17 Bishomofarnesene	0.96 ± 0.80	35 ± 39	GC,Br+
18 Nonadecane	0.53 ± 0.78	18 ± 29	GC,Br-
19 Nonadecene	1.31 ± 0.89	36 ± 16	GC,Br+
20 Nonadecadiene	4.09 ± 2.27	153 ± 96	GC,Br+
21 Trishomofarnesene	2.11 ± 1.19	73 ± 43	GC,Br+

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 17.

TABLE 28

Dufour gland composition of M. rubra, assignments according to WADMAMS (1972).

Compound	Mean % composition by weight (± S.D. n = 10)	mean amount (ng ± S.D. n = 10)
1 Tridecane	0.77 ± 0.25	16 ± 6
2 Tridecene	1.20 ± 1.46	25 ± 30
3 Tetradecane	0.35 ± 0.13	7 ± 4
4 Pentadecane	13.01 ± 3.00	271 ± 92
5 Pentadecene	0.73 ± 0.40	14 ± 7
6 Unknown 1	0.19 ± 0.14	4 ± 3
7 Unknown 2	0.32 ± 0.14	6 ± 3
8 Hexadecane	0.63 ± 0.15	13 ± 5
9 Hexadecene	0.98 ± 0.56	20 ± 10
10 Heptadecane	5.57 ± 1.29	113 ± 33
11 Heptadecene and α-Farnesene	50.38 ± 7.00	1031 ± 277
13 Heptadecadiene	9.83 ± 3.32	204 ± 94
14 Octadecane and Homofarnesene	0.39 ± 0.57	9 ± 16
15 Octadecene	1.19 ± 0.48	28 ± 15
17 Bishomofarnesene	0.54 ± 0.47	9 ± 10
18 Nonadecane	0.50 ± 0.31	9 ± 5
19 Nonadecene	13.31 ± 6.04	274 ± 141

these investigations produce hydrocarbons in their Dufour glands in the C_{12} to C_{23} range.

The Dufour gland of workers of M. ruginodis produce a very similar mixture of substances to the workers of M. rubra (see Figure 28a,b and Tables 27 and 28). In both species the major component is heptadecene which accounts for about half the Dufour gland volatiles in each of the species. The remaining portion of their Dufour gland secretions are made up of both saturated and unsaturated hydrocarbons in the C_{13} to C_{19} chain length range. n-Pentadecane is present as a significant component in both species; 13.2% of the total in M. rubra and 18.4% in M. ruginodis. The compositions of the Dufour gland secretions in these two species differ most noticably with respect to the proportion of their nonadecene component. In M. ruginodis this is a minor component while in M. rubra it is the second most abundant compound, constituting 13.4% of the total volatiles.

While M. rubra and M. ruginodis produce similar mixtures of predominently linear hydrocarbons M. sabuleti and M. scabrinodis produce predominently terpenoid hydrocarbons (Figure 25a,b and Tables 24 and 25). The Dufour glands of these latter two species are dominated by three homologous sesquiterpenoids, α-farnesene, homofarnesene and bishomofarnesene together constituting over 80% of their total glands volatiles. A further homologue trishomofaresene is present but at a much lower level. In M. sabuleti the predominent component is bishomofarnsene, while in M. scabrindis the homofaresene is the major constituent. The minor constituents of the glands have been identified as saturated and unsaturated hydrocarbons in the C_{15} to C_{19} chain length range.

Another Myrmica species examined during the course of this work was M. lobicornis. This species produces fewer individual components than the aforementioned species. Three components namely: α-farnesene, homofarnesene and bishomofarnesene together constitute over 99% of the gland contents. As in M. scabrinodis bishomofarnesene is the major component. The Dufour gland secretion of the leaf-cutting ant A. octospinosus is also dominated by homofarnesene it is however produced in only nanogram amounts. In contrast A. cephalotes produces predominently linear n-alkanes and mono-unsaturated alkenes in the C_{12} to C_{19} chain length range. n-Heptadecane is the most abundant alkane (46.7%) and (Z)-9-nonadecene the most abundant alkene.

The two sub-species of A. sexdens produce predominently linear mono-unsaturated alkenes in their Dufour glands. However they are unusual, as for the first time in the Dufour glands of myrmicine ants higher molecular weight hydrocarbons have been observed in the C_{20} to C_{23} range. It has been proved that these compounds definitely originate from the Dufour gland by analysing single dissected glands and capillary extracts (MORGAN and TYLER, 1977). Tricosene, the most abundant component of the Dufour gland of A. s. sexdens and the second most abundant component of A. s. rubropilosa has been observed previously in ants of the Formicinae (BERGSTROM and LOFQVIST, 1973). The tricosene of the leaf-cutting ants has been positively identified as (Z)-9-tricosene. This same substance also occurs as the sexual attractant of the female house fly (Musca domestica; CARLSON et al., 1971) where it is known as 'muscalure'. It has also been found in the cuticle waxes of a number of species of Periplaneta (JACKSON,

1970) and in the comb and cuticular waxes of Apis mellifera (STREIBL et al., 1966; BLOMQUIST et al., 1980). It is interesting to note that A. cephalotes and A. octospinosus produce none of these higher molecular weight compounds identified in the two A. sexdens sub-species.

The Dufour gland hydrocarbons of the seven myrmicines examined here are, as has been found previously, of two distinct types i.e. linear or terpenoid, and a particular species shows a tendency to produce either predominently one class or the other. Table 29 lists the species studied during the course of this work under the class of hydrocarbons predominating in their Dufour glands. This tendency of the workers of a particular species to produce either linear or terpenoid hydrocarbons would seem to indicate that two distinct biosynthetic pathways are involved in the production of the Dufour gland hydrocarbons.

The Dufour glands of all the Myrmica species studied here are completely filled with microgram quantities of hydrocarbons (summarised in Table 29) and nanogram quantities of volatile oxygenated compounds. The workers of A. cephalotes and A. octospinosus however produce considerably less hydrocarbon material i.e. of the order of nanograms. CAMMAERTS et al. (1978) found that the Dufour gland secretion of M. rubra and M. scabrinodis is used in territorial marking and recruitment in conjunction with the trail pheromone from the venom gland. It is thought that the pheromonal function of this secretion is a secondary evolutionary development and that the primary function is one of sting or egg lubrication (WHEELER, 1910; ROBERTSON, 1968). While the Myrmica species are all

TABLE 29

Catagorising the myrmicine species according to the class of hydrocarbon predominating in their Dufour gland secretion.

Class of hydrocarbon			
terpenoid		linear	
Species	total amount* (ng)	Species	total amount* (ng)
M. sabuleti	1106	M. rubra	2053
M. lobicornis	3687	M. ruginodis	3453
M. scabrinodis	911	A. cephalotes	20 - 254
A. octospinosus	28 - 92	A. s. sexdens	1195 - 6670
		A. s. rubropilosa	685 - 7917

* In the case of the Myrmica species the mean amount of hydrocarbon from ten workers is given; in the Attines, where there is wide range of caste sizes, the range of amounts observed is listed.

stinging ants, the Attines are not, their stings are rudimentary, being used principally to deposit the trail pheromone, originating from their venom gland onto the substratum. At first it was thought that as the Dufour gland's primary evolutionary function was to provide lubricating chemicals for the sting, then the Attines no longer need or are able to produce such large quantities of hydrocarbons. Subsequent examination of the two A. sexdens sub-species showed their Dufour glands to be completely filled with microgram amounts of hydrocarbons (Table 29). Therefore the above suggestion that the production of hydrocarbons is related purely to stinging ability seems not to hold true in this case.

In the leaf-cutting ants there is a charateristically wide variation between the various caste sizes. For example, in the study of the Dufour gland hydrocarbons of A. s. rubropilosa, ants having body weights in the range 5.5 to 51.8mg were examined. In A. cephalotes and the two A. sexdens subspecies there was found to be a linear relationship between the live body weights of the ants and the hydrocarbon content of their Dufour glands (see Figures 15 and 24). Figure 24 inparticular shows there is a variability in the hydrocarbon content at a given body weight which may reflect differences in age or some other factor. This variability may explain the apparent non-linearity in the relationship between hydrocarbon content and the body weight of M. scabrinodis workers (MORGAN et al., 1979). Workers of Myrmica ants do not exhibit such a wide gradation of sizes as the Attines; body weights between 1.1 and 3.1mg were recorded for the seventy determinations performed on M. scabrinodis. Consequently the variation in the hydrocarbon content

is more obvious, and as a result any relationship that there may be between hydrocarbon content and body weight is obscured.

It is interesting to note that there were no significant differences between the glandular compositions of the minims, mediums and soldier castes of the Attines that were examined. Caste variation in exocrine gland composition is known in ants, however significant caste differences have only so far been reported for the venom and mandibular gland secretions of a few species (BRAND et al., 1973; LONGHURST et al.,1978).

Workers of A. s. rubropilosa and A. s. sexdens produce microgram amounts of saturated and unsaturated hydrocarbons in their Dufour glands. This material is composed predominantly of mono-unsaturated alkenes in the C_{15} to C_{23} range (Tables 21 and 22). Although the two sub-species produce the same chemicals in similar quantities there are significant quantitative differences in the relative proportions of the individual components. The differences in the compositions of their Dufour gland secretions are as great as the differences between species in the Myrmica genus e.g. compare M. rubra to M. ruginodis (Figure 28a,b; Tables 27 and 28) or M. scabrinodis to M. sabuleti (Figure 25a,b; Tables 24 and 25). From a chemical taxonomic point of view therefore, they may be considered as separate species. Only one colony of each sub-species was available and so further work with colonies from other areas would be required to establish this possibility clearly. Since the two colonies were obtained from areas geographically far apart, it would be interesting to examine colonies from an intermediate area. However, the work on A. cephalotes and A. octospinosus was carried out on a number of different colonies of

both species and at no time were any differences observed approaching that between the two A. sexdens sub-species.

A much larger number of formicine species have been examined and a much wider variety of compounds found than in myrmicine ants (see Table page 812 in BLUM and HERMANN 1978). Hydrocarbons from nonane to tricosane, methylalkanes and alkenes have been found together with alkanols, alkanones, alkyl and alkenyl acetates, but only for a few species have quantitative comparisons been made. Generally, amoung formicines n-undecane and n-tridecane predominate but the variety of other components makes it possible for each species to have a characteristic blend of substances that renders it detectably distinct. This possible species specificity has been discussed by BERGSTRÖM and LÖFQVIST (1973). Suggestions that the formicine hydrocarbons are alarm pheromones or act as spreading agents for formic acid from the venom gland have been tested by experiment in very few cases.

While some myrmicines employ their Dufour gland secretion as a trail pheromone (for review see PARRY and MORGAN, 1979; VANDER MEER et al., 1981) ethological investigations of the Dufour gland secretion of M. rubra and M. scabrinodis by CAMMAERTS et al. (1978) showed that the volatile fraction acted as a short lived attractant to the workers and caused them to walk more rapidly. The less volatile fraction was demonstrated to serve as a species specific territorial marking agent which causes workers to move more slowly over an area marked with an alien species' Dufour gland secretion (allospecifically marked areas). Normal movement was observed over areas marked by a species' own secretion (conspecific).

Further work by Mme CAMMAERTS at the University of Brussels, Belgium, has shown that the Dufour gland secretion functions in exactly the same manner for the three other Myrmica species studied during this project. Her further investigations into the specificity of the secretions have shown that with the exception of M. rubra and M. ruginodis (which are unable to distinguish between one another's secretions) all of the species studied to date are able to distinguish their own from an alien marking. The ants' territorial marking is specific except between M. rubra and M. ruginodis.

The observed chemical resemblances and differences between the secretions studied parallel the behavioural reactions of the ants to the Dufour gland contents of each species.

These chemical and behavioural investigations have provided data for a taxonomic study of species. The studies on the Dufour gland secretions are complemented by further work on Myrmica Dufour glands (ATTYGALLA, in press) and mandibular glands (also reported in this thesis).

As yet no ethological studies have been performed on the Attine Dufour gland secretions. It would certainly be interesting to test whether or not the secretion acts as a territoral marker pheromone as in the Myrmica species.

Chemical Investigation of Mandibular Gland Substances

The mandibular gland secretion has been shown to produce an alarm response in a large number of Myrmica species. This secretion is released by opening the mandibles wide when workers encounter a prey or disturbance. This allows the secretion to emerge from an opening in the base of the mandibles and form a film or droplet across the mandibles. The substances present cause workers to be attracted to the source of the emission and run with increased speed and less sinuosity. Studies performed on several Myrmica species have shown that 3-alkanones and 3-alkanols in the C_6 to C_{11} chain length range are the major components of the secretion, and that the responses evoked in the workers by the synthetic substance at the glandular level exactly reproduce their responses to a crushed worker head (CREWE and BLUM, 1970; CAMMAERTS et al., 1978).

Colonies of several of the European Myrmica species are often found co-existing in the same habitat. Consequently as their territories overlap the workers may confront each other and emit their alarm pheromone from their mandibular glands. A comparative study of the mandibular gland secretions of M. rubra and M. scabrinodis has already been performed. The results reported in this section extend these studies of mandibular glands to include six further species. The chemical data reported is intended to serve as a basis for complementary comparative ethological studies which should shed light on how the Myrmica workers use their pheromonal message and how they avoid confusion from the other species sharing the same habitat. Several of these closely related Myrmica species

are difficult to distinguish morphologically and so these chemical investigations may provide results which will enable the species to be classified according to chemical taxonomy.

Chemical investigation of the mandibular gland secretion of M. lobicornis

WADHAMS (1972) had previously found in his work on the volatile chemicals of M. rubra that the GC profiles obtained through the solid injection of excised mandibular glands were the same as those obtained from single heads. Analysis of the volatiles from a single head of M. lobicornis revealed significant amounts of twelve components. Figure 30a shows a typical GC profile obtained by the analysis of a single worker's head on a column of 10% PEG-20M (F).

Analysis of a mixture of standard alcohols and ketones showed components 1,2,4,7 and 10 to have the same trs on Apiezon L (H) and PEG-20M phases as a homologous series of 3-alkanones in the C_6 to C_{10} chain length range. These five substances also produced a linear plot of log tr against the number of chain carbons (Figure 31a).

Components 3,5,8 and 11 had tr values corresponding to homologous C_6 to C_9 3-alkanols on the aforementioned GC phases. A plot of log tr against the number of chain carbons suggested that component 12 may be 3-decanol (Figure 31b).

Components 6 and 9 fitted into neither of the homologous series, but did in fact correspond in tr on the two phases to synthetic samples of 6-methyl-3-octanone and 6-methyl-3-octanol respectively.

Ketones are readily reduced to their corresponding alcohols with sodium borohydride. Therefore to test for the presence of ketones in

FIGURE 30

GC analysis of the mandibular gland contents of M. lobicornis workers on 10% Peg 20M (a) before and (b) after treatment with $NaBH_4$ (Nos. refer to TABLE 30)

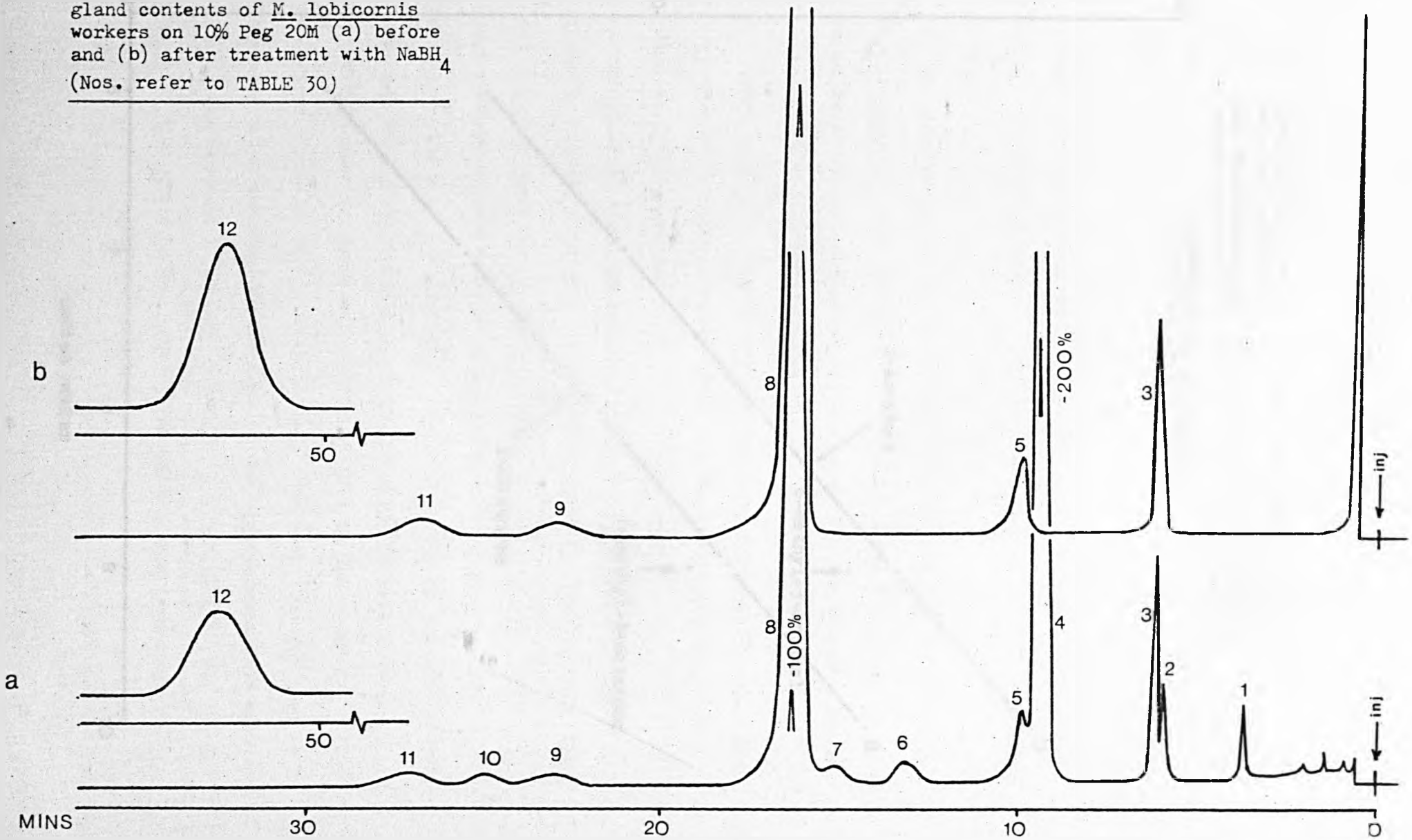
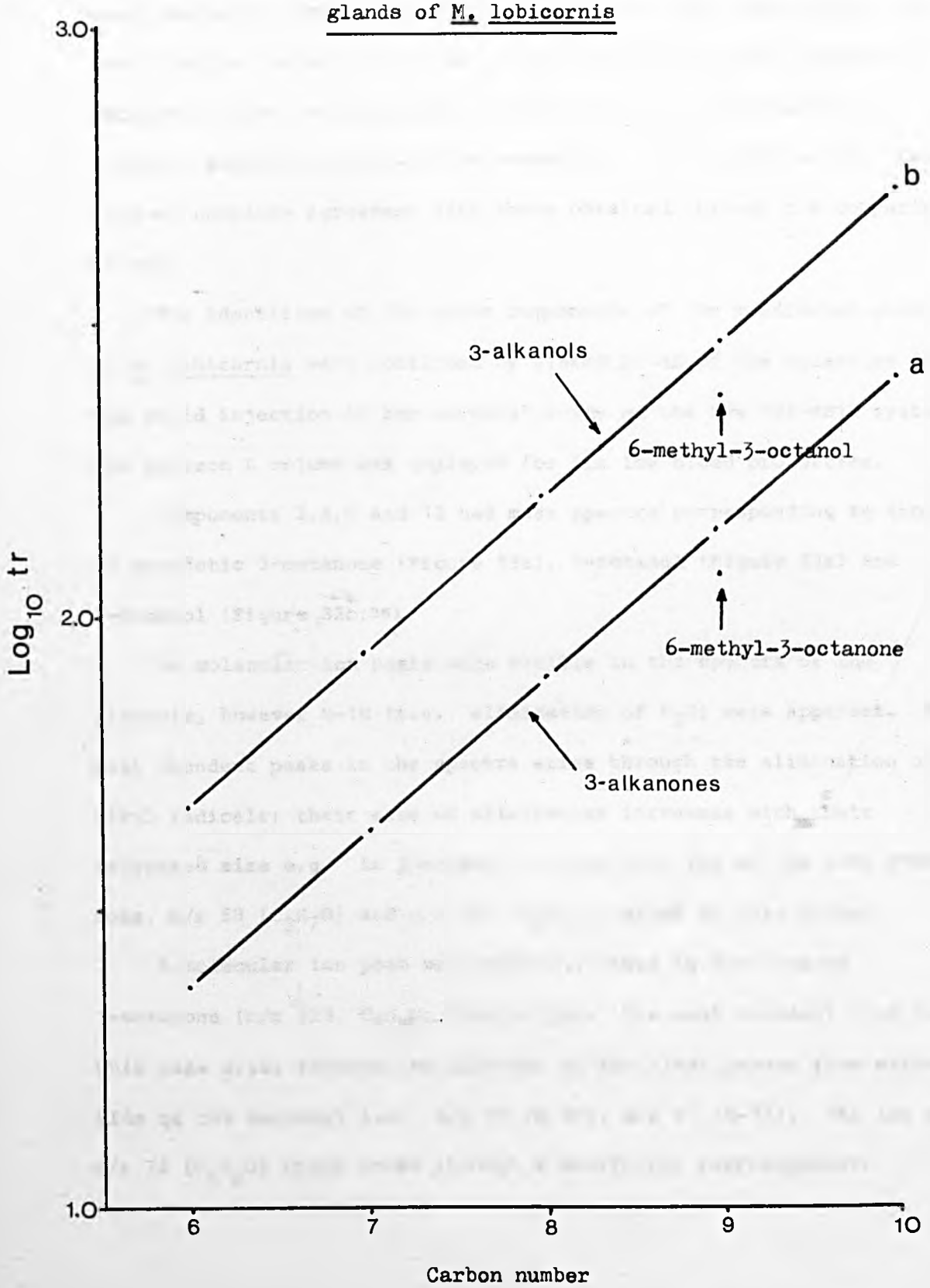


FIGURE 31

Plot of Log_{10} tr vs the number of carbon atoms of the 3-alkanones and 3-alkanols of the mandibular glands of M. lobicornis



the mandibular gland secretion of M. lobicornis, 2mgs of powdered sodium borohydride were added to a single worker's head in a solid sample vial. Figure 30b shows the GC profile obtained from the analysis of the reaction products. Only the peaks corresponding to the alcohols can still be seen as the ketones have been completely reduced to their corresponding alcohols which co-elute with the alcohols already present in the secretion. The results of this test were in complete agreement with those obtained through the comparison of trs.

The identities of the major components of the mandibular gland of M. lobicornis were confirmed by linked GC-MS of the volatiles from the solid injection of ten workers' heads on the Pye 104-MS12 system. The Apiezon L column was employed for its low bleed properties.

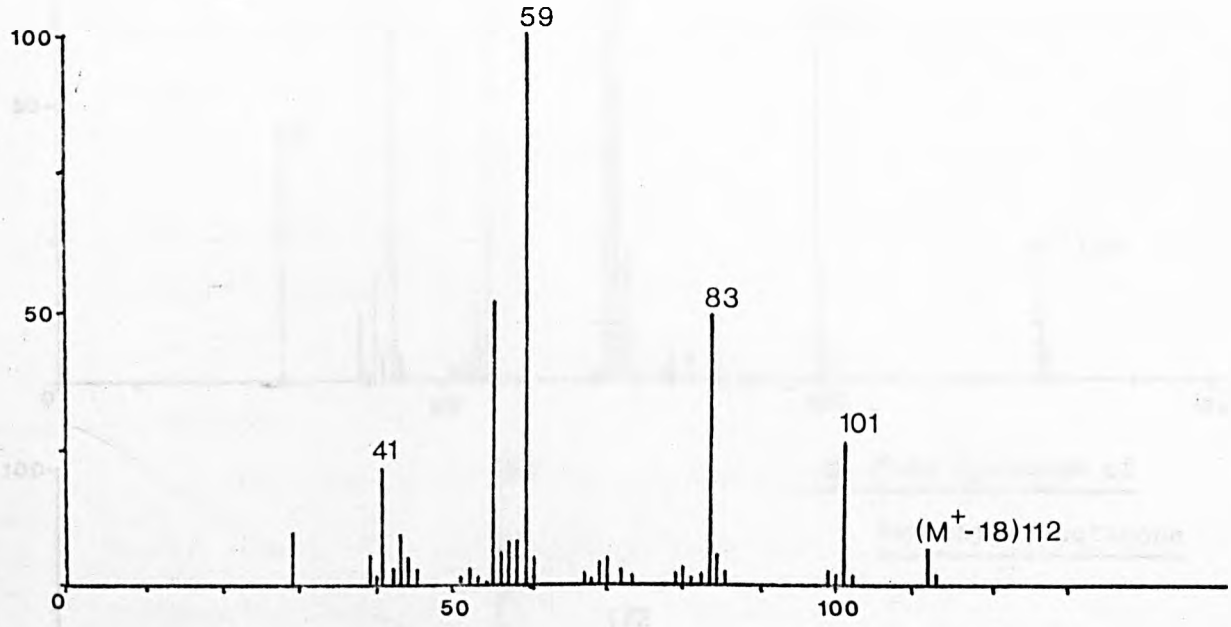
Components 3,4,8 and 12 had mass spectra corresponding to those of synthetic 3-octanone (Figure 33a), 3-octanol (Figure 32a) and 3-decanol (Figure 32b;36).

No molecular ion peaks were visible in the spectra of the alcohols, however M-18 (i.e. elimination of H_2O) were apparent. The most abundent peaks in the spectra arise through the elimination of alkyl radicals; their ease of elimination increases with their increased size e.g. in 3-octanol (Figure 32a) two of the most prominent ions, m/z 59 (C_3H_7O) and m/z 101 ($C_6H_{13}O$) arise in this manner.

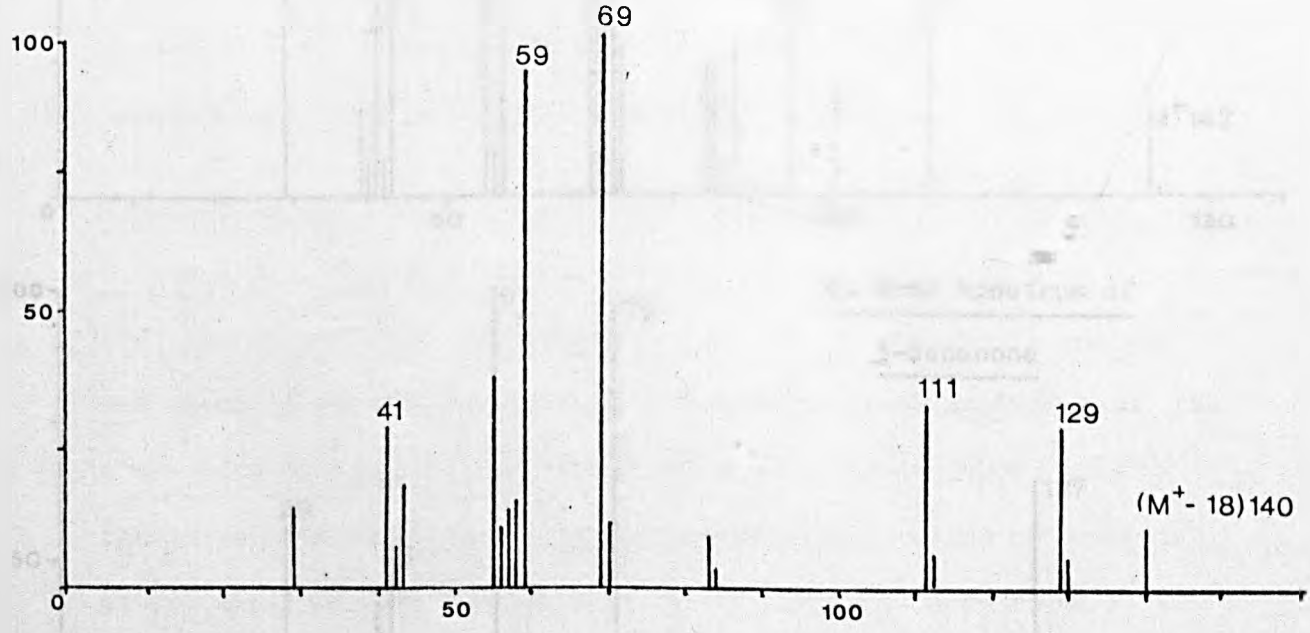
A molecular ion peak was however visible in spectrum of 3-octanone (m/z 128, $C_8H_{16}O$, Figure 33a). The most abundant ions in this case arise through the cleavage of the alkyl groups from either side of the carbonyl i.e. m/z 99 (M-29), m/z 57 (M-71). The ion at m/z 72 (C_4H_8O) would arise through a McLafferty rearrangement.

FIGURE 32

a. Mass spectrum of 3-octanol



b. Mass spectrum of 3-decanol



INTENSITY

m/z

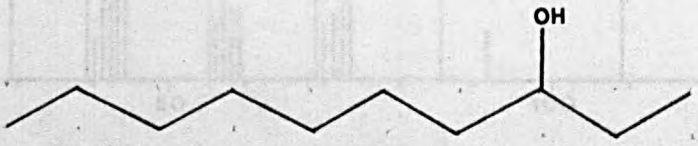
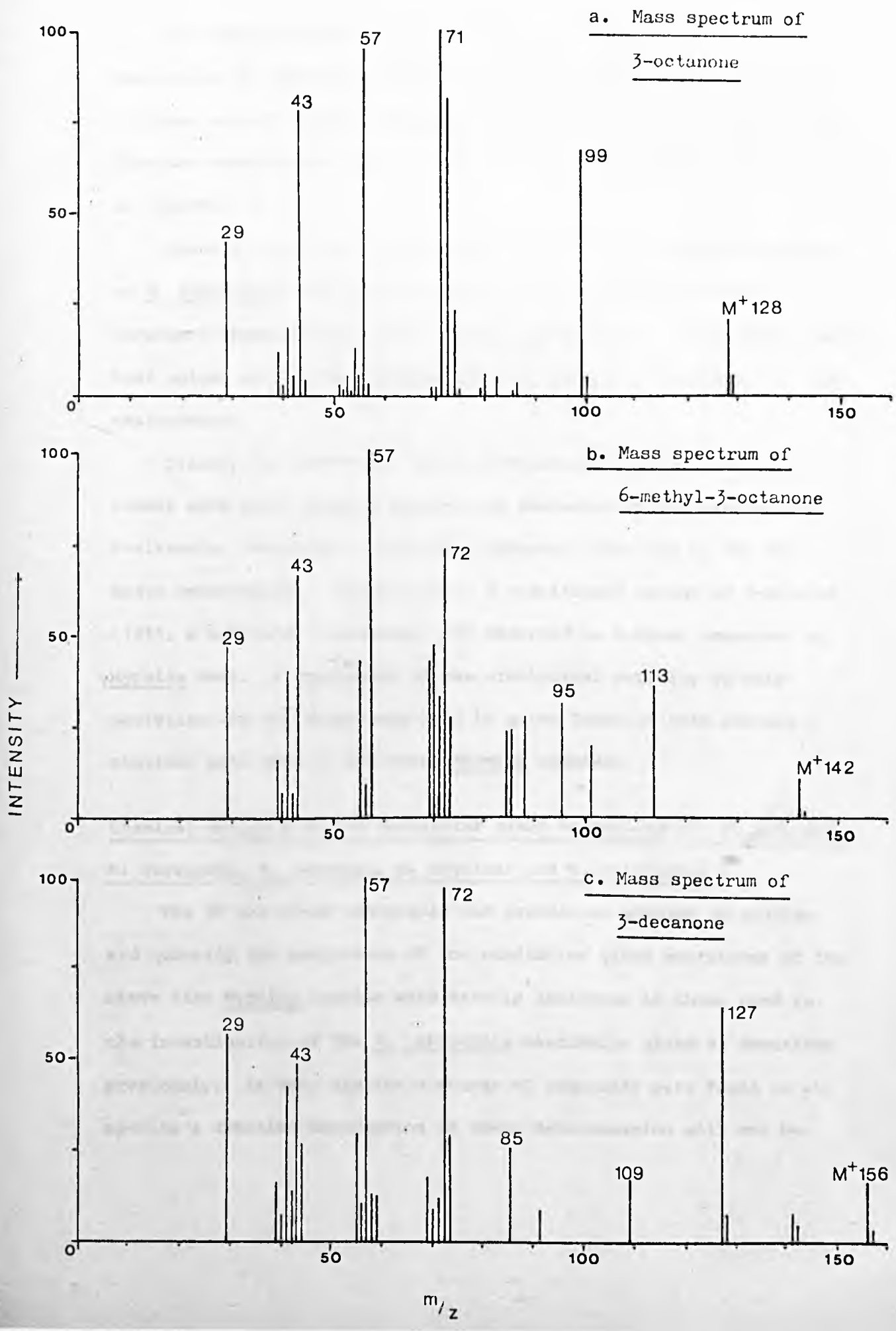


FIGURE 33


a. Mass spectrum of
3-octanone
100
50
0
57
71
43
99
29
M+128
50
100
150
b. Mass spectrum of
6-methyl-3-octanone
57
72
43
29
95
113
M+142
INTENSITY
c. Mass spectrum of
3-decanone
57
72
29
43
127
85
109
M+156
m/z

The absolute amounts of the individual components were determined by comparing the areas of the GC peaks obtained through the analysis of single heads with those of standards of similar size. The raw quantitative data for the ten replicate analyses is presented in Appendix 10.

Table 30 lists the components of the mandibular gland secretion of M. lobicornis with the mean amount and percentage of each component together with their standard deviations. The extreme right hand column of the table summarises the analytical evidence for the assignments.

Clearly the mandibular gland secretion of this species, in common with other Myrmica species, is dominated by 3-alkanones and 3-alkanols. 3-octanone (41%) and 3-octanol (30%) are by far the major constituents. There is also a significant amount of 3-decanol (13%), a substance previously only observed as a minor component in Myrmica ants. A discussion of the ethological activity of this secretion and its components will be given later in this section together with that of the other Myrmica species.

Chemical analysis of the Mandibular gland secretions of M. sabuleti, M. ruginodis, M. schencki, M. rugulosa and M. sulcinodis

The GC and GC-MS techniques and procedures adopted to analyse and quantify the components of the mandibular gland secretions of the above five Myrmica species were exactly analogous to those used in the investigation of the M. lobicornis mandibular gland as described previously. As very similar mixtures of compounds were found in all species a detailed description of their determination will not be

TABLE 30

Mandibular gland composition of M. lobicornis together with the analytical evidence for the assignments (mean of ten replicates).

Compound	mean amount (ng ± S.D. n = 10)	mean % composition by weight (± S.D. n = 10)	Evidence*
1 3-hexanone	118 ± 39	1.32 ± 0.45	$NaBH_4$+,GC
2 3-heptanone	115 ± 37	1.21 ± 0.24	$NaBH_4$+,GC
3 3-hexanol	566 ± 185	5.91 ± 0.80	$NaBH_4$-,GC,MS
4 3-octanone	4028 ± 1277	40.86 ± 2.78	$NaBH_4$+,GC,MS
5 3-heptanol	374 ± 146	3.95 ± 1.67	$NaBH_4$-,GC
6 6-methyl-3-octanone	162 ± 68	1.37 ± 0.37	$NaBH_4$+,GC
7 3-nonanone	42.4 ± 16	0.43 ± 0.11	$NaBH_4$+,GC
8 3-octanol	2913 ± 990	30.16 ± 2.76	$NaBH_4$-,GC,MS
9 6-methyl-3-octanol	31 ± 17	0.3 ± 0.12	$NaBH_4$-,GC
10 3-decanone	32 ± 17	0.31 ± 0.26	$NaBH_4$+,GC
11 3-nonanol	103 ± 41	0.97 ± 0.31	$NaBH_4$-,GC
12 3-decanol	1276 ± 435	13.14 ± 1.90	$NaBH_4$-,GC,MS

*$NaBH_4$+ : signifies peak removed on treatment with sodium borohydride; $NaBH_4$- : peak unaffected by sodium borohydride; GC : indicated that the substance has similar retention times on both PEG 20M and Apiezon phases to the assigned compound; MS : identity confirmed from mass spectrum.

given, however the results of the quantifications and the analytical evidence for the assignments are summarised in Tables 31-35. The raw quantitative data is listed in Appendices 11-15.

Figures 34 and 35 show typical GC profiles obtained through the solid injection of single worker's heads onto a colunm of PEG 20M. Typical profiles for M. rubra and M. scabrinodis mandibular gland volatiles analysed under the same conditions are included for comparison.

Figures 32 and 33 show the mass spectra obtained for the major glandular components in the listed species.

Tables 36 and 37 combine the quantitative data for all the eight Myrmica species studied to date in order to simplify subsequent discussion of the composition.

Analysis of the very volatile fraction

In previous investigations of the volatile components of the mandibular glands of M. rubra and M. scabrinodis (CAMMAERTS et al., 1978; MORGAN et al., 1978) three GC peaks were observed of very short trs and were, from comparison with standard samples identified as ethanal, acetone and ethanol. There was insufficent quantity of these substances for mass-spectrometry. In this present work these assignments were checked on Porapak Q and Chromosorb 102 porous polymer phases. This resulted in better separation and longer trs for these low molecular weight constituents.

The retention data for these three components on the two phases is presented in Table 38; it confirms the identification of ethanal and acetone, but showed no detectable ethanol. The third component

TABLE 31

Mandibular gland composition of M. ruginodis together with the analytical evidence for the assignments (mean of ten samples).

	Compound	mean amount (ng ± S.D. n = 10)	mean % composition by weight (± S.D. n = 10)	Evidence
	unknown	1 ± 1	0.15 ± 0.08	-
d	3-hexanone	2 ± 1	0.19 ± 0.08	$NaBH_4$+,GC
e	3-pentanol	1 ± 1	0.06 ± 0.13	$NaBH_4$-,GC
	unknown	7 ± 8	0.81 ± 1.00	-
	unknown	1 ± 1	0.07 ± 0.10	-
f	3-heptanone	2 ± 1	0.21 ± 0.07	$NaBH_4$+,GC
g	3-hexanol	4 ± 2	0.41 ± 0.08	$NaBH_4$-,GC
h	3-octanone	410 ± 170	40.22 ± 8.41	$NaBH_4$+,GC,MS
i	3-heptanol	18 ± 7	1.88 ± 0.30	$NaBH_4$-,GC
j	6-methyl-3-octanone	11 ± 5	1.11 ± 0.32	$NaBH_4$+,GC
k	3-nonanone	7 ± 2	0.70 ± 0.16	$NaBH_4$+,GC
l	3-octanol	516 ± 180	52.68 ± 7.70	$NaBH_4$-,GC,MS

Explanation of the abreviations in the 'Evidence' column are given in the sub-script to Table 30.

TABLE 32

Mandibular gland composition of M. sabuleti together with the analytical evidence for the assignments (mean of ten replicates).

	Compound	mean amount (ng ± S.D. n = 10)	% (mean ± S.D.)	Evidence
	unknown 1	5 ± 3	0.36 ± 0.12	-
d	3-hexanone	13 ± 1	1.04 ± 0.78	$NaBH_4$+,GC
e	3-pentanol	5 ± 6	0.41 ± 0.14	$NaBH_4$-,GC
	unknown 2	20 ± 23	1.89 ± 2.58	-
	unknown 3	5 ± 8	0.41 ± 0.42	-
f	3-heptanone	16 ± 10	1.21 ± 0.58	$NaBH_4$+,GC
g	3-hexanol	45 ± 30	3.36 ± 1.91	$NaBH_4$-,GC
h	3-octanone	808 ± 3	59.49 ± 8.56	$NaBH_4$+,GC,MS
i	3-heptanol	56 ± 1	4.70 ± 1.78	$NaBH_4$-,GC
j	6-methyl-3-octanone	75 ± 30	3.52 ± 1.55	$NaBH_4$+,GC,MS
k	3-nonanone	12 ± 6	0.86 ± 0.17	$NaBH_4$+,GC
l	3-octanol	240 ± 100	18.72 ± 6.43	$NaBH_4$-,GC,MS
m	6-methyl-3-octanol	4 ± 4	0.31 ± 0.26	$NaBH_4$-,GC
n	3-decanone	7 ± 12	0.42 ± 0.51	$NaBH_4$+,GC

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 30.

TABLE 33

Mandibular gland composition of M. rugulosa together with the analytical evidence for the assignments (mean of ten samples).

	Compound	mean amount (ng ± S.D. n = 10)	mean % composition by weight (± S.D. n = 10)	Evidence
d	3-hexanone	10 ± 8	1.33 ± 1.21	$NaBH_4$+,GC
e	3-pentanol	8 ± 6	1.21 ± 1.03	$NaBH_4$−,GC
f	3-heptanone	12 ± 11	1.47 ± 1.02	$NaBH_4$+,GC
g	3-hexanol	32 ± 22	3.98 ± 1.84	$NaBH_4$−,GC
h	3-octanone	394 ± 173	51.33 ± 11.48	$NaBH_4$+,GC,MS
i	3-heptanol	31 ± 34	4.59 ± 4.89	$NaBH_4$−,GC
j	6-methyl-3-octanone	63 ± 23	8.54 ± 1.41	$NaBH_4$+,GC,MS
k	3-nonanone	14 ± 7	2.21 ± 1.37	$NaBH_4$+,GC
l	3-octanol	119 ± 35	17.76 ± 7.64	$NaBH_4$−,GC,MS
n	3-decanone	31 ± 15	5.58 ± 3.64	$NaBH_4$+,GC
r	unknown	17 ± 20	2.19 ± 1.99	-

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 30.

TABLE 34

Mandibular gland composition of M. schencki together with the analytical evidence for the assignments (mean of ten samples).

	Compound	mean amount (ng ± S.D. n = 10)	mean % composition by weight (± S.D. n = 10)	Evidence
f	3-heptanone	2 ± 1	0.4 ± 0.2	$NaBH_4$+, GC
g	3-hexanol	21 ± 9	3.5 ± 0.8	$NaBH_4$-, GC
h	3-octanone	42 ± 35	7.0 ± 2.5	$NaBH_4$+, GC, MS
i	3-heptanol	28 ± 12	4.4 ± 0.5	$NaBH_4$-, GC
j	6-methyl-3-octanone	10 ± 4	1.6 ± 0.4	$NaBH_4$+, GC
l	3-octanol	335 ± 143	53.7 ± 4.6	$NaBH_4$-, GC, MS
m	6-methyl-3-octanol	20 ± 9	3.5 ± 1.6	$NaBH_4$-, GC
n	3-decanone	3 ± 3	0.5 ± 0.5	$NaBH_4$+, GC
o	3-nonanol	9 ± 6	1.3 ± 0.7	$NaBH_4$-, GC
g	3-decanol	236 ± 270	22.0 ± 3.7	$NaBH_4$-, GC, MS

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 30.

TABLE 35

Mandibular gland composition of M. sulcinodis together with the analytical evidence for the assignments (mean of ten samples).

	Compound	mean amount (ng ± S.D. n = 10)	mean % composition (± S.D. n = 10)	Evidence
d	3-hexanone	213 ± 75	3.8 ± 1.0	$NaBH_4$+, GC
e	3-pentanol	10 ± 4	0.2 ± 0.1	$NaBH_4$-, GC
f	3-heptanone	96 ± 30	1.5 ± 0.4	$NaBH_4$+, GC
g	3-hexanol	210 ± 73	3.7 ± 1.1	$NaBH_4$-, GC
h	3-octanone	3630 ± 1049	62.7 ± 6.5	$NaBH_4$+, GC, MS
i	3-heptanol	212 ± 122	3.9 ± 2.1	$NaBH_4$-, GC
j	6-methyl-3-octanone	131 ± 60	2.4 ± 1.2	
k	3-nonanone	71 ± 35	1.2 ± 0.4	$NaBH_4$+, GC
l	3-octanol	902 ± 357	16.0 ± 6.2	$NaBH_4$-, GC, MS
m	6-methyl-3-octanol	5 ± 1	0.1 ± 0.1	$NaBH_4$-, GC
n	3-decanone	222 ± 92	3.8 ± 1.1	$NaBH_4$+, GC, MS
o	3-nonanol	14 ± 5	0.2 ± 0.1	$NaBH_4$-, GC
q	unknown	10 ± 4	0.1 ± 0.1	
r	unknown	17 ± 8	0.2 ± 0.2	
s	3-decanol	15 ± 5	0.2 ± 0.2	$NaBH_4$-, GC

Explanation of the abbreviations in the 'Evidence' column are given in the sub-script to Table 30.

FIGURE 34

GC profiles of the mandibular gland volatiles analysed on 15% Peg 20M (letters refer to Tables 31,32 and 39)

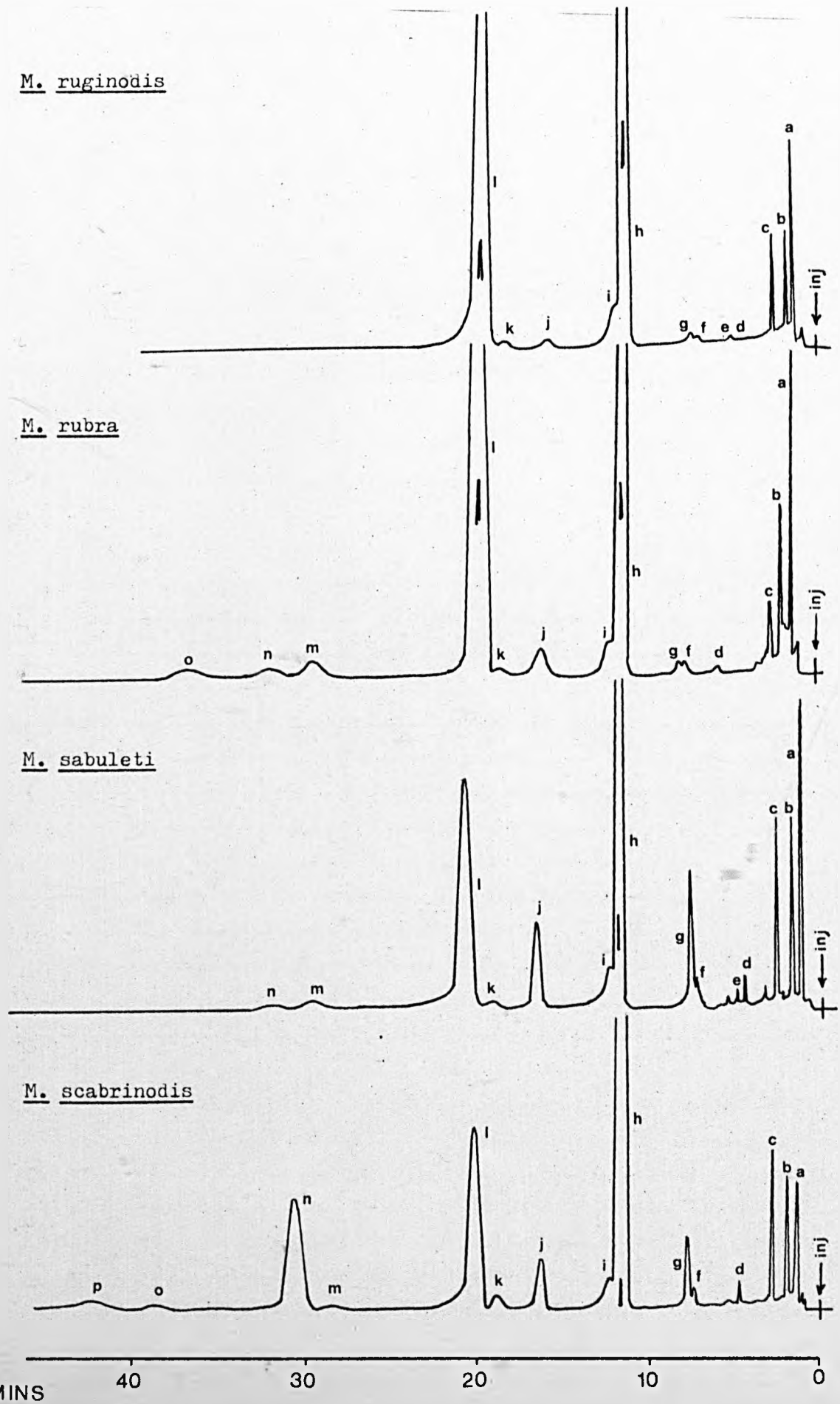


FIGURE 35

GC profiles of the mandibular gland volatiles analysed on 15% Peg 20M (letters refer to Tables 33-35 and 39)

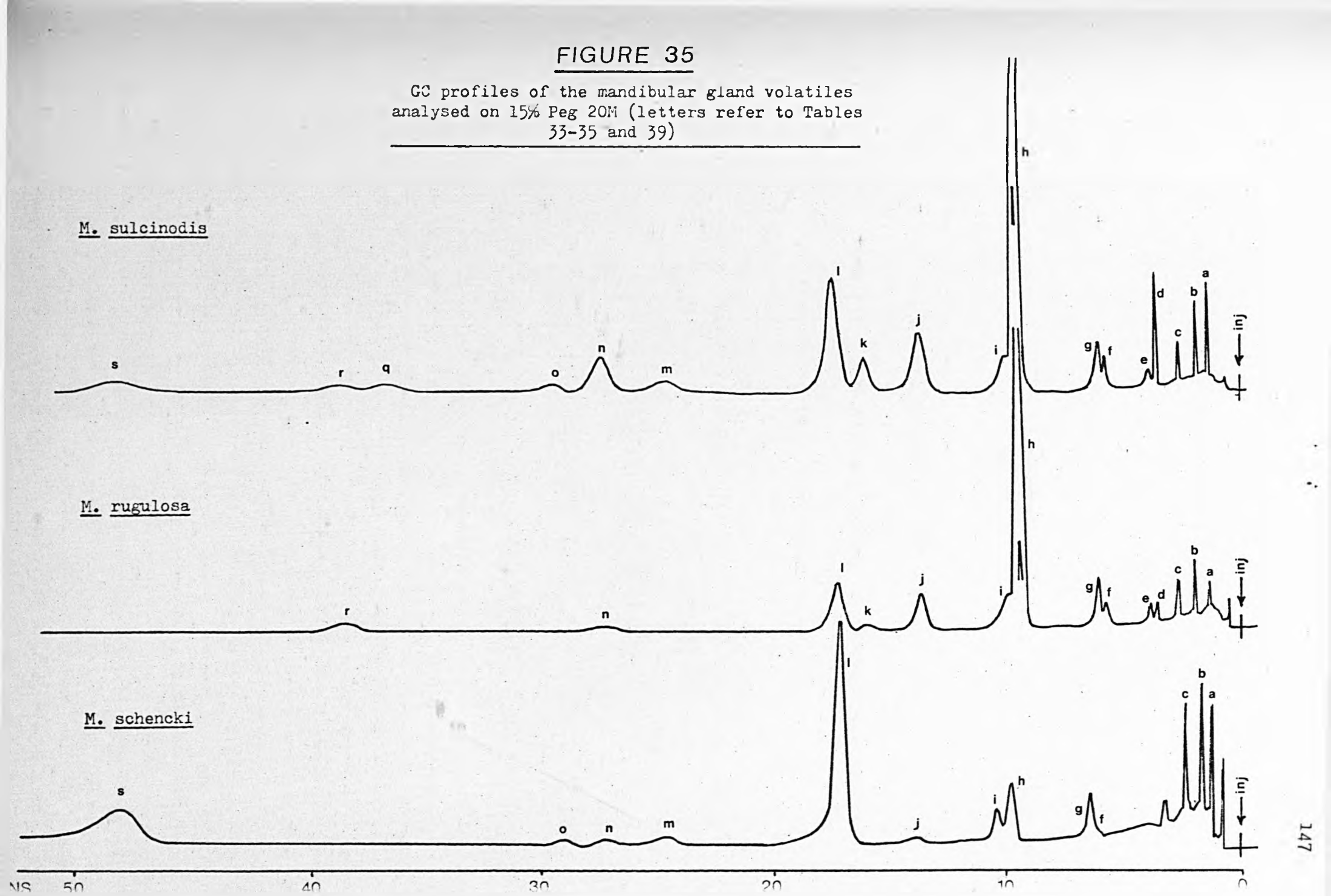


TABLE 36

Summary of % compositions of the mandibular gland secretions of eight Myrmica species.

Compound	Species							
	M. rubra*	M. scabrinodis*	M. sabuleti	M. ruginodis	M. sulcinodis	M. schencki	M. rugulosa	M. lobicornis
	% compositions (mean of ten replicates)							
3-Pentanol	-	-	0.4	0.1	0.2	-	1.1	-
3-Hexanone	0.4	1.1	1.0	0.2	3.8	-	1.5	1.3
3-Hexanol	0.6	5.0	3.4	0.4	3.7	2.9	4.4	5.9
3-Heptanone	0.5	1.4	1.2	0.2	1.5	0.3	1.7	1.2
3-Heptanol	0.9	1.2	4.2	1.8	3.9	3.9	4.2	4.0
3-Octanone	51.1	49.0	61.0	41.3	62.7	5.9	53.6	40.9
3-Octanol	34.8	17.8	18.1	51.9	16.0	46.4	16.3	30.2
6-Methyl-3-octanone	1.3	7.1	5.7	1.1	2.4	1.4	8.5	1.4
6-Methyl-3-octanol	-	0.04	1.1	1.4	0.1	1.8	-	0.3
3-Nonanone	1.1	1.2	0.9	1.5	1.2	-	2.0	0.4
3-Nonanol	-	0.01	-	-	0.2	1.3	-	1.0
3-Decanone	1.4	12.4	0.5	-	3.8	0.5	4.3	0.3
3-Decanol	-	0.01	-	-	-	32.7	-	13.1
3-Undecanone	0.4	2.0	-	-	-	-	-	-
Octanol:Octanone ratio	1:0.68	1:0.36	1:0.29	1:1.26	1:0.26	1:8.37	1:0.30	1:0.74

* Quantified by M.R. INWOOD.

TABLE 37

Summary of absolute amounts of mandibular gland volatiles of eight Myrmica species.

Compound	Species							
	M. rubra*	M. scabrinodis*	M. sabuleti	M. ruginodis	M. sulcinodis	M. schencki	M. rugulosa	M. lobicornis
	Amounts (ng, mean of ten replicates)							
3-Pentanol	-	-	5	1	10	-	8	-
3-Hexanone	12	12	13	2	213	-	10	117
3-Hexanol	19	336	45	4	210	21	32	566
3-Heptanone	16	97	16	2	96	2	12	114
3-Heptanol	28	83	56	18	215	28	31	374
3-Octanone	1590	3300	808	410	3630	42	394	4023
3-Octanol	1080	1200	240	516	902	335	119	2913
6-Methyl-3-octanone	40	472	75	11	131	10	63	161
6-Methyl-3-octanol	-	3	4	-	5	20	-	31
3-Nonanone	35	83	12	7	71	-	14	42
3-Nonanol	-	1	-	-	14	9	-	102
3-Decanone	43	832	7	-	222	3	31	32
3-Decanol	-	1	-	-	-	236	-	1275
3-Undecanone	11	136	-	-	-	-	-	-
TOTALS	2939	6616	1281	971	5725	709	717	9759

* Quantified by M.R. INWOOD

TABLE 38

Comparison of retention times of the C_2-C_4 mandibular gland volatiles with those of standards on Porapak Q and Chromosorb 102 phases.

	GC retention times (mins)			
	Chromosorb 102 (140°C)		Porapak Q (166°C)	
Compound	standard	insect	standard	insect
ethanal	1.3	1.3	2.0	2.0
acetone	4.1	4.1	5.3	5.3
methylpropanal	8.2	8.2	10.1	10.1

was in fact methylpropanal (isobutyraldehyde). The volatile portions of M. rubra and M. scabrinodis have been redetermined; the quantitative data is presented for these two species together with that of three further Myrmica species: M. ruginodis, M. sabuleti and M. lobicornis (Table 39). Their GC profiles obtained through the solid injection of single worker's heads on the column of Porapak Q are shown in Figure 36. Similar profiles were obtained for the remaining three species but these have not been quantified in detail.

The results of these investigations and those reported previously (CREWE and BLUM, 1970; MORGAN et al., 1978) show that the mandibular gland secretions of Myrmica ants are composed of very similar mixtures of relatively simple oxygenated substances. 3-Alkanones and 3-alkanols comprise the major portion of the gland contents. With the exception of M. schencki, 3-octanone and 3-octanol are by far the most abundant components. In M. schencki the most abundant components are 3-octanol (46%) and 3-decanol (32.7%), 3-octanone is the third most abundant constituent (5.9%).

The absolute quantities of each component and the percentage compositions of the secretions were calculated from ten replicate analyses for each species. The standard deviations calculated for the absolute quantity of each component reveal appreciable variation in the amount of material present from one worker to another. The standard deviations in the percentages were smaller than the deviations in the absolute quantities in most cases, particularly with respect to the major components, indicating significantly less variation in the overall composition or blend of the components.

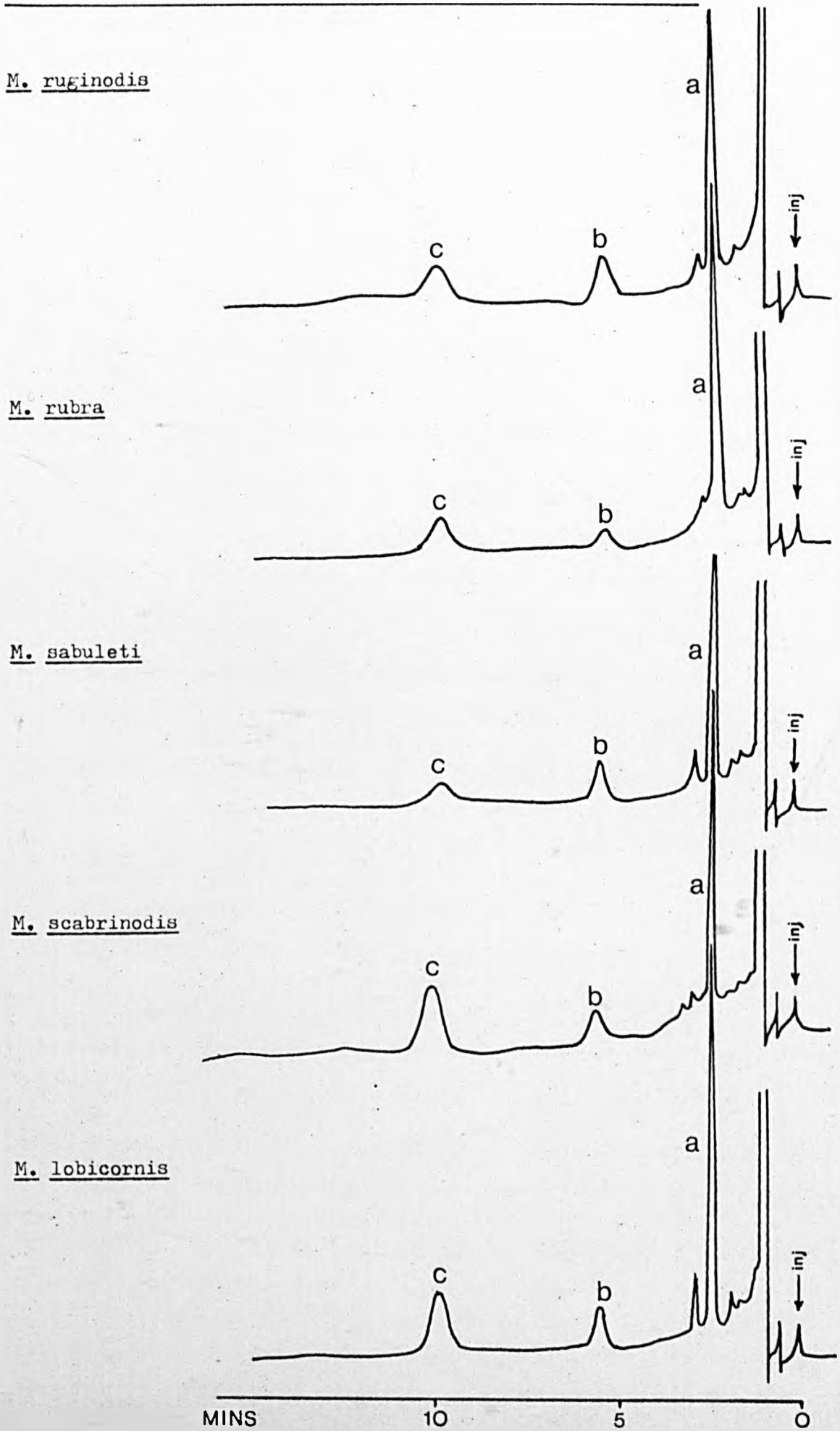
TABLE 39

Comparison between the highly volatile components of the mandibular gland secretion of *M. rubra*, *M. scabrinodis*, *M. sabuleti*, *M. ruginodis* and *M. lobicornis*.

Species	ng/ant (mean ± S.D.) (a) ethanal	(b) acetone	(c) methylpropanal
M. ruginodis	182 ± 143	13 ± 6	29 ± 12
M. rubra	73 ± 29	9 ± 7	20 ± 31
M. sabuleti	149 ± 67	8 ± 4	33 ± 10
M. scabrinodis	127 ± 55	15 ± 9	20 ± 18
M. lobicornis	128 ± 42	13 ± 4	14 ± 2

FIGURE 36

GC profiles of the highly volatile portions of the mandibular gland secretion analysed on Porapak Q (letters refer to TABLE 39)



CAMMAERTS-TRICOT (1974) found in examining the contentsof the mandibular glands of M. rubra that the amount of material varied with the age of the worker, rising to a maximum and falling in very old workers. In these investigations accurate assessment of the ages of the workers have not been made, however, where possible, older workers foraging outside the nest have been used for all these measurements.

Table 36 shows that although all the species produce very similar substances, the composition of the workers' mandibular gland secretion is characteristic of a particular species. CREWE and BLUM (1970) claimed to show a constant ratio of 3-octanol to 3-octanone which varied with species. The ratios of the amounts of 3-octanol to 3-octanone for the species examined during the course of this work are given in Table 36. On the basis of these results it is not possible to distingiush between all eight species from their 3-octanol to 3-octanone ratio alone. However this possibility should not be totally ruled out until further work has been performed on more colonies of each of the various species preferrably originating from a variety of geographical localities.

All the species examined here produce, in addition to the C_5 to C_{10} 3-alkanones and 3-alkanols, nanogram amounts of ethanal, acetone and methylpropanal. The identification of methylpropanal has required that earlier descriptions be corrected (MORGAN et al., 1978; CAMMAERTS et al., 1978). No particular trend or difference between the species was observed. Accurate quantitative determinations have been performed on these components in only five of the eight species under investigation namely: M. rubra, M. scabrinodis, M. ruginodis,

M. sabuleti and M. lobicornis (Table 39).

It is interesting that the very volatile portion of the mandibular, Dufour and poison glands are all quite different and readily recognisable when chromatographed on Poropak Q or Chromosorb Century phases (Figure 37). In other words, from a GC analysis of the very volatile part, the glandular source can be deduced, but not the species. This is in agreement with previous observations that freshly isolated mandibular, Dufour and poison glands display different ethological activities (CAMMAERTS-TRICOT, 1973).

Thorough ethological investigations have so far been carried out by Mme Cammaerts at the Universite de Bruxelles, Belgium on the mandibular glands of seven of the previously mentioned eight species. The work on the eighth, M. sulcinodis, is still in progress. In these investigations the effects of whole crushed heads, synthetic samples of the glandular components (presented both singly and in combination in the glandular proportions and concentrations) and control solvent (liquid paraffin) are quantified and compared in terms of their attractive properties (O), effects on linear speed (V) and sinuosity of movement (S). The responses of the seven Myrmica species to their own and each others mandibular gland secretions are in the majority of cases very similar (Table 40). In general terms the mandibular gland secretions of all these species attracts the workers to the source of the emission and causes them to run faster and less sinuosly. This activity has been attributed to a small number of compounds for most of the species. 3-Octanone and 3-octanol, the two most abundent components are also the principle active constituents, although in certain cases other components do

FIGURE 37

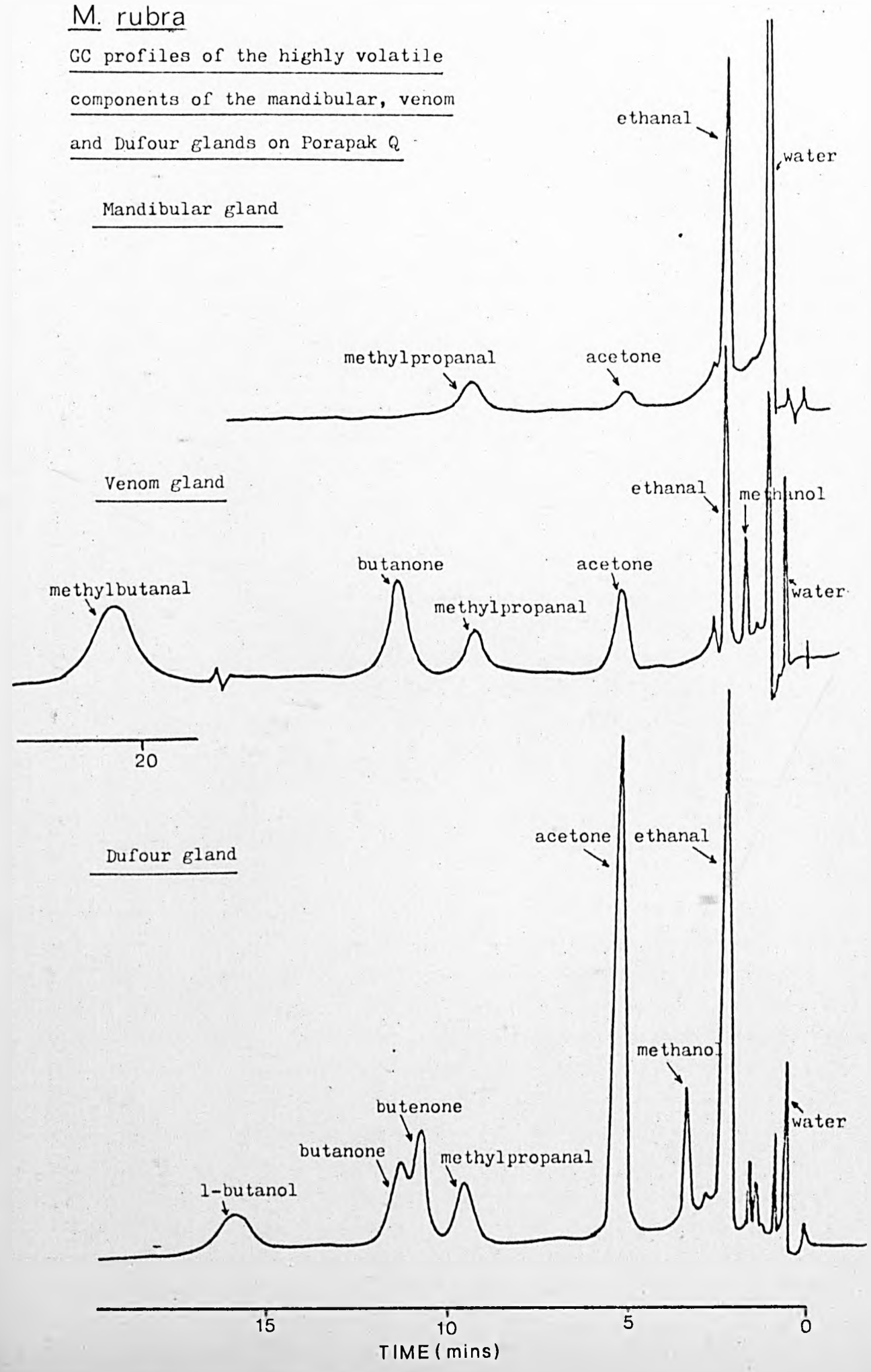

M. rubra
GC profiles of the highly volatile components of the mandibular, venom and Dufour glands on Porapak Q
Mandibular gland
ethanal
water
methylpropanal
acetone
Venom gland
ethanal
methanol
water
methylbutanal
butanone
methylpropanal
acetone
20
Dufour gland
acetone
ethanal
methanol
water
butenone
butanone
methylpropanal
1-butanol
15
10
5
0
TIME (mins)

TABLE 40

Locomotion reactions of seven Myrmica species to one anothers mandibular glands (experiments performed by M.C. CAMMAERTS).
The glands were presented on filter paper (Whatman No.1, 1 cm²) on the foraging area of the colonies. Untreated squares of paper were used for control experiments. For each test species and each test glands the orientation of 30 workers to the paper, the linear and the angular speed of 20 ants reaching the paper were quantified (CAMMAERTS, TRICOT, 1973). Each distribution was characterized by its median. Note that for orientation, the lower the value of the variable, the better is hte orientation towards the test source.

Orientation, O (angular degrees)

Of the seven following species	to control paper	to isolated mandibular glands from M. rubra	M. ruginodis	M. scabrinodis	M. sabuleti	M. rugulosa	M. schencki	M. lobicornis
M. rubra	93	50	52	52	53	48	78	*
M. ruginodis	100	57	49	48	47	54	80	*
M. scabrinodis	100	84	72	49	50	50	53	*
M. sabuleti	95	60	57	46	50	45	59	*
M. rugulosa	92	54	53	60	63	54	72	*
M. schencki	93	56	55	58	83	71	44	*
M. lobicornis	95	54	55	43	44	54	67	47

Linear speed, $\bar{V}$ (mm/sec)

Of the seven following species	near control paper	near isolated mandibular glands from M. rubra	M. ruginodis	M. scabrinodis	M. sabuleti	M. rugulosa	M. schencki	M. lobicornis
M. rubra	13.0	20.0	24.5	23.6	22.0	22.5	24.0	*
M. ruginodis	12.4	28.2	17.2	23.0	17.4	19.7	21.4	*
M. scabrinodis	9.4	17.7	17.7	16.0	20.0	16.3	20.0	*
M. sabuleti	12.0	27.2	21.0	25.5	21.4	21.2	20.8	*
M. rugulosa	12.4	21.2	24.0	22.0	21.7	24.0	22.8	*
M. schencki	11.3	24.0	19.8	22.0	15.0	15.4	19.0	*
M. lobicornis	11.9	20.0	28.0	28.0	27.0	28.6	18.0	36.0

Angular speed, $\bar{S}$ (angular degrees/cm)

Of the seven following species	near control paper	near isolated mandibular glands from M. rubra	M. ruginodis	M. scabrinodis	M. sabuleti	M. rugulosa	M. schencki	M. lobicornis
M. rubra	91	110	132	116	134	138	118	*
M. ruginodis	115	115	120	124	115	136	129	*
M. scabrinodis	168	139	137	115	145	164	140	*
M. sabuleti	105	118	118	119	118	126	135	*
M. rugulosa	145	120	123	125	137	110	125	*
M. schencki	214	133	144	184	172	165	135	*
M. lobicornis	160	128	124	115	120	126	167	95

contribute to the overall activity. Table 41 summarises the results of Mme Cammaerts' ethological investigations using synthetic samples.

CAMMAERTS et al. (1978) were earlier able to rationalise the responses of M. rubra and M. scabrinodis to one anothers mandibular gland secretions in terms of their chemical compositions. These additional observations (Table 40) can be explained to some extent in a similar manner. For example M. rubra, M. ruginodis, M. lobicornis and M. rugulosa are readily attracted to a source of 3-octanone, therefore their weak attraction to an isolated mandibular gland of M. schencki can be attributed to the low level of this compound in the glands of this particular species. The weak response of M. scabrinodis workers to the mandibular gland secretions of M. rubra and M. ruginodis is due to the lack of 3-nonanone and 3-decanone in the secretions of these two species, which in combination with 3-octanone serve to attract M. scabrinodis workers. Further inspection of Table 40 reveals that workers of M. schencki exhibit weak attractive behaviour and little change in linear speed when encountering mandibular glands of M. sabuleti and M. rugulosa. This probably results from the low level of 3-octanol (the major active constituent of M. schencki) present in their secretions (Table 37).

These studies have revealed a trend for the ethological activities of 3-octanone and 3-octanol (which is especially pronounced with respect to their attractive properties) in which the contribution by 3-octanone to the overall activity of the mandibular secretion steadily decreases from M. ruginodis where this compound is the major attractive constituent, to M. scabrinodis, where it has only a synergistic effect, while the role of 3-octanol increases from

TABLE 41

Summary of the effects of substances found in Myrmica mandibular glands on orientation, speed and angular velocity (sinuosity). The substances show either a statistically significant activity along, +, or other substances are active, a, or other substances act as synergists, b.

Species	Orientation (O)		Velocity (V)		Sinuosity (S)		
	octanone	octanol	octanone	octanol	octanone	octanol	other active substances
M. ruginodis	+b	-	+	+	-	-	ethanol (O,b)
M. rubra	+b	-	+	-	+	-	
M. rugulosa	+	-	+	+	-b	-b	6-methyl-3-octanone (S,b)
M. lobicornis	+	-	+a	+a	-b	-b	3-decanol (V,a;S,b)
M. sabuleti	+a	+	+	+	-	-	
M. schencki	-b	+b	+	+b	-b	-b	
M. scabrinodis	-b	+a,b	+a,b	-a,b	-	-	3-hexanone (V,a) 3-nonanone (O,b;V,a) 3-decanone (O,b;V,b)

Explanation of symbols: ethanal (O,b) means ethanal acts synergistically to produce a statistically significant effect on the workers orientation.

having only a moderating effect (in *M. ruginodis*) to being the only true attractant among the mandibular gland components of *M. scabrinodis*.

OVERVIEW

In reporting the results of these chemical and ethological investigations of myrmicine ants it has become apparant that further work is required in a number of areas. In addition to describing a number of these in more detail the following section is intended to draw attention to some of the more general aspects of ant pheromones, with examples drawn from these investigations.

This project, and the complementary studies performed by Mme Cammaerts, have provided several interesting examples of the characteristic ways in which exocrine secretions and pheromones have evolved to fulfill their particular roles. For example, when an M. rubra worker encounters a prey or disturbance it opens its mandibles wide and releases a portion of the contents of its mandibular glands. The secretion forms a film across the mandibles from which evaporation occurs. Diffusion of the volatile chemicals results in the formation of a steadily expanding, approximately hemispherical, cloud of odour which is intended to trigger 'alarm' behaviour in fellow workers moving into the 'active space' (that is: the space within which the pheromone concentration is equal to or greater than the threshold concentration). Workers are attracted to the source of the emission from upto 5cm away (i.e. approximately ten body lengths) with attraction reaching its maximum in two minutes.

This type of alarm message is said to be "localised in time and space" (WILSON, 1971). If the disturbance continues, further emissions of the alarm pheromone are made to sustain the alarm behaviour. In order that the response threshold concentration can be

maintained the ants must store relatively large quantities of these alarm substances. Workers of M. rubra contain on average 1.6 micrograms of 3-octanone, their principal alarm component. Through evolution Nature has arranged that the alarm pheromone is sufficently highly volatile that it quickly disperses once the disturbance has passed.

In marked contrast M. rubra workers contain on average only 5.8 nanograms of their trail substance, 3-ethyl-2,5-dimethylpyrazine. This is a slightly less volatile substance which is emitted in much smaller quantities than the alarm substances. It is applied directly to the substratum via the sting lance. The slow evaporation of this trail substance ensures that a discrete tunnel of scent develops; owing to the highly localised nature of the trail, trail following is only evoked in those workers actually crossing the trail, although other more volatile components of the Myrmica venom act as short range attractants to recruit workers to a freshly laid trail. Clearly the trail pheromone must be rather more persistant than the alarm pheromone to ensure that an 'active' trail is maintained long enough for the workers to locate and reinforce, however the trails must not be so persistant that they remain for too long once the food souce has been exhausted.

M. rubra workers produce approximately three hundred times more alarm pheromone (1.6µg per worker) than trail phermone (5.8ng per worker), the reason for this relates to the contrasting modes of dispersal which have evolved (described above).

Pheromone systems possessing many of the features described above are common amongst the Formicidae and WILSON (1971) has

discussed the effects of such pheromones in terms of their "Q/K ratio" i.e. the ratio of the number of pheromone molecules released to the response threshold concentration (in molecules per cubic centimetre).

Thus by careful design Nature ensures that pheromones intended to fulfil a particular communicative role are composed of substances having optimum volatility; furthermore the components are produced, stored and secreted in the quantities required for them to produce the necessary response. WILSON (1971) pointed in relation to the above, that if the Q/K ratios for alarm pheromones were the same as the values calculated for trail pheromones, then the alarm message would only travel a few millimetres and fade out in seconds. As a result the signal would not travel beyond the distance within which other ants could perceive the disturbance directly. Conversely, a trail pheromone having the same Q/K ratio as an alarm substance would form a very broad rapidly fading trail which would be impossible for workers to follow over any appreciable distance.

The work performed by RILEY et al. (1974b) on the principle alarm component of A. cephalotes and A. texana emphasised the importance of stereochemistry in pheromone perception. They found that the 4-methyl-3-octanone produced by these two Attines was biosynthesised stereospecifically. Furthermore the workers were able to distinguish between the S(+) and R(-) forms; the S(+) form was about four hundred times more active than the R(-) form in this example.

As a number of the alarm components of the Myrmica ants possess

chiral centres, it would be interesting to determine whether the stereospecificity observed for the Attines is also appparent in the Myrmica species. Methods are available for determining enantiomeric compositions by GC e.g. mixtures of enantiomers of chiral alcohols can be separated by GC after forming diastereomeric esters of them with N-trifluroacetyl-L-alanine (FRANKE and KRUSE, 1979) or chrysanthemic acid (BROOKS et al., 1973).

The identification of 3-ethyl-2,5-dimethylpyrazine from the venom of M. rubra is the first characterisation of a trail pheromone from a non-tropical ant. As with the mandibular gland alarm pheromone it has been possible to exactly reproduce the trail activity of a single M. rubra venom gland with the synthetic pheromone at the glandular level. There is therefore no reason to suspect the presence of further trail pheromone components in the venom of M. rubra.

The synthetic trail pheromone also satisfies another criterion by which 'true' trail pheromones are sometimes judged: it was capable of inducing a mass exodus from nests of M. rubra, M. scabrinodis and M, ruginodis (the only Myrmica species tested to date) when 1ng of the synthetic material was placed at the nest entrance on a filter paper disc.

The solid sampling techniques employed in the analysis of the Myrmica trail pheromone have also been used to determine the absolute amounts of the major trail pheromone components of three Attine species (including two sub-species) for the first time. TUMLINSON et al. (1972) and RILEY et al. (1974a) in identifying methyl

4-methylpyrrole-2-carboxylate as the major trail component of A. texana and A. cephalotes noted the presence of several other trail active fractions of gaster extracts. Perhaps the techniques employed here could be used to investigate these unidentified components.

Although the alarm and trail pheromones of the above Attine ants have been thoroughly investigated, considerably less is understood regarding the functions of their Dufour gland secretions. The first chemical investigations of Attine Dufour glands have been reported here, and they show that in common with other myrmicine ants the secretion consists mainly of hydrocarbons. Still more work is required to establish the role of these hydrocarbons; it is quite possible that they possess pheromonal properties as in other myrmicines. The Dufour gland secretion of several of the Myrmica species has been shown to contain a volatile fraction which elicits a short lived attraction; while the less volatile part of the secretion acts as a territorial marking pheromone in foraging (CAMMAERTS et al., 1978). Although the substances responsible for the attractive properties of the Dufour gland secretion have been identified, the components involved in territorial marking have not. It would seem likely that the hydrocarbons are responsible for this, however until synthetic samples have been rigorously tested by bioassay this should not been presumed, as this marking effect may be produced by unidentified minor component(s). Although in certain species of ant the Dufour gland secretion has been found to elicit trail following, in M. rubra this is not the case. It has been shown that the trail activity of the venom is in fact supressed when presented with the

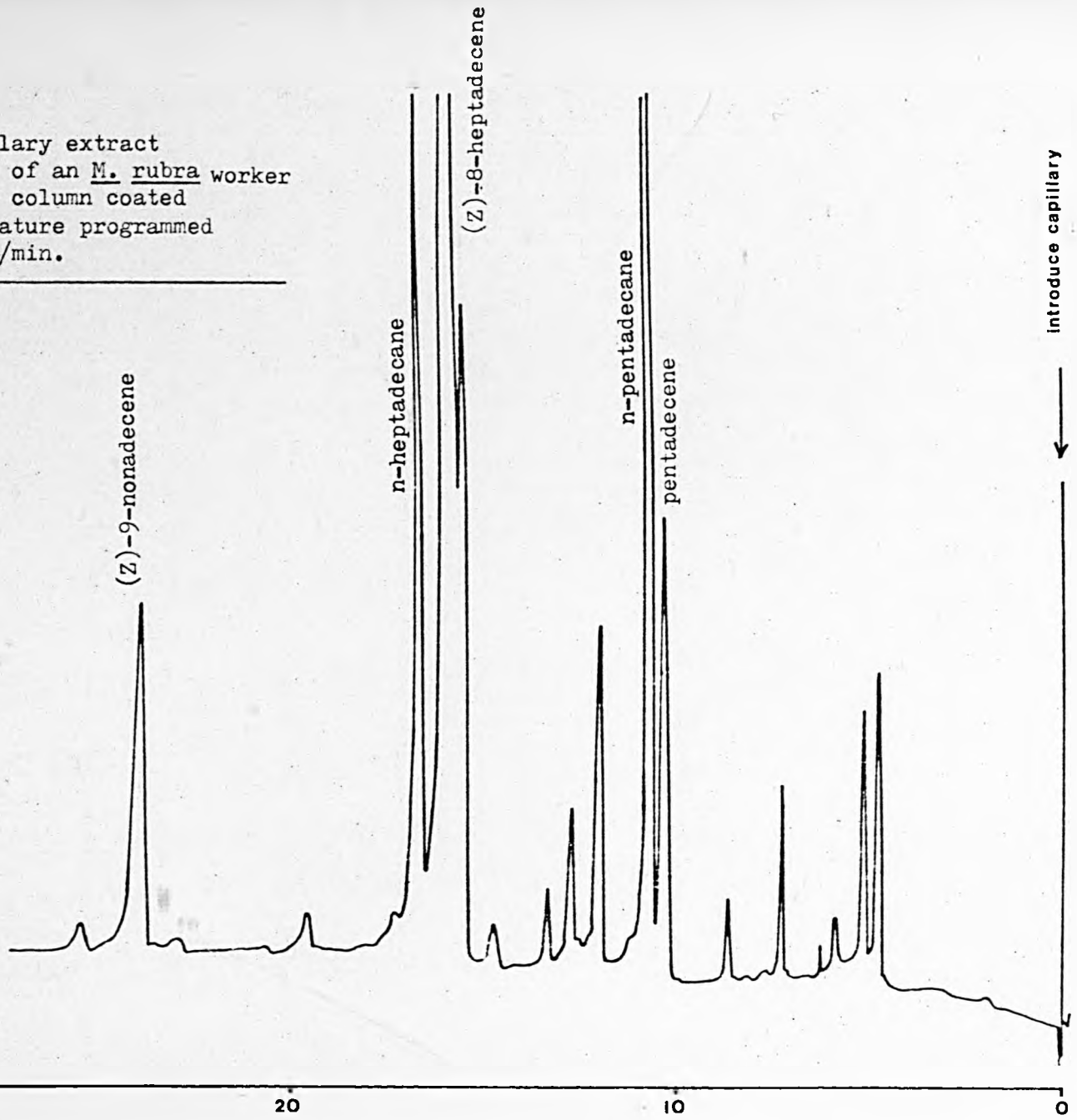
Dufour gland secretion (Table 16). This result does not however eliminate the possibility of a positive interaction between the two exudates in other species.

In recent years considerable effort has been focused on characterising exocrine gland constituents of ants assisted by rapid developments in separation and spectroscopic techniques. In spite of this progress there are few reports of investigations of the biosynthetic pathways by which their pheromones and other exocrine components arise. Further work is therefore required in this area to establish these pathways more fully. It may be possible to control pest species by interfering with their communicative processes through a disruption of their pheromone production. Only when the details of their biosynthesis are known can this approach be seriously contemplated.

During the course of this work attempts were made to adapt the packed column GC solid sampling techniques to use with capillary columns in order to take advantage of their superior sensitivity and separating properties. Although capillary extracts (MORGAN and TYLER, 1977) were analysed effectively (Figure 38) by employing the capillary sampler (Figure 42) this technique is of little use where the glands are inaccessible or where there is too little liquid to withdraw using a fine capillary. Ideally it would have been convenient to employ the existing packed column solid sampler (Figure 41) with a capillary system incorporating a wider bore pre-column to accommodate the sampler. It appears however from a number of trials that were performed that the dead volume of this type of system is

FIGURE 41

Analysis of a capillary extract of the Dufour gland of an M. rubra worker on a WCOT capillary column coated with OV-101, temperature programmed from 165-198°C @ 2°/min.



far too great to enable sufficiently efficient separations to be performed in the splitless mode. Clearly it is advantageous to avoid split injection in order that the quantitative aspect of this form of GC analysis be retained. Further work is therefore required to develop an efficient solid sampling system for use with capillary columns preferrably in the splitless mode.

EXPERIMENTAL

EXPERIMENTAL

SECTION I : GENERAL PROCEDURES

1. Sources of ant colonies

a. Attine ants

The species used in this work, and their origins are listed in Table 42. Live colonies were transported by air in boxes with a portion of their fungus garden and, as quickly as possible, established in the laboratory. The colonies were made available through the help of Rothamsted Experimental Station and Dr. Cherrett of University College of North Wales, Bangor.

b. *Myrmica* ants

The species and their origins are given in Table 43. In general the ants were identified in the field, then a large piece of soil containing the nest was transported to the laboratory where the ants were separated from the soil and transferred to an artificial nest. Most of the colonies used were collected locally, some were kindly supplied from other parts of England, and some through the cooperation of Mme. Cammaerts (Belgium) and Dr. Nielsen (Denmark).

2. Maintenance of ant colonies

a. Attine ants

The live colonies of *A. cephalotes* and *A. octospinosus* were maintained in the laboratory in 22.0cm x 16.5cm x 4.0cm wooden nest boxes with sliding glass tops (Figure 39). Four 1.5cm diameter holes in the two longest sides of the nest box provided ventilation and exits or entrances for the foraging workers. These nest boxes were placed on small wooden tables, the legs of which were immersed in

TABLE 42

Countries of collection of the Attine species

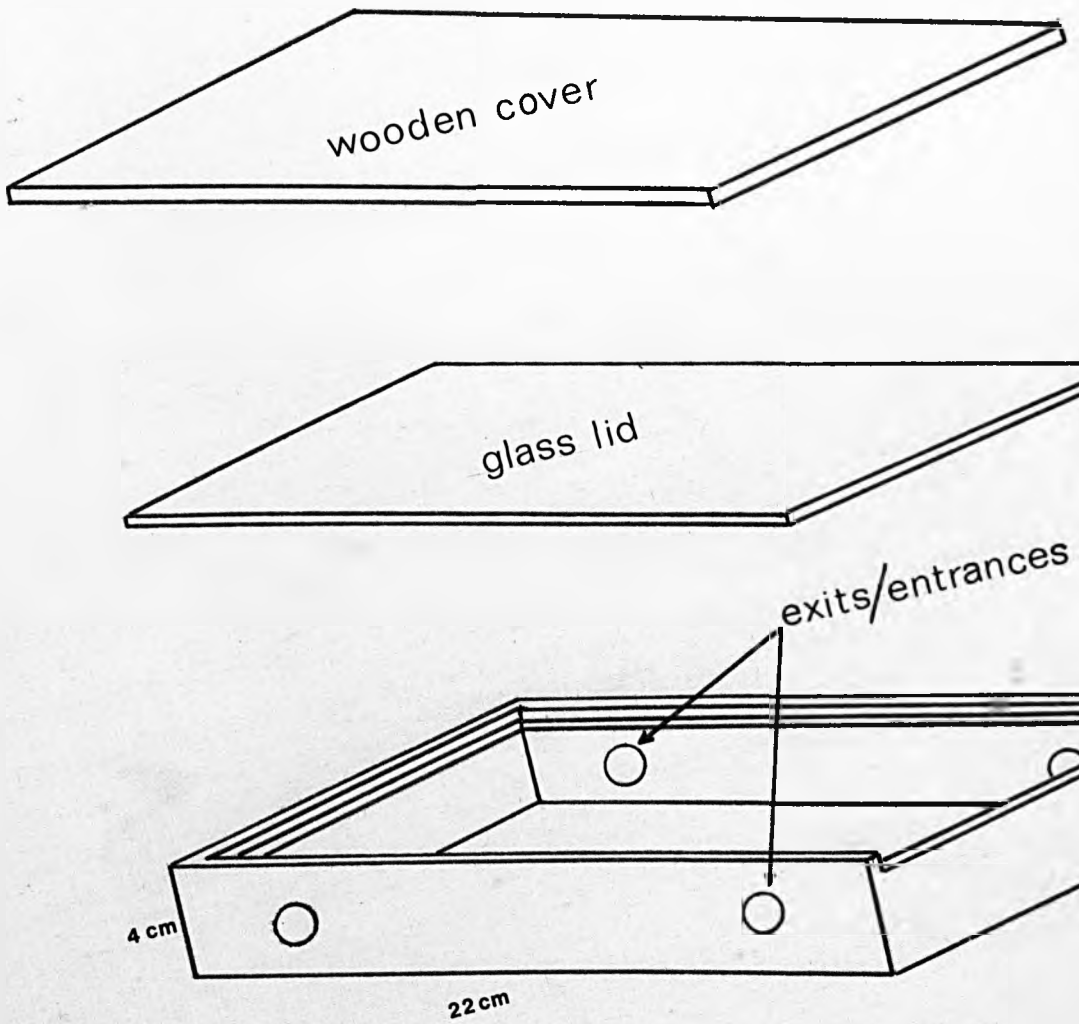
Species	Region
Atta cephalotes	Trinidad and S. America
Acromyrmex octospinosus	Trinidad
Atta sexdens sexdens	Guyana
Atta sexdens rubropilosa	Paraguay

TABLE 43

Regions of collection of the Myrmica species.

Species	Region
Myrmica rubra	Chesterton, N. Staffordshire.
Myrmica scabrinodis	Chesterton, N. Staffordshire.
Myrmica sabuleti	Dion, Belgium.
Myrmica sulcinodis	Furzbrook, Dorset.
Myrmica ruginodis	Keele and Leek, N. Staffordshire.
Myrmica schencki	S. Lincolnshire, England and Jutland, Denmark.
Myrmica rugulosa	Belgium.
Myrmica lobicornis	Heptonstall, W. Yorkshire.

FIGURE 39 Attine nest box



cups of oil in order to prevent the ants escaping. The nests were maintained at 26°C-28°C and at 85-95% relative humidity and supplied with a variety of plant material for their fungus cultivation. The temperature was very carefully monitored as it was found that at 33°C -35°C the ants began to 'dump' the fungus very rapidly. Allowing this to continue resulted in the colony eventually perishing.

b. Maintenance of Myrmica ants

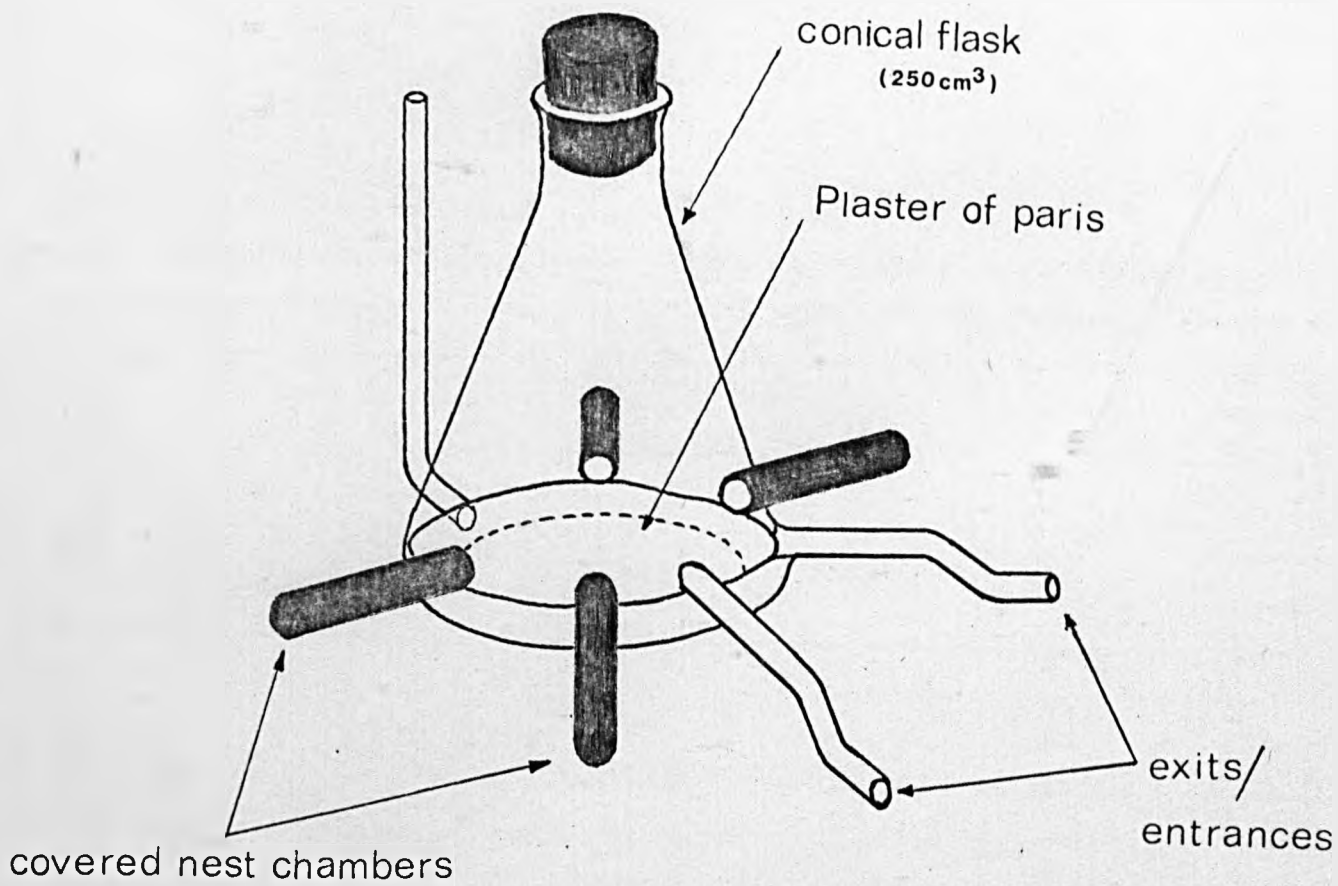
The Myrmica ants were maintained in the laboratory at room temperature in artificial nests constructed from 250cm^3 conical flasks partially filled with plaster of paris (BDH calcium sulphate, technical grade) (Figure 4O; WADHAMS, 1972). These nests were usually contained in plastic washing up bowls with their vertical inner walls coated in polytetrafluoroethylene (PTFE, ICI 'fluon') to prevent the ants escaping. A 60cm x 35cm x 7.5cm aluminium tray, again with the vertical inner walls coated with in PTFE provided a larger foraging enclosure for colonies used in bioassay experiments.

The ants were fed on a diet of mealworm larvae (Tenebrio molitor), desert locusts (Schistocerca gregaria) and a 10% w/w aqueous sugar solution dispensed from 5cm^3 specimen tubes with cotton wool wicks.

3. Identification

The species M. rubra, M. scabrinodis, and M. ruginodis collected by R.P.E. were identified in the laboratory by microscopic examination of the workers' antennal scapes, epinotal spines and petiole (COLLINGWOOD, 1964). M. schencki and M. lobicornis were identified by C.A.Collingwood, Ministry of Agriculture, Lawnswood,

FIGURE 40 Myrmica nest



Leeds; M. sulcinodis was identified by workers at Furzbrook Experimental Station, Dorset; M. rugulosa and M. sabuleti were characterised by M.C. Cammaerts, Universite Libre de Bruxelles, Belgium. The identifications of the Attines were made in the field, chiefly by Dr. Cherrett, Bangor.

4. Gas liquid chromatography (GLC)

a. Instrumentation

Throughout this work the analyses were performed on Pye 104 series gas chromatographs (GC), fitted with flame ionisation detectors (FID). The results were recorded on a Servoscribe 1s potentiometric recorder (Smiths Inds. England).

b. Columns

The columns used in these investigations are listed in Table 44 together with their specifications. All these columns were prepared in the laboratory.

c. Preparation of column packings and columns

(i) Preparation of conventional column packings

The solid support material and the liquid phase were weighed out in the required proportions, the quantity of material required to fill the column depended upon the column length, internal diameter and the mesh range of the support (approx. 10g/m). The liquid phase was dissolved in the solvent recommended by the manufacturers in a $500cm^3$ ribbed rotivapour flask. Sufficient solvent was used to cover the solid support, which was slowly added to the solution through a filter funnel to form a slurry. The flask and slurry were then transferred to a rotary evaporator and the solvent removed slowly

TABLE 44

Specifications of the separation columns employed in the GC analyses.

Column	liquid phase	% loading	support	mesh range	support treatment	column dimensions length (m)	i.d. (mm)
A	OV-101	3	Chromosorb W	100-120	AW-HMDS	1.5	4
B	OV-101	5	Chromosorb W	100-120	AW-HMDS	1.5	4
C	PEGA	20	Chromosorb P	80-100	AW	2.75	4
D	PEGA	10	Diatomite M	100-120	AW-HMDS	2.75	4
E	PEG 20M	15	Chromosorb P	80-100	AW	2.75	4
F	PEG 20M	10	Chromosorb W	100-120	AW-HMDS	2.75	4
G	DEGS	5	Supersorb	100-120	AW-HMDS	2.75	4
H	Apiezon	5	Phasesep	100-120	AW-HMDS	2.75	5
I	Porapak	-	-	120-150	-	1.5	4
J	Chromosorb-102	-	-	100-120	-	1.5	4
K	Carbowax 20M	bonded*	Chromosorb W	100-120	AW	1.5	4
L	OV-101	WCOT	Capillary	3090 PLATES m^{-1}	-	30	0.5
M	SE-30	5	Chromosorb W	100-120	AW-HMDS	1.5	4

* See experimental section I.4.6. and AUE et al., 1973.

under vacuum. Hand turning of the flask was employed to ensure an even coating of the support with stationary phase and to avoid bumping and excessive movement of the fragile support which would have caused it to fragment. This would have resulted in a support with a wide mesh range and, consequently, poor chromatographic properties. Once dry, the freely flowing packing was transferred to a fluidised bed drier. A steady stream of nitrogen was passed through the packing for one hour whilst maintaining the bed at approximately 100°C, to complete the drying process and free the packing from any very fine particles.

Before filling the column, its inner surfaces were deactivated by washing with either a 10% solution of hexamethyldisilazane or 20% dimethyldichlorosilane in dry toluene. After emptying the columns of any residual liquid, they were heated in an oven at 120°C for one hour to complete the deactivation. Columns were then packed by applying a vacuum at one end through a column fitting; a silanized glass wool plug at this end of the column ensured the retention of the particulate packing. The packing was added through a small funnel with gentle tapping of the column to aid even packing and the flow of material. Once the column was full, the very minimum of vibration from a Pifco body massager was applied to it, ensuring a tight, even packing of the particles. A 6cm gap between the top of the column and a 1cm silanized glass wool plug, included to retain the packing, was left to accomodate the solid sampler.

All the columns were conditioned overnight (15hrs) at 10°C below the maximum isothermal operating temperature for the phase, using a nitrogen carrier gas flow rate of $50cm^3$/min or $60cm^3$/min. Columns

prepared in this way performed well giving no peak tailing.

(ii) Preparation of the bonded Carbowax 20M packing

The bonded Carbowax 20M packing was prepared by the technique of AUE et al. (1973). Diatomaceous earth support, Chromosorb W (50g) was thoroughly washed for one week with hydrochloric acid (36%) at boiling temperature in a glass thimble in a soxhlet apparatus. Holes in the lower end of the thimble allowed the constant percolation of acid, while a glass wool plug retained the support. This was continued until new portions of HCl remained free from a yellow colouration after several hours of extraction. After washing to neutrality and drying in an oven at 120°C, the support was coated with Carbowax 20M to 6% loading by rotary evaporation as described above. After separating from the fines with a stream of nitrogen in a fluidised bed drier, the packing (30g) was transferred to a 2.75m x 4mm i.d. glass GC column. This column was connected to the nitrogen carrier in a GC oven with a flow rate of 10ml/min. The temperature was then programmed from room temperature to 280°C at 10°C/min and maintained at that temperature overnight (15hrs). The packing was subsequently removed from the column, poured into a paper thimble and exhaustively extracted with methanol for one week in a soxhlet apparatus. The packing was then dried in an oven at 120°C and packed into a 1m x 4mm i.d. glass column and conditioned at a temperature of 250°C and a nitrogen carrier gas flow rate of 50ml/min.

The column prepared in this way gave symmetrical peaks and had very low bleed characteristics. Consequently it was particularly useful for temperature programmed analyses over a wide temperature range at high sensitivities and linked GC-MS.

(iii) Preparation of Porapak and Chromosorb 102 columns

Both Porapak Q and Chromasorb 102 are cross-linked polyaromatic resins. They are capable of separating volatile low molecular weight compounds on the basis of molecular weight and shape. The columns of these two phases were prepared simply by filling the deactivated glass column with the powdered polymer of the appropriate mesh range by the technique described in 4c(i). No coating with stationary phase was required. In this way 1.5m x 4mm i.d. columns of 120-150 mesh Porapak Q and 100-120 mesh Chromosorb 102 were prepared.

(iv) Capillary columns

These were prepared by Mr. T. Alston using a static coating technique as used in the laboratory of Prof. Verzele, at the University of Ghent.

5. Solid sampling

Solid sampling was employed in these analyses in order to avoid contaminating or losing the tiny amounts of material under investigation.

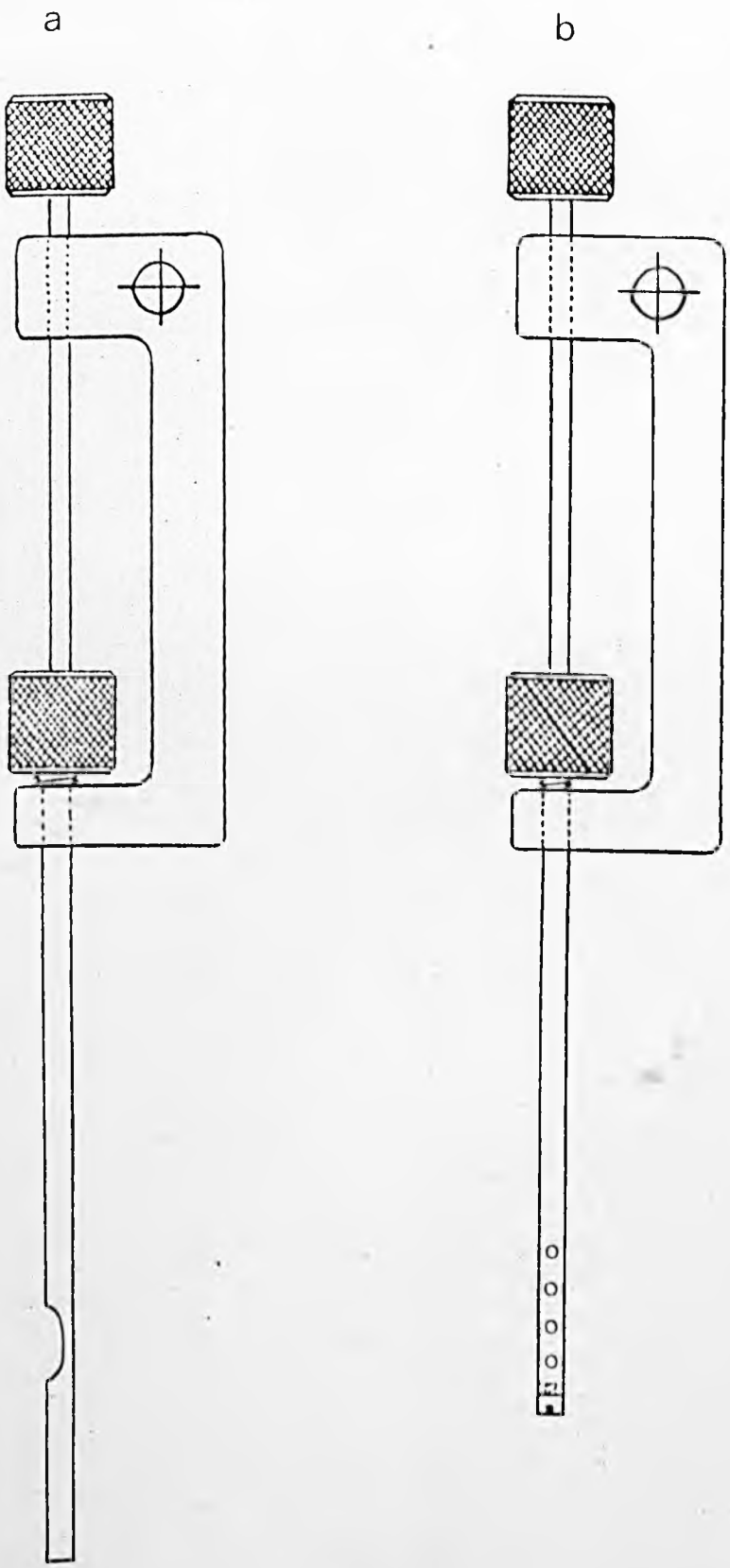
a. Solid sampling with packed columns

The technique employed was based on that described by MORGAN and WADHAMS (1972a). The configuration of the original apparatus is shown in Figure 41a. In these analyses either single dissected glands, capillary extracts (MORGAN and TYLER, 1977), whole heads or gasters were sealed in a thin walled soda glass vial approximately 2cm x 2mm i.d. The glass vial containing the sample was then introduced into the sampler mounted in the modified injection port of a Pye 104 model GC and allowed to heat for a short period (5mins at

FIGURE 41 Solid samplers

a. Solid sampler developed by WADHAMS (1972)

b. Solid sampler modified for use with glass columns



an injection port heater temperature of 200°C was sufficient for most samples. These conditions were however altered according to the stability and volatility of the compounds under investigation). Depressing the plunger crushed the vial and released the volatile material which was carried onto the separation column by the carrier gas.

The original solid sampler was developed for use with robust stainless steel packed GC columns. In this situation it proved perfectly adequate, however, a problem was encountered when operating this form of the sampling device with the more fragile glass columns. During the crushing operation small fragments of glass became jammed between the column wall and sampler, causing the glass columns to crack on a number of occasions. Thus the sampler was modified (Figure 40b) to retain the larger glass fragments and prevent this damage occuring.

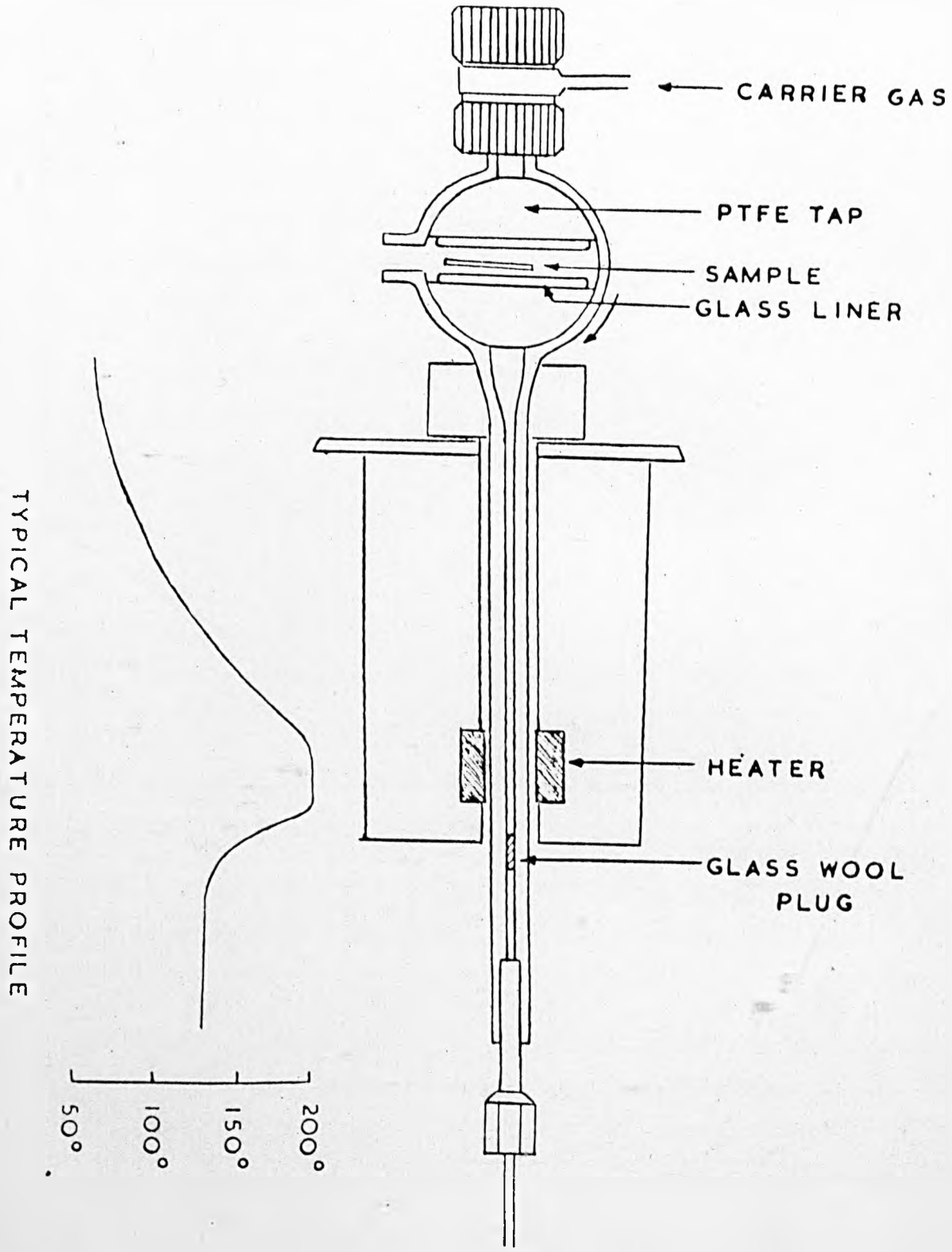
The stem of the sampler was shortened from 9.5cm to 7.0cm and the opening in the stem, which originally facilitated the release of the volatiles from the sample vial into the carrier gas, was replaced by a series of eight 1.5mm diameter holes. These retained the larger fragments of glass and tissue that might otherwise have damaged the GC column. The removable screw in the base of the sampler allowed the retained debris to be removed at intervals.

b. Capillary sampler for capillary GC

Capillary extracts (MORGAN and TYLER, 1977) of exocrine glands can be efficiently analysed by capillary GC using the sampler illustrated in Figure 41. This sampler consisted of a three-way PTFE

FIGURE 41

Capillary sampler



tap with a glass liner connected to a pre-column made of 1mm i.d. capillary glass with a glass wool plug 5cm below the injection port heater. To analyse the sample, the tap was rotated to interrupt the carrier gas, the sample capillary was then placed in the bore of the tap which is then turned so that the carrier gas stream is reopened allowing the sample to drop into the heated zone of the pre-column where flash volatilisation occurs.

Extracts of venom glands and Dufour glands have been analysed in this way by capillary gas chromatography.

6. Quantitative analysis

The quantity of material represented by a peak in the GC profile obtained through the analysis of a single gland was determined by comparing its area with that of a similar sized peak of a known amount of synthetic standard injected as a solution in hexane, acetone or carbon disulphide. The determinations were made on ten replicate analyses of both the unknowns and the standards. The areas of the peaks were determined by the geometric method, which assumes that a normal peak approximates to an isosceles triangle; the area is measured by multiplying the height by the width at half height.

SECTION II : INVESTIGATION OF TRAIL PHEROMONES

1. Dissection and preparation of samples

The worker ants were killed by momentary immersion in liquid nitrogen. The poison glands and vesicles were then removed by dissecting the ants in distilled water under a Vickers Zoomax binocular microscope (mag. x35). In the case of *M. rubra* the poison apparatus was removed by holding the ant by the petiole with fine forceps (Idealtek No.4 Trady's Switz.) and gently pulling on the everted sting; the Dufour gland can be dissected away with the forceps to leave the poison vesicle with its two glandular filaments. Pulling on the extruded sting of workers of the other *Myrmica* species causes the poison vesicle to rupture, consequently in the investigations of these other species the poison vesicle was isolated by parting the tergites and sternites of the fourth and fifth segments and gently pulling away the anterior portion of the gaster. The ovaries, hindgut, Malpighian tubules, Dufour gland and muscular tissue were removed until only the sting, a little muscular tissue and cuticle remained attached to the poison glands and vesicle.

Glands and vesicles isolated by either of the above techniques were subject to either thin layer chromatography (TLC) or gas chromatography (GC). Solvent extracts of poison glands were prepared for TLC, bioassay or chemical analysis by macerating five glands with 125µl or 500µl of distilled hexane, acetone or reagent in a small tissue grinder. A suitable tissue grinder was prepared from a $5cm^3$ pyrex test tube and a piece of glass rod. Abrasive surfaces were produced on the base of the test tube and on the end of the rod by

grinding the two together by hand with a slurry of carborundum and water.

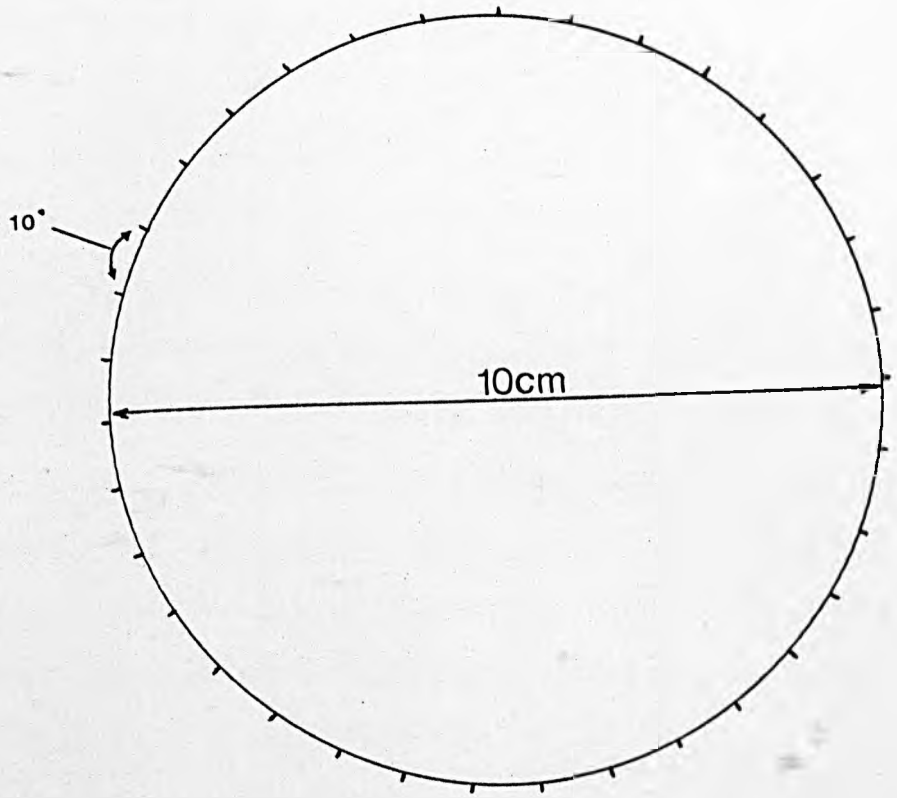
2. Bioassay of trail following behaviour

Two versions of the trail following bioassay devised by PASTEELS and VERHAEGHE (1974) were employed to test for ethological activity in test solutions:

a. A synthetic trail was formed by depositing a 100µl aliquot of the test solution onto the circumference of a 10cm diameter circle, pencil-drawn on graph paper. The trail was laid from a melting point tube with one end drawn out to a fine tip, and mounted in a pair of geometric compasses. The circumference of the circle was marked off into divisions of 10° (Figure 43). The substances under test were laid onto the circle usually as a solution in hexane or acetone. The solvent was allowed to evaporate for two minutes and the circular trail presented to the foraging worker ants. The reactions of either twenty-five or fifty workers crossing the trail were noted and quantified in terms of the number of arcs walked by the individual workers.

b. In this second version of the trail following bioassay 25µl of the solution under test was laid around the circumference of the same type of circle using a normograph pen. The solvent was allowed to evaporate, and the reactions of workers crossing the trail were recorded for 15mins, and quantified as above.

FIGURE 43 Bioassay trail



3. Analysis by thin layer chromatography (TLC)

TLC of the pheromone was performed on 20cm x 5cm x 0.3mm thick silica gel layers. The freshly removed poison glands or glandular extracts (prepared by the technique described above) in distilled hexane or acetone (100µl) were applied to the origin of a TLC plate. The plates were developed once either with hexane-acetone (85:15) or toluene-diethylether (60:40). After air drying, five bands of silica were removed from the plates. These bands corresponded to the R_f regions 0.0-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8 and 0.8-1.0. Each of these bands was then eluted with acetone (500µl) in a vertically mounted Pasteur pipette plugged with glass wool. These extracts were then tested for trail following activity by the bioassay described in II,2, along with control samples of acetone washings of clean silica.

Samples of simple reference compounds were chromatographed under the same conditions as the gland extracts; visualisation of these substances was achieved by exposing the plate to iodine vapour, by examination under a UV source or by spraying with 20% sulphuric acid followed by baking in an oven at 120°C for 15mins. The R_f values of these standard compounds were compared to the R_f region in which the trail activity was observed. By these comparisons, an indication of the polarity and chromatographic properties of the trail pheromone could be deduced.

4. Chemical analysis

Glandular extracts prepared by the technique described above were treated with a number of reagents to test for the presence of certain functional groups. The reaction mixtures were then subjected

to TLC and the silica bands scraped from the plate and bioassayed as described above. If the appropriate R_f band still showed a similar level of activity, it was concluded that no reaction had occured. Samples of a number of synthetic standards were treated with the test reagents in order to determine suitable reaction conditions for the microgram sample sizes used.

a. Treatment with chromic acid

Five freshly dissected venom glands of M. rubra were mounted on a small piece of glass and transferred to the tissue grinder, where they were homogenised with distilled acetone (50µl). Chromic acid (10µl), prepared from $Na_2Cr_2O_7$ (2g), 98% H_2SO_4 (1.5ml) and distilled water (10ml), was added to the tissue extract and grinding continued for 10mins. The reaction mixture was then spotted onto a 5cm x 20cm x 0.3mm TLC plate and developed with an eluent of hexane and acetone (7:3). After air drying, bands of silica were removed from the plate, extracted with acetone and bioassayed as described previously (II,2a).

b. Treatment with bromine

A 1% v/v solution of bromine in carbon tetrachloride was substituted for the chromic acid solution in the above procedure. The bromine solution was added dropwise from a Pasteur pipette until the red colouration remained. The reaction mixture was subsequently spotted onto the origin of a TLC plate developed and bioassayed as described above.

c. Treatment with sodium borohydride

Five poison glands were ground together in the tissue grinder with methanol (100µl) and powdered $NaBH_4$ (1mg). The mixture was

allowed to stand for 30mins at room temperature and then chromatographed and bioassayed as described above.

d. Treatment with acetylating mixture

Five freshly dissected poison glands were treated with a solution of acetic anhydride and pyridine in ether (1:1:18, 10µl) (HUWYLER, 1972) in a 500µl Reacti-vial for four hours in an oven at 120°C. After allowing the vial to cool, its interior was washed with acetone (100µl). This reaction mixture was subjected to TLC and bioassayed as described above. Results of these experiments are given in Table 6, Section I of the Discussion.

5. Gas chromatography

a. GC-Trapping

Trapping of the volatile compounds from the solid injection of five poison glands was performed using a micro-preparative apparatus (BAKER et al., 1976) employing an FID to trap split ratio of 1:100 at a carrier gas flow rate of 50ml/min. The 5% OV-101 and 5% DEGS columns (Table 44, columns B and G) were employed in the trapping experiments. The effluent from the columns was allowed to pass through acetone (0.5ml) contained in a 2cm diameter spherical trap cooled in a slurry of liquid nitrogen and ethylacetate (-80°C). Aliquots (0.1ml, 1 gland equivalent) of the trapped material were tested for ethological activity by the bioassay (II,2). A mixture of n-alkane standards was analysed under the same chromatographic conditions to act as markers and allow calculation of Kovat's retention indices.

Trapping was carried out successively under the following

conditions: (The results are shown in Table 7.)

(i) The 5% OV-101 column was employed with the GC oven temperature held isothermally at 130°C for 8mins, then temperature programmed up to 190°C at 4°C/min. The splitter outlet heater was maintained at 150°C and the effluent from the columns collected as a single fraction in the Kovat's index range 0-2000.

(ii) Whilst maintaining exactly the above operating conditions the effluent from the column was collected as five separate fractions in the 0-2000 KI range i.e. 0-1200, 1200-1400, 1400-1600, 1600-1800, and 1800-2000.

(iii) The effluent from the OV-101 column was collected as six fractions covering the KI ranges 0-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300 and 1300-1400. This was achieved by temperature programming from 68°C-103°C at 4°C/min. The splitter outlet heater was maintained at 130°C.

(iv) The effluent from the 5% DEGS column was fractionated, by maintaining the GC oven temperature isothermally at 93°C and the spitter outlet at 130°C. Fractions were collected over the KI ranges 0-1300, 1300-1400 and 1400-1500.

b. Analyses with FID

Single dissected glands and capillary extracts were also analysed under the conditions described in (iii) and (iv) above. No splitter was employed and the GC was operated with its FID detector (amplifier system set to the maximum practical sensitivity; x10).

c. GC with post column boric acid loop

Single dissected glands were analysed by GC with a 15cm x 4mm i.d. post column reaction loop of powdered 20% w/w boric acid and 5%

SE-30 (Table 44, column M) on 100-120 mesh Chromosorb W (AW-HMDS) positioned between the detector and the end of the analytical column.

6. Linked gas chromatography-mass spectrometry (GC-MS)

Linked GC-MS was performed on a Pye 104 series GC with FID linked to an AEI MS12 mass-spectrometer through a Watson-Biemann separator. Helium was used as the carrier gas at a flow rate of 15ml/min. The detector and accelerator potentials were set at 3KV and 8KV respectively and the MS sensitivity at its maximum; x10. The source and detector slits were set at their widest to achieve maximum sensitivity.

The analysis was carried out on the capillary extracts of poison gland reservoirs of fifty workers sealed into a soda glass vial and introduced into the GC-MS system via a solid sampler.

To minimise the background interference, a low bleed 5% SE-30 column (Table 44, column M) was used, and to allow a manual background subtraction to be performed, background spectra were recorded before and after sampling the peak of interest.

7. Quantitative analysis

The mean absolute amount of trail pheromone in the poison apparatus of the individual worker ants was calculated from the mean of ten replicate analyses of single poison glands and vesicles on the bonded Carbowax column (Table 44, column K). The GC oven temperature was maintained at 84°C and the carrier gas flow rate at 50ml/min. The absolute amount of material was calculated by comparing the GC peak areas with those of standards of known concentration (I,6).

8. Bioassay of ethological activity of synthetic 3-ethyl-2,5-dimethylpyrazine

a. Determination of behavioural thresholds

The threshold concentrations at which the worker ants display trail following reactions were determined by presenting them with a range of concentrations of synthetic trail pheromone in the circular trail bioassay (II,2b). Seven separate circular trails were formed from 10^{-3}, 10^{-2}, 10^{-1}, 1, 10, 10^{2}, and 10^{3} nanograms of the synthetic 3-ethyl-2,5-dimethylpyrazine in hexane (25µl). The trail following reactions of the worker ants were recorded and compared (by non-parametric χ^2 tests, SIEGEL, 1956) with their responses to a trail formed from pure hexane (25µl). Results are given in Table 11.

b. Comparison of the ethological activity of the synthetic trail pheromone and poison gland extracts

Five worker poison glands were extracted and macerated with hexane (25µl) in a tissue grinder (II,1). Portions of this extract (25µl, 1 gland equiv) were then used to form artificial trails. The trails were presented to the worker ants and their trail following reactions noted.

Trails made with synthetic 3-ethyl-2,5-dimethylpyrazine (5.8ng, the mean amount of pyrazine per poison gland, determined by GC) were presented to the foraging ants and their trail following reactions again recorded.

The reactions of the ants to the synthetic 3-ethyl-2,5-dimethyl pyrazine at the glandular level and the gland extracts were compared statistically using the Mann-Whitney U test (SIEGEL, 1956). Results are given in Discussion, Section I, Table 10.

SECTION III : CHEMICAL INVESTIGATION OF DUFOUR GLAND SUBSTANCES

1. Sample preparation

a. Dissection of glands

The Dufour gland is found in close association with the poison gland in all the myrmicine species. Therefore after killing the workers by momentary immersion in liquid nitrogen or by contact with solid carbon dioxide, exactly the same procedure is adopted for the dissection of the Dufour and poison glands (II,1).

b. Solid Sampling

Either single or multiple dissected glands, capillary extracts (MORGAN and TYLER, 1977) or whole gasters (containing the Dufour gland) sealed in small glass vials were introduced into the GC for analysis by the solid sampling technique (I,5).

c. Solvent extraction

This technique was used where larger amounts of insect material were required for linked gas chromatography-mass spectrometry, trapping experiments or thin layer chromatography.

A number of insect gasters (e.g. 10-100) were simply ground together with a minimum amount of volatile solvent e.g. carbon disulphide, methylene chloride, hexane etc. (200-500µl) in a small tissue grinder. The solvent was decanted from the residual tissue and reduced in volume under a stream of nitrogen at room temperature.

d. Ampoule distillation (TYLER, 1977)

This is a further technique which can be used conveniently for obtaining extracts from large amounts of insect material. It does however suffer from the disadvantage of extracting a relatively large

quantity of water along with the volatile chemicals of interest. The volatile chemicals may be separated from the aqueous phase by extraction with a suitable immiscible solvent e.g. methylene chloride, carbon disulphide etc.

The apparatus used for this extraction is shown in Figure 44. Typically, two hundred Acromyrmex octospinosus workers were killed by momentary immersion in liquid nitrogen and the gasters separated from the rest of the body. The gasters were placed in the bulb of the ampoule, the ampoule sealed under reduced pressure (10^{-3}-10^{-5} Torr), and the bulb was heated in a detergent bath at 200°C for 30mins while the drawn out arm was chilled in a coolant slurry of acetone and carbon dioxide. The aqueous distillate obtained was extracted with methylene chloride (100µl). The organic extract was concentrated by evaporation under a stream of nitrogen. The extract was split into two portions which were used for linked GC-MS.

2. Gas chromatography

Five types of GC phase were used for the analyses of the hydrocarbons in the Dufour glands of the eight species studied. The columns employed in the investigations of the various species together with the appropriate operating conditions are listed below:

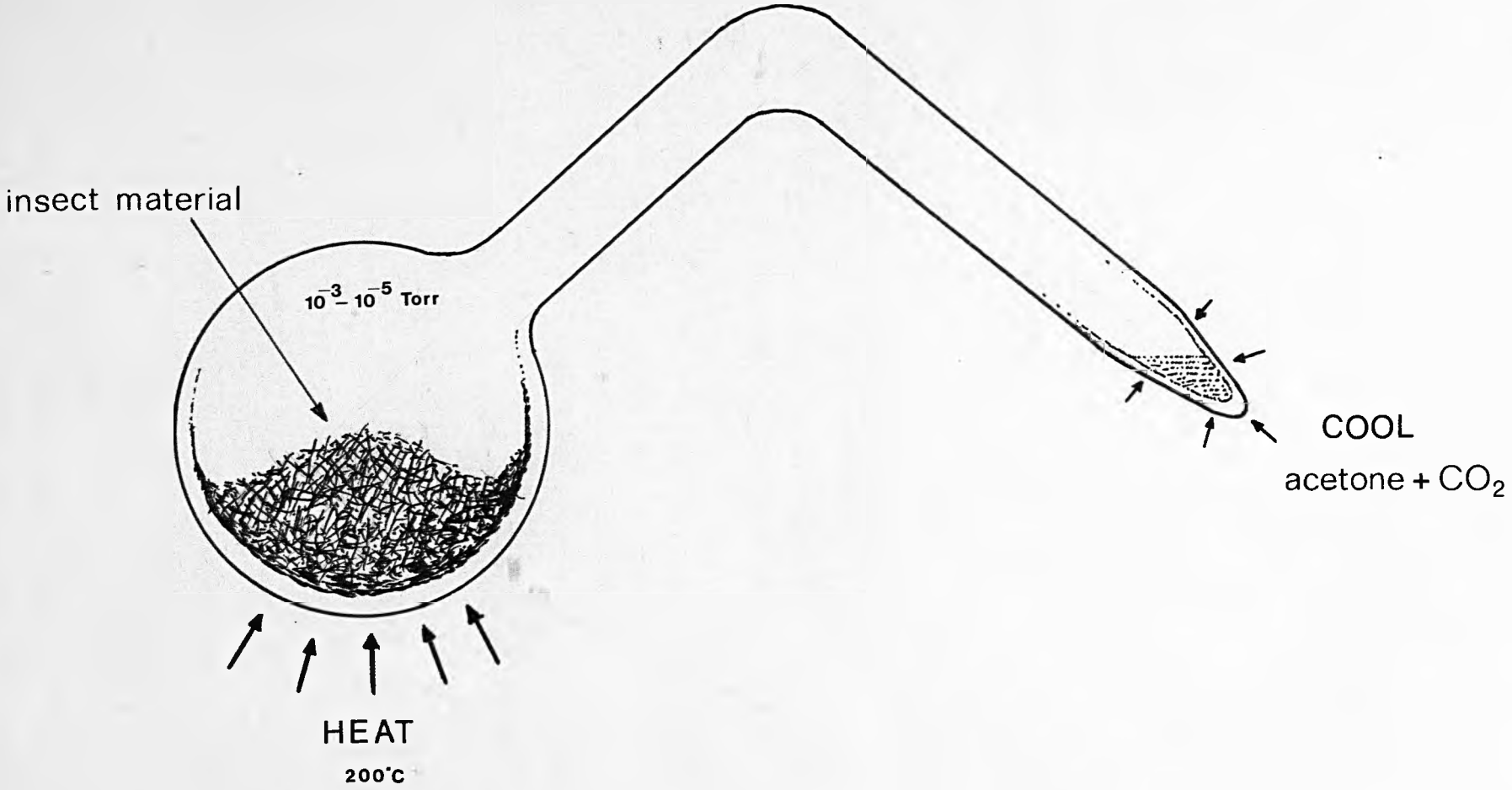
a. A. cephalotes and A. octospinosus

The GC columns used were:

(i) 3% w/w OV-101 (Table 44, column A) operated isothermally at a GC oven temperature of 180°C and a nitrogen carrier gas flow rate of 60ml/min.

(ii) 5% w/w OV-101 (Table 44, column B) operated isothermally at

FIGURE 44 Ampoule distillation



165°C and at a carrier gas flow rate of 50ml/min.

(iii) 5% w/w diethyleneglycolsuccinate (DEGS, Table 44, column G) operated isothermally at 125°C and at a carrier gas flow rate of 60ml/min.

b. A. s. sexdens and A. s. rubropilosa

(i) 5% w/w OV-101 (Table 44, column B) temperature programmed from 180°C-210°C at 2°C/min; the carrier gas flow rate was maintained at 60ml/min.

(ii) 10% w/w polyethyleneglycoladipate (PEGA Table 44, column D) operated isothermally at 174°C with a nitrogen carrier gas flow rate of 50ml/min.

(iii) 10% w/w polyethyleneglycol (PEG 20M, Table 44, column F) operated isothermally at 165°C with a carrier gas flow rate of 50ml/min.

(iv) 5% w/w DEGS (Table 44, column G) held isothermally at 140°C for 30mins then temperature programmed at 8°C/min to 210°C; the carrier gas flow rate was maintained at 50ml/min.

c. Myrmica species

(i) 5% w/w OV-101 (Table 44, column B) operated at 165°C isothermally with a carrier gas flow rate of 50ml/min.

(ii) 10% PEGA (Table 44, column D) temperature programmed from 107°C to 162°C at 2°C/min., with a carrier gas flow rate of 50ml/min.

(iii) 20 % PEGA (Table 44, column C) with the temperature programmed from 130°C to 170°C at 2°C/min, with a carrier gas flow rate of 75ml/min.

3. Gas chromatography-mass spectrometry (GC-MS)

Linked gas chromatography-mass spectromtry (GC-MS) was used to characterise a number of the glandular components from worker ants. Three systems were used during the course of these investigations:

a. Pye 104 series GC with FID linked to a Hitachi-Perkin Elmer RMU 6E mass spectrometer through a Watson-Bieman separator.

b. Pye 104 series GC with FID linked to an AEI MS12 mass spectrometer through a Watson-Bieman separator.

c. Pye 204 series GC linked to a VG Micromass 7070F mass spectrometer through a glass jet separator. This system incorporated a VG 2000 data system.

All the spectra were obtained with the ionisation potential set at 70eV. In order to achieve maximum sensitivity the source and detector slits of the spectrometers were operated at their maximum opening and their senstivities at x10; the highest possible.

Background spectra were recorded to enable manual subtractions to be performed where no data system was available to carry this out automatically i.e. in the cases of the AEI and Hitachi systems.

4. Bromination

Micro-scale bromination of the glandular components was performed by adding a 1:1 solution of bromine in carbon disulfide (0.5µl) to a single Dufour gland in a solid sample glass capillary tube (MORGAN and WADHAMS, 1972a). The sealed capillary was then placed in the solid sampler with the injection port heater maintained at 200°C. Five minutes were allowed to elapse before crushing to ensure complete bromination of any unsaturated components. This

analysis was performed on a column of 5% OV-101 (Table 44, column B) in the cases of the two A. sexdens subspecies, a nitrogen carrier gas flow rate of 60ml/min was employed, and the oven temperature programmed from 180°C to 210°C at 2°C/min. In the investigation of A. cephalotes, the GC oven temperature was maintained isothermally at 165°C. In the case of the Myrmica species the temperature was programmed from 130°C to 192°C at 4°C/min.

5. Ozonolysis

The positions of the double bonds in the alkenes were determined by ozonolysis of fractions trapped from the GC using a micropreparative apparatus (BAKER et al., 1976). The trapping was performed on extracts obtained through the grinding of twenty-five whole gasters with redistilled hexane (200µl). This volume was reduced to 2µl under a stream of nitrogen at room temperature before fractionation by a GC. A 1:100 (FID:trap) split ratio was employed to avoid unnessecary loss of sample to the detector. The samples were trapped in 20cm 'U' tubes of 0.5mm i.d. cooled in liquid nitrogen. It was possible to achieve greater than 85% recovery of microgram samples under these trapping conditions. The trapped material was washed from the 'U' tube with dichloromethane (100µl). Ozonolysis was then performed by exposing the alkene solution, cooled in an ice bath, to a slow stream of ozone fron a microozone generator (BEROZA and BIERL, 1969) for a few minutes. The ozonides were reduced by addition of a small crystal of triphenylphosphine, in order to yield a the carbonyl compounds.

The micropreparative work was carried out on a column of the 5%

OV-101 column (Table 44, column B). Analysis of the ozonolysis products was performed on a column of the 5% DEGS (Table 44, column G).

6. Thin layer chromatography

Thin layer chromatography was employed to determine the configurations of the double bonds of the unsaturated glandular components of the two A. sexdens subspecies and A. cephalotes. Silica gel plates impregnated with 10% $AgNO_3$ of 0.3mm thickness were used in conjunction with an eluent of 1% diethylether in light petroleum (b.p. 40°C-60°C). In the analysis of the alkenes in the two subspecies of A. sexdens a tissue extract of poison gland complexes from five workers was chromatographed together with synthetic samples of (E) and (Z)-9-nonadecene and (Z)-9-tricosene. An extract from the gasters of sixty workers of A. cephalotes was also analysed under similar conditions. The plates were visualised by spraying with 20% sulphuric acid followed by baking in an oven at 120°C for 15mins.

7. Determination of glandular volume

The dimensions of the glands were determined using an eye graticule. The glands appear as gently tapering sacs, being hemispherical at their posterior (see Figure 20). On this basis the glandular volume (V) was calculated from the following formula, where r is the radius at greatest cross section and h is the length of the gland :

$$V = {}^{1}/_{3}\pi r^{2}h + {}^{2}/_{3}\pi r^{3}$$

8. Quantitative analysis

The mean absolute amounts of the individual gland component were calculated by comparing the areas of the peaks on the GC profile with those of standards of known concentration (I,6).

9. Photography

Photographs of the venom apparatuses of *M. rubra* and *A. octospinosus* were taken on a Wild M20 microscope using a micro-photo automat.

SECTION IV : CHEMICAL INVESTIGATION OF MANDIBULAR GLAND SUBSTANCES

1. Preparation of samples

The worker ants were killed by momentary immersion in liquid nitrogen. Care was taken to ensure that the ants did not become too disturbed and so discharge their mandibular gland contents. The heads were quickly separated from the thoraces and abdomens and sealed in a solid sampling vial in preparation for gas chromatography.

It had been demonstrated earlier that the solid injection of whole workers' heads gave the same GC profile as that given by the injection of excised mandibular glands alone (WADHAMS, 1972).

2. Gas chromatography

The higher molecular weight compounds from the mandibular glands were analysed initially on a column of 15% PEG 20M (Table 44, column E) on 80-100 mesh Chromosorb P, with a GC oven temperature of 130°C and a carrier gas flow rate of 50ml/min.

Improved separations were obtained by employing a column of 10% PEG 20M (Table 44, column F) on 100-120 Chromosorb W. The GC oven temperature was maintained at 100°C and the nitrogen carrier gas flow rate at 50ml/min.

Further analyses were carried out on a column of 5% Apiezon L (Table 44, column H). The GC oven temperature was held at 129°C and nitrogen carrier gas flow rate at 50ml/min.

For the analyses of the highly volatile constituents of the mandibular gland secretions two porous polymer columns were employed,

namely, Porapak Q and Chromosorb 102 (Table 44, columns I and J). The GC oven temperatures were maintained at 166°C and 140°C respectively and the nitrogen carrier gas flow rate at 50ml/min.

3. Gas chromatography-mass spectrometry (GC-MS)

Linked GC-MS investigations were performed on a Pye 104 series GC with FID linked to an AEI MS12 mass spectrometer through a fritted Watson-Bieman separator. The ionisation potential was maintained at 70eV. To ensure that maximum sensitivity was achieved the source and detector slits were set at their widest and the spectrometer sensitivity at x10.

The analyses were performed on five workers heads, introduced into the GC by the solid sampling technique (I,5a). The separations were carried out on a column of 5% Apiezon L (Table 44, column H) as this column gave little bleed and hence low background interference. The helium carrier gas flow rate was held at 18ml/min while the GC oven temperature was maintained isothermally at 100°C for 12mins, then programmed at 10°C/min for 10mins.

4. Treatment with sodium borohydride reagent

To test for the presence of ketones in the mandibular gland secretions single heads were sealed in the solid sampling vial together with excess sodium borohydride (1mg). The vial was then introduced into the solid sampler and heated for 10mins at 200°C by the injection port heater ensuring complete reduction of any ketones having a molecular weight at least as high as that of 3-undecanone.

5. Quantitative analysis

Quantification of the individual glandular components was achieved as described previously (I,8).

SECTION V : PREPARATION OF REFERENCE COMPOUNDS

1. Preparation of (E) and (Z)-9-nonadecene

1-nonanol (50ml, 0.35mole) was refluxed with a slight excess of 36% hydrobromic acid (40ml, 0.2 mole) and 98% sulphuric acid (4.5ml). The progress of the reaction was followed by GC. After eight hours refluxing an 80% conversion of the alcohol to the alkylhalide had occured. The refluxing was stopped at this point and the aqueous and organic layers separated. The alkylbromide was separated from the remaining unreacted alcohol by distillation at atmospheric pressure using a Claisen still head. The purity of the distillate was checked by GC. Five fractions were collected between 211°C and 222°C. The latter three fractions were pure 1-bromononane by GC.

1-bromononane (23.2g, 0.1 mole) was then refluxed with triphenylphosphine (28g, 0.1 mole) for five hours in dimethylformamide (DMF, 50ml, dried by five hour reflux over P_2O_5, followed by distillation from P_2O_5). The DMF was removed by rotary evaporation under reduced pressure, and the residual oil triturated with dry diethylether (50ml) and left overnight at 0°C-2°C. The oil solidified to give a white solid which was filtered from the ether. Drying to a constant weight gave 1-nonyltriphenyl phosphonium bromide as a white solid (38.9g, 89%, m.p.58°C-60°C).

The phosphorium salt (14.0g, 0.04 mole) was treated with n-butyllithium (0.05 mole as a solution in hexane) in freshly distilled tetrahydrofuran (THF, 75ml, distilled from sodium) in dry glassware under a nitrogen atmosphere at 0°C. The n-butyllithium solution was added in 5ml portions with rapid stirring. After

completing the addition, stirring was continued for 15mins to ensure the formation of the red ylid complex. Decanal (6ml, 0.03 mole) was then added dropwise over a five minute period. The red colouration dispersed completely as the last drops of aldehyde were added.

Light petroleum (boiling range 40°C-60°C, 100ml) was added to precipitate the salts. These were removed by filtration on a Buchner over 'Hyflow'.

The petroleum solution was then washed with distilled water (2 x 15ml), dried ($MgSO_4$) and distilled, to give 9-nonadecene (5.8g, 68%) as a colourless oil (b.p.136°C/0.5mm).

2. Preparation of (E) and (Z)-8-heptadecene

8-heptadecene was prepared by exactly the method employed above except that octanal was substituted for the decanal in the Wittig condensation. Distillation of the product gave 8-heptadecene (3.1g, 57%, b.p.90 C-93°C / 0.08mmHg). The thin film IR spectrum again revealed the presence of (E) and (Z) isomers.

3. Separation of (E) and (Z) isomers by medium pressure column chromatography (MPCC)

The separation of the (E) and (Z) isomers of the two hydrocarbons was achieved by medium pressure column chromatography on a 100cm x 15mm i.d. glass column (Whatman, Maidstone) packed with

Kieselgel 60 (Merck, Darmostadt, 40-63 m diameter, 230-300 mesh, ASTM), coated with 20% of its weight in silver nitrate. A portion of hydrocarbon (1g) was injected onto the column and eluted with light petroleum (b.p.40°C-60°C) at a flow rate of 1ml/min. The eluent was collected in 1ml portions and analysed by GC on a 5% DEGS column (Table 44, column G) isothermally at 125°C and carrier gas flow rate of 50ml/min and by IR to monitor the separation. The GC analysis showed the relative proportion of (Z) to (E) isomers to be 4:1 under the experimental conditions described.

4. Preparation of 3-ethyl-2,5-dimethylpyrazine

An ethereal solution of ethyllithium was prepared according to the method of GILMAN et al. (1949) from ethylbromide and lithium metal

Freshly cut lithium (1.4g, 0.2 mole) was added to 100ml of diethylether (dried over sodium) in a 250ml three necked flask while flushing with dry nitrogen. Initially a few drops of an ethereal solution (50ml) of ethylbromide (10.8g, 0.1 mole) were added to the lithium with rapid stirring, while cooling to -10°C with a bath of ethylacetate and liquid nitrogen. The solution became cloudy and bright spots appeared on the lithium. The remainder of the ethylbromide solution was added over a 30 minute period while maintaining the internal temperature at -10°C. Stirring was continued for two hours while the temperature was allowed to increase to 0°C-10°C. The ethereal solution of ethyllithium was freed from all particulate matter by filtration under nitrogen through an angled glass tube plugged with glass wool, into a second three necked flask

in preparation for a the next stage of the synthesis. The 2,5-dimethylpyrazine (5g, 0.05mole) (Aldrich Chemical Co. Ltd., England) in diethylether (15ml, dried over sodium) was added under nitrogen to the solution of ethyllithium over a period of thirty minutes with constant stirring and cooling to 0°C. After completing the addition the stirring was continued for one hour while the temperature was allowed to rise to 15°C-20°C. The red complex was cautiously decompossd on 150g of ice/water with vigorous stirring. The aqueous solution was saturated with sodium chloride and extracted diethylether (4 x 5ml, KLEIN and SPOERRI, 1951).

The solvent was removed by rotary evaporation under reduced pressure to leave a mixture of unreacted starting material and products which was purified by micro-preparative gas chromatography using an all glass splitter (BAKER et al., 1976).

Portions of the synthetic 3-ethyl-2,5-dimethylpyrazine (0.5-0.2mg) were trapped into 10cm x 1mm i.d. glass capillaries at room temperature. The purification was performed on the analytical 10% PEG 20M column (Table 44, column F) with the nitrogen carrier gas flow rate held at 50ml/min. The GC oven temperature and splitter heater were maintained at 140°C and 150°C respectively. The yield was determined by GC (1.2g, 19%). Chromatographic properties: TLC on silica plates R_f = 0.44 (7:3 hexane/acetone), R_f = 0.34 (6:4 toluene/diethylether). GC, KI.= 1060 (SE-30, Table 44, column H).

5. Preparation of 3-decanol and other 3-alkanols

3-Decanone(1g, 0.01 mole) was refluxed with excess sodium borohydride in methanol (25ml). The progress of the reaction was

monitored by GC using a column of 10% PEG 20M (Table 44, column F) at an oven temperature of 120°C and a carrier gas flow rate of 50ml/min.

Once complete conversion of the ketone to the alcohol had occured the solids were removed by Buchner filtration. The methanol was removed by rotary evaporation under reduced pressure and the 3-decanol finally distilled at normal pressure, fractions being collected between 209°C-213°C. The purity of the distillate was tested by GC.

Other 3-alkanols that were not commercially available were prepared from their corresponding 3-alkanones by exactly the above procedure.

6. Preparation of 6-methyl-3-octanone and 6-methyl-3-octanol

A sample of 6-methyl-3-octanone was prepared in the laboratory at Keele by Mr.J.Clews following the method of SKAIKH and THAKAR, (1966).

A portion of this ketone was converted to the corresponding alcohol by treatment with sodium borohydride as described above.

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APPENDICES

APPENDIX 1

Absolute amounts of the major trail pheromone components from the venom of workers of A. cephalotes, A. octospinosus, A. s. sexdens and A. s. rubropilosa , together the workers live body weights (mg).

A. cephalotes		A. octospinosus		A. s. sexdens		A. s. rubropilosa	
body wt.	pyrrole*	body wt.	pyrrole	body wt.	pyrazine†	body wt.	pyrazine
74.0	2.45	9.8	6.5	23.8	4.9	3.1	0.9
39.8	1.78	4.0	1.7	23.0	4.5	16.0	2.8
7.2	0.4	6.8	3.4	12.4	3.1	0.5	0.1
4.3	0.3	14.2	6.1	11.6	1.9	8.6	3.1
26.6	1.8	8.4	4.1	5.7	2.6	71.6	7.3
81.5	2.7	12.4	4.8	5.0	1.4	9.7	2.8
9.0	0.4	1.1	0.9	65.0	8.0	12.6	2.5
-	-	7.0	3.0	3.5	0.5	80.0	6.1
21.8	0.9	2.6	1.0	0.9	0.2	13.7	3.2
53.8	2.9	2.4	1.1	56.4	6.3	25.7	5.9
2.3	0.2	-	-	36.0	7.7	19.1	3.6
3.8	0.2	-	-	52.7	9.1	25.4	5.1
11.8	0.4	-	-	42.6	9.1	4.0	1.2
-	-	-	-	-	-	9.1	1.8
-	-	-	-	-	-	45.1	8.8
-	-	-	-	-	-	49.0	7.6
-	-	-	-	-	-	19.1	2.5

* pyrrole is methyl 4-methylpyrrole-2-carboxylate

† pyrazine is 3-ethyl-2,5-dimethylpyrazine

APPENDIX 2

Absolute amounts of hydrocarbons in the Dufour glands of ten A. cephalotes workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
C_{12}	1.1	0.1	0.4	0.2	0.2	0.3	0.2	0.1	0.4	0.1
C_{13}	0.8	1.0	1.0	3.4	3.5	2.5	1.5	1.6	6.0	2.8
C_{14}	1.0	0.6	0.2	0.4	0.8	0.7	0.5	0.3	2.0	0.5
$C_{15:1}$	0.4	0.2	0.3	0.6	0.8	0.5	0.8	1.2	4.6	0.8
C_{15}	0.8	1.0	2.5	3.5	5.3	6.1	1.8	3.5	14.2	2.5
homofarnesene	0.1	0.3	0.3	0.5	0.3	0.6	1.2	0.4	2.2	0.3
C_{16}	0.8	0.5	1.2	2.4	1.3	2.6	1.5	2.3	5.4	1.0
$C_{17:1}$	1.2	0.2	0.6	0.8	2.3	1.6	1.0	1.6	4.0	1.0
C_{17}	12.1	12.2	17.1	28.2	37.3	37.9	39.9	43.3	104.2	31.1
$C_{18:1}$	0.7	0.3	1.2	0.9	1.2	1.8	1.1	1.2	3.6	1.4
C_{18}	0.7	0.6	2.0	1.5	1.8	2.5	1.4	2.1	4.2	1.6
$C_{19:2}$ $C_{19:1}$	1.8	2.4	10.0	10.1	13.7	19.5	19.2	25.0	78.0	23.6
C_{19}	2.0	2.5	5.0	6.6	9.1	9.1	10.2	12.1	25.2	7.6

* C_{12} is n-dodecane, $C_{13:1}$ is tridecene, etc.

APPENDIX 3

Absolute amounts of hydrocarbons in the Dufour glands of ten A. s. rubropilosa workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
$C_{15:1}$	1	4	1	1	4	1	3	6	8	2
C_{15}	5	20	2	4	24	1	3	20	4	12
homofarnesene	3	18	3	5	24	8	10	22	10	11
C_{16}	7	17	5	6	19	14	7	41	28	19
$C_{17:1}$	11	30	9	12	41	24	7	22	6	32
C_{17}	129	283	65	121	315	220	16	373	226	312
unknown	2	8	2	2	24	5	16	24	6	12
$C_{18:1}$	33	98	24	89	205	79	2	167	90	90
C_{18}	14	49	10	10	32	26	35	69	35	43
$C_{19:2}$ / $C_{19:1}$	437	1340	454	675	3100	1107	1063	2432	1126	1471
C_{19}	65	266	71	79	532	142	236	310	260	179
unknown	0	0	0	0	0	0	0	3	0	0
$C_{20:1}$	6	32	8	0	138	16	331	41	20	22
C_{20}	3	8	6	0	39	16	14	14	10	6
$C_{21:1}$	16	148	50	53	354	130	102	230	83	130
C_{21}	8	39	16	20	21	16	26	67	24	20
unknown	0	0	0	0	0	0	0	4	0	0
$C_{22:1}$	10	35	16	22	205	47	59	138	27	47
unknown	0	0	0	0	0	0	0	8	0	0
$C_{23:1}$	106	1122	213	354	2598	661	630	2066	541	591

* See sub-script to Appendix 2 for explanation of symbols.

APPENDIX 4

Absolute amounts of hydrocarbons in the Dufour glands of ten A. s. sexdens workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
C_{15}	6	6	8	2	0	16	2	4	1	2
homofarnesene	4	4	4	1	0	12	2	4	2	3
C_{16}	5	4	5	1	0	9	2	5	3	3
$C_{17:1}$	9	12	18	2	0	25	2	12	6	4
C_{17}	98	87	95	29	32	142	33	55	71	28
unknown	25	39	39	7	5	74	8	24	15	4
$C_{18:1}$	49	66	83	23	14	84	19	47	55	19
C_{18}	0	59	69	20	16	95	22	39	71	17
$C_{19:2}$ $C_{19:1}$	913	1120	1200	425	472	1192	410	780	788	354
C_{19}	709	903	898	460	413	999	236	799	753	145
unknown	16	22	0	0	0	10	0	0	10	2
$C_{20:1}$	32	35	28	7	14	21	9	63	20	6
$C_{20:1}$	47	57	63	11	28	55	13	35	39	14
$C_{21:1}$	226	268	425	87	142	357	61	236	230	87
$C_{21:1}$	177	165	256	39	89	271	36	158	165	36
unknown	10	16	0	0	0	22	0	0	0	0
$C_{22:1}$	98	102	154	39	56	193	28	79	124	28
unknown	0	12	0	0	0	12	0	0	0	0
$C_{23:1}$	1831	1547	2945	784	1043	2658	683	2658	1998	485

* See sub-script to Appendix 2 for explanation of symbols.

APPENDIX 5

Absolute amounts of hydrocarbons in the Dufour glands of ten M. sabuleti workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
C_{15}	10	8	7	3	4	2	12	12	3	12
$C_{15:1}$	15	15	23	17	21	3	28	28	12	25
C_{16}	25	17	17	19	23	3	25	8	10	25
C_{17}	7	2	4	4	5	0	6	3	5	5
α-farnesene	223	282	352	256	388	111	526	267	367	350
$C_{17:2}$	3	6	2	2	4	2	17	5	0	6
homofarnesene	119	202	359	223	180	52	290	168	219	189
$C_{18:1}$	21	26	48	21	19	8	59	36	28	33
bishomofarnesene	173	391	772	516	377	118	669	296	519	419
C_{19}	58	8	3	10	4	12	46	7	36	109
$C_{19:1}$	39	5	6	23	0	13	8	7	42	59
trishomofarnesene	63	6	13	63	0	19	9	8	79	132

* See sub-script to Appendix 2 for explanation of symbols.

APPENDIX 6

Absolute amounts of hydrocarbons in the Dufour glands of eight *M. scabrinodis* workers.

Compound*	Amounts (ng)							
	1	2	3	4	5	6	7	8
C_{15}	5	10	34	8	55	12	13	26
$C_{15:1}$	10	12	66	13	66	20	13	26
C_{16}	5	6	26	9	13	7	12	19
C_{17}	12	7	20	5	20	7	6	25
α-farnesene	69	41	256	46	206	182	66	241
$C_{17:2}$	3	7	25	5	22	6	18	13
homofarnesene	168	165	345	305	610	323	281	630
$C_{18:1}$	6	9	27	6	64	11	9	16
bishomofarnesene	158	172	333	111	425	116	236	277
C_{19}	5	9	10	3	19	30	5	35
$C_{19:1}$	0	12	74	11	19	19	8	85
trishomofarnesene	0	12	50	3	13	13	7	50

* See sub-script to Appendix 2 for explanation of symbols.

APPENDIX 7

Absolute amounts of hydrocarbons in the Dufour glands of ten *M. lobicornis* workers.

Compound	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
α-farnesene	231	229	179	167	229	160	171	262	119	171
homofarnese	727	1541	1226	973	668	1121	715	1402	620	792
bishomofarnesene	1111	3521	2793	2043	3045	2513	2748	2371	2003	3011

APPENDIX 8

Absolute amounts of hydrocarbons in the Dufour glands of ten M. ruginodis workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
C_{13}	79	81	58	41	119	89	45	65	111	71
$C_{13:1}$	12	8	50	45	33	3	74	96	165	121
C_{14}	17	17	17	12	16	17	4	8	3	17
C_{15}	587	705	346	629	993	663	351	743	371	990
$C_{15:1}$	32	46	13	46	66	26	29	26	26	81
unknown	3	5	18	6	13	8	3	5	3	6
unknown	12	6	6	4	8	11	10	10	8	19
C_{16}	35	46	58	28	52	35	13	35	14	35
$C_{16:1}$	35	46	20	35	46	33	15	25	14	35
C_{17}	231	363	165	297	347	310	132	281	116	314
α-farnesene	1650	1878	858	899	3152	1733	818	1931	776	1878
$C_{17:1}$										
unknown	8	3	4	4	4	8	12	3	4	19
$C_{17:2}$	153	93	41	227	198	116	62	103	83	173
C_{18} homofarnesene	74	21	41	206	144	62	144	726	289	25
$C_{18:1}$	10	23	83	159	124	223	59	50	15	149
$C_{18:2}$	5	23	10	15	40	15	25	20	15	35
bishomofarnesene	12	10	29	15	29	58	17	144	12	25
C_{19}	7	7	33	12	7	99	0	7	0	6
$C_{19:1}$	40	33	26	46	40	40	13	59	5	53
$C_{19:2}$	150	272	139	109	215	107	22	322	0	193
trishomofarnesene	111	74	82	67	82	111	22	22	7	149

* See sub-script to Appendix 2 for explanation of symbols.

APPENDIX 9

Absolute amounts of hydrocarbons in the Dufour glands of ten M. rubra workers.

Compound*	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
C_{13}	7	18	17	15	13	7	13	19	26	20
$C_{13:1}$	9	85	68	53	3	4	15	4	2	2
C_{14}	4	8	7	10	5	3	8	5	17	7
C_{15}	208	280	270	270	232	110	321	210	475	336
$C_{15:1}$	14	14	17	27	16	4	8	3	20	20
unknown	2	12	6	3	3	2	3	5	1	1
unknown	4	13	7	5	5	3	17	7	5	3
C_{16}	12	13	14	6	18	5	18	13	20	12
$C_{16:1}$	17	17	17	12	19	6	18	17	30	46
C_{17}	99	152	152	99	109	56	150	145	92	79
α-farnesene	894	1063	1053	1045	1224	665	1606	639	1280	825
$C_{17:1}$										
$C_{17:2}$	140	273	231	89	196	109	400	309	132	163
homofarnesene	2	5	0	11	7	2	0	0	54	6
$C_{18:1}$	22	20	24	23	12	12	58	58	30	26
bishomofarnesene	8	11	11	3	0	3	3	11	36	8
C_{19}	5	12	10	8	9	16	3	4	17	9
$C_{19:1}$	295	140	196	113	129	227	465	495	462	218

* see sub-script to Appendix 2 for explanation of symbols.

APPENDIX 10

Absolute amounts of volatile components in the mandibular glands of ten M. lobicornis workers.

Compounds	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
3-hexanol	531	538	531	310	497	739	234	856	656	766
3-henanone	91	124	140	107	124	107	66	103	91	269
3-heptanol	386	580	538	165	248	442	110	469	414	386
3-heptanone	105	138	110	88	96	127	38	129	121	193
3-octanol	3983	2515	2417	1433	2223	3384	1121	4563	3645	3326
3-octanone	4475	3190	2828	2928	3469	5292	1381	5226	4983	600
6-methyl-3-octanol	44	22	22	22	22	33	0	33	44	66
6-methyl-3-octanone	121	136	149	116	82	116	33	165	165	232
3-nonanol	165	99	49	66	99	33	149	132	99	132
3-nonanone	58	38	38	19	48	48	9	58	66	38
3-decanol	1657	1044	663	994	1381	1657	442	1769	1602	1545
3-decanone	49	12	12	24	49	49	0	37	49	37

APPENDIX 11

Absolute amounts of volatile components of the mandibular glands of ten M. ruginodis workers.

Compound	1	2	3	4	5	6	7	8	9	10
3-pentanol	3	3	0	0	0	0	0	0	0	0
3-hexanol	5	2	5	5	6	4	7	2	2	3
3-hexanone	2	1	2	1	4	3	1	1	1	2
3-heptanol	25	11	25	23	17	23	28	12	12	10
3-heptanone	2	1	4	3	3	2	2	1	1	1
3-octanol	935	460	567	386	567	521	690	294	400	335
3-octanone	525	216	612	534	566	484	557	236	168	200
6-methyl-3-octanone	19	6	11	10	13	6	21	5	10	8
3-nonanone	8	5	7	7	7	6	9	4	7	5
unknown 1	3	2	1	1	1	0	1	1	1	1
unknown 2	19	14	11	4	0	0	7	0	0	19
unknown 3	3	2	1	1	0	0	0	0	0	0

APPENDIX 12

Absolute amounts of volatile components of the mandibular glands of ten M. sabuleti workers.

Compounds	Amounts (ng)									
	1	2	3	4	5	6	7	8	9	10
3-pentanol	6	3	5	2	6	4	4	6	5	10
3-hexanol	116	53	19	37	74	20	19	29	18	54
3-hexanone	30	31	6	17	10	6	5	7	4	15
3-heptanol	82	44	41	55	49	78	90	42	40	41
3-heptanone	34	25	7	18	7	11	11	7	12	23
3-octanol	267	115	254	125	316	206	159	325	177	455
3-octanone	1205	703	361	635	466	786	710	1039	470	1706
6-methyl-3-octanol	3	1	3	2	4	2	1	11	1	11
6-methyl-3-octanone	115	42	56	73	84	77	46	125	31	100
3-nonanone	14	10	6	6	8	10	8	17	9	28
3-decanone	2	1	2	2	1	1	1	14	5	42
unknown 1	5	2	3	2	5	5	2	6	4	12
unknown 2	19	3	70	54	5	1	0	1	21	25
unknown 3	2	2	6	11	3	1	0	21	2	2

APPENDIX 13

Absolute amounts of volatile mandibular gland components of ten M. schencki workers.

Compound	Amounts (ng) 1	2	3	4	5	6	7	8	9	10
3-hexanol	37	11	23	11	15	31	31	12	13	23
3-heptanol	40	16	33	16	19	42	28	14	16	52
3-heptanone	4	2	2	1	1	3	3	1	0	2
3-octanol	396	208	396	181	249	604	403	171	221	522
3-octanone	63	46	33	27	19	52	63	14	7	126
6-methyl-3-octanol	12	2	12	12	12	25	0	12	8	24
6-methyl-3-octanone	9	9	12	6	6	15	12	6	3	19
unknown	31	15	15	10	36	18	26	10	10	31
3-nonanol	11	5	11	0	11	15	11	0	5	22
3-decanol	140	90	170	70	100	210	157	50	90	383
3-decanone	5	5	5	0	5	0	2	0	0	7

APPENDIX 14

Absolute amounts of volatile mandibular gland components in ten M. rugulosa workers.

Compound	Amounts (ng) 1	2	3	4	5	6	7	8	9	10
3-pentanol	2	10	7	4	4	3	2	16	8	21
3-hexanol	12	50	42	30	53	12	79	18	11	11
3-hexanone	2	13	5	7	5	3	4	15.8	18	31
3-heptanol	6	8	15	12	14	42	14	47.0	29	5
3-heptanone	3	19	7	7	15	12	42	7.9	3	6
3-octanol	106	142	147	127	71	104	85	78.9	140	191
3-octanone	230	648	493	294	633	353	589	309.5	141	244
6-methyl-3-octanone	34	84	52	63	75	63	115	57.9	42	42
3-nonanone	9	6	12	21	26	10	13	12.6	26	6
3-decanone	66	9	14	36	19	31	23	37.9	39	33
unknown	12	21	6	15	0	10	13	12.6	0	25

APPENDIX 15

Absolute amounts of volatile mandibular gland components of ten *M. sulcinodis* workers.

Compound	Amounts (ng) 1	2	3	4	5	6	7	8	9	10
3-pentanol	12	6	12	3	12	6	9	16	19	6
3-hexanol	169	314	88	177	339	169	218	137	258	226
3-hexanone	271	129	206	84	329	290	135	258	245	187
3-heptanol	96	96	161	129	371	113	350	452	193	193
3-heptanone	129	96	72	56	153	20	80	72	37	80
3-octanol	1027	1038	1077	583	1045	1635	426	1122	581	484
3-octanone	4586	5103	1989	2106	4974	3501	2997	3268	4354	3423
6-methyl-3-octanol	0	0	7	5	4	3	3	7	0	3
6-methyl-3-octanone	124	159	86	108	287	109	53	154	142	88
3-nonanol	0	5	15	0	15	10	12	25	15	15
3-nonanone	137	92	46	46	132	69	35	68	53	35
unknown 1	0	13	6	0	20	6	6	6	13	6
unknown 2	0	14	14	0	36	11	12	21	14	9
3-decanol	0	14	9	0	9	19	16	19	9	23
3-decanone	192	262	100	95	406	262	246	286	123	248

APPENDIX 16

Absolute amounts of the highly volatile mandibular gland substances of ten M. rubra workers.

	Compound		
	ethanal	acetone	methylpropanal
1	35	2	7
2	95	5	12
3	104	6	18
4	82	6	16
5	64	8	36
6	121	25	58
7	35	4	15
8	89	7	21
9	35	7	10
10	68	16	18

Amounts (ng)

APPENDIX 17

Absolute amounts of the highly volatile mandibular gland substances of ten M. scabrinodis workers.

	Compound		
	ethanal	acetone	methylpropanal
1	268	20	28
2	149	21	37
3	87	12	8
4	80	15	10
5	86	16	67
6	141	30	16
7	98	9	15
8	104	8	10
9	166	13	10
10	91	8	9

Amounts (ng)

APPENDIX 18

Absolute amounts of the highly volatile mandibular gland substances of ten M. sabuleti workers.

	Compound		
	ethanol	acetone	methylpropanal
1	60	6	14
2	100	4	21
3	132	5	33
4	104	10	38
5	132	4	36
6	181	7	30
7	237	20	38
8	284	9	53
9	84	9	21
10	177	7	47

Amounts (ng)

APPENDIX 19

Absolute amounts of the highly volatile mandibular gland substances of ten M. ruginodis workers.

	Compound		
	ethanal	acetone	methylpropanal
1	295	20	26
2	166	16	33
3	63	14	20
4	67	6	14
5	120	26	41
6	49	4	10
7	111	7	27
8	326	13	56
9	318	13	33
10	108	13	29

Amounts (ng)

APPENDIX 20

Absolute amounts of the highly volatile mandibular gland substances of six M. lobicornis workers.

	Compound		
	ethanal	acetone	methylpropanal
1	16	179	15
2	7	86	12
3	11	70	15
4	10	77	11
5	12	150	15
6	18	180	15

Amounts (ng)