

BEHAVIOURAL, CHEMICAL AND ECOLOGICAL INTERACTIONS
BETWEEN WEST AFRICAN ANTS AND TERMITES.

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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BEHAVIOURAL, CHEMICAL AND ECOLOGICAL INTERACTIONS BETWEEN WEST
AFRICAN ANTS AND TERMITES

by Christopher Longhurst

A survey of southern Guinea Savanna at Mokwa, Nigeria revealed the presence of 63 species of ants; 56% were recorded as being predators of termites. The majority of these ants were opportunist predators.

The two obligate termite predators, Megaponera foetens (Fab.) and Decamorium uelense (Santschi) showed specific adaptations in their recruitment and foraging regimes to secure their prey. Macrotermes bellicosus (Sm.) and Odontotermes spp. were the main prey of M.foetens and Microtermes spp. of D.uelense. Both ant species used a scout ant to locate foraging termites. M.foetens scouts then recruited columns of 200-500 ants which were guided by the scout ant to the termites along scent trails originating from the poison gland. D.uelense scouts recruited small groups of ants which attacked and immobilized their prey; a mass recruitment phase then occurred to secure the prey.

The scout ants of M.foetens located foraging termites through chemical cues contained in the protective soil sheeting over their prey. The chemical cues were solvent extractable and originated from the anterior region of worker termites. Chemicals were also involved in the co-ordination of attacks on termites. Ants finding groups of termites release alkyl sulphide pheromones from their mandibular glands which attract sister workers who dig, in response to other pheromones, into the termite galleries.

The specialized predator, M.foetens, took 141 M.bellicosus per square metre per year and 42 Odontotermes spp. per square metre per year. With a termite standing population of 128 M.bellicosus and 260 Odontotermes spp. per square metre, M.foetens can turn-over the worker-soldier population of these two species in 0.9 and 6.2 years respectively. Other Macrotermitinae accounted for only a small proportion of the prey (7 Ancistrotermes, 3 Microtermes and <1 Macrotermes subhyalinus per square metre per year). It was concluded that the efficiency of predation on different termite species was controlled through termite foraging regimes and density, the response of ants to termite soldier defence secretions and the responses of termites to ant secretions.

The repellent responses of termites to ants were partially controlled through glandular secretions from the predator. Successful specialised predators, such as D.uelense, produce non-repellent compounds (aliphatic alcohols) in their mandibular glands, whereas less successful opportunist predators produce compounds (aliphatic ketones, aldehydes and alkyl pyrazines) which are repellent to termites. It was concluded that inter-specific pressures were one of the reasons for the great diversity of exocrine secretions in the Formicidae.

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SECTION 1. INTRODUCTION

1.1 Termites: the Order Isoptera

Termites are hemimetabolous insects thought to be closely related to cockroaches (Blattoidea), and are sometimes referred to as social cockroaches (Cleveland et. al. 1934). Termites live in colonies with a complex social system; there are several castes, each with a different role. In the lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae and Serritermitidae) the workers are not a strictly defined caste; nymphs of various age groups maintain the colony; soldiers, replacement reproductives and winged imagoes are also present (Miller 1969) along with the dealate queen and king who founded the colony. In the higher termites (Termitidae), which constitute 74% of extant species (Wilson 1974), the worker castes are more easily recognised and in many of the Macrotermitinae both major and minor worker castes are recognised, with the major workers responsible for food gathering and the minor workers for building nests and other soil structures (Noirot 1969). The soldier castes in termites are either specialised for chemical defence, with enlarged glands and structures for applying the chemicals (Moore 1963, Quennedey et al. 1973) or for physical defence with enlarged mandibles (in Howse 1971).

In the lower termites digestion of the food, which contains a large percentage of cellulose, is facilitated by flagellate protozoa in the gut. The higher termites all possess gut bacteria to assist digestion, and in addition the Termitinae contain ciliate and amoeboid protozoa, the Nasutitermitinae amoeboid protozoa and the Macrotermitinae utilize external fungi to digest their food (Grassé and Noirot 1959). The Macrotermitinae have an inefficient digestive system but use their faeces to construct combs where symbiotic fungi (Termitomyces spp.) degrade the complex plant polysaccharides; fungal spores and degraded parts of the comb are re-injected, resulting in a high utilisation of injected food.

The higher termites all reproduce by swarming; a large proportion of the colonies energy input goes into the production of male and female winged reproductives. Swarming usually takes place at the start of the wet season. Female reproductives appear to possess a sex attractant pheromone, and call to the males (Nutting 1969) to initiate a tandem pair, which lose their wings and dig into the soil where the colony is initiated. Egg production is prolific, a physogastric Macrotermes natalensis queen producing 36,000 eggs every day (Hegh 1922) and Odontotermes badius 4,000 a day.

1.2 Termites in Tropical Ecosystems

Termites have long been regarded as important pests in tropical agro-ecosystems, and, although quantitative figures on their damage levels are only just becoming available (ODM Report 1977), their importance should not be underestimated. Sands (1973) makes the comment that " because the order Isoptera includes such a wide range of behaviour and feeding habits it is probably true to say that practically every plant used by man has been attacked by at least one termite species at some stage in its growth in some part of the tropics ". Sands (1973) also points out that the subfamily Macrotermitinae includes the most important crop pests in Africa and Asia, the majority of the pest species (in the genera Microtermes, Ancistrotermes, Pseudacanthatermes, Odontotermes and Allodotermes) being predominately subterranean nesters. The fungus growing Macrotermitinae attack the whole range of cash, tree and food crops, including the important African diet foods, yam and cassava (Harris 1961). Studies on damage levels on an experimental farm at Mokwa, Nigeria (ODM Report 1977), in an area where termites are not considered important pests (R.A.Johnson, pers. comm.) revealed the following damage figures in 1975: yam, 2% heavy damage; sweet potato, 2% unmarketable tubers and 12 - 18% of tubers moderately damaged with an effect on storage; and loss in yeild of maize by Microtermes spp., 4 - 10%. Examination of plots of upland sugar cane indicated that 21 - 23% of the stools failed to germinate due to termite attack, mainly by Odontotermes smeathmani (Fuller) and

Macrotermes subhyalinus (Rambur).

Mound nesting termites can be effectively controlled by destroying the nests mechanically, or by mound poisoning (Harris 1961), but subterranean pests require more sophisticated control regimes. Applications of insecticides, especially aldrin and dieldrin, into the soil have been successful where mechanical application methods are available and larger scale cultivation is practised (Harris 1961). Local applications of insecticides as seed dressings (Sands 1973) are also useful as they do not need special application equipment. The most effective and economical insecticides (aldrin and dieldrin) are those which leave long-term residues which may adversely affect other arthropod fauna. Termites themselves are implicated in soil aeration, soil movement and water penetration, and by degrading dead plant material increase carbohydrate turnover (Lee and Wood 1971). In view of the large populations in savanna (3500 m^{-2} , Wood et.al. 1977) the termites are likely to achieve the beneficial effects attributed to them. Persistent use of insecticides on the termite fauna may therefore have deleterious effects on the fertility of tropical soils (Sands 1973). Broadcast treatment with insecticides is also likely to affect the innocuous and probably beneficial soil feeding termites because such a large quantity of soil passes through their guts.

The use of repellent seed dressings might be advantageous in termite damage control programmes. A number of sources of repellents are available, including those from natural sources in plants and animals. Some timbers are known to contain termite repellents and antifeedants; Woolcott (1934) found that coating susceptible timber with tectoquinone from teak prevented further attack by Coptotermes spp. and Floyd (1975) found that the heartwood of Afzelia bipedensis was repellent to Reticulitermes lucifugus santonensis (Feytaud). Howse (1975) and Wadhams (L.J.Wadhams, pers. comm.) have implicated ants, including predatory species, as containing substances repellent to termites which might have a potential for development for agricultural use. In this present study a number of ants have been assayed for compounds repellent to Macrotermitinae so that

generalizations on the types and variety of repellents and the termites responses to them can be made.

1.3 Methods for Bioassay of Repellents

Responses of termites, which are in the main cryptic foragers which shun the light, are difficult to bioassay without disturbing the foragers. The standard method for testing repellents and antifeedants is to impregnate wood blocks with the chemicals being assayed and push these blocks into the soil where termites occur; the attacks of the termites can be quantified by examining the blocks after a period of time (Williams 1973). Unless laboratory colonies are available, the tests with impregnated blocks do not distinguish attacks by different species of termites. Neither can the behaviour of termites as they approach the stimulus be observed with these routine assays, which makes them of limited value when quantitative scores, to discern specific differences in behaviour to repellents, are required. The methods are also of limited use if initially only very small quantities of natural product extracts are available. Laboratory colonies of the lower termites are easily established and Howse (1965) and Floyd (1975) have carried out behavioural and repellency studies on termites foraging from laboratory colonies into observation chambers. The Macrotermitinae are difficult to establish in culture, but can be collected from bait blocks to be bioassayed in observation chambers (5.1.3.).

Electrophysiological techniques, such as electroantennography (EAG), in which the overall slow response of the antenna to chemicals is recorded, has been used by Abushama (1964) and Floyd (1975) for studies on termites and other insects. Compounds found to be active by EAG can be subsequently bioassayed behaviourally to test if the response was that of a repellent. The unavailability of most Macrotermitinae, other than Macrotermes bellicosus (Sm.), in the laboratory precluded the detailed use of EAG in this study. A Gas chromatograph (GC) - EAG system was set up (Appendix C), but

was not developed beyond the initial stages because of lack of material to use on it.

1.4 Microscale Methods for Chemical Analysis

To examine the chemicals that are found in ants, often at levels of less than 100 ng per individual, the analytical techniques used must be especially adapted. Morgan and Wadhams (1972) developed solid-sample gas chromatographic (SSGC) techniques for analysing small quantities of biological material, and various workers (Baker et. al. 1976) have developed an all-glass micro-preparative system to allow the collection of nanogram quantities of compounds in GC effluent fractions. Higgs (1976) and Bradshaw (1976), working in the Chemical Entomology Unit at Southampton University, have modified equipment and defined the conditions for use of instrumentation to analyse small quantities of natural products. A GC - Mass Spectrometer (MS) link-up was set up with a Watson-Biemann all glass separator interface which is capable of obtaining spectra on less than 100 ng of hydrocarbons and less than 400 ng of other compounds. Higgs and Bradshaw also carried out studies on fourier Transform-Proton Nuclear Magnetic Resonance Spectroscopy (FT-NMR) and concluded that in conjunction with the GC micro-preparative system (Baker et.al. 1976), proton NMR spectra could be obtained on 20 µg samples if water was carefully excluded. Apparatus was also set up for ozonolysis (modified from Beroza and Bierl 1966), post loop GC analysis (modified from Bierl et.al. 1969), microscale reactions (modified from Hoff and Feit 1964, Fredericks and Taylor 1966) and solid sample GC on larger biological preparations (modified from Bergström 1963).

1.5 Ants: the Family Formicidae

Wilson (1974) regards ants as the premier social insects; they are wide ranging in geographical distribution with an estimated 1×10^{15} individuals present at any one time. Ants are holometabolous

insects in the order Hymenoptera, and are thus related to the social bees and wasps and the abundant solitary ichneumonid and braconid parasitoids. Ants are thought to have evolved from a tiphioid type wasp ancestor in the mid - Cretaceous period (Wilson et.al. 1967). These authors consider that ants may have originated twice in evolution, to give the 'poneroid complex' (Ponerinae, Myrmicinae, Dorylinae, Leptanillinae and Cerapachyinae) and the 'myrmecoid complex' (Formicinae, Dolichoderinae, Myrmecinae and Pseudomyrmecinae). In the Formicidae there is usually a wingless sterile worker caste, and male and female reproductives. After fertilizing the female, the haploid male dies, and the colony is established by the diploid queen alone (Wheeler 1910). The nesting habits of formicids are diverse. The majority nest directly in the soil; but Oecophylla spp. construct arboreal nests from leaves bound together by larval silk, many Camponotus and Crematogaster spp. build nests of wood carton in trees, and the myrmicine, Cardiocondyla wroughtoni, may nest in cavities left in dead leaves by leaf-mining caterpillars (Janzen 1967).

The ancestors of the present ant fauna are thought to have been predators of arthropods (Sudd 1967), and many extant ants still exploit this source of food; some ants being specialised for certain groups of arthropods, Megaponera foetens (Fab. .) feeds only on termites and Leptogenys diminuta F. Smith feeds only on terrestrial isopods (Fletcher 1971). Some ants (e.g. Messor spp.) take only seeds, others (e.g. Atta spp., Weber 1966) have developed a symbiotic relationship with fungi and others tend only Homoptera, to obtain their honeydew secretions (Way 1966). The majority of extant species have fairly catholic diets (Sudd 1967), taking plant and animal food in a wide variety of forms.

1.6 Ants as Predators of Termites

The importance of ants as predators of termites has long been recognised. Arnold (1915) noted the importance of ponerines, especially Brachyponera sennaarensis (Mayr) and Megaponera foetens

as termite predators. Many observers in the early part of this century, including Ghesquire (1922), Hegg (1922) and Collart (1927), gave preliminary descriptions of the organised raids of M.foetens on termites. Wheeler (1936) collated the known information on the interactions between ants and termites, including observations of his own collected on expeditions to Africa (Wheeler 1922). In the period after Wheeler's synthesis work very little work appeared on the subject of predation of termites by ants until near the beginning of the present decade. Levieux (1966) studied M.foetens in the Ivory Coast, Sheppe (1970) studied predation on termites by ants and other invertebrates in east African savannas and Fletcher (1973) studied ponerines in southern Africa. Bodot (1966, 1967) observed Dorylus spp. as predators of the brood centres of Macrotermitinae and concluded that the large proportion of unoccupied Macrotermes bellicosus mounds in her study area in the Ivory Coast were due to predation by Dorylus dentifrons Wasm. Maschwitz and Mühlenberg (1975) have studied recruitment of Leptogenys ocellifera in Sri Lanka, but have not quantified predation of termites and other arthropods and annelids by this species.

Two laboratory studies on predation on termites by ants have also appeared in the past decade. Smythe and Coppel (1964,1973) studied Solenopsis molesta (Say) as a brood predator of Reticulitermes flavipes (Kollar) and Beard (1973) recorded the destruction of colonies of R.flavipes by Lasius alienus (Foerster), L.umbratus (Nylander) and Tetramorium caespitum (L) under experimental conditions. Beard also recorded opportunist predation on R.flavipes by Camponotus spp. and Aphaenogaster spp.

Despite the wealth of observations of interactions between ants and termites, no attempts have been previously been made to quantify these interactions. It became apparent from preliminary observations in Guinea savanna at Mokwa that predation on the Macrotermitinae, one of the most important groups of Ethiopian termite pests of agriculture (Sands 1973), was an important factor influencing the behaviour and ecology of this subfamily. With the availability of population data and biomass information (ODM Report 1977, Wood and

Sands 1977), detailed studies of predation by M.foetens and the myrmicine Decamorium uelense (Santschi) were carried out.

1.7 Other Behavioural and Chemical Interactions between Ants and Termites

Although considerations of the repellent responses of termites to ants (1.3) may give some insight into the factors governing the efficiency of predation, it is likely that other factors are also involved. These are likely to include communication between sister workers to improve the co-ordination of predation, the response of predatory ants to termite soldier defence secretions and the responses of ants to termites and their artefacts, such as nest structures and soil enclosed surface foraging runways. These factors have been investigated by following up field observations of pertinent interactions.

SECTION 2. THE ANT AND TERMITE FAUNA OF MOKWA

2.1. Introduction

In this section the ant and termite faunas, principally of primary savanna woodland, are described. Information is given on the biology, food preferences and colony density of ants, collated from field notes compiled over six visits to Mokwa, during September - November 1974, April - May 1975, October - November 1975, April - May 1976, September - November 1976 and April - May 1977. Additional notes are provided on the termites of major importance in the southern Guinea savanna at Mokwa. These are compiled from ODM Termite Team data (Wood et.al. 1977, ODM Report 1977, Collins 1977b) collected from 1974 onwards, and from personal observations. The information given in this section will help to clarify the interactions of the ants and termites when they are discussed in later sections on predation, chemical interactions and behaviour.

2.2. Study Area and Sampling Methods

2.2.1. The study areas at Mokwa, Nigeria.

Mokwa is located in the southern Guinea savanna of West Africa (Fig. 2.1). Five major biotypes can be recognised. In Primary Savanna Woodland Burkea africana Hook, Detarium microcarpum Guill. et Per. and Afzelia africana Sm. are the dominant cover trees (Collins 1977a). There are also dense stands of the grasses Andropogon gayanus Kunth. and Eragrostis tremula (Hochst ex. Steud) which reach heights of up to three metres. Local bush fires destroy these grass stands in January and February. Primary woodland has never been cultivated or cut for firewood. Secondary savanna woodland has been lightly cultivated by local farmers prior to 1948 with various systems of land use (ODM Report 1977).

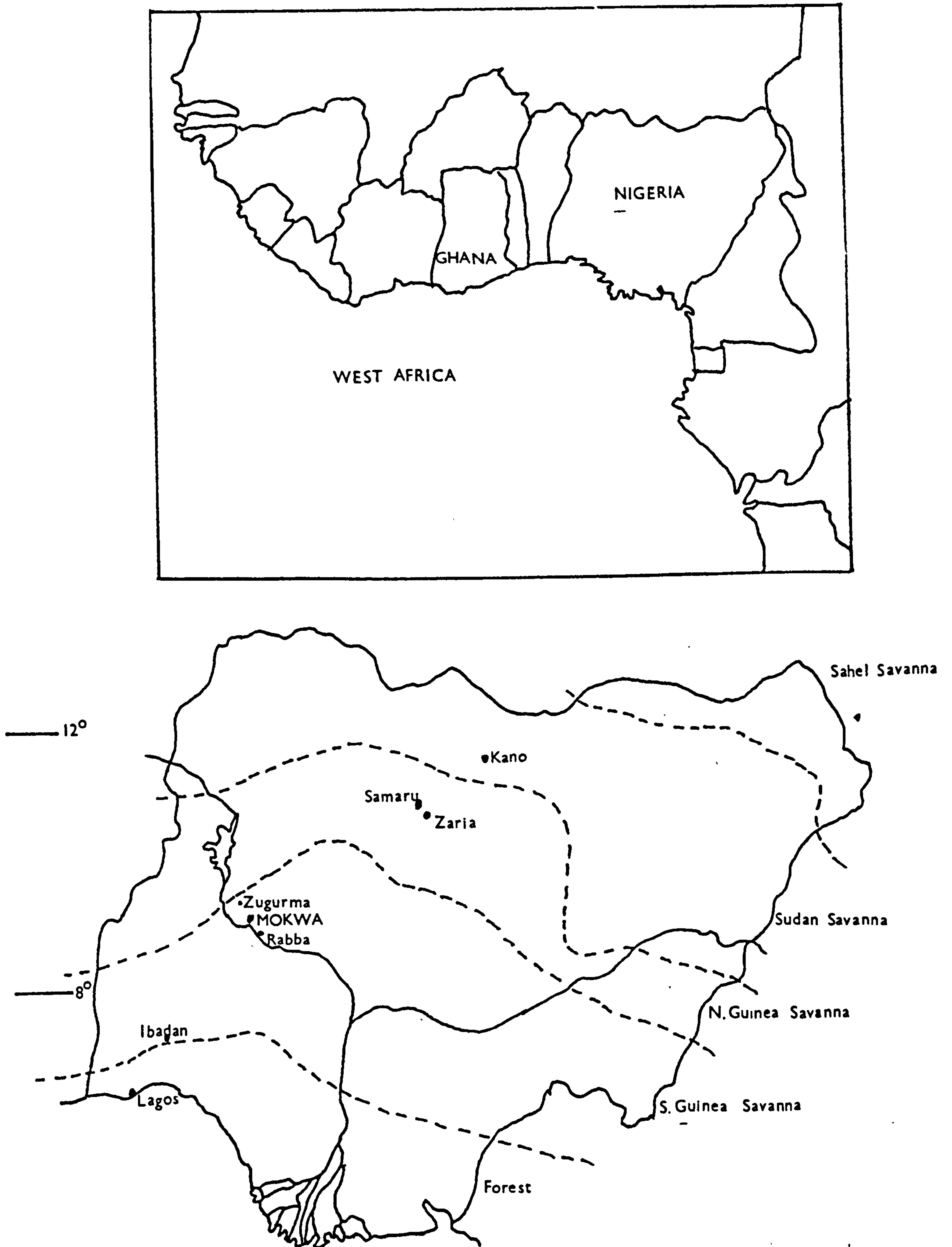
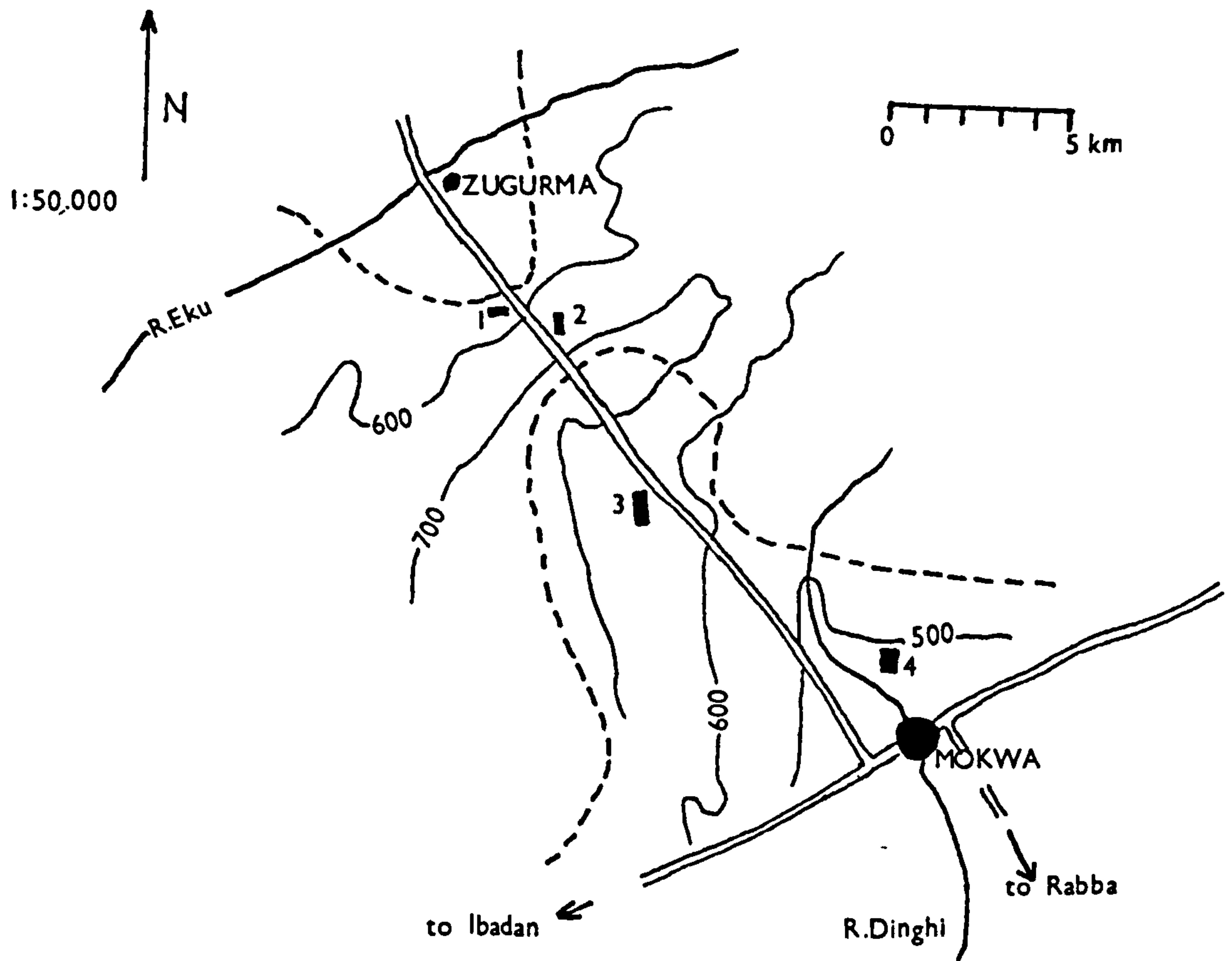


Figure 2.1. Nigeria with vegetation zones
Inset (above) West Africa



- cultivation boundary
- 1 Megaponera study area
 - 2 Macrotermes study area
 - 3 Termite Team Experimental Farm
 - 4 Parkland

Figure 2.2. Map showing the location of study areas north of Mokwa

Cultivated woodland has been cultivated by local farmers manually on a 'shifting' basis of 3 - 4 years, followed by 5 - 8 years 'bush fallow'. Parkland is the vigorously maintained area around areas of human habitation, with short grass and a few large, often introduced, trees. Intensively cultivated land is farmed with agricultural machinery and sophisticated farming methods.

Collections of ants were from primary savanna woodland, parkland and the intensively cultivated farmland areas. The areas collected were parkland at kilometre 1.5 (Fig 2.2), primary savanna woodland at kilometre 17 and the Termite Team Experimental Farm at kilometre 3.

In the primary savanna woodland area at kilometre 17 two adjacent one hectare sites were selected and marked out with stakes. Collections of ants and nest density studies were made on these areas, as well as in the study area used by Collins (1977b, area 2 on figure 2.2) to work on Macrotermes bellicosus at kilometre 15. The predation studies (Section 4) were carried out on the sites at kilometre 17.

The mean annual rainfall, monitored at the Agricultural Research Station, kilometre 7, is 10,807 mm. Rainfall greater than 100 mm per month is recorded between April and October, with peaks in July (1905 mm) and September (2953 mm). Dust laden winds (harmattan) blow from the north between December and February, coinciding with low night temperatures (ca. 12°C). The mean maximum temperature at the hottest part of the year (March - April) is 39°C.

2.2.2. Sampling methods

An indication of the species diversity of the ant fauna was obtained by hand sampling throughout the study periods at Mokwa. Nest density of Megaponera foetens and Paltothyreus tarsatus (Fabre.) were obtained by intensive searching of the one hectare study areas and are described in 4.2.1a. The nest density of Decamorium uelense was determined by nearest neighbour techniques (Blackith 1958) and

is described in 4.2.2a. The density of the other ground nesting ants with distinctive nest sites was determined by counting in a sample area. A sample strip 100 m long and 25 m wide was marked out through the centre of a study area at kilometre 17, the long axis running in an east - west direction. This area was carefully searched on three visits to Mokwa, in April 1975, April 1976 and October 1976. Any nests discovered were noted and from the figures obtained an estimate of colony density per hectare was obtained. Observations were easiest during the April sampling as the long savanna grasses had been burned down earlier in the year by bush fires. With the start of the rains and subsequent excavation activity of the ants, nests were readily observed on the bare ground. Estimates of the nest density of Pheidole sp.A, Crematogaster sp.E, Anochetus sedilloti Emery and Bothroponera soror Emery were obtained in this manner. The density of tree-dwelling Crematogaster spp. was obtained by counting the number of trees in the sampling area which had large numbers of ants on them carrying food up into the tree. The tree canopy is rarely continuous in the primary savanna woodland at Mokwa, and the nests did not appear to occupy a number of trees as they do in denser forest areas (Majer 1972, Leston 1973); it is therefore likely that ants would carry food only up the trees they were nesting in.

2.3. Ants of the Southern Guinea Savanna at Mokwa

2.3.1. Introduction

Formicines are well represented in the Ethiopian region and a number of field workers have published monographs on various areas (e.g. Arnold 1915, Wheeler 1922). Recently there have been two West African studies in which the ant faunas were comprehensively examined. Studies on Ghanaian cocoa farms by Leston, Majer and their co-workers (e.g. Leston 1971, 1973, Majer 1972) have established the identity of three dimensional mosaics and identified the dominant and sub-dominant ants in these mosaics. Studies by a

Ministry of Overseas Development (U.K.)/International Institute of Tropical Agriculture Team at Ibadan, Nigeria (ODM Reports 1974, 1975) have examined the ant fauna in the context of faunal changes after clearing secondary regrowth forest and the treatment of these cleared plots with insecticides. In the studies at IITA, Ibadan over fifty species of ants belonging to 44 of the 96 African genera were found. Mokwa, where this present study was carried out is in southern Guinea savanna (Fig. 2.1) and a different complement of ants might be expected, contrasting to the forest and forest regrowth fauna of Ghana and IITA Ibadan.

Identification of the ants collected at Mokwa was made difficult by the confused state of the taxonomy of many genera. This is partly a result of earlier workers creating subspecies, races and varieties from a knowledge of the ants of only a small area, with little regard to the status of the species throughout its range. These faults are being rectified. Bolton (1973a, 1973b, 1974, 1975 & 1976) has published revisions of a number of genera. Unfortunately, the important and ubiquitous genera, Crematogaster, Pheidole, Bothroponera and Camponotus are still in urgent need of revision. In this present study ants have not been ascribed to species without consultation with Bolton (ant taxonomist, British Museum, Natural History) and have, in the absence of specific identification been ascribed a letter code, e.g. Crematogaster sp.B. Specimens of ants have been deposited in the British Museum, Natural History. The code numbers following the ants in 2.3.2 may be quoted if reference ants in the museum collection need to be examined.

2.3.2. Notes on the biology of savanna ants

2.3.2.1. Ponerinae

Tribe: Ponerinii

Brachyponera sennaarensis (Mayr). 9/2/D; 12/5/C; 18/3/C; 11/5/A;
8/5/A.

This small black ant is found foraging throughout the day and night. It is more common in the open parkland at kilometre 1.5, where a nest density of ca. 5 per hectare is reached. In this area it is often found foraging inside human dwellings. In the undisturbed primary savanna (Km 17) the nest density is approximately 1 per hectare, and here it is found only in the more open areas of bush. B.sennaarensis is the only ponerine found abundantly on the Termite Team Experimental Farm.

The nest system is subterranean and diffuse; entrance holes from one colony spreading over three square metres, as estimated from the aggressive response of ants from different colonies to each other in petri dishes. Complete nests have never been successfully excavated, and workers and brood, brought back to Southampton have not survived more than a few weeks. Swarming has been observed at the end of the wet season in October, with both males and females leaving the nest together.

B.sennaarensis has catholic feeding habits, taking dead animal material, small seeds and live insects, including termites (4.2.3.).

Bothroponera soror (Emery) 27/4/A; 21/5/B; 11/4/C; 3/4/A; 9/2/C

Superficially this ant resembles B.sennaarensis, but closer examination reveals that the petiole is a thick node and that the ant is 1 mm longer than Brachyponera. It is more abundant in the primary woodland savanna (nest density 8 per hectare) than in the open areas at kilometre 1.5.

B.soror nests in the ground, often among the roots of small shrubs. The nest system is fairly discrete, a typical nest extending downwards 0.6 m. From 120 - 207 workers have been found in the six nests excavated. Alate males have been found during both April and October excavations, but alate queens have not been recorded. This ant keeps well in culture in perspex nest boxes (9.7) when

fed on Drosophila larvae and Reticulitermes lucifugus.

B.soror takes a large proportion of soil surface arthropods (4.2.3.), probably both dead and alive; but has not been observed taking vegetable material as B.sennaarensis does. Tandem running (Maschwitz et.al. 1974 for Bothroponera tesserinoda) has been observed as a method of worker recruitment to leaf litter rich in foraging arthropods.

Bothroponera sp.B (? silvestri) 28/4/C

This ant has similar habits to B.soror, although its nest system is more diffuse and shallow. It is a rare ant, with a nest density of probably less than 1 per hectare.

Paltothyreus tarsatus (Fab .) 28/4/B

This large black ponerine ant is the "stink ant" of the African savannas. It is common throughout the primary savanna (4 colonies per hectare) but has not been recorded from parkland and appears to be rare on the Experimental Farm.

The nest system of P.tarsatus is very diffuse and consequently excavation is difficult, and an intact colony has never been obtained. Foraging occurs throughout the day and night, and recruitment of small groups (8-14 ants) may occur to damaged termite mounds, areas of intense termite foraging or to millipede cadavers. Usually single foragers are encountered taking living and dead animal material, especially millipedes and termites (4.2.3.) and occasionally large seeds. Sudd (1967) has also recorded P.tarsatus taking the pericarp of fallen oil - palm nuts.

Megaponera foetens (Fab .) 20/1/A

The habits of this obligate termite predator are described in detail in 3.3. M.foetens is absent from parkland and cultivated areas but in primary savanna attains a density of 9 colonies per hectare. M.foetens utilizes a number of nest site types; of 73 nests examined 37 were in deserted Macrotermes bellicosus mounds, 20 directly in the ground under bushes and trees over 1 metre high, 6 in the ground under bushes less than 1 metre high, 7 directly in the ground and 3 in the deserted mounds of Trinervitermes spp.

M.foetens is usually regarded as a dimorphic ponerine (Arnold 1915, Bolton 1973). Measurements of the scape length, head width across the eyes and head length of 384 workers from a complete colony showed that this ant exhibited monophasic allometry, confirming Wilson's (1953) earlier measurements on a smaller sample of 23 ants. A bimodal distribution of individuals was found, with major workers (head width across the eyes 2.40 - 2.96 mm) predominating as 52% of the colony, the minors (1.39 - 1.99 mm) 25% and the rest forming intermediates (Fig. 2.3). Hand sorting of ten other colonies revealed a similar distribution of castes, with good agreement between percentages from head measurements and hand sorting. (Table 2.1).

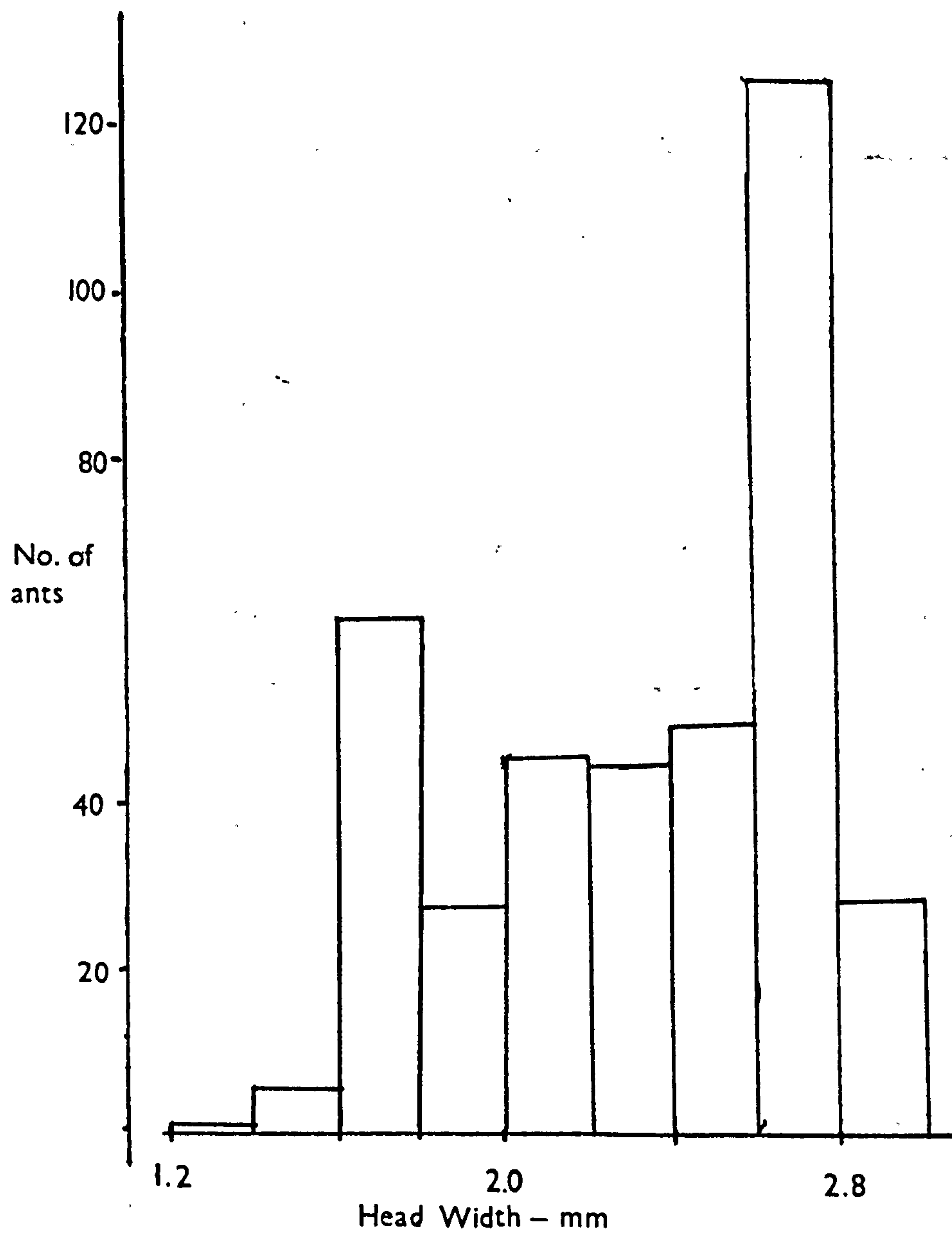
Tribe: Odontomachinii

Anochetus sedilloti Emery

24/5/A.

Anochetus is a tropicopolitan genus closely related to Odontomachus; Bolton (1973a) regards Anochetus as "at best a very weak genus, hardly if at all separable from Odontomachus". A.sedilloti is found both in primary savanna woodland and parkland, with a nest density in the savanna of 12 colonies per hectare. A.sedilloti nests in hard packed soil, with the nest chamber 0.2 - 0.6 metres below the soil surface. Colonies are small, consisting of 20 - 70 workers (5 nests examined). Alate males and females were found in nests excavated in April, and swarming takes place in the evening

Figure 2.3. Distribution of individuals of a colony^a of Megaponera foetens workers^b.



^a Colony 29 (Table 2.1)

^b Majors 2.40 - 2.96 mm; Intermediates 2.00 - 2.39 mm;
Minors 1.39 - 1.99 mm.

Table 2.1 Numbers of worker ants and their size classification
in Megaponera foetens colonies^a

Colony number	Major workers	Inter- mediate workers	Minor workers	Totals
2	364	218	146	728
5	322	153	140	615
6	249	190	150	589
12	213	77	67	357
17	279	160	128	567
28	141	106	73	320
29	210	93	81	384
42	332	159	197	688
54	234	125	98	457
64	359	266	231	856
65	239	117	76	432
29 ^b	201	89	94	384
Mean percent. composition: hand sorting	50%	27%	23%	
Percent. composition: measurement	52%	23%	25%	

^a hand sorting of workers unless otherwise stated (b)

^b worker classification determined from the maximum head width across the eyes; Figure 2.3

at the start of the wet season.

Anochetus is an important predator of small arthropods in leaf litter; its slender jaws with three teeth at their tips may assist in the picking out of its prey from interstices in the litter. Its role as a termite predator is described in 3.2.2. and 4.3.3. This ant is of further interest as it appears to mark the nest entrance with a secretion which is repellent to the common Pheidole sp.A, which has been observed attacking A.sedilloti and relieving it of its prey.

Anochetus sp.A

27/5/C

This is a larger ant than A.sedilloti which in this present study has been found only in primary savanna woodland. The nest density is estimated to be one colony per hectare, and although the nests are found in the hard-packed soil in similar conditions to those of A.sedilloti, the nest chambers are much deeper, 0.5 - 1.2 metres below the soil surface. In the two nests excavated 29 and 43 workers were found; it is possible that more ants from the same colony are found at depths greater than 1.2 metres which were not excavated. The foraging habits of Anochetus sp.A are probably similar to those of A.sedilloti, although species A has never been recorded foraging after dark (ca. 1900 hours). Anochetus sp.A may also forage underground, as single foragers have been found in sample pits, well away from known nests.

Odontomachus troglydytes Santschi

25/5/A; 21/5/A.

This ponerine is not found in the drier savanna areas to the north of Mokwa, but is found in the riverain area at Rabba, south of Mokwa (Figure 2.1). Nests are found either in the soil at the base of trees or in rotten logs which contain large numbers of workers and brood.

Only a few casual observations have been made on this ant, it has been recorded bringing back Ancistrotermes cavithorax, Amitermes evuncifer and Noditermes, along with other small arthropods, to its nest.

Other Ponerinae

The following ponerines have been recorded as single foraging workers in 0.5 metre squared litter samples from the study area in primary savanna woodland:

Amblyopone pluto Gotwald and Lévieux.

Mesoponera cafferaria F.Smith.

2.3.2.2. Dorylinae

Specimens of Dorylus species have been deposited with W.H.Gotwald Jr. (Utica College, Syracuse, New York, USA), who is currently revising the subfamily.

Dorylus (Rhogmus) sp.A 19/3/A; 4/4A/A; 23/5/D; 13/1/A.

This subterranean army ant has been encountered in all parts of the Mokwa area. Nothing is known of the population density or foraging cycles of this doryline. It has been encountered in sample pits, where it was attacking Microtermes sp.A on a fungus comb and a nest of Crematogaster sp.E (one observation of each). In April 1976 an attack on Odontotermes pauperans (Silvestri) fungus combs in a deserted Macrotermes bellicosus mound was observed. The ants emerged from the soil at five points about 0.2 metres from the mound, and on reaching the mound walls, climbed up the sides of the mound, entering through weak points in the walls. Destruction of the mound wall revealed O.pauperans

fungus combs which were being attacked by the ants. Dorylus sp.A has also been observed driving earthworms out of the soil and killing them. (P.A.Johnson pers.comm., Pers.observations).

Dorylus (Dorylus) sp.B A6/1/A.

This doryline is known only from alate males caught at light traps in October (1974, 1975).

Dorylus (Anomma) sp.C 25/5/D.

This black doryline is found crossing clearings in the primary savanna, often under a protective soil covering. No observations have been made on its predatory behaviour. Gotwald (1974) has examined the predatory behaviour of Dorylus (Anomma) nigricans Illiger in Ghana, where the major prey was insects, especially Orthoptera, Hymenoptera and Coleoptera. D.(Anomma) gerstaeckeri Emery preyed mainly on annelids.

Aenictus sp.A 4/5/A.

This monomorphic doryline is usually encountered in columns moving in single file over the savanna floor whilst in the process of emigrating. Although Aenictus spp. have previously only been recorded as predators of the immature stages of ants (Gotwald 1976) one observation has been made of an attack on Microtermes sp.A. The ants emerged from a hole in the ground and ascended a fallen tree trunk where they encountered a gap in the edge of some termite protective soil sheeting (2.4.3). Microtermes workers (mainly minors) were carried by returning worker ants. Another branch of the same colony was observed to attack a Pheidole sp.A colony from which immature ants were taken.

Aenictus sp. B

25/6/C

This smaller Aenictus species has been encountered once while it was emigrating, it was carrying brood and moved in single file like Aenictus sp.A.

2.3.2.3 Myrmicinae

Tribe: Crematogastrini

Crematogaster sp.A

26/6/C

Crematogaster sp.A is an aboreal tree-nesting ant which forages both aboreally and on the ground. The nest density in primary savanna is 4 colonies per hectare; this species has not been recorded from cultivated land or parkland around Mokwa. Foraging occurs throughout the day. Records made at Mokwa indicate that its prey includes termites taken from leaf- and wood-litter and from under soil sheeting on the ground and on trees.

Crematogaster sp.B

26/5/B

Nests of this species are found in rotten wood and large seed pods on the ground. Colonies are small, 40 - 80 workers with queen and brood were found. No evidence was found that these groups were part of a polycalic system. Colony reproduction may be by budding as small colonies of a queen, 10 - 15 workers and brood have been found in abandoned termite baits (5.1.2) and seed pods on three occasions. The nest density is at least 24 colonies per hectare.

Crematogaster sp.C (?bequaerti)

31/6/E; 22/5/A

This ground-nesting species is common in cultivated areas, and in the primary woodland savanna it attains a density of eight colonies

per hectare. The colonies appear to be single nest systems consisting of 50 - 200 workers. Swarming occurs in May and June and a large number of alates have been caught on sticky boards laid in the Experimental Farm (R.A. Johnson, pers. comm.). Foraging occurs throughout the day and into the early evening.

Recruitment to foraging termites has been observed; and this species appears to have a specialised foraging behaviour when preying on termites. When termites are located in wood, plant stems or bait blocks, the foraging worker ants return to their nest to recruit additional workers which invade the foraging area of the termites. Observations in perspex assay chambers (Fig. 5.1) indicated that the termites are not alarmed by this species of Crematogaster, consequently a raid could continue until all termites had been seized. The termites are not stung by the ants but are seized alive in the ants mandibles and carried back to the nest. This ant also has other food sources; foraging has been observed to carrion and plant juices and Homoptera are tended on grasses and shrubs.

Crematogaster sp.D

26/1/A.

This aboreal Crematogaster species is found foraging on trees; it may also descend to the ground and forage. It is found in the primary savanna, cultivated areas and parkland. The density in savanna woodland is estimated to be 8 per hectare. No observations have been made on this ants feeding habits.

Crematogaster sp.E

25/6/A.

This ground-nesting Crematogaster is the most abundant species in this genus in the primary savanna and in cultivated areas, but it is rare in the parkland at kilometre 1.5. The nest system is polycalic which has made assessment of the colony density difficult, in

primary savanna the density is estimated to be 12 colonies per hectare. Nests are more common in open areas, especially at the edge of drainage channels and the more permanent paths. Nest entrances are at the bases of shrubs and large clumps of savanna grasses. Several hundred workers are found in each nest. Alates are present in April and May and have been collected at light during these months after rain.

The ants forage throughout the day, even in bright sunlight. They explore the leaf litter for food, as well as climbing small shrubs and grasses where Homoptera are tended under protective coverings. Termites are taken when encountered by single foragers but no recruitment has been observed to foraging termites.

Crematogaster sp.F

4/6/A.

This species builds carton nests on savanna trees. It is not common and did not occur in the study area at kilometre 17. Observations suggest it to be a general scavenger of animal and plant remains. Termites are also taken when encountered, but no recruitment to them has been observed.

Crematogaster sp.G

ZR1

This aboreal tree-nesting species is uncommon and no observations have been made on its habits.

Tribe: Meranoplini.

Meranoplus inermis Emery

16/2/A; 16/5/A.

Meranoplus nests directly in the ground, usually at the base of trees. It is not a commonly encountered species because of its

cryptic habits. Only two nests have been encountered; one in parkland and the other in the primary savanna woodland, although single foragers have been encountered in litter samples in the primary savanna. The nest in savanna was approximately 50 mm below the soil surface and contained ca. 170 workers with brood. The colony only survived a few days in culture although it was presented with a variety of food.

Foragers are found throughout the day and night and are primarily predators of small arthropods, including Microtermes spp.

Tribe: Myrmecinni

Atopomyrmex mocquerysi André

11/3/A.

This is an aboreal species nesting in tall trees in the parkland, cultivated areas and savanna. Foragers have been seen on the ground taking dead insects. Wheeler (1936) reports that this species attacks foraging Megaponera foetens and relieves them of their prey; this behaviour has not been observed in the savanna at Mokwa.

Tribe: Myrmicariini.

Myrmicaria eumenoides Gerst.

19/1/A.

Myrmicaria is very common in the parkland at kilometre 1.5 and in the secondary woodland and bush fallow surrounding the Experimental Farm. It appears to be an ant of cleared savanna, and counts in the parkland revealed that densities of 16 colonies per hectare were reached in some areas. In the primary savanna it is a locally abundant ant, especially in areas which have been subject to some disturbance. In the study area at kilometre 17 no nests

were found, although two nests were found within 400 metres of the area.

This myrmicine forages mainly throughout the day, and is abundant even in bright sunlight. It is essentially a scavenger on animal and plant remains on the ground, but occasionally climbs grasses and shrubs to take plant juices. It has not been observed tending Homoptera. M.eumenoides takes termites when they are encountered; it has been observed breaking open the protective soil sheeting of Microtermes spp. and Odontotermes smeathmani and securing a few termites before they have retreated into their galleries (3.2.1)

The nest is made directly into the soil, usually at the base of a shrub. The nest chambers are found 30 - 600 mm below the soil surface, the queen usually being in one of the lower chambers. Foraging colonies contain 50 to several thousand ants. The crater nests of the larger colonies are surrounded by a pile of soil up to 50 mm high and 1 m in diameter. Emigration has been observed in large colonies of this ant twice; in both cases colonies transferred to larger deserted nest sites. Arnold (1914) has also observed nest-changing habits in this ant.

Tribe: Pheidolini.

Messor regalis Emery

19/1/B

This polymorphic myrmicine is abundant in primary woodland savanna and bush fallow areas around cultivated land. In the primary savanna a density of 4 colonies per hectare is attained. This ant is a seed harvester, and foragers may travel 20 - 30 metres to obtain seeds. The nest is made directly in the soil, the brood chambers being 50 - 200 mm below the surface. Seeds are stored in chambers, spheres of 20 - 50 mm diameter, at a similar depth but peripheral to the brood chambers.

Messor sp.B

27/5/B; 15/1/A; 9/2/A; 2/5/A; 30/4/C.

This ant is smaller than Messor regalis, the largest workers being smaller than the smallest workers of M.regalis. It is found in similar habits to M.regalis but appears to take smaller seeds. The nest is usually found in harder packed soil than that of the previous species; the density is estimated to be 8 colonies per hectare.

Pheidole sp.A

29/6/B; 29/6/C; 29/6/D.

Pheidole sp.A is the most abundant ant in this genus in the primary woodland savanna. It is also found on cultivated land. The nest system is polycalic, usually with groups of 3 - 4 nests approximately 0.4 metres apart. The overall density is 16 colonies per hectare. The brood chambers are over 0.5 metres below the surface of the soil; there is usually a single tunnel connecting these with the soil workings at the surface; the depth of the brood chambers may ensure colony survival in agricultural areas.

Foraging occurs throughout the day and night, and a wide variety of food is taken. Grass seeds are harvested and animal and plant materials are also taken. This Pheidole species is an opportunist predator of termites, observation of a nest for a few minutes will usually reveal worker ants returning with termites.

Pheidole sp.B

11/6/A; 27/6/A

This ant is more common in parkland than the primary savanna, where the nest density is probably less than 1 per hectare. Pheidole sp.B forages throughout the day and night. Seeds are rarely harvested and its nest is not characterised by the piles of seed husks which mark those of Pheidole sp.A. Dead animal and vegetable material is taken, but it has not been found taking termites.

Pheidole sp.C

12/5/A

The minor workers of this Pheidole species are only 1 mm long. They are only rarely encountered in primary savanna woodland or parkland where the species seems to be primarily a harvester of small seeds. Colonies are small (60 ants) and the nest chambers are found 20 - 150 mm below the surface of the soil. Nests are difficult to locate and consequently no estimate of nest density is available.

Pheidole sp.D

24/6/C.

The minor workers of this species are superficially similar to Pheidole sp.A, but the major workers are smaller (ca. 4mm shorter) with orange-brown heads which contrast with their brown thorax and gaster; the major workers of Pheidole sp.A are the same brown colour all over. Pheidole sp.D has been recorded only occasionally from primary savanna; no observations have been made on its feeding and foraging habits.

Tribe: Solenopsidini.

Monomorium bicolor

25/4/B; 16/2/A; 13/5/G.

This is the most abundant Monomorium species to be found in the primary savanna woodland, parkland and cultivated areas. M.bicolor nests directly in the soil, the nest chambers being 50 - 400 mm below the surface. In the primary savanna the density of this species is 16 colonies per hectare.

M.bicolor forages throughout the day and night and is mainly a scavenger on decaying animal and plant material. Small arthropods, including termites, are taken when encountered; the termites are killed with the sting.

Monomorium pharaonis (L.)

19/5/A

M.pharaonis is found in primary savanna, parkland and cultivated areas. It is not a dominant ant in human habitations in the parkland as might be expected from its pest status (Winteringham 1973); in these areas Tapinoma melanocephalum (Fabre.) and Pheidole sp.B are the most common small ants. M.pharaonis nests directly in the soil or under stones or pieces of fallen timber. No information was obtained on nest density or foraging, the small size of this ant may preclude it from preying on even the smaller Microtermes spp.

Monomorium sp.C

30/6/B.

This small black Monomorium is found nesting in the walls of Macrotermes bellicosus (primary savanna) and M.subhyalinus (Rambur) (parkland) mounds. It has not been encountered in leaf-litter samples but has been found on the fungus combs of M.bellicosus and M.subhyalinus and the food stores of M.bellicosus, it is therefore assumed to prey on the early instars of termites, their eggs or small termitophiles in a similar manner to Carebara spp. (in Wheeler 1936).

Monomorium sp.D (scabriceps group) 29/4/B; 6/3/A.

This polymorphic Monomorium species, belonging to the scabriceps group, was incorrectly referred to as Sylophosis sp.A in an earlier research report (Longhurst 1976). This species was found nesting in termite mounds, decaying wood and large seed cases. Possibly two varieties or species occur, one in termite mounds, where it is intimately associated with the termites, and the other free living. Observations on ants in perspex assay chambers (Fig. 5.1) indicated that the ants could kill termites larger than themselves with their stings, and that the cadavers of Microtermes sp.B workers and those of Odontotermes smeathmani (Fuller) and M.bellicosus minor workers could be carried by one ant.

Colony size is small, 50 - 100 ants being recorded in M.bellicosus and Trinervitermes spp. mounds, with fewer (20 + queen and brood) in the hard packed soil around a fungus comb of Microtermes sp.A.

Monomorium spp.E & F

31/6/D & 31/1/B2.

These two species have been recorded from leaf litter samples from primary savanna, nothing is known of their habits.

Carebara sp.A

10/2/D.

Carebara is a genus of ants recorded mainly as inhabitants of termitaria. The queen is up to 5000X the volume of the minute workers (Wheeler 1936) who are assumed to prey on the workers and brood in the termite nest. This ant has been recorded once in the primary savanna, from the hard packed soil below the soil surface level of a living M.bellicosus mound. Several hundred workers were taken for bioassay (5.1).

Tribe: Tetramoriini.

Decamorium uelense (Santschi)

13/5/B

D.uelense has only been found in primary savanna woodland where the nest density is ca. 11 per hectare (4.2.2a). This species is a termite predator with specialised foraging and recruitment regimes to allow it to secure its prey (3.4). The nest is found in the soil at a depth of 0.3 - 0.8 metres; the colonies are small with 30 - 120 workers and brood. No alates have been recorded during excavations in either November or April/May.

Tetramorium sericeiventre (sp.A)

29/6/A; 11/4/A.

The workers of this ant bear a superficial resemblance to the

minor workers of Pheidole sp.A on whom they prey. T.sericeiventre has only been found in primary savanna woodland where a nest density of 4 per hectare is attained. The nest is made directly in the soil, with the brood chambers 0.1 - 0.5 metres below the surface. A large number of de-alate queens were present in the nests, and these participated in foraging on the surface. Alate females were present in April, and swarming occurred at the start of the rains.

This species forages throughout the day, it has been recorded as preying only on Pheidole species, both major and minor workers. Usually single foragers are taken, but if a Pheidole recruitment trail is located, T.sericeiventre workers are recruited to the trail and attacks on the foraging parties and nests of Pheidole occur.

Tetramorium guineense Fab .

24/6/A.

This species is found in parkland, cultivated areas and primary savanna. In the primary savanna the nest density was 4 per hectare. The nests are made directly into the soil, the brood chambers being 0.3 - 0.6 metres below the surface. Up to 250 workers and de-alate females are present, the de-alate females foraging on the surface in a similar manner to those of T.sericeiventre. Alate females are found in the nest in April and May at the start of the wet season.

T.guineense has a catholic diet, taking dead animal and plant material and live arthropods, including worker and alate termites.

Tetramorium angulinode (Santschi)(sp.C)

21/4/C

T.angulinode nests directly in the soil, both in parkland and the primary woodland savanna. The nest chambers are 40 - 100 mm below

the soil surface, and 200 - 300 workers are present.

This Tetramorium species forages in the leaf-litter where it secures small arthropods; termites are included in its diet.

Tetramorium sp.D

15/5/E; 13/5/I.

This species resembles T.angulinode in appearance, and Bolton (B.Bolton, pers.comm.) places it in the angulinode group of Tetramorium. The colonies are small, of 20 - 40 ants, and the brood chambers are found 20 - 60 mm below the surface of the soil in the parkland. Its habits are not known but probably resemble those of T.angulinode.

Tetramorium sp.E

28/4/A.

This species, which is also a member of the angulinode group has been found in litter samples collected in primary savanna.

Tetramorium sp.F

22/6/A.

Tetramorium sp.F has been found on one occasion from a living mound of Macrotermes bellicosus in the primary savanna woodland. A small chamber in the termite mound wall contained 35 workers and brood; other workers were found on the termite fungus comb.

Tetramorium termitobium

24/6/B.

This species has been recorded from mounds of M.bellicosus in the primary savanna, and also from mounds occupied by Promirotermes sp., Cubitermes spp, and Noditermes sp. in riverain areas near Rabba and Zugurma (Fig. 2.1). Colonies were small, consisting of 20 - 40 workers with brood. This species probably preys on the termites in whose mounds they reside.

2.3.2.4 Dolichoderinae

Tribe: Tapinomini

Tapinoma melanocephalum (Fab .)

23/1/A.

This ant is primarily a parkland/human habitation species and is a common house ant. T.melanocephalum is less common in the primary savanna woodland where it makes nests in rotten wood in the soil. Foragers are encountered in the leaf litter. Few observations have been made on the habits of this species, although it has been recorded as taking termites in the parkland at kilometre 1.5.

Technomyrmex sp.A

Single foragers of this species have been taken from leaf litter samples in the primary woodland savanna.

2.3.2.5 Formicinae

Tribe:Camponotini

Camponotus maculatus (Fab .)

C/A/1

Camponotus maculatus is common throughout the parkland, cultivated areas and savanna. It forages at night and occasionally in the early morning after rain if it is still overcast; exposure to bright sunlight usually proves fatal. No measurements of nest density are available for the primary savanna where it nests directly in the soil or in deserted termite mounds.

This formicine is a catholic feeder, taking dead plant and animal material; it is also a common household ant, taking sugar and other sweet foods. It has been recorded taking both foraging

termites as well as workers and soldiers of M.subhyalinus and M.bellicosus engaged in mound building extensions and repairs.

Camponotus sericeus (Fab .)

C/B/1.

This day foraging ant is easily recognised by the gold pubescence on its gaster. It is found in all areas at Mokwa, and in the primary savanna the nest density is 24 per hectare. Nests are made directly into the soil. A single entrance tunnel leads to the brood chambers, 0.3 - 0.5 metres below the surface.

Foraging to both dead animal and plant material has been observed, with tandem running being used as a method of recruitment.

C.sericeus ascends grasses and shrubs where it takes plant juices and visits Homoptera; termites have also been recorded in its prey.

Camponotus sp.F (? vesticus)

C/F/1

This fast-running ant is found nesting directly in the soil in all areas at Mokwa. Foraging is diurnal but may extend into the early evening, foragers having been observed up to 2000 hours GMT+1. Camponotus sp.F is primarily a ground forager, occasionally ascending shrubs and grasses to take plant juices. Dead animal and vegetable material is taken, and termites, including Trinervitermes spp., have been recorded in its prey.

Camponotus sp.D (? viticola)

C/D/1.

This Camponotus species nests in rotten wood on the ground and under the loose bark of trees. It is commonly found foraging by day on grasses and shrubs where it takes plant juices and tends Homoptera. Camponotus sp.D has not been recorded as a termite predator.

Camponotus sp.E (subgenus Dinomyrmex)

C/E/1.

This species has been recorded only from the primary savanna woodland where it is commonly encountered nesting in deserted M.bellicosus mounds. It is essentially a nocturnal forager, but may be found in the mornings after rain when it is still overcast. No observations have been made of its feeding habits, excepting for one record of it as a secondary predator (5.3.1b) of Megaponera foetens columns returning with M.bellicosus.

Camponotus sp.C (? perrisii Forel)

C/C/1.

This species has been found nesting under the loose bark of trees. It is a diurnal forager and has been collected in all biotypes at Mokwa. Camponotus sp.C forages on the ground, trees and shrubs and appears to be a general scavenger.

Camponotus sp.G

C/G/1.

This small and pubescent Camponotus species is usually found nesting in deserted M.bellicosus and Trinervitermes spp. mounds. It forages mainly during the day, but has occasionally been encountered at night. No other information on its habits at Mokwa is available.

Camponotus acvapimensis Mayr

C/H/1

This species has been recorded from the primary savanna woodland where it is found nesting under bark and in crevices in decaying wood. It has been observed foraging on trees and shrubs where it takes plant juices and visits Homoptera.

Levieux (1967) has described the place of this ant in the food chain in the Ivory Coast savannas; he found that as well as

tending a wide range of homopterans the ant took termites, chilopods, crickets, coleopteran larvae and the brood of other ants. He noted that in the dry season, after the destruction of vegetation by bush fires, that the ants obtained their food primarily by predation.

Camponotus sp.J

C/J/1.

At first glance this camponotine resembles the myrmicine, Myrmicaria eumenoides, when foraging, as it is of a similar chocolate brown colour and also has the habit, like Myrmicaria, of holding its gaster directly under the thorax. It nests in close proximity to M.eumenoides and foragers appear to take food, such as termites, from Myrmicaria foragers returning to the nest. Camponotus sp.J has only been recorded from the parkland where Myrmicaria is abundant.

Polyrachis viscosa F.Smith

28/9/C.

P.viscosa nests either directly in the ground or in rotten wood on the ground. It is found in primary woodland savanna and in bush fallow near cultivated areas. It forages throughout the day and takes dead animal and vegetable material, it also ascends grasses and shrubs to take plant juices and visit Homoptera. Termites are recorded in its prey and it has been observed taking foraging Trinervitermes spp., including T.geminatus (Wasmann) (C.E.Ohiagu, pers.comm.).

Tribe: Formicini

Cataglyphis seticornis

28/4/D.

This long-legged fast-running formicine is usually encountered in bright sunlight in the parkland. It has also been recorded at the

Experimental Farm and in bush fallow, but not in the primary savanna woodland. It nests directly in the ground. The feeding and foraging habits of this ant have not been studied.

Tribe: Lasiini

Paratrechina longicornis

18/5/B.

Workers of the ant have been taken from leaf-litter samples in the primary savanna woodland.

Tribe: Oecophyllini

Oecophylla longinoda (Latreille)

0/6/A

The tailor ant, Oecophylla, is most abundant in the parkland where it builds leaf nests in mango and citrus trees. It is occasionally encountered in bush fallow where tall trees remain, but has not been recorded from the primary savanna woodland.

O.longinoda forages throughout the day and night, descending to the ground to take dead animal and plant material. Termites are taken from both the ground and trees, and Oecophylla major workers are able to break open the protective soil sheeting of the termites on the trunks of trees. This formicine also tends Homoptera on its colony trees, including scale insects on mango and citrus fruits.

Tribe: Plagiolepidini

Acantholepis sp.A

25/1/A; 30/6/F; 6/6/A.

This, the larger of the two Acantholepis species at Mokwa, is found nesting both in rotten wood on the ground and in live mounds of M.bellicosus, M.subhyalinus and Trinervitermes spp. This

species has been recorded from parkland, cultivated land and primary savanna woodland. It forages mainly on the ground but also ascends blades of grass to visit Homoptera. This species of Acantholepis has been observed taking foraging termites.

Acantholepis sp.B

13/4/A; 20/4/C; 28/4/F; 30/1/C

This species makes its nest in pieces of rotten wood on the savanna floor. It is an abundant ant in litter samples in primary savanna and parkland but little is known of its habits; this species has not been observed taking termites.

Plagiolepis sp.A

20/4/B.

This small yellow ant is abundant in leaf-litter samples taken in primary savanna. No observations have been made on its habits although nests have been found in the soil when digging sample pits.

2.4 Notes on the Biology of Savanna Termites

2.4.1 Introduction

The O.D.M./A.B.U. Termite Research Team has been working in the southern Guinea Savanna at Mokwa since early 1974, studying the populations, biology and economic significance of termites in savanna ecosystems. The notes on savanna termites in the following section have been collated from personal communications with and publications (e.g. Collins 1977b, Wood 1976, Wood et.al. 1977, Wood and Ohiagu 1977 and ODM Report 1977) of the termite team supplemented by my own field observations. The notes are intended as a guide to the habits of the termites preyed on by ants; a comprehensive picture of the termites can be obtained by reference to the termite team publications. A diagrammatic representation

of the distribution of termites in primary savanna woodland can be found in figure 2.4.

2.4.2. Rhinotermitidae

Coptotermitinae

Coptotermes spp.

The foraging galleries of this termite are frequently found up the sides of trees in all areas at Mokwa. Wood (1976) reports that at Mokwa Coptotermes feeds on live wood, dead wood and roots and wood-litter. The nest system is located within standing or fallen trees.

2.4.3. Termitidae (subfamilies according to the revision of Sands 1972).

Termitinae

Amitermes evuncifer (Silvestri).

In primary savanna woodland, subterranean populations of A.evuncifer are 37 per square metre (Wood 1976), although on cultivated land this species becomes more abundant (2463 m⁻² on maize, 262 m⁻² on yam, ODM Report 1977). A.evuncifer builds above-ground carton mounds in nearby riverain areas, but in savanna at Mokwa the nest system is usually subterranean. Foragers are rarely encountered as this termite feeds largely underground on living roots (eg. yam) and dead, partially decomposed wood and roots. A second species, A.stephensoni Harris has been recorded once from primary savanna woodland.

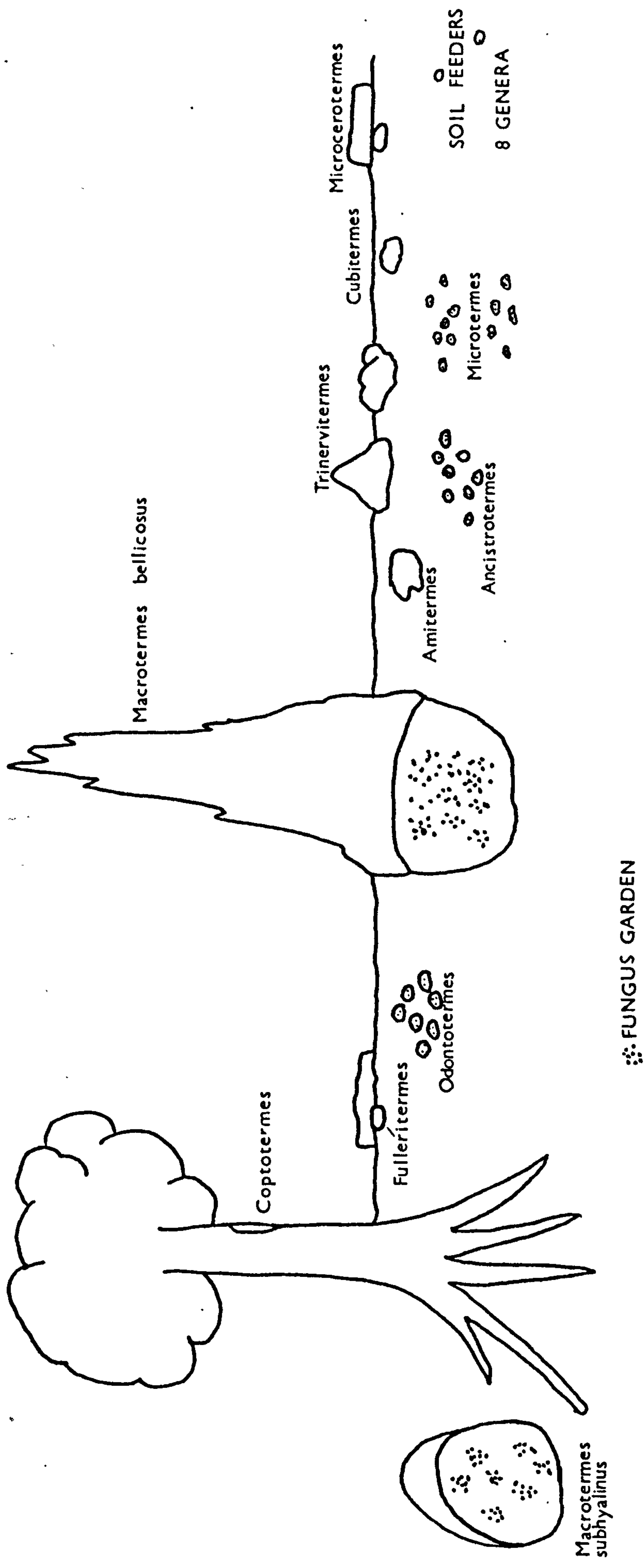


Figure 2.4 Diagrammatic representation of the distribution of termites in the primary savanna woodland (adapted from Wood et.al. 1977).

Microcerotermes spp.

This genus, provisionally consisting of two species, is reported as having high populations in primary savanna ($1,034 \text{ m}^{-2}$) where it feeds mainly on dead wood, roots and fresh wood litter (Wood 1976). The carton nest is usually found just beneath the surface of the soil, usually at the base of trees. Foraging galleries may extend from the nest up the trunk of the tree.

Soil Feeding Termitinae.

Basidentitermes sp., Cubitermes sp., Noditermes sp., Pericapritermes sp. and Promirotermes sp. are all soil feeders which are only rarely encountered in soil sample cores.

Apicotermitinae.

Three genera from this subfamily are encountered in soil sample cores at Mokwa; Adaiphrotermes sp., Anentotermes sp. and Astalotermes sp. They are all soil feeders; the interactions of ants with these genera have not been recorded.

Macrotermitinae.

Macrotermes bellicosus (Smeathman). (synonomous with Bellicositermes natalensis, cited in Ruelle 1970).

The energetics and population biology of this species in primary savanna woodland have been studied in detail by Collins (1977b). M.bellicosus builds cathedral type mounds, exceptional examples of which may be over 7 metres high. The mound population of M.bellicosus at Mokwa is 52 per square metre (workers and soldiers) or 128 per square metre if the larvae of the sterile castes are also included.

M.bellicosus consumes mainly standing dead wood, bark and fresh wood litter including leaves. Foraging occurs throughout the year with minimum activity at the end of the dry season. Foraging parties on trees, surface wood and leaf litter feed under sheeting constructed of soil particles. The soil sheeting, constructed by all Macrotermitinae as well as Coptotermes spp. and Microcero-
termes spp., is thought to provide protection from small predators, desiccation and possibly light.

Macrotermes subhyalinus (Rambur). (synonomous with Bellicositermes bellicosus, cited in Ruelle 1970).

This is the largest of the Macrotermitinae at Mokwa, the nest system is subterranean in the primary savanna, although low, irregularly domed mounds are found in the parkland at low elevations. Core soil samples indicate a low population density of 37 per square metre (Wood and Sands 1977) for this species in the savanna.

M.subhyalinus takes more dead standing grass and grass litter than M.bellicosus, but also takes dead wood and wood litter; foraging parties have also been encountered in leaf litter in primary savanna woodland. M.subhyalinus exhibits a peak of foraging activity in January and is scarcest as a surface forager in the wet season. M.subhyalinus constructs soil sheets for protection when foraging. In open areas the soil sheeting takes the form of galleries, from the ends of which termites emerge and forage on grass and leaves.

Odontotermes spp.

Two species, O.pauperans (Silvestri) and O.smeathmani (Fuller) reach a combined density of 260 per square metre in primary savanna woodland. Both species are wood-, grass- and leaf-litter feeders; O.pauperans showing a preference for grass litter and O.smeathmani for wood litter. Both species have subterranean

nest-systems. Odontotermes forage principally in the dry season, doing so evenly throughout. Odontotermes also construct soil sheeting to forage under, that on the ground is often very powdery in appearance compared with that of other genera.

Anoistrotermes cavithorax (Sjöstedt).

This species reaches high populations (881 m^{-2}) in primary savanna woodland where it feeds primarily on wood litter; the bark of trees and grass roots may also be attacked. Foraging to surface wood litter occurs in the wet season and at the end of the dry season (March/April); the foraging activity is fairly even throughout this period. The nest system is diffuse and subterranean.

Microtermes spp.

There are probably five species of Microtermes in primary savanna (R.A.Johnson, pers.comm.), but they are difficult to distinguish and the taxonomy of the genus is poorly known. The two species assayed in this study (Section 5), species A (? usambaricus) and species B correspond to the usage of Johnson (in ODM Report 1977). In some cultivated areas the populations of this genus rises to 4,700 per square metre, compared to the primary savanna population of 850 per square metre. The subterranean nest system is a diffuse collection of fungus combs connected to the queen cell by foraging galleries.

The principal food of Microtermes is dead wood and roots and fresh wood litter; living roots are sometimes attacked. There is a seasonal variation in foraging habits (Wood and Johnson, in press). In the dry season foraging termites and their fungus combs are found at greater depths in the soil than in the wet season. The genus is rarely found on the soil surface during the dry season. Microtermes feed on surface wood litter in the wet season, with

a peak at the start of this season. Microtermes and Ancistrotermes forage less often under soil sheeting than do Macrotermes and Odontotermes. They forage principally within their food sources of wood litter, roots and grasses, entering at points of contact with the soil.

Allodotermes sp.

This wood and litter feeder is a very rare subterranean nester in primary savanna woodland. It has never been encountered as prey of ants.

Nasutitermitinae.

Fulleritermes tenebricus (Silv.).

This wood-feeding nasute builds a carton nest below the soil surface, often at the base of trees. Soil enclosed foraging galleries may be found up the trunks of trees, from which it takes bark and wood. It also forages in the leaf litter.

Trinervitermes spp.

Five species of Trinervitermes; T.geminatus (Wasmann), T.trinervius (Rambur), T.togoensis (Sjöstedt), T.occidentalis (Sjöstedt) and T.oeconomus (Trägårdh) are found at Mokwa. T.geminatus, T.trinervius and T.oeconomus are all mound builders, their domed shaped mounds reaching over 200 per hectare in primary woodland. The former two species have food stores in the mounds (Sands 1961a, 1961b, Wood and Ohiagu 1977). T.oeconomus and T.togoensis occupy the abandoned mounds of other termite species. The diet of the five termite species is composed of the following (Wood 1976, Wood and Ohiagu 1977);

<u>T.geminatus</u>	grass
<u>T.trinervius</u>	grass
<u>T.togoensis</u>	grass + small pieces of plant debris and seeds
<u>T.oeconomus</u>	grass + other plant material including wood litter
<u>T.occidentalis</u>	as <u>T.oeconomus</u>

All Trinervitermes species may forage in the open, unprotected by soil coverings. The foraging behaviour of T.geminatus has been studied by Ohiagu and Wood (1976) who found that it forages in the late afternoon, evening and early morning.

SECTION 3 FORAGING AND RECRUITMENT IN ANTS PREDATORY ON TERMITES

3.1 Introduction.

Except for those ants, such as some cryptic ponerines, which live in rotten wood in very close association with their arthropod prey, the majority of ants have to leave their nests and search for food. Their methods of foraging will partly be affected by the food source being utilized. Ants such as Monomorium pharaonis (Sudd 1960) and Solenopsis saevissima (Wilson 1962), which can utilize relatively large food units which are spatially and temporally discontinuous, have developed recruitment methods which allow large numbers of ants to find the food and secure it for the colony before other scavengers find it.

Searching 'scout ants' of M.pharaonis leave the nest and search for food. After locating it they return to the nest laying a scent trail and then excite sister workers which follow the trail to the prey (Sudd 1960). Recruitment along the scent trail continues until the food source has been completely utilized. In the case of some Pheidole species a similar recruitment mechanism leads to large prey items being dragged back to the nest (Sudd 1967).

Larger ants can utilize prey items which can be dealt with by single foragers. Daceton armigerum is a large-eyed ant of the canopy of South American rainforests. Single ants of this species hunt insect prey, although if other single foragers are near to the hunting ant they are visually stimulated to assist the hunter (Wilson 1971).

A party of foraging termites can be envisaged either as an aggregate prey or single small prey items which are discontinuous in space and time. The difference between foraging termites and the food utilized by (eg) M.pharaonis is that the living termites may be disturbed by the predator and retreat to their subterranean

galleries to evade predation. If the predatory ants adopt the strategy of treating the termites as single prey items, single foraging ants could secure a termite and then return to the nest with their prey. The disturbed termites could retreat after the attack and recruitment of further ants to the termite foraging area would not be useful. This opportunist predation is wasteful of food resources (termites), but it is a suitable strategy to be adopted by ants with suitable alternative food sources, such as honeydew from Homoptera. Camponotus spp., Crematogaster sp.E and Myrmicaria eumenoides all utilize termites in this way.

A more efficient predation strategy is to locate the termites, with the minimum of disturbance, with a 'scout ant', and for this scout ant to recruit more ants to the termites. The strategies adopted by the recruited ants may then depend on their size relative to their prey, the reactions of the termites to them and the foraging regimes of the termites.

Another efficient predation regime would be to mount a full-scale attack on the termites brood centres. This method would require large resources in the form of numbers of ants in order to breach the termites defences. It is probably only feasible for dorylines, where several hundreds of thousands of ants (Schneirla 1971) are to be found in each colony.

Termite predators can also live among their prey (Wheeler 1936)- a strategy which probably depends on physical and chemical crypsis rather than sophisticated recruitment and foraging methods.

In the following sections the foraging strategies used by termite predators are described and discussed. The predation methods of two opportunist predators, Myrmicaria eumenoides and Anochetus sedilloti are described, as are the foraging regimes of the specialized termite predators Megaponera foetens and Decamorium uelense.

3.2 Foraging and Predation by Opportunist Predators.

3.2.1 Myrmicaria eumenoides

The following observations were made on M.eumenoides foraging in the parkland in May 1975. Odontotermes smeathmani were foraging under fresh soil sheeting for about 0.5 metres up the side of a tree, 2.4 metres from the ants nest. Single ant foragers were exploring the area around the nest and two recruitment trails were established, one to a large dead orthopteran and another to a decaying mango fruit; pieces of orthopteran and fruit were being taken back to the nest.

At least eight foraging ants were running over the Odontotermes sheeting (area ca. 1.5 m^2), stopping occasionally to explore it with their antennae and mandibles. One foraging ant, possibly after finding a weak area in the sheeting, broke through the soil sheeting and after ca. two seconds secured a major termite worker. Two other foraging ants came to the breach in the protective sheeting (perhaps attracted either visually or chemically) and one ant seized a minor soldier which had appeared at the edge of the sheeting. More ants appeared but no more termites were taken.

Forty five seconds after the first termite had been taken a piece of soil sheeting was removed from the base of the tree; it was observed that the termites were moving downwards, away from the initial breach 0.4 metres above. It was concluded that these termites were retreating from the original disturbance. Myrmicaria workers continued to explore the original break for about five minutes after the first entry. No more termites were taken and eventually workers ignored the breach but continued to move over the sheeting, exploring it.

Other large ants which have been observed breaking open soil sheeting and securing single termites include Paltothyreus tarsatus, Crematogaster sp.E and F, Polyrachis viscosa, Camponotus

maculatus, Camponotus sericeus, Camponotus sp.E and Oecophylla longinoda.

3.2.2. Anochetus sedilloti.

This ponerine forages in the litter and is usually very difficult to observe feeding as any movement of the obscuring litter disturbs both ants and termites. One predatory interaction was observed to illustrate how opportunistic predation occurs in this and other ant species.

Macrotermes bellicosus were observed foraging over a dead leaf. Soil sheeting was being established, but was open at the leading edge; worker and soldier termites being present at this edge. An A.sedilloti worker was observed exploring the leaf surface and then reach under the edge of the sheeting and seize a minor worker termite. The building termites retreated but approximately 15 seconds later extension of the soil sheeting recommenced in the region from which the termite had been taken. The Anochetus worker returned to its nest which was 0.6 metres away from the termites foraging area. Over a period of one hour the same ant returned thirteen times (or thirteen different ants) to repeat the actions described above; there being no mass recruitment of ant workers to the termites. Although the termites retreated for a short interval after each interaction, they eventually resumed extending the soil sheeting.

Four other species of ants were observed to attack the termites during the one hour observation period, and in all instances the ants attacked the termites at the edge of the soil sheeting in a similar manner to that described for A.sedilloti. Acantholepis sp.A, took 1 minor worker; Camponotus sp.F took 5 minor workers, 7 major workers and two minor soldiers; Pheidole sp.A took 1 major and 1 minor worker and Crematogaster sp.E took 3 minor workers, 2 major workers and a minor soldier. The 13 interactions

between A.sedilloti and the termites secured 9 minor workers, 3 major workers and a minor soldier. Thus in one hour a total of 35 termites (18 minor workers, 13 major workers and 4 minor soldiers) were taken. After each interaction between opportunist predatory ants and building termites, the termites nearest to the area under attack retreated under the soil sheeting. There did not appear to be any widespread communication of alarm and the termites returned to build the sheeting after 10 - 15 seconds.

3.3 Foraging, Recruitment and Emigration in Megaponera foetens

3.3.1 Activity Cycles.

Foraging in M.foetens colonies was found by continuous day and night observations during November 1974 and April/May 1975 to occur in the early morning (0700 - 0930 hours GMT+1) and late afternoon/early evening (1630 - 1830 hours GMT+1). Established foraging parties sometimes stayed away from the nest longer; in the morning until 1000 hours and in the evening until 1930 hours. These known patterns of foraging were used to study predation (4.2.1) and foraging in detail from October 1975 until October 1976. Four colonies were selected for sampling and they were observed in sequence from 0700 - 0930 and 1615 - 1830 hours on alternative days (except Sunday). If emigration occurred a new colony was selected for sampling. Information was collected on the foraging activities of single ants and columns of ants and on foraging distances, times and areas. The data were derived from over 350 observations of M.foetens nests and 200 columns of foraging ants during the whole period of study.

3.3.2 Recruitment of Megaponera foetens to Termites.

At Mokwa foraging termites (Macrotermitinae) were the only prey of M.foetens (4.3.1). These termites were located by single major workers which are referred to as "scout ants" (Collart 1927,

Fletcher 1973). Scout ants left the nest between 0600 - 0900 and 1500 - 1800 hours and searched in an area of up to 95 square metres around the nest. Foraging termites protect themselves from desiccation, smaller predators and perhaps also light, by constructing soil sheets or runways. During the searching phase, scout ants moved slowly (ca. 0.03 m s^{-1}), actively palpating the litter and any termite soil sheeting with their antennae, while searching through leaf litter and under wood. This searching phase could last for more than one hour, an unsuccessful ant generally returning to the nest by a circuitous route.

The scout ant changed its behaviour for the return journey if termites were found (6.1). The gaster was bent downwards with the sting partially extruded, and the ant moved forward more rapidly (ca. 0.045 m s^{-1}) with the antennae held upwards and away from the soil surface. Occasional pauses, sometimes followed by slight changes in direction were observed. The ant returned to the nest by a more direct route than on the outward journey. Returning scout ants displaced 2 metres to the left or right of their position showed no disorientation, but continued in the same forward direction (7 observations), suggesting that a scent trail was not being followed back to the nest.

If recruitment was successful, a column of ants, containing all worker castes, emerged from the nest 60 - 300 seconds (mean = $203 \text{ s.d.} \pm 116 \text{ s}$, $N = 67$) after the return of the scout ant, which led them, accompanied by 5 - 12 major workers. Leadership by returning scout ants was confirmed by marking with polymer paint (Rownhams Orange or Yellow.). Of 12 returning scout ants marked, 5 recruited columns were led by 5 marked scout ants. Scout ants marked on previous occasions were also found in the group of major workers which headed the foraging column, and these marked ants were never found in other parts of the column (17 observations), suggesting that there may be some form of polyethism in the major workers of M.foetens.

The route taken by the outward column followed the return journey

of the scout ant. Other workers in the column were observed to extrude their stings, suggesting that they were laying trails. When the column of ants reached the area from which the scout ant had started the column halted and the ants spread out around the end of the trail, presumably because they had reached the end of the recruitment scent trail (Fig. 3.2). On re-discovering the termites, the ants formed into small groups at the entrances to the termites foraging galleries. Major workers broke open the protective soil sheeting constructed by the termites.

All worker castes went under the sheeting, but only the minor caste were observed to enter any exposed galleries and capture termites. The ants re-entered the galleries repeatedly and obtained more termites which were meanwhile piled around the entry points. Attacks lasted for about 540 seconds (mean $542 \pm \text{s.d. } 203 \text{ s}$; $N = 143$); they presumably ceased when the termites had retreated far enough into the galleries to become inaccessible to even the minor workers of M.foetens. At the end of the raid the ants, predominately major workers, gathered up termites from the piles and packed them in their jaws, as Levieux (1966) observed. The ants then returned to the end of the recruitment trail and the column reformed.

On three occasions all the major workers (10, 7 and 8 ants) at the head of the column were marked on their outward journey. This was done with the minimum of disturbance; the scout ant had been marked distinctly before the recruitment of the column. The reformation of the column appeared to be initiated by the marked ants which left those attacking termites and moved to the end of the original recruitment trail. The remaining ants then also moved to there and the column set out on its return journey. The column was led by the marked major workers, but during one observation three other unmarked major workers were also included in the group of ants at the head of the column (Table 3.1). The scout ant was always in this group of leader ants, but did not always lead the column.

Table 3.1 Fate of marked major workers of Megaponera foetens on the re-formation of the recruitment column after a raid on termites.

Number of major workers at the head of the column on route to the termites	Number of marked major workers at the head of the column returning from a raid	Number of unmarked major workers at the head of the column returning from a raid
10	10	0
7	7	0
8	8	3

The column of ants returned to the nest along the same route as that taken on the outward journey. The ants usually kept close formation, the column being 3 - 6 ants wide with the ants following in close files. Some major workers left for short intervals to drive off Pheidole spp. and Camponotus spp. who were trying to relieve M.foetens of its prey. The return journey was completed at a faster pace than the outward journey; the mean outward speed being 0.038 m s^{-1} and the return speed 0.046 m s^{-1} ($t = 2.38$, $p < 0.001$ for $N = 220$).

Plates 3.1 - 3.4 and the diagrams (Figs. 3.1 & 3.2) summarize the events occurring during a raid by M.foetens on surface foraging Macrotermitinae.

3.3.3 The Role of the Scout Ant.

Collart (1927), working in the former Belgian Congo, found that removal of the leading ('guide') ant resulted in the disruption of the raiding column on its outward journey. The ants halted as if

they had come to the end of the trail, milled around in the area from which the scout ant had been removed and then returned to the nest. Removal of the leading ant had no effect when the column was returning to the nest after a raid on termites. In southern Africa, Fletcher (1973) found that the "scout ant" was the only major worker at the head of an attacking column. Minor workers followed immediately behind and the remainder of the column had a mixture of all castes. The removal of the leading ant on the outward journey led to the disruption of the column. Neither Fletcher (1973) nor Collart (1927) gave details of any marking experiments to confirm the identity of the scout ant as the "guide ant" leading columns of ants to attack termites.

The foraging behaviour of M.foetens in savanna and savanna-forest interfaces in the Ivory Coast, where the nest density was as great as twenty colonies per hectare, has been described by Levieux (1966). He made no mention of the scout ant, and careful removal of the major workers at the head of the column on its outward journey did not result in any disruption of the raiding party. Prey was located by columns of workers that attacked the first parties of foraging termites they encountered. Scent trails laid on the outward journey were used by the returning ants.

At Mokwa the scout ant is immediately followed by 5 -12 other major workers, which in turn were followed by the bulk of the column consisting of all castes. Careful removal of the leading ant from a column of ants on its way to attack termites resulted in the ants returning to the nest (11 observations). Removal of the leading ant did not disrupt columns of ants returning from attacking termites (8 observations), but some alerting behaviour with faint stridulation (Markl 1973) was observed in some of the ants following the leader ant.

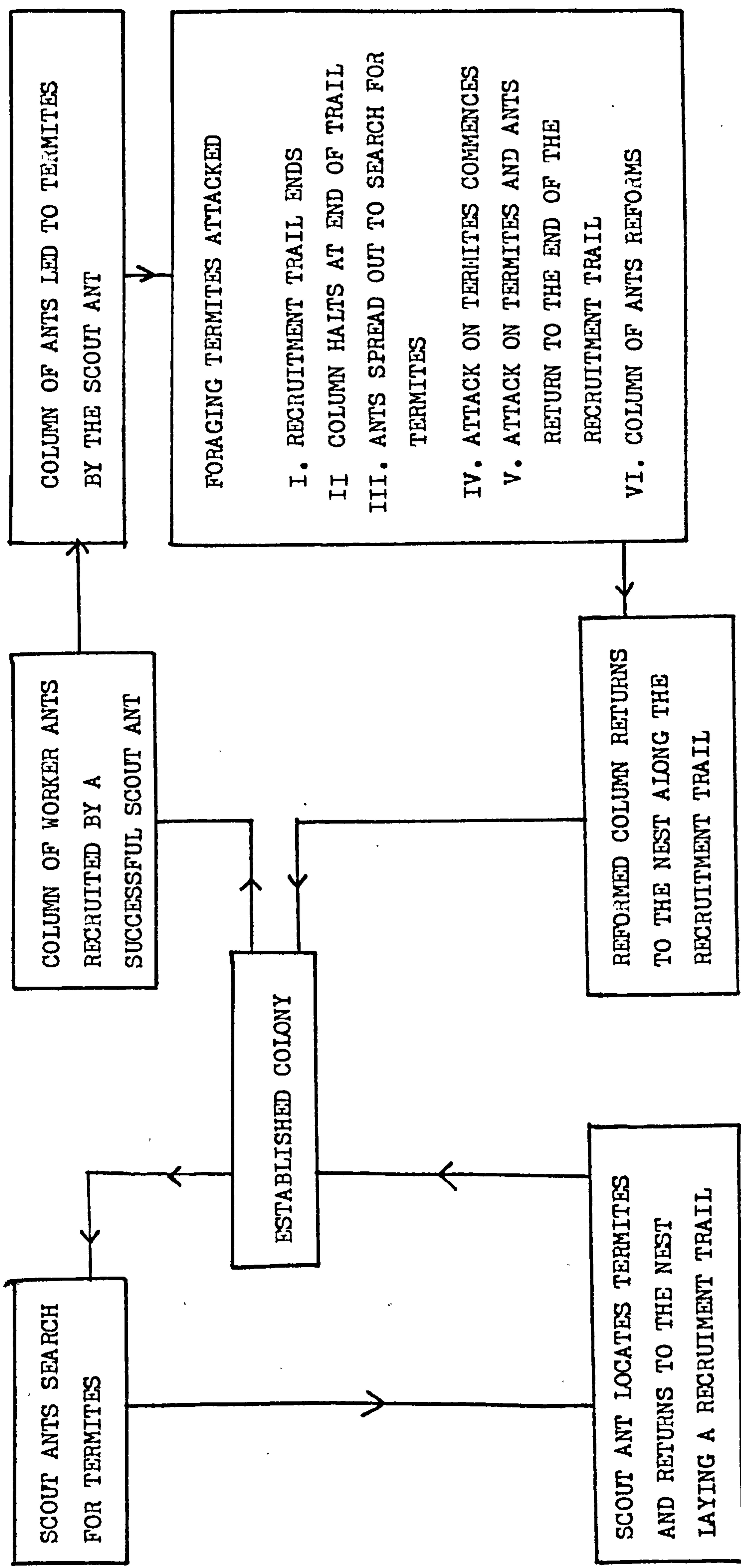


Figure 3.1 Events occurring in the detection and predation of termites by Megaponera foetens

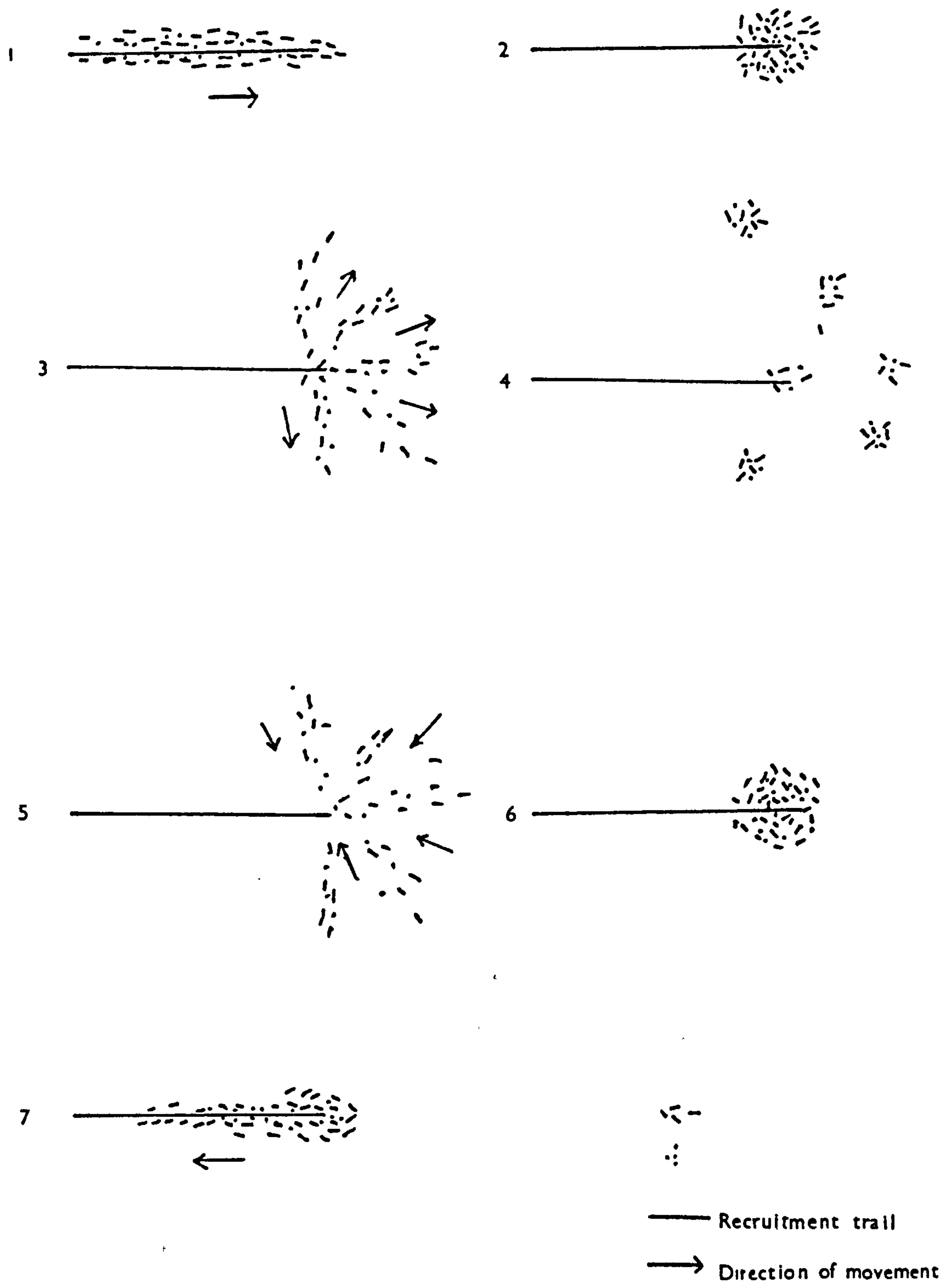


Figure 3.2 Events occurring during an attack by *Megaponera foetens* on foraging *Macrotermitinae*.

1. Recruitment trail ends. 2. Column halts at end of trail.
3. Ants spread out to search for termites. 4. Attack on termites commences. 5. Attack on termites ceases and ants return to the end of the recruitment trail. 6. Column of ants reforms.
7. Reformed column returns to the nest along the recruitment trail.

PLATE 3.1 Recruitment in Megaponera foetens.

I. The column of ants on its outward journey
to attack foraging termites

PLATE 3.2 Recruitment in Megaponera foetens.

II. At the termite foraging area the ants are
attracted to foraging termites (6.3).

PLATE 3.1



PLATE 3.2



PLATE 3.3 Recruitment in Megaponera foetens.

III. The ants dig into the termite foraging galleries.

PLATE 3.4 Recruitment in Megaponera foetens.

IV. The ants gather up the termites (arrowed) at the end of the raid and the column reforms.

PLATE 3.3



PIATE 3.4



3.3.4 Foraging Areas and Distances.

M.foetens at Mokwa forages in an area of up to 95 metres from the nest. The nest density was 9 colonies per hectare (4.2.1a) on the study area in primary savanna woodland. There was thus an overlap of the foraging areas of colonies, despite which encounters between columns of ants were rare. Inter-colony aggression, resulting in the disruption of the foraging columns and the death of some of the ants was observed in three encounters. The surviving ants returned to the nest after the re-location of the recruitment trail, although some of the ants were apparently lost in the surrounding bush.

In the Ivory Coast Levieux (1966) observed that the same recruitment trail was used on several consecutive raids. This did not happen at Mokwa, where a fresh trail was laid by a scout ant for each foray. In the Ivory Coast it appears that only trails from previous raids were available to guide the raiding columns to areas where termites had been foraging previously.

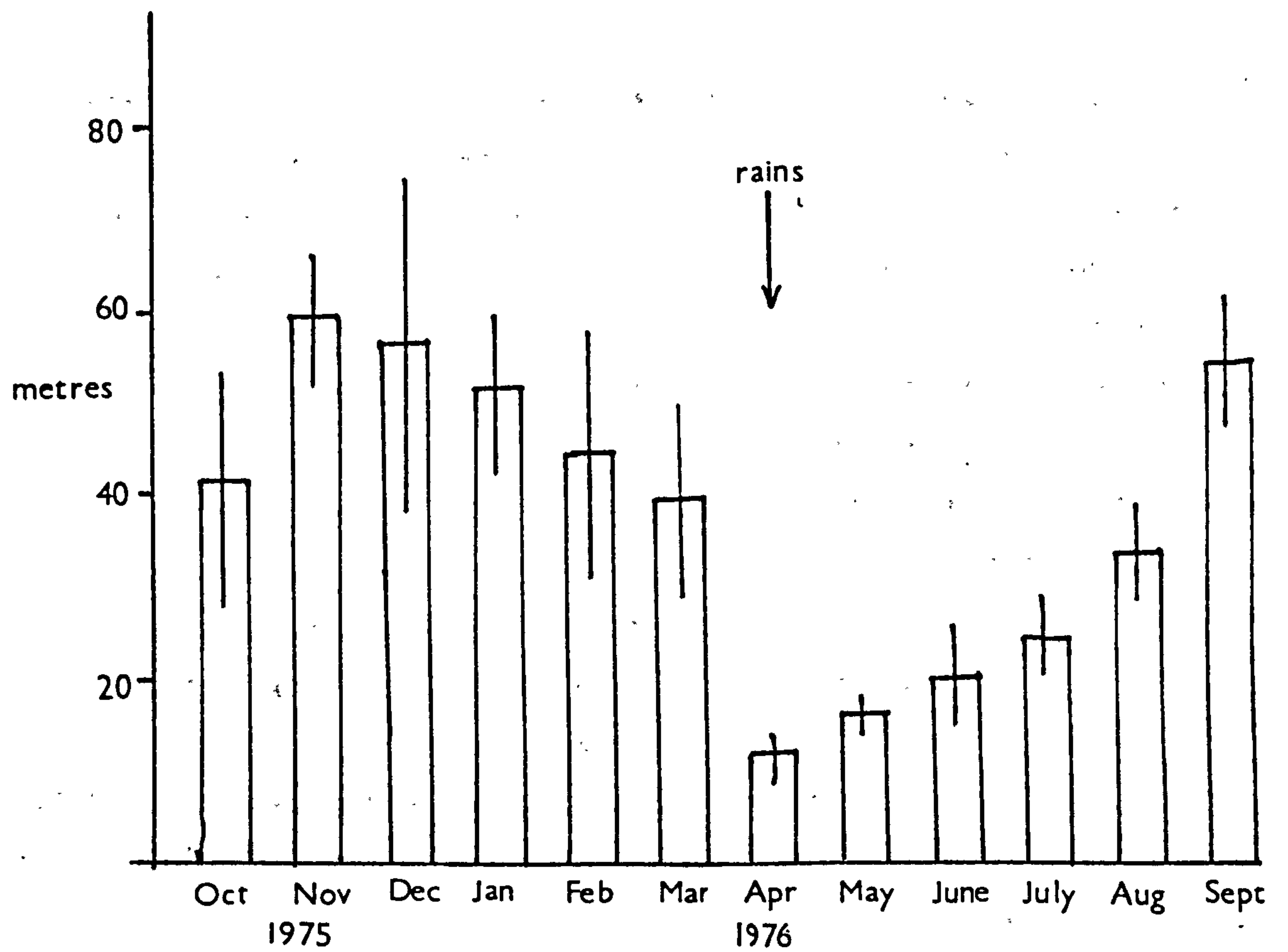
At Mokwa the length of the recruitment trail varied throughout the year, and was shorter during the early part of the wet season (April - July) than at other times of the year. (Fig. 3.3).

3.3.5 Emigration of Colonies.

Although Arnold (1914) described the emigration of a M.foetens colony and associated myrmecophiles in southern Africa, Fletcher (1973) did not regard this as sufficient evidence to classify this ponerine as an emigratory species.

At Mokwa emigration occurred throughout the year (Table 3.2) but colonies were observed for up to eight months without emigration occurring. However, on one occasion emigration occurred only five days after the colony had moved to the new site. Indirect evidence of emigration was also obtained from the identification of marked

Figure 3.3 Foraging distances of Megaponera foetens columns
($\pm$ standard error of the mean)



scout ants and the inactivity of known nests followed by the appearance of ants at new sites, where vigorous excavation was in progress. Excavation of previously active nests did not reveal the presence of living or dead ants (14 old nest sites).

All completed emigrations were observed in the evening (1630 - 2000 hours) and those at Mokwa resembled the description given by Arnold (1914). One such emigration was observed in April 1976.

Workers emerged at 1720 hours carrying eggs, larvae and pupae which they deposited in piles near to the nest entrances. Workers of all castes then picked up eggs and brood and left the old nest site at 1738 hours. They were led by a single major worker who appeared to have a function analogous to the scout ant at the head of foraging columns. The column of ants went to the base of a tree 5.2 metres away where they stopped and put down their loads, some returning to the old nest site, while others stayed and tended the eggs and brood. Not all the ants carried eggs and brood from the old nest site to the new. Eventually a continuous two-way traffic of ants was established, with ants carrying eggs and brood from the old (Site 1) to the new (Site 2) site, while ants with empty mandibles moved in both directions. By 1806 hours all eggs and brood had been moved to Site 2 and all the ants were massed at this site around the brood. A number of myrmecophiles were still moving from Site 1 to Site 2 at this time.

At 1814 hours the column moved again in a similar manner to a third site (3) which was a deserted mound of Macrotermes bellicosus 9.7 metres from Site 2. A number of the ants deposited their brood outside the mound before returning to Site 2, whilst others entered the nest directly. By 1911 hours all the ants and their brood, and the majority of myrmecophiles had entered the new nest. The new nest site was watched on the following morning and a raiding column of ants was seen to be recruited to attack foraging M. bellicosus.

Table 3.2 Monthly occurrence of emigration by Megaponera
foetens on two 1 hectare sites. (18 colonies).

Month	Number of emigrations occurring	Percentage of colonies emigrating per month
Oct. 1975	3	17%
Nov.	5	28%
Dec.	2	11%
Jan. 1976	3	17%
Feb.	2	11%
Mar.	3	17%
Aprl.	4	22%
May	2	11%
June	1	6%
July	2	11%
Aug.	0	0%
Sept.	2	11%
Oct.	3	17%

It was interesting to observe that after the completion of emigration, groups of major workers appeared to mark an area of approximately 750 mm in diameter around the nest entrance. This was achieved by the major worker standing with the gaster curved downwards and forwards with the sting partially extruded. The ant then moved backwards rapidly for 50 - 200 mm, dragging its sting across the soil surface. This marking behaviour may help in the location of the nest of this emigratory species by scout ants searching for termites subsequent to emigration.

3.4 Foraging and Recruitment in Decamorium uelense.

Columns of small ants carrying termites, and groups of 10 - 20 worker ants were observed on the savanna floor between 0700 - 1800 hours GMT+1, being most common between 0700 - 1100 hours. These ants, identified as Decamorium uelense, were not discovered until the penultimate visit to Mokwa, consequently only limited observations could be made on them. Bolton (1973a, 1976) reported that Decamorium species have been observed progressing "through the leaf litter in single file" and that the genus had been recorded as preying on termites, but did not give any further details on their habits. Observations indicated that the behaviour of D. uelense in securing its termite prey could be divided into four distinct stages.

- i) Searching by "scout" ants.
- ii) Recruitment of groups of 10 - 30 ants to foraging termites.
- iii) Locating and immobilizing the prey.
- iv) Mass recruitment to retrieve the prey.

3.4.1 Searching by Scout Ants.

Single worker ants emerged from the nest and searched in an area of up to 10 metres around the nest, although 6 out of 8 ants that were followed did not travel more than 8 metres from the nest. Whilst searching, the scout ants palpated with their antennae, leaf-litter, fallen grass stems and exposed roots and wood. After

Table 3.3 Examination of sites by searching scout ants of Decamorium uelense; comparison of searching time at the last and previous sites.

Location sequence	Ant No.	1	2	3	4	5	6
1		2	4	3	3	7	3
2		3	2	2	5	4	3
3		2	2	2	14	2	2
4		3	2	2		2	2
5		2	8	2		4	2
6		6		2		2	3
7		2		8		12	4
8		2		4			2
9		2		3			2
10		2		5			3
11		12		2			2
12				7			3
13				4			15
14				2			
15				2			
16				18			
Termites at last location		+	+	+	-	+	+
Ants recruited		Yes	No	Yes	No	Yes	Yes

Mean time spent at final location = 13.16 $\pm$ 3.29 s (N=6)

Mean time spent at all other locations = 2.86 $\pm$ 1.57 s (N=51)

't'- test for difference, unequal variances.

$$t = 7.39; p < 0.001.$$

investigating some locations for longer than usual (Table 3.3) the scout ant returned to the nest, by a direct route. Examination of some of the sites which had elicited longer investigation by the ant revealed the presence of termites (Table 3.3).

Observation of the returning scout ant with a 20X magnifying glass revealed that the tip of the gaster was being dragged along the ground for lengths of up to ca. 10 mm, with gaps of less than ca. 5 mm between. It was not possible to observe if the sting was extruded while the gaster was being wiped along the ground. On five occasions the scout ants route back to the nest was marked with small wooden stakes, approximately 300 mm apart and 10 mm to the side of the trail, for further observations.

3.4.2 Recruitment of Columns of Ants to the Termites.

Within ten seconds (mean = 5.6 ± 2.3 , $N = 17$) of a scout ants return to the nest a column of 10 - 30 ants (mean = 19.7 ± 6.9 , $N = 23$) emerged from the entrance. The group of ants travelled in single file (Plate 3.5) to the termite location, following the route taken by the scout ant, suggesting that a scent trail was being followed. Destruction of the trail, by raking over the sand between two marker stakes 300 mm apart, caused the column of ants to halt at the beginning of the disturbed area. The ants searched for a few minutes but did not relocate the trail and returned to the nest, either singly or in small groups (6 Observations). No marking experiments were carried out to ascertain if the scout ant guided the column of ants on their outward journey, as is the case in Megaponera foetens (3.3.2).

3.4.3 Localization and Immobilization of the Termite Prey.

This third stage in the predation sequence lasted from 15 minutes to three hours. The column of ants, after arriving at the termite foraging area, dug into the termite foraging galleries, this sometimes entailed digging through several millimetres of soil. The galleries of the termites in grass or wood stems may have a

Table 3.4 Reactions of Decamorium uelense to termites in perspex chambers.

Time elapsed from the ants initial entry into the termite chamber until the first termite was killed (s)	Time elapsed from the killing of the first termite until the death of the 30 th termite (s)	Species of termite used
138	233	<u>Microtermes</u> sp.A
47	204	"
267	332	"
174	212	"
126	374	"
321	176	<u>Ancistrotermes</u> <u>cavithorax</u>

layer of soil sheeting over them which facilitated the ants entry, otherwise they had to cut directly through the grass stem or wood to the termite galleries.

During the localization and immobilization stage, a grass stem which the ants had entered was taken and examined. The stem, 4 mm in diameter, was lying on the ground, although still fixed by its roots into the soil. The stem was in direct contact with the ground at three points, 150, 280 and 515 mm from the root system. The ants entered at a point 280 mm from the roots by biting through the outer wall of the grass stem.

Approximately 480 seconds after the ants had first gained entry to the stem, an ant emerged, carrying a worker termite, and returned to the nest. 180 seconds later the stem was lifted and inspected. The stem was carefully slit lengthways and the numbers and state of any ants and termites present inside was examined. The stem was hollow (ca, 3 mm internal diameter) for 512 mm of its length. The termites had probably gained access from the roots, which showed signs of termite damage. 28 Dead Microtermes sp.A (5 soldiers, 8 minor workers and 15 major workers) were present in the stem, along with 14 D.uelense workers; 9 ants from the initial column of 24 ants being unaccounted for. The hole which had contained the grass roots was observed and 4 more ants emerged before single foragers arrived (3.4.4.), recruited by the worker ant which had returned to the nest 340 seconds earlier. No termites were taken by the ants from the disturbed area and they returned to the nest.

In some cases more columns of ants were recruited; single ants, not carrying termites, returned from the initial foraging party to the nest and another column of ants emerged. Up to four extra columns have been observed to be recruited in this way. They probably assist the first column in immobilizing the prey.

When the Decamorium workers were in the termite galleries it is probable that a rapid slaughter of termites occurred; as observations in perspex bioassay chambers (Fig. 5.1) indicated. Thirty major

worker termites (Microtermes sp.A or Ancistrotermes cavithorax), collected from baits and handled as described in 5.1.2 were placed in the chamber. Two ants were placed in chamber C (Fig. 5.1) and the chamber left for five minutes for the ants and termites to settle down. It was not possible to carry out the observations under 'red-light' conditions at Mokwa, instead observations were carried out in a darkened room, with enough light for observation from a desk lamp, fitted with a 40 W bulb and screened with white cloth.

The gate connecting the ant and termite chambers of the bioassay apparatus was removed and the behaviour of the ants observed. Termites were either exploring the chamber or small groups were standing together in it. The ants entered the chamber containing the termites and explored it; the subjective impression being of the ants moving slowly and carefully, examining the chamber with their antennae and palps. The ants also approached the termites, initially with their antennae tucked into depressions at the sides of their heads, but eventually exploring the termites with their antennae. The termites were not usually alarmed by the ants; occasionally if antennal contact was made between ant and termite, the termite jerked backwards, but no oscillatory alarm displays (5.1.3) were observed.

After a period of exploration (Table 3.4) the ants started to kill the termites. A termite was usually seized from the rear in the ants mandibles, the ant then curved its gaster, with the sting extruded, downwards and forwards between its legs into the termite. The termite was released within a few seconds (mean = 2.81 ± 1.07 seconds, $N = 21$) and was immobile, presumably killed by a potent poison. Two ants were able to kill 30 termites in less than 300 seconds (Table 3.4); once the slaughter had begun the ants moved from termite to termite until all were immobilized. The ants appeared to be able to distinguish dead termites from living ones easily, possibly a marker pheromone is involved. On four of five occasions when only one ant was used no termites were killed, the reasons for this are discussed in 5.3.2 (p.190).

3.4.4 Mass Recruitment.

Ants, some carrying termites, but others unladen, returned to the nest from the area of localization and immobilization. Recruitment of single and small groups of ants occurred, a typical example is illustrated in Figures 3.4a and 3.4b. This mass recruitment phase appeared to be essentially the same as the later stages of recruitment recorded in the myrmecines Monomorium pharaonis (Sudd 1960) and Solenopsis saevissima (Wilson 1962). Recruitment continued until the supply of termites had been taken to the nest (Figs. 3.4a & b).

In some cases, the mass recruitment phase recommenced at the same or nearby sites (foray 3b, Fig. 3.5). It then seems probable that some of the last ants recruited had located fresh sources of termites and initiated subsequent mass recruitment phases.

The species of termite taken, and the predation pressure exerted by D.uelense on termites are described in 4.2.2.

3.4.5 Foraging Distances in Decanorium uelense.

Intensive observations of one colony during October 1976 revealed that D.uelense exploited termites in an area of up to 10m radius around the nest entrance (Fig. 3.5).

Figure 3.4a Mass recruitment in Decamorium uelense. Numbers of ants leaving the nest per 5 minutes.

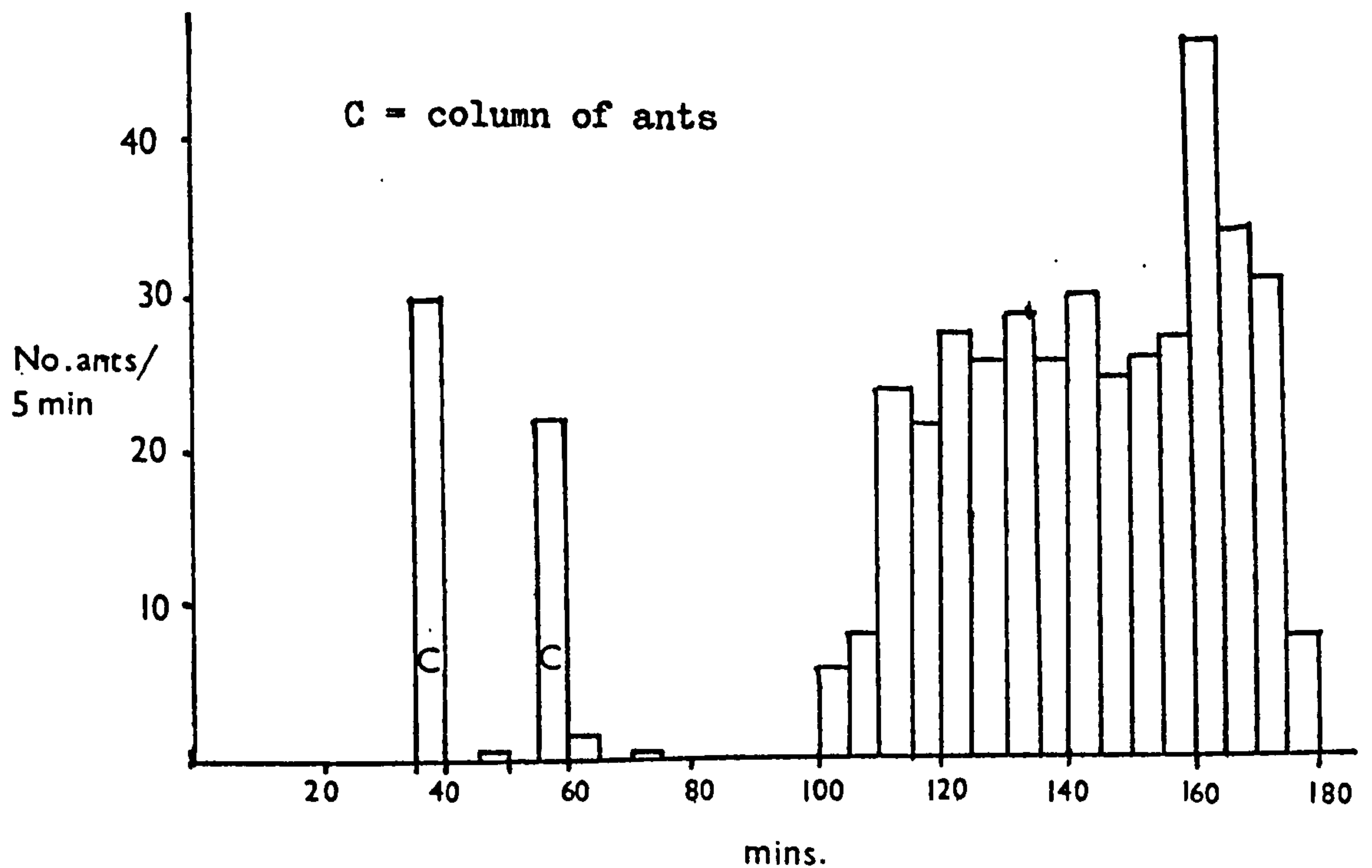
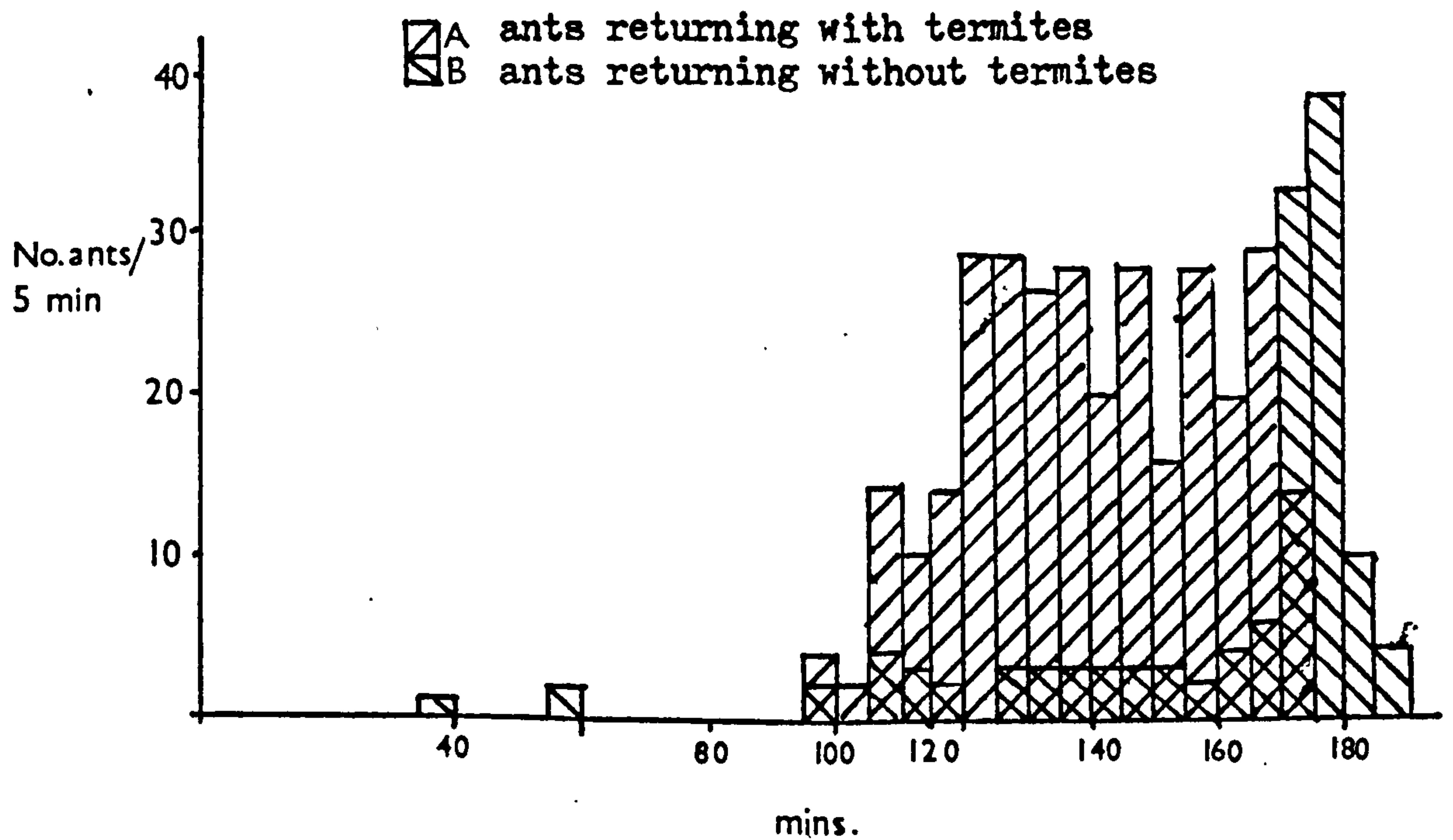


Figure 3.4b Mass recruitment in Decamorium uelense. Numbers of ants returning to the nest per 5 minutes.



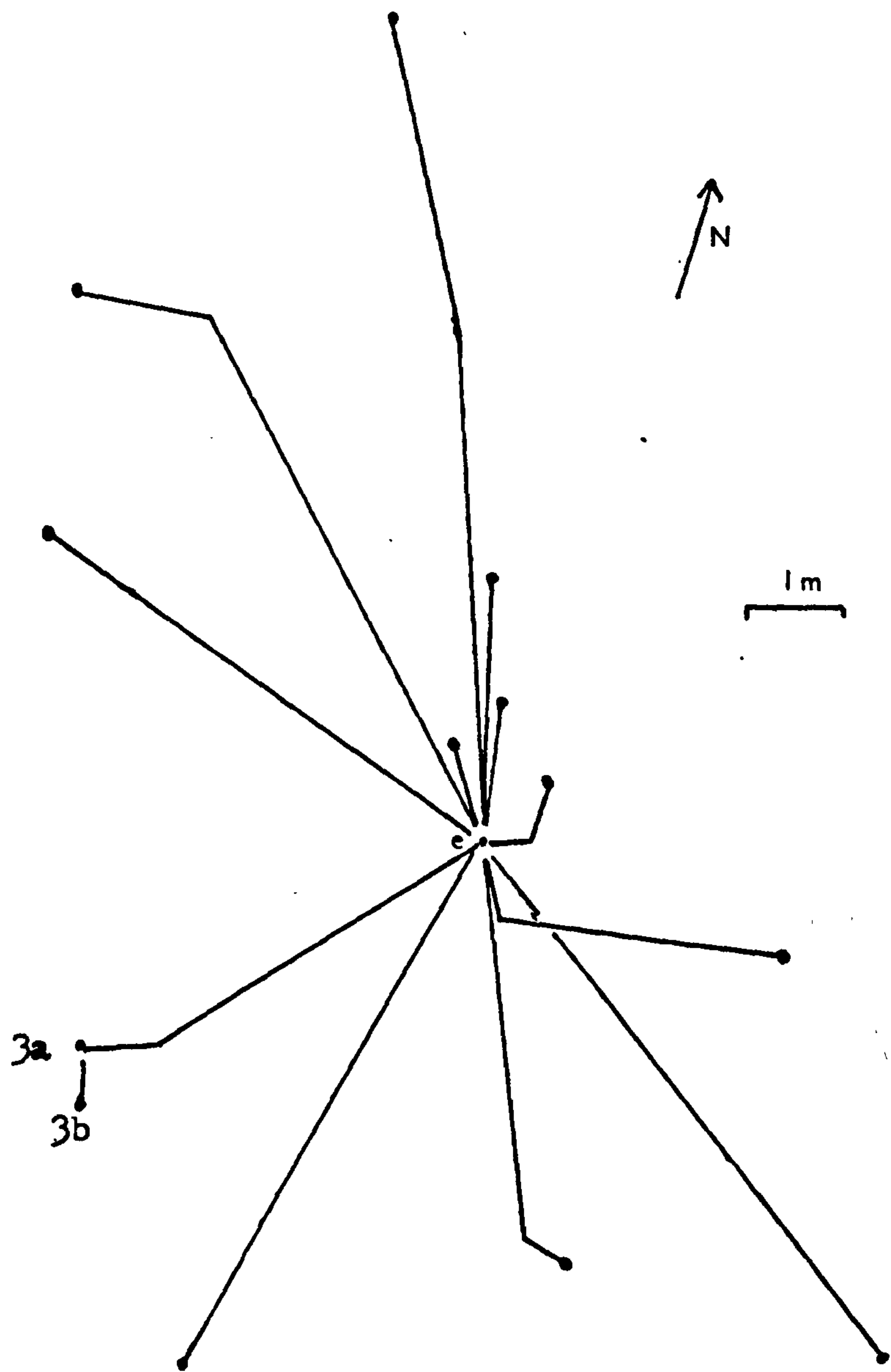


Figure 3.5 Foraging distances and directions on Decamorium.
(Colony No. 1, October 1976)

PLATE 3.5 Recruitment in Decamorium uelense.

I. A column of ants on its way to the foraging termites.

PLATE 3.6 Recruitment in Decamorium uelense.

II. Ants retrieve the immobilised termites (arrowed) at the end of the mass recruitment stage.

PLATE 3.5



PLATE 3.6



3.5 Discussion.

3.5.1. Foraging, Recruitment and Predation.

Observations at Mokwa indicated that opportunist predators could be divided into two broad categories which were not mutually exclusive. Large ants, with powerful mandibles, such as Myrmecaria eumenoides, Paltothyreus tarsatus, Camponotus spp., Polyrachis viscosa, Oecophylla longinoda and some Crematogaster spp. were capable of breaking open the termites protective soil sheeting and retrieving the prey. The smaller, less powerful ants, which were less able to break open the protective soil sheeting, were able to take termites which were in the process of constructing soil sheeting and were thus exposed to the hostile environment at the edge of this sheeting. The larger ant species can probably also take termites in the second manner; Crematogaster sp.E has been observed both breaking open soil sheeting and taking termites from the edge of the soil sheeting under construction. Camponotus maculatus has been observed at night breaking open sheeting and attacking M.subhyalinus rebuilding their mounds, a situation analogous to that at the edge of protective soil sheeting. It is also probable that many of the smaller predators can secure termites from the interstices of wood litter. Microtermes spp. and Ancistrotermes cavithorax are commonly found foraging in such situations and if ants could gain access to them they would represent a fruitful source of prey.

The two major specialised termite predators, Megaponera foetens and Decamorium uelense have both developed predatory regimes to cope with the habits of their different major prey items (4.3.1, 4.3.2). An effective predation strategy is to use scout ants to locate foraging termites and for the scouts to recruit more ants to the undisturbed termites. In M.foetens, the scout ant, utilizing cues in the prey termites soil sheeting (6.1), returns to the nest to recruit sister workers. Recruitment cannot be the same as that found in Monomorium pharaonis (3.1, Sudd 1960); a continuous two-way

traffic of ants is not feasible when dealing with a living prey such as foraging termites which have the ability to escape from their predators and which are repelled chemically by their attackers (Table 5.5). The sister workers must be recruited as a single bloc and carry out an highly organised raid in the minimum amount of time. This strategy, as adopted by M.foetens, involves the ants being recruited as a column, which when marching to the termites is up to three metres long (Levieux 1966, pers. observations Mokwa). This column halts at the end of a recruitment trail (Fig. 3.2) so that the eventual attack is carried out by the maximum number of ants in the minimum amount of time.

If the column of ants did not clump at the end of the recruitment trail, but went immediately into the attack, the last ant in a three metre long column (speed 0.03 m s^{-1}) would arrive at the edge of the termite foraging area 100 seconds after the attack had commenced. By forming a clump ca. 400 mm in diameter the last ants arrive only 13 seconds after the attack has commenced. The ants are also deployed over a broader initial front (ca. 300 mm opposed to a column ca. 100 mm wide) at the onset of the attack. Co-ordination of the attack on the termites by chemicals (6.3.3a) also serves to increase the efficiency of predation, as only a proportion of ants need to search for termites in the foraging area.

Decamorium uelense has utilized a similar recruitment method to M.foetens but has modified it to suit the foraging methods of its main species of termite prey. Macrotermes and Odontotermes, the main prey of M.foetens (4.3.1) forage mainly on leaf-, grass- and wood-litter under soil sheeting; their foraging areas extending over 0.5 square metres or more (N.M.Collins, pers.comm.). As such they represent a suitable prey for the large ponerine, but cannot be so easily utilised by the small (2-3 mm long) D.uelense.

Microtermes spp., and to a certain extent Ancistrotermes cavi-thorax, forage predominately inside grass roots, wood, roots and other litter and only occasionally utilize soil sheeting (T.G. Wood, pers.comm.). These termites represent an highly localized

prey source, for example wooden bait blocks in the later stages of attack by Microtermes spp. may contain several hundred termites (R.A.Johnson, pers. comm.). To secure this highly localised prey the ants could either attack en masse in a similar manner to the raids of M.foetens against M.bellicosus, or a few ants could immobilize the termites and recruit more ants to carry the cadavers back to the nest. The first alternative, although feasible, would probably be made difficult by the small size of termite galleries. Several hundred ants, even as small as Decamorium, would quickly block the galleries and the efficiency of predation would be reduced.

If, as in D.uelense, the ants are not repellent to their prey (Table 5.5), a few ants can infiltrate among the termite foragers and rapidly kill the termites (3.4.3), the efficient poison from the ant greatly assisting the process. To return the cadavers to the nest the assistance of extra sister workers is useful as each ant can carry only one termite. Rapid recruitment to remove the prey may prevent other scavenging ants (e.g. Pheidole spp.) from taking the dead termites.

Specific adaptations of the foraging regimes of ants to achieve efficient utilization of their prey have been studied in other specialised ponerines. Examples from Leptogenys spp., ants related to M.foetens, illustrate how differences have developed within the genus to secure their prey.

Fletcher (1971) studied Leptogenys spp. in southern Africa. The main food of L.nitida F.Smith was the terrestrial isopod Philoscia (Setophora) mina Budde-Lund. Successful foragers of L.nitida, equivalent to the scout ant of M.foetens, lay well defined odour trails to foraging sites where groups of up to five ants co-operate to capture each isopod. In effect miniture raids of a M.foetens (Mokwa) type are conducted, smaller numbers of ants being recruited as a corollary of the smaller numbers of isopods present compared with termites. As Fletcher observes, this recruitment method is

ideal for the utilization of prey which, although plentiful, is spatially localized by its specialized microclimatic and food requirements.

Maschwitz and Mühlenberg (1975) studied the sociobiology of Leptogenys ocellifera in Sri-Lanka. This ponerine lives in colonies of several tens of thousands of workers. The ants construct a number of elaborate permanent routes from the nest which are used continuously. Workers leave the main trails singly or in small groups, and if prey (earthworms and arthropods including the termites Odontotermes redmanni and Hypotermes obscuriceps) is encountered the ant returns to the nearest permanent trail, releasing a secretion from the poison gland. This secretion recruits more workers who reinforce the secondary trail laid by the explorers. When the prey has been exploited the secondary trails fade away. The availability of ants for predation at all times due to the permanent trail systems and large colony size, is another method of exploiting prey such as termites. It shows some similarities to the regime used by M.foetens in the Ivory Coast where recruitment trails are used more than once (Levieux 1966, 3.3.3, 3.5.2). The method used by L.ocellifera probably ensures that enough ants are recruited to prey foraging areas before the prey has retreated. It would be interesting to compare the efficiency of this predation strategy to that used by M.foetens, but unfortunately quantitative figures on the proportions and numbers of termites in its prey are not yet available.

3.5.2 Regional Variations in Foraging by Megaponera foetens.

The variations between southern Africa, central Africa and West Africa (Mokwa; 3.3.3) are relatively minor, and no obvious advantage can yet be suggested for either major or minor workers following the scout/guide ant. In the Ivory Coast recruitment does not involve a scout ant (Levieux 1966). Scout ant trails might be obscured by re-inforced trails from other foraging colonies where a high nest density exists, as in the Ivory Coast (22 colonies ha⁻¹).

Observations at Mokwa suggest that this is not the case. Scout ants leading columns of ants on the outward journey are not disorientated when they cross trails made by other colony columns which had passed less than two hours earlier (6 observations), suggesting that colonies have distinctive odour trails. Confusion only occurs in the rare instances when another column leaves the same nest after an earlier column. The second scout ant leads along its own scent trail, but some of the workers respond to the first recruitment trail, resulting in a split second column with ants going along the first and second trails (2 observations). The ants not led by the scout ant who are on the first trail return to the nest before progressing more than a metre.

Bernstein (1975) studied the foraging strategies of the "seed predatory ants" Veromessor pergandei and Pogonomyrmex spp. She found that foraging regimes could be grouped into three categories:

- i) "Individual foraging", where the individuals search and collect food independent of each other;
- ii) recruit foraging, where individual scouts search independently, but the actual collection of food is a highly coordinated group effort; and
- iii) group foraging, where individuals collect food independently, but travel along defined colony trails, or columns."

Investigations revealed that the foraging regimes could change in response to seed ("prey") density, with individual foraging at high seed densities and group foraging at low seed densities. An idealized summary of the foraging strategies of V. pergandei as studied by Bernstein is given in Figure 3.6a.

If the foraging strategies of M. foetens are examined in the light of Bernstein's finding a similar pattern may be distinguished. The foraging strategies of M. foetens throughout Africa can be grouped in a similar manner to the classification given for seed harvesters.

- i) Opportunist (=individual) foraging, where single ants attack termites. This has not been observed at Mokwa, but has been observed in East Africa (Kenya, W.A. Sands, pers.comm.);
- ii) recruit foraging, where a scout ant searches for termites which are collected by an highly coordinated column of ants. This is the only foraging regime observed at Mokwa;
- iii) group foraging, where individual travel along defined colony trails as observed in the Ivory Coast by Levieux (1966).

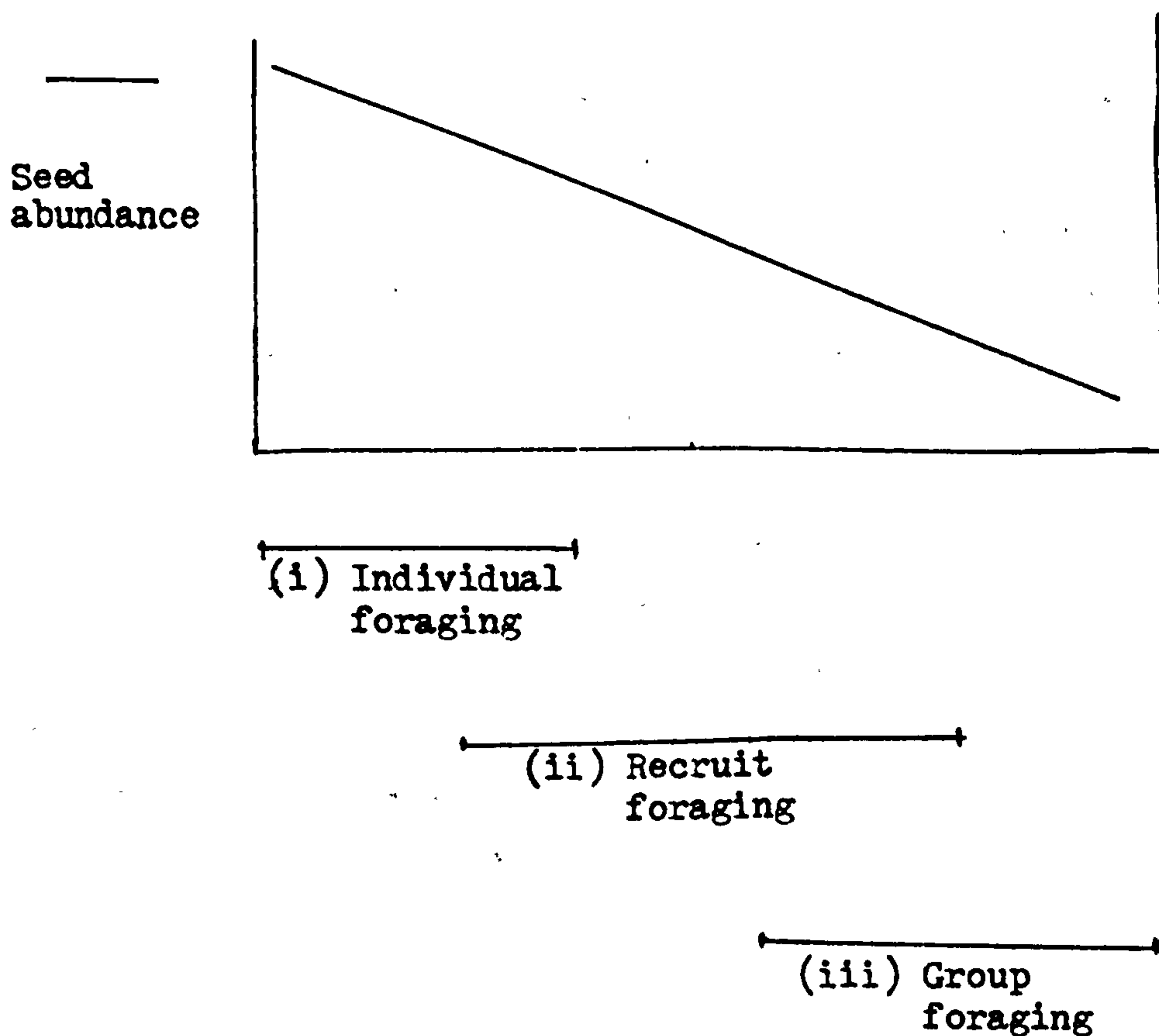


Figure 3.6a Foraging regimes of Veromessor in relation to seed abundance. (idealised from Bernstein 1975).

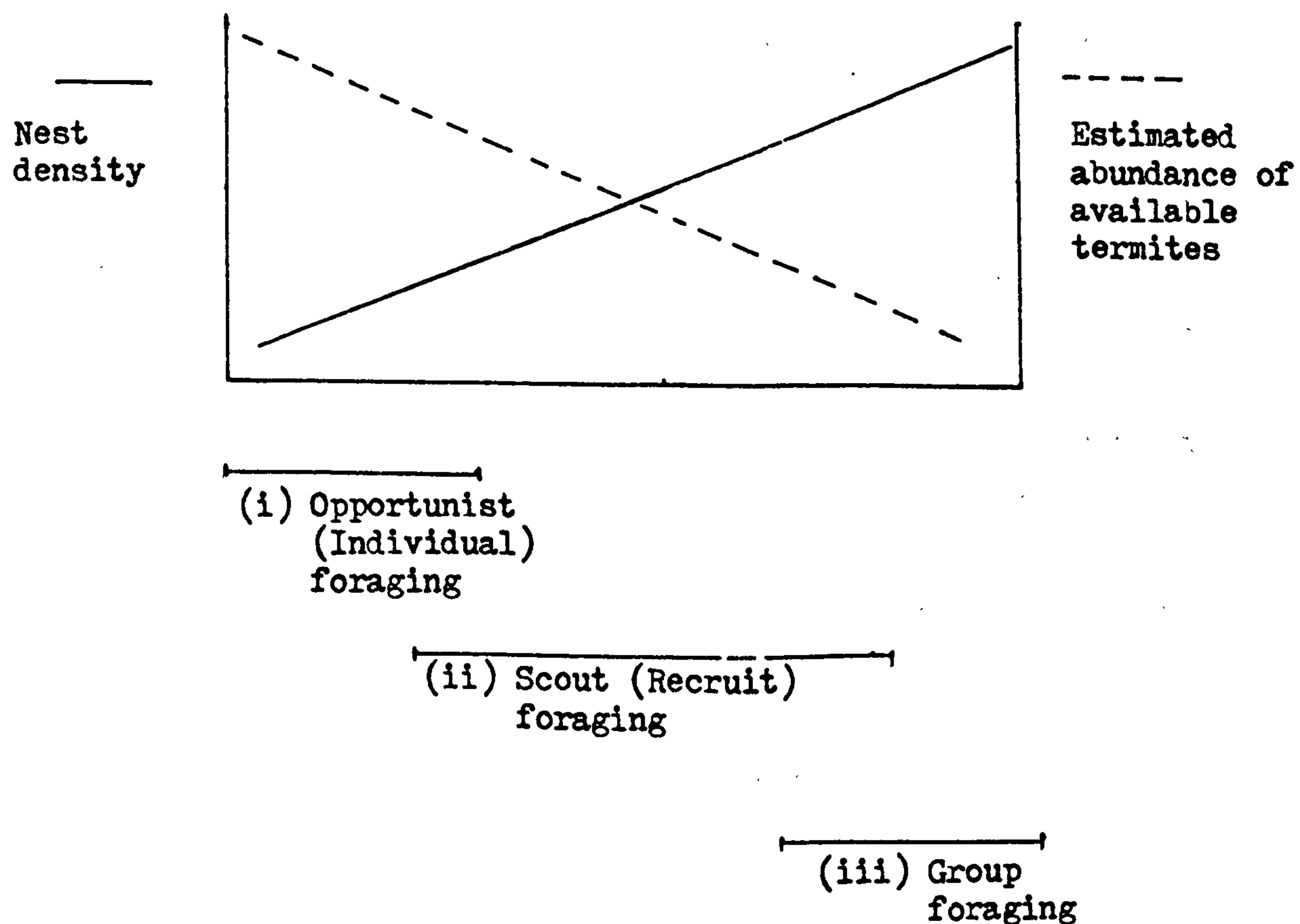


Figure 3.6b Foraging regimes of Megaponera in relation to abundance of available termites and nest density.

There are no figures available to compare the abundance of available termite tissue in the three areas. If colony density is taken as an indice of the pressure on available food, then in East Africa (Kenya, colony density ca. 4 ha^{-1} , Lepage pers.comm.) there is less pressure than in the Ivory Coast (colony density 22 ha^{-1} , Levieux 1966), which may allow a switch to type i foraging in the former area. The foraging strategies of M.foetens are summarized in Figure 3.6b in a similar fashion to those of Vero-nessor (Fig. 3.6a) to allow comparision. It seems that there may be a connection between food abundance (as expressed in predation pressure related to colony density) and foraging strategy in M.foetens. Unfortunately there is not enough data available from other parts of Africa to draw definâte conclusions.

3.5.3 Predators of Termite Brood Centres.

Although predation on brood centres has not been quantified in this present study it is a subject worthy of consideration. Brood centre predators can be divided into two categories, those which are nomadic and attack the brood when encountered during foraging, and those which live intimately in the brood centres, corresponding to Wheeler's (1936) lestobiotic ants.

The nomadic brood centre predators are exemplified by subterranean Dorylus spp. which are adapted to penetrate through soil spaces. Bodot (1961, 1967) concluded that the large proportion of unoccupied M.bellicosus mounds in her study area in the Ivory Coast were due to decimation by Dorylus dentifrons (q.v. 4.3.4). Macrotermes spp. do not flee when attacked by these ants (Wood and Sands 1977) but stay in the nests to fight and defend the relatively immobile queen. The marks of the ants mandibles have been found around the enlarged openings to the queen cell in both the Ivory Coast (Bodot 1961, 1967) and Mokwa, Nigeria (N.M.Collins, W.A.Sands pers. comm.). The large size of doryline colonies (Schneirla 1971) makes nomadic predation, coupled with complete destruction of the

colonies of their prey feasible and contrasts with strategies used by the predators living permanently in the termite nests.

No observations of predation by termitolestic ants have been made, but at Mokwa Carebara sp. have been found in M.bellicosus nests and these ants are assumed to prey on termite brood and early instars (Wheeler 1936, Lepage pers.comm.). Monomorium spp C and D, Tetramorium sp.F and T.termitobium have been recorded as living in the walls of termitaria and have been found on the fungus gardens and food stores of M.bellicosus; these ants probably prey on eggs and early termite instars. Those 'lestobiotic' ants assayed do not release repellent antagonisms in Macrotermitinae (Table 5.5 , 5.3.1b) and this factor must help them to move amongst and secure their prey.

3.5.4 Emigration by Megaponera foetens.

Arnold (1914) considered that M.foetens emigrated to areas of fresh sources of termites. In this study Colony 50 emigrated over short distances but (Fig. 3.7b) the foraging areas of the three sites overlapped, suggesting that some other factor, such as nest site flooding by early rains was responsible. Colony 10 (Fig. 3.7a) however, emigrated in the middle of the dry season. The emigration was then over a greater distance (50 m) and the new foraging area only slightly overlapped with the previous one. In an area where M.foetens colonies number 9 per hectare, exploitation of termites is likely to be so intense that competition would render emigration fruitless. It is then tempting to consider other possibilities, such as attacks by the subterranean burrowing Dorylus spp. These ants are known to attack M.bellicosus colonies (3odot 1967) and in their wanderings would also be likely to encounter Megaponera nests.

While observations on emigrating colonies did suggest that a leader ant was used in guiding the colony, no experiments were carried out to ascertain if this guide ant played any role in selecting the new nest site. The crustacean feeding ponerines, Leptogenys attenuata and L.nitida were shown by Fletcher (1971) to use a scout ant to guide colonies to new nest sites along odour trails. It seems likely that M.foetens would use similar mechanisms.

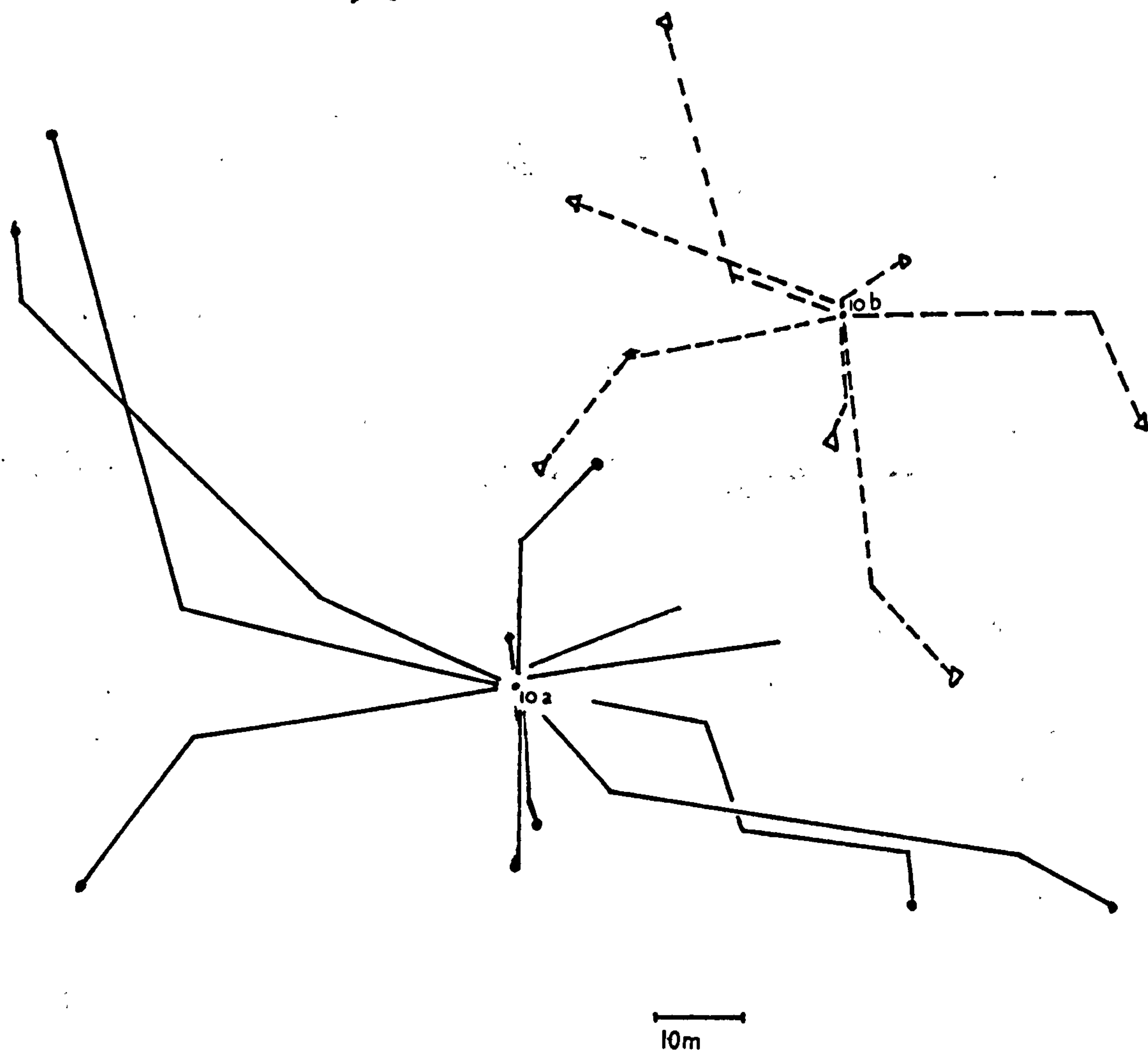


Figure 3.7a Foraging trails of Colony 10 at nest sites 10a and 10b.
(October 1975 -10a; April 1976 - 10b)

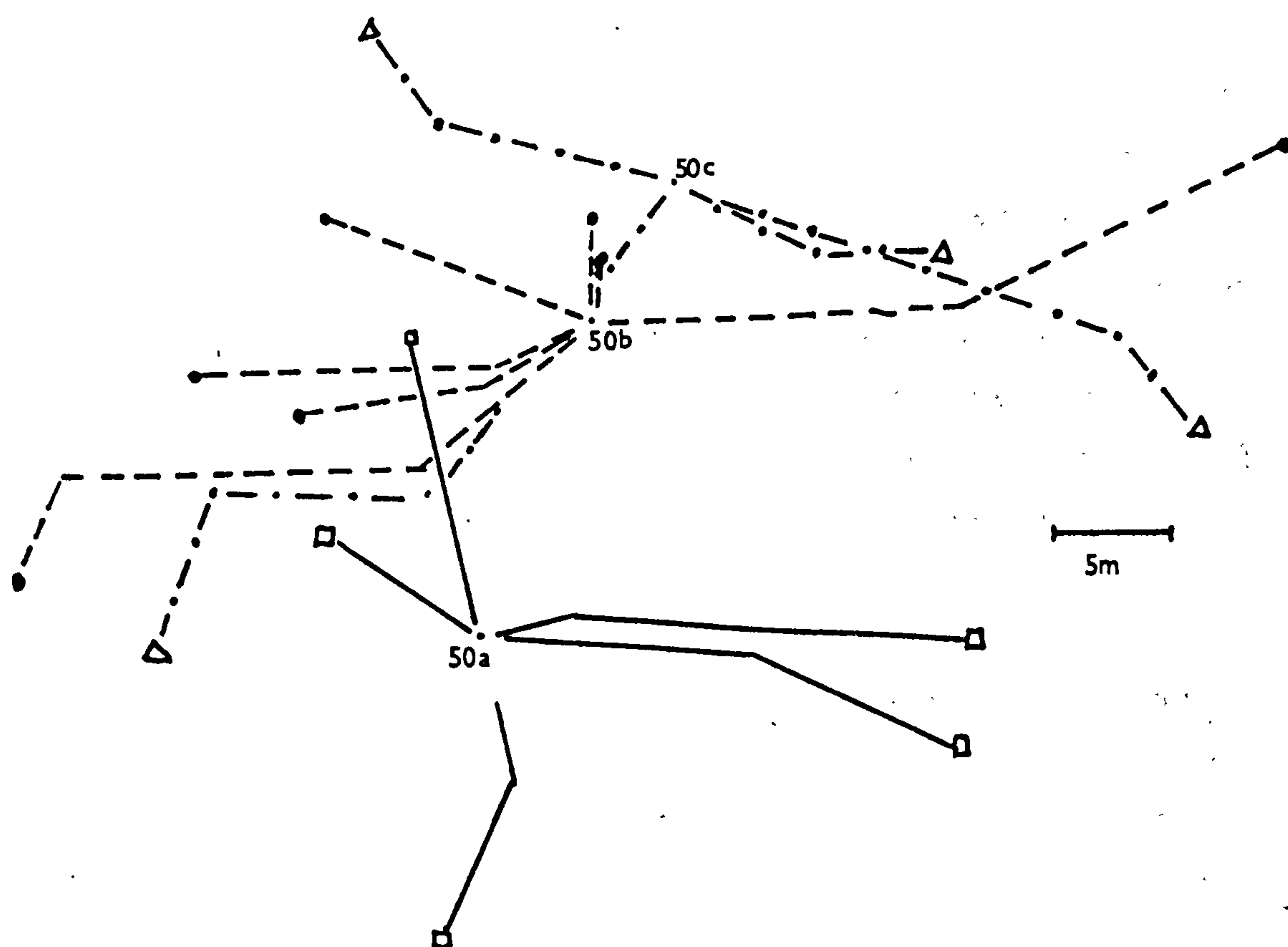


Figure 3.7b Foraging trails of Colony 50 at nest sites 50a, 50b
and 50c. (April/May 1976).

SECTION 4. ECOLOGICAL IMPACT OF ANTS ON TERMITES.

4.1 Introduction.

Ants have always been regarded as important predators of termites. Arnold (1915) notes that for Brachyponera sennaarensis the "economic value of this little species can hardly be overestimated, since it is exceedingly plentiful and preys unceasingly on termites". Wheeler (1936) gives a list of ant predators and their termite prey (5.3.1b) and attempts to indicate which are the most important predators, but no figures were available for him to give substance to his ideas. Forty years after Wheeler's (1936) classification of the ecological interactions between ants and termites, data is still not available to quantify the predatory interactions, although workers have continued to make observations on ant/termite interactions both in the field (Levieux 1966, Sheppe 1970 and Fletcher 1973) and the laboratory (Smythe and Coppel 1964, 1973, Beard 1973).

There are a number of reasons for this lack of quantitative data on the predation of termites by ants. The biology and taxonomy of many of the groups of potential ant predators is still badly in need of revision (2.3.1). This may partly be a reflection of the great diversity of ant species in the tropics. Lack of knowledge of the basic foraging regimes of ants must have discouraged the setting up of complex sampling programmes to monitor predation, especially of the opportunist predators. To sample adequately, a source of trained manpower is required and such scientist-technicians are only now becoming available in the tropical countries where the majority of interactions between ants and termites take place.

Studies on predation are of little value if parallel research has not been carried out on the population biology and ecology of the termite prey. Such studies are only just reaching fruition through work in the Ivory Coast (Josens 1971), Senegal (Lepage

1974) and Nigeria (ODM Report 1977, Wood et.al. 1977, Wood and Sands 1977). This present study on ant predation was greatly assisted by the availability of population data and information on termite foraging from the Nigerian Guinea Savanna.

In the Nigerian Guinea Savanna at Mokwa termites represent a live weight biomass of 10.59 g m^{-2} , of which 6.39 g m^{-2} is accounted for by Macrotermitinae (Wood and Sands 1977) - a rich potential source of energy for predators. The return of this energy to the ecosystem can occur via a number of routes; constructional saliva, faeces or termite tissue (Wood 1976). At Mokwa returns via termite tissue are the most important, and of this, returns via oophagy and cannibalism are considered much less important than returns via predation.

The most suitable ants for studies on predation are those with predictable foraging regimes. Megaponera foetens fits into this role well (3.3.1), as observations indicated that at Mokwa it foraged only in the early morning and late afternoon/early evening. Decanorium uelense was also chosen for predation studies; as a specialised termite predator its behaviour shows both similarities to and differences from that of M.foetens (3.4). Because of its small size, the difficulty of locating nests and less predictable foraging periodicity, D.uelense was not ideally suited for studies on predation. The large number of ant species which have been observed preying opportunistically on termites (2.3) makes it necessary for quantitative figures on predation to be obtained from them. Four ponerine genera, representing a range of specializations, were chosen to contrast and compare opportunist predation with that of the specialised M.foetens and D.uelense.

4.2 Materials and Methods.

4.2.1 Predation by M.foetens on Savanna Macrotermitinae.

4.2.1a Nest Density

The abundance of M.foetens was estimated to be 9 colonies per hectare. Abundance of nests was estimated during April 1976, after the tall savanna grasses had been burnt; observations were facilitated by these conditions (2.2.2). Nests were located by three methods - following columns of ants returning from attacks on termites, following scout ants or looking for discarded pupal cases in the vicinity of likely nest sites (2.3.1 M.foetens).

4.2.1b Sampling Regime.

From information obtained on the activity cycles of M.foetens (3.3.1), nests were sampled between 0700 - 0930 and 1615 - 1830 hours GMT+1 on alternate days. Four nests were selected for sampling from two 1 hectare sites and they were observed in sequence until emigration (3.3.6) occurred, when another nest was selected. During June 1976 eight sampling days were lost.

4.2.1c Information Collected.

Data were collected on the number of ants in a raiding column, the number of returning ants carrying termites and the number of termites per ant in a sample of ten ants from each column. The termites were identified but information on their caste composition was not collected as preliminary observations indicated wide variations, probably due to the age of the termite foraging party; more soldiers probably being present in food-searching than food-consuming parties.

4.2.1d The Effect of Emigration on Sampling.

Emigration is described in 3.3.5. Nests were classified as non-functional if two consecutive observations on the same nest revealed no activity (columns of ants, scout ants, excavation); the colony was then assumed to have emigrated after the last observed active period. In the calculation of monthly predation figures (4.2.1e), because a proportion of the total observations were on non-functional nest sites, a correction was made during

the calculation of R (Equation 2, 4.2.1e). If O_t is the number of observations made during the calendar month, and O_i the number of observations made on non-functional nests, then O_v , the number of valid observations per calendar month, is equal to $O_t - O_i$.

4.2.1e Analysis of Predation.

Predation (P), expressed in termites per square metre per day, was calculated for each calendar month using Equation 1.

$$P = \frac{A \cdot T \cdot R}{10,000} \quad \text{termites} \cdot \text{metres}^{-2} \text{ day}^{-1} \quad (\text{Equation 1})$$

where:

A = mean number of ants carrying termites per raid for the calendar month,

T = mean number of termites carried per ant per raid for the calendar month, and

R = the number of raids on the selected species of termite for the calendar month, calculated from Equation 2.

$$R = \frac{R_s \cdot C \cdot 2}{O_v} \quad \text{raids} \cdot \text{hectare}^{-1} \text{ day}^{-1} \quad (\text{Equation 2})$$

where:

R_s = the number of observed raids on the species of termite for the calendar month,

C = the number of colonies per hectare,

2 = the estimated number of raids per observation day, and

O_v = the number of valid raids per calendar month (4.2.1d)

4.2.1f Treatment of raids in which more than one species of termite were taken.

Raids were sometimes recorded when more than one prey species was taken, especially in the wet season. For example Ancistrotermes cavithorax appeared with Macrotermes bellicosus on twelve occasions (Table 4.4). In such cases the two termite species were apportioned equally between the number of ants carrying termites for that raid, as the mean number of termites per ant (T, Equation 1) was sampled without regard to the termite species. M.bellicosus and Odontotermes spp. do not differ significantly for the value of T (M.bellicosus mean = 2.42, Odontotermes spp. mean = 2.88; $p > 0.05$ for 't'-test with equal variances) and the value of T for two columns with A.cavithorax alone was 4.5 and 2.2. For Microtermes spp. the value of T can be somewhat higher (8 - 10 per ant) which may result in a slight underestimate for predation on this genus.

4.2.1g Assumptions made in the calculation of predation.

In the assessment of emigration and colony density it is assumed that any colonies leaving the study area are replaced by new, immigrating colonies. This is reasonable as the nest density in the area surrounding the study plots was approximately 9 colonies per hectare and emigration occurs throughout the year (Table 3.2).

Colonies for sampling were selected at random and as the foraging columns varied in size it might be expected that there would be variation between months in mean column size. This was not found to be so (Table 4.1) and may be due to the rarity of M.foetens columns of under 100 and over 500 ants. When the biology of the alate male of M.foetens was considered (Appendix A) it was concluded that new colonies could arise by the splitting of larger colonies, this might explain the lack of very small or very large columns (Table 4.1) or colonies (Table 2.1)

Table 4.1 Mean Monthly Numbers ($\pm$ standard error of the mean)
of Megaponera foetens in Raiding Columns
(October 1975 - October 1976)

Month	Mean monthly column size ($\pm$ standard error of the mean)	Probability of a significant difference between months ^a
Oct. 1975	214.4 $\pm$ 20.3	>0.05
Nov.	328.4 $\pm$ 82.0	>0.05
Dec.	410.4 $\pm$ 65.1	>0.05
Jan. 1976	445.0 $\pm$ 20.0	>0.05
Feb.	398.3 $\pm$ 35.2	>0.05
Mar.	443.6 $\pm$ 26.8	>0.05
Apr.	349.4 $\pm$ 40.6	>0.05
May	277.9 $\pm$ 51.1	>0.01 - 0.05
June	407.8 $\pm$ 27.7	>0.05
July	417.3 $\pm$ 27.4	>0.05
Aug.	445.5 $\pm$ 29.3	>0.05
Sept.	417.7 $\pm$ 26.6	>0.05
Oct.	323.3 $\pm$ 32.1	>0.05

^a 't'- test for data with unequal variances (Clarke 1969)

4.2.2 Predation by Decamorium uelense on Savanna Termites.

4.2.2a Nest Density.

The entrance to the nest of Decamorium uelense is a small hole, 1 - 1.5 mm in diameter, in the soil. There are rarely any signs of soil workings around the nest entrance and as the hole resembles that of a number of other ant species, it is not possible to search for colonies in the absence of foraging ants. The ants are only ca. 2 - 3 mm long and the location of single foragers is also very difficult. Fortunately the columns of ants setting out on raids (3.4.2) and workers returning carrying termites (3.4.4) are more easily seen, and colonies have therefore been located by searching for ants in either of these two stages of their attacks on termites. Intensive searching in the study area in the primary savanna woodland between 0700 - 1700 hours resulted in the location of a substantial number of nests, and from the spacing of these nests the colony density was calculated.

The nearest-neighbour technique (Blackith 1958) was used to calculate nest density, with the modification that the starting point for searching was not randomly selected, but was the first nest encountered in the study area. The density of nests was calculated from:

$$m = \frac{1}{4 \cdot d^2} \cdot 10,000 \text{ nests hectare}^{-1} \quad (\text{Equation 3})$$

where m = density per unit area, and

d = mean distance between nearest-neighbours (14.88 m).

The nest density of D.uelense in the study area was therefore 11 (11.29) nests per hectare.

4.2.2b Sampling Regime.

Observations indicated that foraging was not as predictable as in M.foetens (3.3.1), but as the majority of colonies did forage in the mornings, it was decided that observations should be carried out between 0700 - 1000 GMT+1, or longer if foraging was in progress. Observations were made once a week: the study area was searched at 0700 hours and the foraging status of the nests was determined. The first active nest encountered on the assessment round was returned to and observed until 1000 hours. At this time all other nests were re-examined and their foraging status re-established.

4.2.2c Information Collected

The number of ants returning and leaving the nest during five minute intervals was recorded, and those ants returning with termites were noted. The number of separate raids during each observation period was determined as each colony may mount a number of raids each day, sometimes simultaneously. The distance between the nest and the termite foraging area was also recorded.

4.2.2d Analysis of Predation

Predation pressure (P) was calculated from the formula;

$$P = \frac{(C \cdot 2.2 \cdot R \cdot T)}{10,000} \quad \text{termites} \cdot \text{metre}^{-2} \cdot \text{day}^{-1} \quad (\text{Equation 4})$$

where

P = predation pressure in termites per metre squared per day,

T = mean number of termites taken per raid per calendar month,

C = the proportion of active nests per calendar months, calculated

from $\frac{\text{Total number of active nests per month}}{\text{Total number of observation periods per month}}$,

R = the number of raids per colony per observation period for

the calendar month, calculated from

$$\frac{\text{Total number of raids on the termite species for the month}}{\text{Total number of observation periods per calendar month}}$$

and

2.2 = a correction factor for the proportion of colonies sampled with respect to the total number of colonies per hectare

$$\text{when } \frac{\text{Number of colonies ha}}{\text{Number of colonies sampled}} = \frac{11}{5} = 2.2$$

4.2.3 Opportunist Predation by Ponerines

4.2.3a Species of Ponerines Studied

Four opportunist ponerine predators, Paltothyreus tarsatus, Brachyponera sennaarensis, Bothroponera soror and Anochetus sedilloti were selected for observation. B.sennaarensis was rare in the primary savanna woodland and only one nest was available for observation. These four ponerines were selected because preliminary observations suggested that, although detailed information on foraging habits was not available, they foraged throughout the day and night.

4.2.3b Nest Densities.

The nest density of P.tarsatus was obtained by following the conspicuous single ants back to the nest and searching for signs of ant activity at likely nest sites. The nest density in two areas at kilometre 17 was 2 and 2 colonies per hectare and at kilometre 15 (N.M.Collins study area, Collins 1977b) on two 1 hectare sites the density was 3 and 7 colonies per hectare. These figures were averaged to the nearest whole colony, to give a density of 4 per hectare for use in calculations of predation. The estimation of colony density in B.soror, B.sennaarensis and A.sedilloti has been described in 2.2.2. and was found to be 8, 1 and 12 colonies per hectare respectively.

4.2.3c Sampling Regimes.

A random sampling technique for known active nests of the four species could not be conveniently used and in lieu of this, nests were sampled when time was available for observation. Observations were carried out for one hour periods before another nest was selected for observation. Eventually a comprehensive picture of foraging was built up between 0700 - 2000 hours for each species. Because of the lack of time available only a limited number of observations could be made, but a total of 75 hours monitoring was achieved over the six visits to Mokwa.

During observation periods the number of ants returning to the nest and those returning with termites and other food was noted. In those cases when the termite could not be identified in the ants mandibles (size, colour of the head capsule and shape were useful clues), the termites were taken from the ants for identification. The ant was trapped in a glass specimen tube and the termite prey taken from the ant with fine forceps. The ant was then released near to the place it was taken from. Ants returning with arthropods other than termites, or other prey items, were not relieved of their prey.

4.2.3d Analysis of Predation.

Predation (P) was estimated with the following formula:

$$P = \frac{\left(\frac{N}{h}\right) \cdot 24 \cdot C}{10,000} \text{ termites } \cdot \text{metre}^{-2} \text{ day}^{-1} \quad (\text{Equation 5}),$$

where:

P = predation in termites per metre squared per day,

N = mean number of termites taken over the total period of observation for the ant species,

h = total number of hours observation on the ant species

24 = hours per day, and

C = number of colonies per hectare for the ant species.

4.2.3e Assumptions made in the calculation of predation.

The expression ($\frac{N}{h}$) in equation 5 assumes that foraging occurs with an even frequency throughout the day. There is evidence for this from casual observations. No information is available on the seasonality of foraging. Personal observations have only been made in September, October, November, April and May in any year, which precludes observations at the peaks of the wet and dry seasons. It is probable that seasonal differences and even those throughout the day would become apparent during a long-term detailed study, but in the absence of further information equation 5 has been used to estimate opportunistic predation.

4.3 Results and Discussion

4.3.1 Predation by Megaponera foetens on Macrotermitinae.

The mean daily predation of Macrotermitinae at Mokwa was 0.5286 termites per square metre (Table 4.2), of which 73.2% was accounted for by predation on Macrotermes bellicosus and 21.8% on Odontotermes spp. Predation on M.bellicosus resulted in an annual turnover slightly exceeding the standing crop population. However, predation is on foraging parties, which consist of mature soldiers and workers, whereas these castes make up only 41% of the non-reproductive population, the rest consisting of larvae (Collins 1977b). Thus predation by M.foetens results in the turnover of the M.bellicosus foraging worker/soldier population at the rate of 2.7 times per year. This is likely to be an underestimate as an unknown proportion of these castes may be confined to the nest chamber and never take part in foraging. Wood and Sands (1977) noted that whereas the Production:Biomass ratio of termites other than Macrotermitinae was in the region of those recorded for other invertebrates (approximately 3:1), the ratio for the Macrotermitinae is in the region of 5:1 to 10:1. They indicated that these high levels were due to the production of neuter castes

Table 4.2 Predation by Megaponera foetens on Macrotermitinae;
October 1975 - October 1976. (termites $m^{-2} day^{-1}$)

Month	Macrotermes bellicosus	Odontotermes spp.	Ancistrotermes cavithorax	Microtermes spp.	Macrotermes subhyalinus	Monthly totals
Oct. 1975	0.1730	0.0380			0.0001	0.2111
Nov.	0.2359	0.0220				0.2579
Dec.	0.2820	0.2620				0.5440
Jan. 1976	0.7087	0.1347				0.8434
Feb.	0.3469	0.1676				0.5145
Mar.	0.7040	0.3234				1.0274
April	0.4863	0.0659		0.0001	0.0017	0.5540
May	0.2373	0.1156	0.0275			0.3809
June	0.2741	0.1790	0.0702			0.5233
July	0.2897	0.0976	0.0295			0.4168
Aug.	0.4671	0.0075	0.0403			0.5149
Sept.	0.4870	0.0236	0.0303	0.0356		0.5765
Oct.	0.3387	0.0608	0.0450	0.0613	0.0008	0.5066
Total	5.0312	1.4977	0.2428	0.0970	0.0026	6.8713
Mean	0.3870	0.1152	0.0187	0.0075	0.0002	0.5286

Table 4.3 Termite Population and Turnover by Megaponera foetens

Termite species	Predation: termite $m^{-2} a^{-1}$	Worker/Soldier Population termite m^{-2}	Predation as a percentage of abundance
Macrotermes bellicosus	141.26	128 ^a	110.4
Odontotermes spp.	42.05	260 ^b	16.2
Ancistrotermes cavithorax	6.82	881 ^b	0.8
Microtermes spp.	2.72	850 ^b	0.3
Macrotermes subhyalinus	0.07	37 ^b	0.01

^a From Collins (1977b). This figure includes larvae; the workers/soldiers alone account for 52 termites m^{-2} .

^b From Wood and Sands (1977). Core samples, southern Guinea Savanna, Mokwa

(workers/soldiers) and may well have been a response to heavy predation, such as that by M.foetens on M.bellicosus.

Several factors lead to M.bellicosus being the principal prey of M.foetens. These include the abundance of termites, their seasonal foraging pattern, foraging behaviour, relative size and the reactions of the ants to the termites and their products, and the response of the termites to the ants.

Termite abundance is shown in Table 4.3, indicating that, with the exception of Macrotermes subhyalinus, M.bellicosus is the least abundant of the prey species. The consistent monthly predation on M.bellicosus (55% taken in the six month dry-season, November - April) compared to the more seasonal predation on Odontotermes spp. (65% taken during November - April) is probably a reflection of the seasonal foraging behaviour of the termites; M.bellicosus forages all-year round, whereas Odontotermes spp. forage principally in the dry season (Collins 1977b). Ancistrotermes cavithorax and Microtermes spp., although very abundant (Table 4.3) are an insignificant prey (5.3% of the total) and as they rarely forage on the surface during the dry season they are not preyed on during this period.

Foraging behaviour of the termites influences predation through its effect on their availability. Macrotermes spp. and Odontotermes spp. forage principally on wood-, grass- and leaf-litter, covering it with a thin layer of soil and feeding beneath this protective cover. Foraging M.foetens can break open this soil sheeting and remove the termites. A. cavithorax and Microtermes spp. forage less frequently in this way. They forage principally within their food source, of roots, grasses and wood-litter, entering it at points of contact with the soil. They are therefore largely unavailable to M.foetens which are too large to enter the termites foraging tunnels.

Size also favours predation on Macrotermes spp. A catch of 200 termites would (based on the weights of major workers, Wood and

Sands 1977) supply approximately 3.5 g from M.subhyalinus, 2.0 g from M.bellicosus, 0.6 g from Odontotermes spp., 0.4 g from A.cavithorax and 0.2 g from Microtermes spp.

None of these factors explain the low rate of predation on M.subhyalinus which is larger in size than M.bellicosus. The occasional raids on this species are usually very inefficient with few termites being taken; for instance, one raid of 215 ants recovered only 5 termites. The larger soldiers appear to be physically capable of defending themselves against the ants, and there is evidence (6.2.4a) that the soldier defence secretion is obnoxious to the ants.

A.cavithorax and Microtermes spp. sometimes appear in columns of ants carrying M.bellicosus (Table 4.4), whereas A.cavithorax has only been recorded as the sole catch on two occasions and Microtermes spp. never. There is evidence (6.2.4a) that soldiers of both of these genera are obnoxious to M.foetens and that the scout ants do not find their protective soil sheeting as attractive as that of Macrotermes spp. or Odontotermes smeathmani (6.1.4, Table 6.3). Mixed catches occurred when different species of termite were foraging close together. When an attack on M.bellicosus commenced the ants spread out (3.3.2, Fig. 3.2) and thereby encountered other termite species as the raid progressed.

In other areas of Africa raids of M.foetens have been observed on the termite nests, with both mature workers and larvae appearing in the prey. Sheppe (1970) recorded a raid of M.foetens on Odontotermes latericus (Haviland) in which the ants entered the nest through an open chimney. Raids of this type were not observed at Mokwa during the study period, but Collins. (N.M.Collins, pers. comm.) reported a raid on a M.bellicosus nest which had been damaged by rain; both adults and larvae were taken.

At Mokwa, no species other than Macrotermitinae have been recorded as prey. Sheppe (1970), noted that in Zambia Macrotermitinae were

Table 4.4 Prey Composition of M.foetens Columns
Raiding Macrotermitinae

PREY SPECIES	NUMBER OF COLUMNS
Macrotermes bellicosus	133
Odontotermes spp.	46
Ancistrotermes cavithorax	2
Macrotermes subhyalinus	3
Microtermes spp.	0
M.bellicosus + Odontotermes spp.	4
M.bellicosus + A.cavithorax	12
M.bellicosus + Microtermes spp.	2
M.bellicosus + M.subhyalinus	1
M.bellicosus + A.cavithorax + Microtermes spp.	1

the most common prey species. Of 15 columns observed 9 were carrying Odontotermes spp., 3 Pseudacanthotermes militaris (Hagen), 2 mixed catches of these two species, and only one column a non-Macrotermitinae, Hodotermes mossambicus (Hagen). In Tanzania, Sheppe observed M.foetens preying on Macrotermes subhyalinus. Weber (1964) recorded the following Macrotermitinae as the prey of M.foetens collected by Emerson in the former Belgian Congo (Zaire); Odontotermes sladeni Emerson, Microtermes calvus Emerson, Odontotermes planiceps (Sjöstedt), Pseudacanthotermes militaris, Acanthotermes militaris (Sjöstedt) and Protermes sp.

Trinervitermes spp. (Nasutitermitinae) are grass-harvesting, surface-foraging termites, which like Hodotermes spp. in East and southern Africa, might be expected to occur as prey of M.foetens. This has not been observed at Mokwa despite the abundance of Trinervitermes spp. (680 m^{-2} , Wood et.al. 1977). Observations on M.foetens columns which encountered foraging T.geminatus and T.togoensis, indicated that the ants' columns were disrupted (6.2.1) and that minor worker ants which came into contact with soldier termites were mechanically disabled or killed (6.2.1) by the termites sticky defence secretion. Monoterpene hydrocarbons and alcohols from the frontal gland of the termite soldiers repelled the ants from their own recruitment trails (6.2.4c).

4.3.2 Predation on Termites by Decanorium uelense.

The major prey species taken by D.uelense is Microtermes. Analysis of prey captured from foraging ants indicated that ca.80% of the raids were on M.usambaricus (sp.A). Reference to Table 4.5 reveals that there was a decline in the amount of prey taken by D.uelense in the dry season, decreasing from 0.2050 termites per square metre per day in October (1976 wet season continued into November) to 0.0004 termites per square metre per day in March at the end of the dry season. The figure of 0.0011 termites per square metre per day for April represents termites taken after the start of the rains; D.uelense was not foraging early in the month. During



Table 4.5 Predation on Termites by Decamorium uelense
(termites $m^{-2}day^{-1}$)

Month	Microtermes spp.	Ancistrotermes cavithorax	Microcerotermes spp.
Oct. 1976	0.2050		
Nov.	0.0876	0.0015	
Dec.	0.0517		0.0033
Jan. 1977	0.0575	0.0022	
Feb.	0.0487		
March	0.0004		
April ^a	0.0011		
May	0.4969	0.0012	
June	0.3203		
July	0.3763		

^a figure obtained after the start of the rains at the end of April.

the period of heavier rains between May and July the predation increased again (Table 4.5).

Ancistrotermes cavithorax and Microcerotermes spp. were both taken as alternative prey items, principally in the dry season, but their total contribution to termite intake is less than 2% of the predation on Microtermes spp. These three genera of termites are all of a similar size, the major workers being only slightly larger than the workers of D.uelense. Other genera, such as Macrotermes and Odontotermes, are larger and would be less easily handled by D.uelense. The latter two genera also forage over a wider area, whereas Microtermes, Ancistrotermes and Microcerotermes represent a concentrated prey (4.3.1), foraging inside roots and woody litter.

Total predation by D.uelense on Microtermes spp. is in the region of 60 termites per metre squared per year. With a Microtermes spp. population of 850 per square metre in primary savanna (Wood and Sands 1977), turnover of the worker/soldier population of this termite could be achieved in 14 years, compared with a turnover rate of 312 years for Microtermes by Megaponera foetens (Table 4.3)

4.3.3 Predation by Opportunist Ponerines on Termites.

Anochetus sedilloti.

Thirty hours of observation were undertaken on A.sedilloti between 0700 - 2000 hours GMT+1. During the total observation period 318 foragers returned to the nest; 184 of these carried termites, 87 "other arthropods" and 21 (7%) unidentifiable plant or animal material. The remainder of the foragers were not carrying anything in their mandibles, but could have been carrying liquid in the crop. The figures indicate that 53% of foragers return with termites and 27% with other arthropods.

The major prey termites were Microtermes spp. (Table 4.6), if it is assumed that these are only available for half of the year

(2.4.3), predation is 21 termites per metre squared per year. M. bellicosus is also an important prey item (10.5 termites $m^{-2}a^{-1}$) and other Macrotermitinae account for 8.8 termites per metre squared per year.

Brachyponera sennaarensis.

Thirteen hours of observations were made on this ant between 0600 - 1900 hours. During the observation period 1460 ants returned to the nest, 306 carrying termites and 193 other arthropods. Another 537 ants were carrying unidentifiable plant or animal material. These figures represent 25% with termites, 13% with other arthropods and 40% with other plant and animal material. The remaining 22% of foragers did not have any solid material in their mandibles.

Although B. sennaarensis is an ant with a catholic diet (Arnold 1915) it is also an important termite predator, taking 20 termites per metre squared per year in the primary savanna, an area where it is not common. In open savanna and parkland where nest densities are higher and other ponerines are scarcer the impact of this species must be greater. Thus despite its unspecialised habits B. sennaarensis is still an important opportunist predator of termites

Bothroponera soror.

B. soror was observed for 25 hours, and during the observation period 772 foraging ants returned to the nest. 275 (36%) of these carried termites, 205 (27%) other arthropods and 117 (15%) unidentified plant and animal material. The remaining 22% of foragers returned to the nest without material in their mandibles.

B. soror, like A. sedilloti, has a high nest density (8 colonies ha^{-1}) in the primary savanna. In consequence its overall contribution as a termite predator is substantial. 56 Termites per metre squared per year are taken, a figure comparable with the total termite consumption by A. sedilloti (43 termites $m^{-2}a^{-1}$).

Table 4.6 Predation on termites by opportunist ponerines (termites $m^{-2} day^{-1}$).

Termite Species	Anochetus sedilloti	Brachyponera sennaarensis	Bothroponera soror	Paltothyreus tarsatus
Microtermes spp.	0.1152	0.0240	0.1229	0.0905
Macrotermes bellicosus	0.0283	0.0059	0.0530	0.1152
Macrotermes subhyalinus		0.0006	0.0008	0.0247
Ancistrotermes cavithorax	0.0115	0.0035	0.0054	0.0590
Odontotermes spp.	0.0125	0.0080	0.0092	0.1097
Trinervitermes spp.	0.0038	0.0048	0.0123	0.0178
Other species	0.0048	0.0091	0.0092	0.0400

If the three species, A.sedilloti, B.soror and Br. sennaarensis are compared, a gradation in specialisation as small arthropod (including termites) predators can be discerned. A.sedilloti, with colonies of less than 100 workers, take over 80% of their prey as termites and small arthropods. Observations on foraging Anochetus revealed that their slender trident mandibles were adept at extraditing small animals from crevices (eg, 3.2).

Bothroponera, with colonies of 100 - 200 workers, take 63% of their prey as small arthropods including termites. A greater proportion of unidentifiable plant and animal material is taken. Observations on B.soror revealed that recruitment by tandem running, similar to that observed by Maschwitz et.al. (1974) in B.tesserinoda, to carrion food sources occurs, as would befit a less specialized predator of more catholic tastes than A.sedilloti.

Colony size in Br. sennaarensis is not known, but probably exceeds 300 ants. Only 38% of the prey of this species is whole animal in origin, a greater proportion (40%) of ants take unidentifiable plant and animal material. The recruitment mechanisms of this ant are poorly understood, but observations suggest that broad recruitment trails are laid to bring workers to large food sources.

Paltothyreus tarsatus.

P.tarsatus was only observed for 7 hours in a manner comparable with the previous three opportunist ponerines. During the observation period 532 ants returned to the nest entrance holes under observation. A total of 266 termites, 197 other whole arthropods and 73 pieces of animal and plant remains (including sections of millipedes) were brought back to the nest.

In addition to opportunist predation by single foragers, Paltothyreus has been observed attacking a rain damaged M.bellicosus mound. In this case recruitment occurred to the mound, and during the four hour attack 775 ants carried 877 termites to their nest. A number of ants carried more than one termite. If raids of this

type are frequent, they would boost the status of P.tarsatus as a predator of M.bellicosus considerably. Unfortunately the occurrence of predation of this type is not known, and would require a very rigorous sampling programme to quantify.

Glover (1967) concluded that in the arid areas of Somalia P.tarsatus fed exclusively on termites; it is probable that these were the most abundant invertebrates available in the area. Kalule-Sabiiti and Banage (1977) analysed the prey remains in the "kitchen middens" of P.tarsatus in Uganda. They concluded that insects constituted 31.8% of prey; termites accounting for 11.5% of this total, with Hymenoptera (16.3%), Dictyoptera (11.9%) and Coleoptera (11.0%) other important prey orders. Millipedes accounted for 15.1% of the ants prey. The composition of prey remains in the kitchen middens did not vary significantly throughout the year for monthly samples of the major prey groups. At Mokwa termites were the most abundant prey group (52%), other arthropods accounted for 39% of the prey and millipedes and parts of millipedes 9%. As an opportunist predator P.tarsatus will prey on the most abundant groups available. This is borne out by Kalule-Sabiiti and Banges's findings that Hymenoptera, Diplopoda and Dictyoptera were the most abundant organisms in litter samples and in the prey of P.tarsatus in Uganda.

4.3.4 Predation by Dorylus on Termites.

No direct quantitative data were available from Mokwa on predation by dorylines on termites. Dorylus sp.A has been observed taking Macrotermitinae (2.3.2.2), and its method of attack on Macrotermes bellicosus at Mokwa (W.A.Sands, N.M.Collins, pers.comm.) appears to be the same as attacks observed on this termite (cited as Bellicositermes natalensis Hav.; synonymy by Ruelle 1970) by Dorylus (Typhlopone) dentifrons Wasmann in the savannas of the southern Ivory Coast (Bodot 1961, 1966, 1967). Whole M.bellicosus colonies were attacked and the queen cell destroyed; distinctive marks were left on this cell by the doryline mandibles.

Bodot (1966, 1967) found that the density of active epigeal termite

colonies was 1.94 per hectare. She recorded that 60 "young" epigeal nests on a 24 hectare site were destroyed by Dorylus, as indicated by marks on the termite queen cell, over a four year study period. This represents a nest mortality of 0.63 colonies per hectare per year, or a turnover of 34.24% of the colonies per year.

Collins (1977b) found that the density of M.bellicosus in southern Guinea Savanna at Mokwa was 5.44 epigeal colonies (<2 m high) per hectare. If 34.24% of these colonies were attacked by Dorylus, as Bodot found in the Ivory Coast, 1.86 colonies per hectare per annum would be taken. This would account for 81.27% of the total mortality of epigeal nests that was reported by Collins (1977b).

Collins (1977b, his table 3.6.2) calculated the mortality of M.bellicosus by death of entire colonies. In epigeal nests (<2 m high) 26.6687 termites per metre squared per year were destroyed. Assuming a level of predation of 34.24%, this represents 9.1314 termites per metre squared per year taken by Dorylus. If it is further assumed that there is a similar level of predation on hypogeal nests (45 colonies ha⁻¹, 4.1683 termites m⁻²a⁻¹ destroyed), 1.4272 termites per metre squared per year would be taken by Dorylus. This represents a total predation by Dorylus on M.bellicosus of 10.5586 termites per metre squared per year (0.0289 termites m⁻² day⁻¹) at Mokwa.

Although annual predation by Dorylus on M.bellicosus appears to be low when compared with M.foetens (142 termites m⁻²a⁻¹), it should be realised that doryline predation usually results in the destruction of whole colonies, including the reproductive pair. M.foetens are predators of foraging parties consisting of sterile workers and soldiers. Although Dorylus has been observed taking Microtermes and Odontotermes at Mokwa, estimates of predation on these genera are not possible using the method used for M.bellicosus because of the diffuse nature of their colonies. It is worth considering that the diffuse nest systems of many Macrotermitinae could be an adaptive response to subterranean ant attack. An attack on a discrete fungus comb would still leave the queen cell intact.

SECTION 5. REPELLENCY OF ANTS TO TERMITES.

5.1 Bioassay of Ants as Sources of Repellents.

5.1.1 Introduction.

Predator-prey interactions mediated through chemicals have been studied in a number of insects. Remold (1962,1963) found that pentatomid bugs produced a secretion consisting of n-tridecane and $C_6 - C_{10}$ aliphatic aldehydes. The secretion was repellent to Formica sanguinea, F.cinerea, Tapinoma sp., Tetramorium caespitum and Lasius alienus, all of which are known predators of hemiptera, but was not repellent to the scavengers Lasius affinus and L. emarginatus.

A number of genera of tropical wasps are reported as producing ant repellents to protect their nests. The neotropical Mischocyttarus drewensi (Jeanne 1970) and the Ethiopian Belonogaster sp. and Ropalidia sp. (Edmunds 1974) all build pendant nests and smear an ant repellent on to the nest pedicel.

Howse (1975) studied the repellency of ants to termites and concluded that forest (Thoracotermes macrothorax) and forest-fringe (Amitermes evuncifer) species were repelled by a wider range of ants than two savanna species (Macrotermes bellicosus and M.subhyalinus). He suggested that the results reflected, at least in part, predator-prey relationships; especially the likelihood of ants and termites encountering each other in the field. He recorded that Monomorium spp., Odontomachus haematodes (= troglydytes), Dorylus sp. and Paltothyreus tarsatus were all termite predators and released strong antagonisms in termites. Wadhams (L.J.Wadhams, unpublished data) found that the repellency of O.haematodes to Amitermes evuncifer was due to mandibular gland compounds (n-alkyl pyrazines) which leaked onto the cuticles of the ants.

In this present study the interactions between savanna ants and

savanna Macrotermitinae were examined, and the glandular secretions of both repellent and non-repellent genera were identified and tested as repellents in order to elucidate some of the principles governing the behavioural responses. These results, coupled with other findings, may help to explain the differing predation of ants on some termites.

5.1.2 Sources of Material.

Macrotermitinae of the genera Microtermes, Ancistrotermes and Odontotermes live in diffuse subterranean nests, and consequently cannot be collected in adequate numbers for bioassay by digging up the nests. Wood et.al. (O.D.M. Report 1977) have used wooden bait blocks to monitor foraging termites and collecting termites for insecticide studies. These bait blocks were also utilized to capture Macrotermitinae for this present study.

Ceiling board (Cellotex, $100\text{ mm}^{-2} \times 20\text{ mm}$) and softwood (various wood types, 6" (153 mm) $\times$ 1" (25.4 mm) $\times$ 1") baits were laid on the surface of the soil after clearing away vegetable debris. The baits were attacked from approximately one week after laying, depending on the termite species and season. Baits were usually collected early in the morning, before field work was carried out, and stored in polythene bags for 2 - 3 hours until 10.00 hours when termites were taken for bioassay. When the baits were lifted, some of the topsoil was also taken with a trowel, as this was also a useful source of the species attacking the bait. In the case of Microtermes spp. baits could be collected later in the day as, during the later stages of bait attack, termites tend to remain in the blocks throughout the day.

Macrotermes bellicosus and M. subhyalinus were less easily obtained at baits, and these two species were also collected from their nest mounds. Both Macrotermes spp. build discrete mounds (M. subhyalinus only in the parkland). The fungus garden, complete with termites, can be lifted out of the mound of M. bellicosus in its entirety

(Collins 1977b). M.subhyalinus mounds have a more diffuse fungus garden/nest system. Blocks of fungus comb containing worker termites were obtained by breaking open the top of the mound and digging down into the fungus garden area.

Whole fungus gardens of M.bellicosus were transported from Mokwa to Southampton where they were kept at 26 - 29°C, 65 - 85% RH (9.5). This was the only species of Macrotermitinae suitable for laboratory work in the U.K.. Colonies lasted two - four months in culture. Termites for assay were obtained from catchment vessels; 100 ml glass beakers filled with damp cotton wool and placed in the nest box, or from wood blocks placed in the foraging arena (9.5).

5.1.3 Bioassay Techniques.

Ideally a laboratory bioassay should meet the following requirements:

- a) it should not disrupt normal behaviour,
- b) it should be quick and efficient to ensure the rapid screening of potential repellents,
- c) it should record the behaviour of different castes, and in the case of this present study it should
- d) be adaptable to laboratory conditions where power and electronic recording equipment could not be guaranteed.

If laboratory cultures of insects are being used the age of insects used should be the same, this could not be achieved when the biological material was collected from the field.

A search was made for a suitable technique in the laboratory at Southampton, on a colony of M.bellicosus. A number of bioassay techniques were investigated and they are described in the following sections.

5.1.3a Bioassay Chambers

- i) The simplest method available did not use a chamber of any sort,

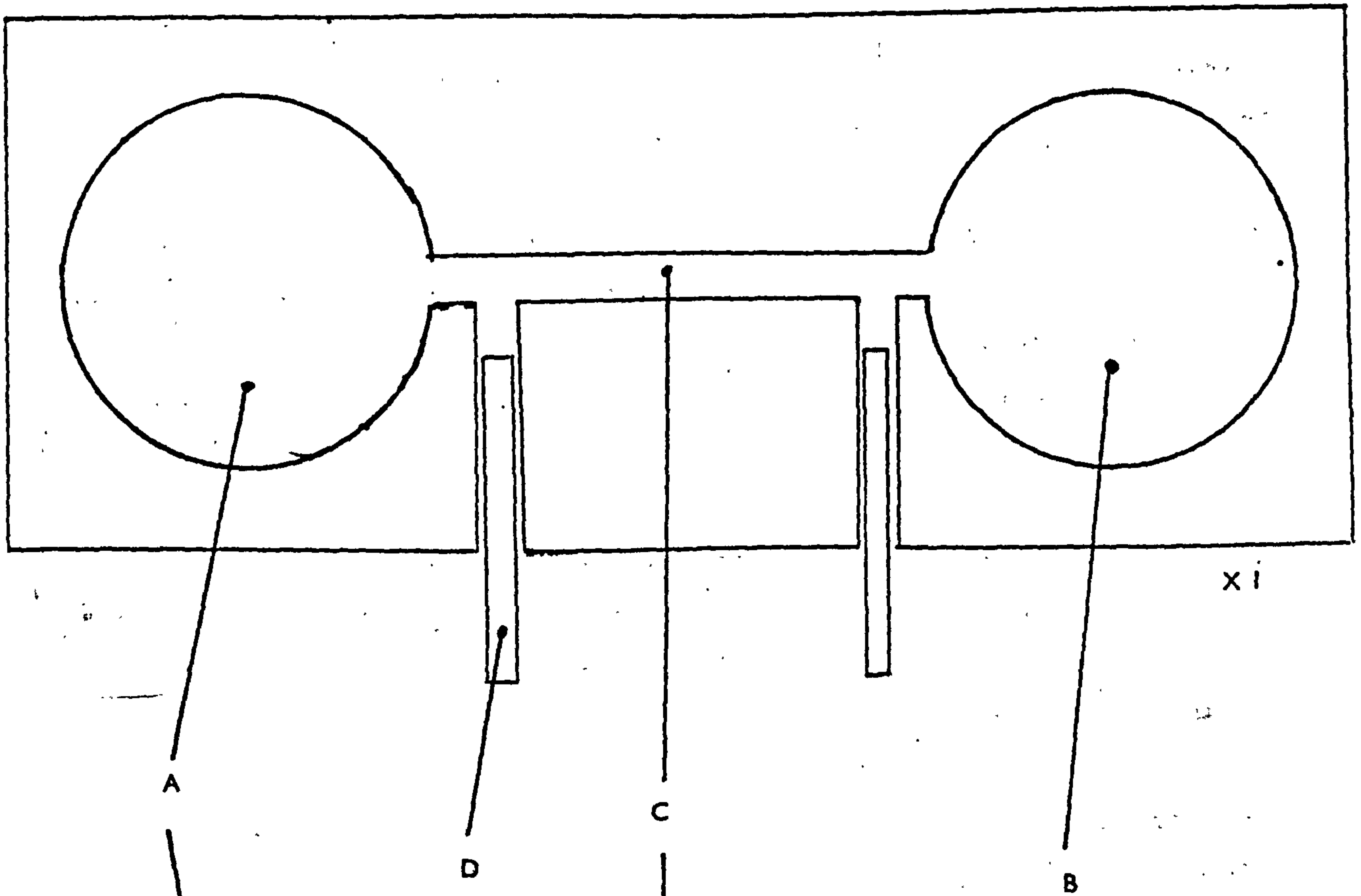
but relied on presenting stimuli to termites emerging from the ends of foraging galleries when they were in the process of extending them. It was only of use if whole nests were available in the laboratory and termites could be encouraged to forage. This method was used by Howse (1975, and pers.comm.) to test the reactions of termites to whole ants. The main problem with this bioassay was that it was difficult to quantify and that the termites were easily disturbed. No detailed tests were carried out using this method.

ii) Perspex Assay Chambers. Bioassay chambers (Figs. 5.1a & 5.1b), tooled out of perspex blocks were easily manufactured and prepared for tests. The smaller version (Fig. 5.1a) was most suitable for Microtermes spp., Odontotermes spp., Ancistrotermes cavithorax and Macrotermes bellicosus. The larger Macrotermes subhyalinus were best tested in the larger chamber (Fig. 5.1b). The different castes were separated for bioassay.

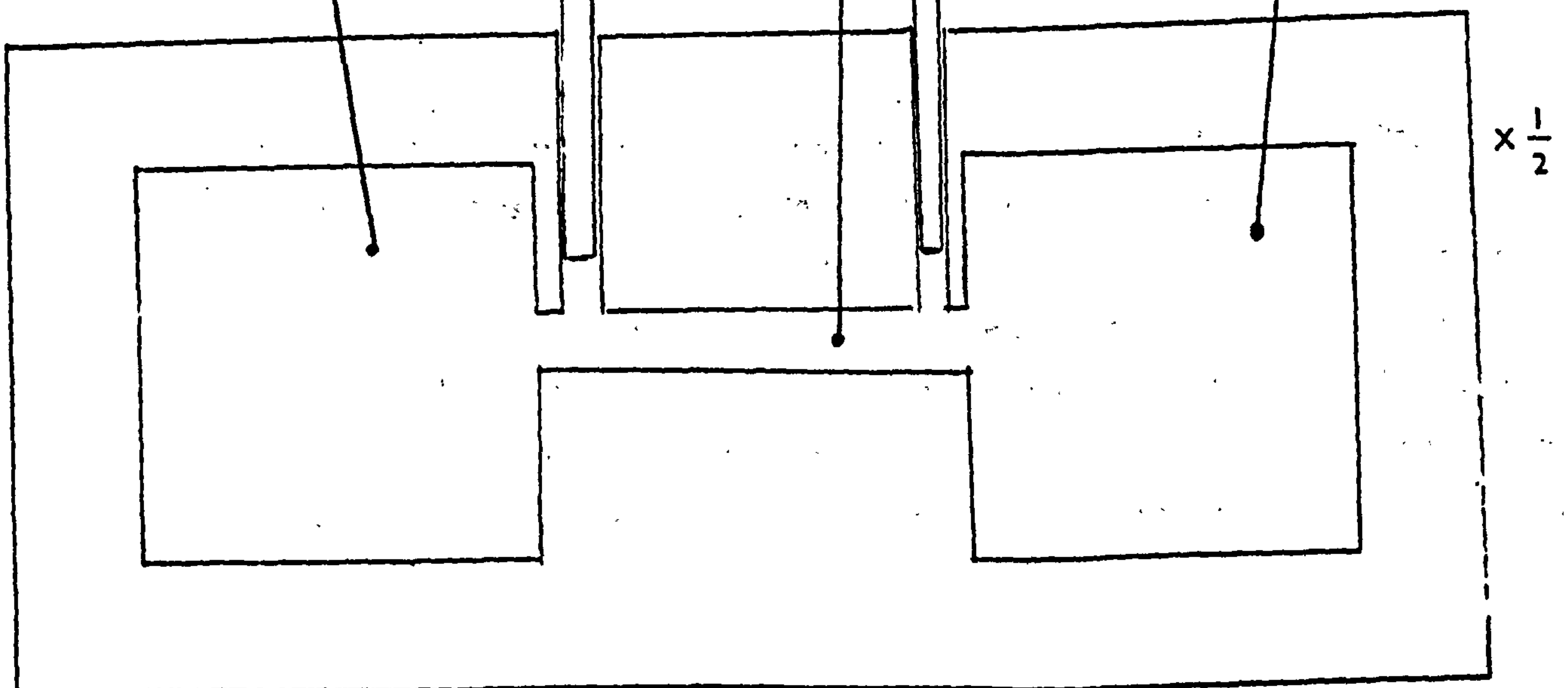
Before use, the floor of the chamber was covered with filter papers and moistened with distilled water. The bioassay chamber was used in one of two ways. The termites were taken from the bait, using a capillary pooter against the head capsule, and placed in specimen tubes. When 25 termite workers had been taken they were introduced into chamber A (Fig. 5.1), with the sliding partitions closed. The assay chamber was covered with black paper or polythene for 5 - 10 minutes and the termites allowed to settle. The black covering was then slid across to expose runway C, and the material to be assayed was placed in the runway. Chamber B, and the runway C on the 'B' side of the stimulus were covered, the sliding partitions (D) opened and A exposed to light.

a. The termites were observed and scored (5.1.3c) as they approached the stimulus. The first ten termites were scored, no attempt was made to distinguish individual termites; consequently in some instances the same termite was scored more than once. An alternative method b., for use with this bioassay chamber, was to count the number of termites remaining in the exposed chamber, A, at the end of a fifteen minute period.

a.



b.



Figures 5.1a & 5.1b Perspex Bioassay Chambers.

A. Termite chamber. B. Darkened chamber. C. Substance under test. D. Sliding gates.

iii) The testing of whole anaesthetised ants against termites in assay chambers 5.1a & b was not always possible, because of the physical obstruction caused by the ants. To test large ants (Megaponera, Paltothyreus, Camponotus, Oecophylla, Polyrachis, Myrmicaria) a perspex box was modified with plaster of Paris to a similar design to Fig. 5.1b, but with a wide central chamber. The termites were observed in the manner described in ii) above. With smaller ants, assays were carried out in the standard assay chambers if enough space was available to give the termites free access past the ants.

iv). A potential bioassay chamber for use with termites collected from baits is the multiple choice assay chamber (Fig. 5.3). Candidate compounds were placed in the arms of the chamber, which were covered with black paper. Fifty termites were placed in the central chamber (A). The distribution of the termites in the arms $B_1 - B_n$ was then noted after thirty minutes. This bioassay did not give repeatable results with M. bellicosus (Table 5.1). Termites are social animals and show gregarious behaviour (Harris 1961) and thus tend to clump in one of the chambers, even if non-repellent substances are also present in some of the other chambers. A modification with two arms, one for the potential repellent and one for the control situation might prove to be more efficacious, but was not tested.

v). When whole segments of fungus garden were available from M. bellicosus nests, they could be placed in a perspex assay box (Fig. 5.2). This chamber was used by Floyd (1975) and Floyd et.al. (1976) to test repellents against the subterranean termite Reticulitermes lucifugus santonensis. Potential repellents were placed at C and the reactions of the termites noted. This bioassay chamber was used in conjunction with the presentation method 5.1.3b.ii with M. bellicosus, but was not investigated in greater detail.

5.1.3b Presentation of Stimuli.

i). Repellent compounds and extracts were presented on 10 mm² filter paper squares. Compounds and extracts were made up in purified dichloromethane and injected onto the filter paper. The

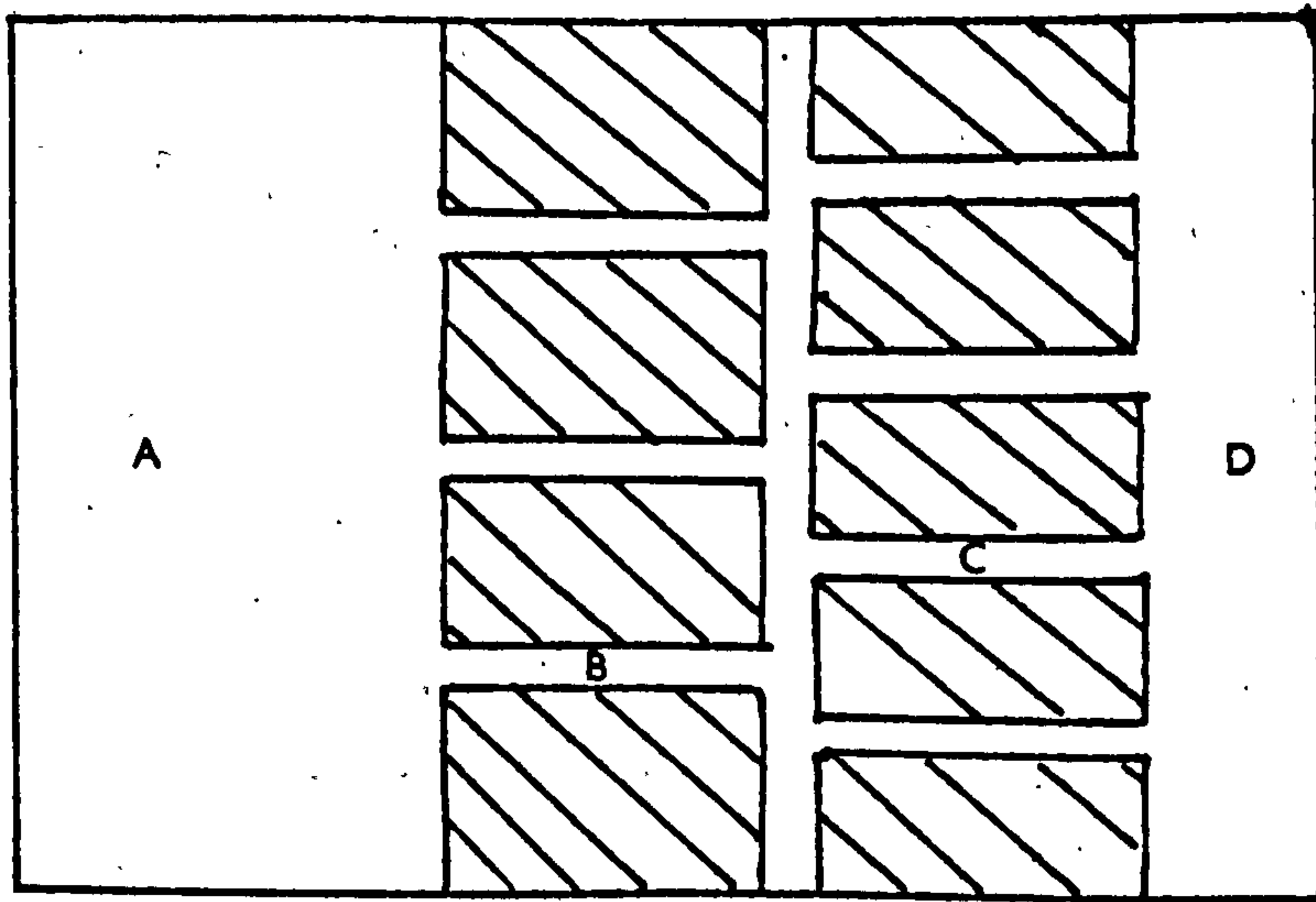


Figure 5.2 Bioassay Chamber.

A. Nest material. B. Foraging galleries.
C. Substance under test. D. Feeding chamber.

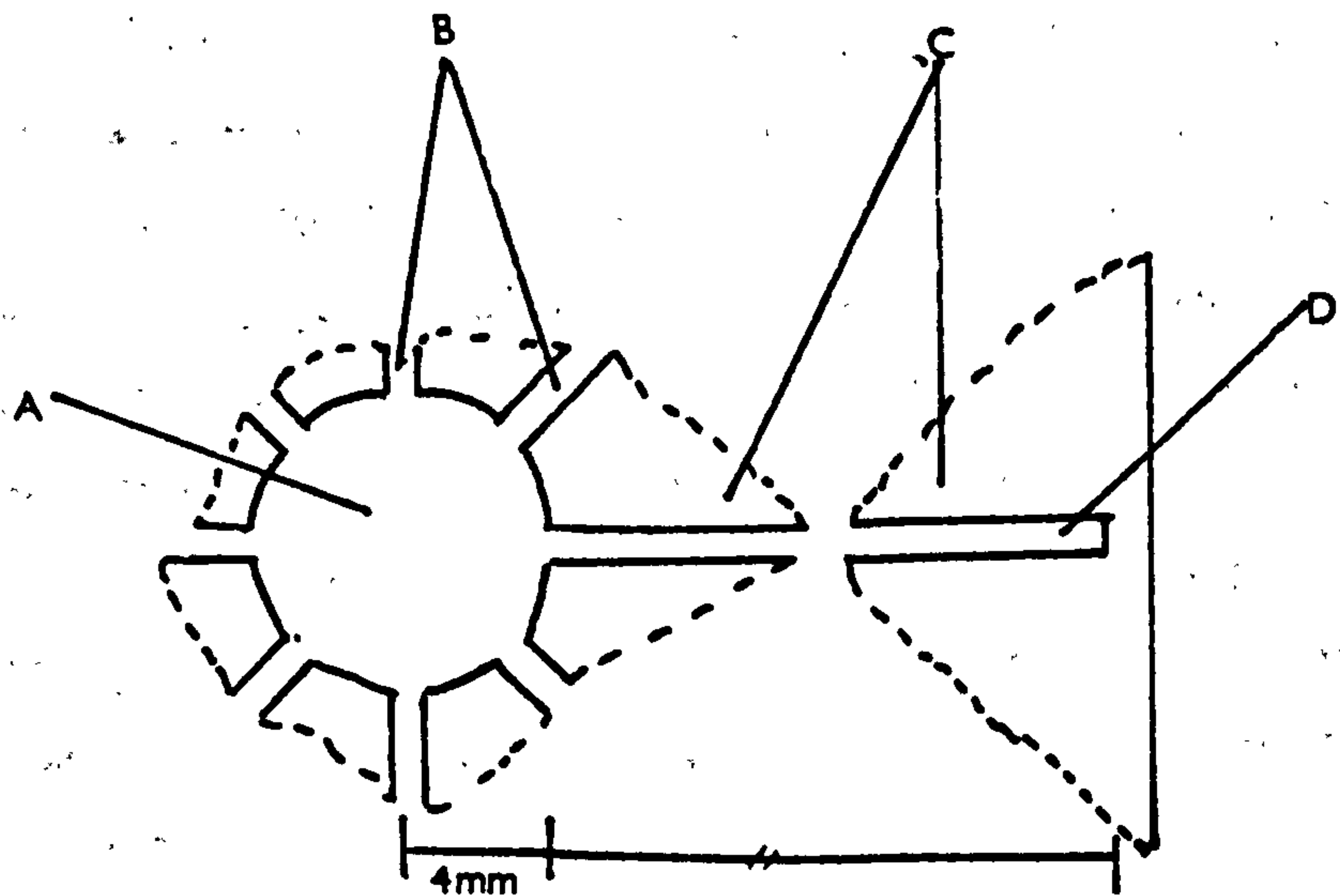


Figure 5.3 Multiple-choice Bioassay Chamber.

A. Termites. B. Choice chambers. C. Plaster of
Paris. D. Substance under test.

filter paper was left for 1- 2 minutes to allow the solvent to evaporate before it was placed in the bioassay chamber. Filter papers were similarly treated with dichloromethane for control tests.

ii) A novel presentation technique was used with bioassay chamber v. (5.1.3a.v; Fig. 5.2). Potential repellents were adsorbed onto gas chromatography column packing (80 - 100 mesh Diatomite C; J.J's Chromatography; refluxed overnight in ethyl alcohol) and placed at point C (Fig. 5.2) in a circular mould. The mould was removed, leaving a circle of column packing. Control (untreated) packing was taken by minor workers and incorporated with soil into foraging galleries and other soil structures. Observations were made on the disappearance of the packing and diagrams made of the shape of the pile of packing onto graph paper. From measurements of the area of packing disappearing in treated piles and untreated control piles a measure of repellency, or more correctly 'anti-pickup potential', of the test extracts could be obtained.

This method was time consuming and only one series of assays was carried out. Whole ants, major workers of M.foetens and Camponotus maculatus and large workers of Myrmicaria eumenoides, were extracted in 500 µl of dichloromethane. 100 mg of column packing was shaken with the extract for ten minutes; the solvent was then removed under reduced pressure. The packing was then dried for thirty minutes at room temperature. The results of one series of assays using this method are presented in Table 5.2. This method was probably especially useful for assaying repellent compounds in the 'cuticular' (5.1.3d) fraction of M.foetens as the packing was relatively untouched by the termites for over 24 hours (Table 5.2).

ii) With large ants (eg. M.foetens, P.tarsatus, C.maculatus) it was possible to take scrapings of cuticular wax from the gasters or top of the head for assay. The wax scrapings were taken with a sliver of glass cover-slip. This treated glass was presented to the termites in perspex bioassay chambers (5.1.3.ii, Fig. 5.1a). Although this method allowed bioassay without the use of solvent extraction, they were difficult to quantify and could only be used

Table 5.1 Responses of Macrotermes bellicosus to whole ants in a multiple choice bioassay chamber.

Contents of chamber	Number of termites in chamber for test No:-					Totals
	1	2	3	4	5	
Blank	20	0	2	5	1	28
Megaponera foetens	0	0	0	0	0	0
Camponotus maculatus	0	0	0	16	0	16
Megaponera foetens	0	0	0	0	0	0
Blank	5	0	13	4	21	43
Megaponera foetens	0	0	0	0	0	0
Camponotus maculatus	0	25	10	0	3	38
Megaponera foetens	0	0	0	0	0	0

Table 5.2 Responses of Macrotermes bellicosus workers to whole ant extracts absorbed onto gas-chromatography column packing

	Percentage Removal in Times (hours)		
	5	25	32
Control	9	48	74
<i>Myrmicaria eumenoides</i>	0	27	68
<i>Megaponera foetens</i>	0	10	26
<i>Camponotus maculatus</i>	16	32	83

Table 5.3 Responses of Macrotermes bellicosus (major workers) to wax cuticular scrapings from three ant species. (N = 5 for all presentations).

		Repellency Rating	
		30 min.	5 hours
<i>Megaponera foetens</i> (majors)	Head	++	+
	Gaster	+	+
<i>Camponotus maculatus</i> (majors)	Head	o	o
	Gaster	o	o
<i>Paltothyreus tarsatus</i>	Head	++	+
	Gaster	+	+

with those large ants which had waxy cuticles. A summary of the results obtained from the presentation of cuticular waxes to M. bellicosus major workers is presented in Table 5.3. This method was not used extensively, but might be considered in future studies when "waxy" insects are being used as sources of potential repellents; or when studies are being made of inter- and intra-specific signals on the cuticles of insects.

iii) Live whole ants for presentation were anaethetised with diethyl ether. Large ants were presented in the chamber described in 5.1.3a.iii, and smaller ants in bioassay chamber 5.1.3a.ii (Fig. 5.1b). Ants were always presented in a standard manner, with the anterior of the ant pointing towards chamber A, and the folded legs out of the termite path.

5.1.3c. Analysis of Results.

Behavioural scores used in the bioassay (5.1.3a.ii).:-

Termites were observed, without magnification, and their behaviour noted as they approached the stimulus. Their behaviour was scored according to the following scheme:

Score	Behaviour
0	No response to stimulus
1	Pause < 1 second, resume forward
2	Pause > 1 second, resume forward
3	Pause > 1 second, retreat
4	Pause < 1 second, retreat
5	Immediate retreat (< 1 second pause) and alarm behaviour, or alarm behaviour at or near the stimulus, with subsequent retreat.

The time interval (> 1 second) was judged subjectively as it was not possible to observe a stop watch and the termites behaviour simultaneously. Alarm behaviour (Score 5) in the Macrotermitinae

consisted of vertical and horizontal oscillatory head movements, similar to those described by Howse (1965) for Zootermopsis. No attempt was made to distinguish different oscillatory movements, any were noted down as alarm behaviour. No claim is made that the scoring method used represents an hierachial series of responses to repellents. The lower scores are more properly defined as measures of exploratory behaviour, but the time spent exploring would appear to give at least some indication of the "strangeness" of the stimulus. True repellency is measured from Score 3 (Pause > 1 second and retreat). It is assumed here that the exploratory time for a weaker repellent is longer than that for a strong one; hence, 3 = pause > 1 second; 4 = pause < 1 second.

The first ten termites to encounter the stimulus were scored using the above method and the scores analysed according to:

$$\text{Degree of Repellency (DR)} = \frac{\text{Total Behavioural Score Realised}}{\text{Total behavioural Score Possible (50)}}$$

In presenting the data (Tables 5.3, 5.5 - 5.11), the DR was grouped into classes to give Repellency Ratings (RR) in the following was:

DR	Repellency Rating (RR)
0.80 - 1.00	+++
0.60 - 0.79	++
0.40 - 0.59	+
0.20 - 0.39	o

If, instead of scoring the termites behaviour (p.122) the number of termites remaining in chamber A was counted the DR was calculated

from
$$\frac{\text{Number of termites remaining in chamber A}}{\text{Total number of termites used}}, \text{ and the RR}$$

was calculated as above.

Each of the Repellency Ratings presented in Tables 5.5 - 5.11 represents at least three presentations of the stimulus to groups of termites.

5.1.3.d Preparation of Samples for Bioassay.

The preparations described in this section are for the bioassay described in 5.1.3a.ii (Figs. 5.1a & 5.1b).

i) Whole ants were prepared and analysed as described in 5.1.3b.ii.

ii) Extracts of whole ants. Whole ants were immobilised on a bed of crushed dry ice (Southampton) or by freezing (Mokwa) and placed in dichloromethane to give approximately 3 'Myrmicaria equivalents' (ME) per 1 ml, in an attempt to standardise the amount of material presented according to the weight of the ants; it does not take into account surface area or gland size which would also affect the amounts of compounds going into solution. The numbers of ants used per ml are given in Table 5.4. The ants, in dichloromethane, were crushed with a glass rod and filtered through glass wool. 1 µl aliquots of the whole ant extracts were taken up in a Hamilton fixed needle 10 µl syringe and presented to the termites on filter papers.

iii) Extracts of heads, gasters and cuticles. Ants were either frozen on dry ice or in a freezer; the heads and gasters were then broken off and placed in dichloromethane. The 'Myrmicaria equivalent (5.1.3d.ii) was used, substituting 10 X the numbers of heads or gasters if GC fractionation was carried out, or 2 X the numbers if assay of the unfractionated extract was carried out. The heads or gasters used were crushed with a glass rod and filtered through glass wool.

In some cases (Table 5.8) 'volatile' and 'non-volatile' compounds were separated on a 4 mm X 1.5 m 10% E 301 silicone gum GC column (column H), programmed from 80° - 300° at 8°C per minute. The

Table 54 'Ant equivalents' used to prepare solvent extracts of ants.

Ant species	Mean weight (N = 10)	Number of ants per ml
<i>Myrmicaria eumenoides</i>	0.0130	8
<i>Brachyponera sennaarensis</i>	0.0044	24
<i>Bothroponera sczori</i>	0.0063	16
<i>Anochetus sedilloti</i>	0.0058	16
<i>Megaponera foetens</i> (minor)	0.0160	8
<i>Dorylus</i> sp.A	0.0107	8
<i>Carebara</i> sp.A	0.0010	104
<i>Crematogaster</i> sp.B	0.0018	56
" sp.C	0.0020	56
" sp.D	0.0015	72
" sp.E	0.0036	32
<i>Pheidole</i> sp.A	0.0024	40
<i>Decamorium uelense</i>	0.0015	72
<i>Tetramorium sericeiventre</i>	0.0028	40
<i>Tetramorium angulinode</i>	0.0010	104
<i>Tapinoma melanocephalum</i>	0.0010	104
<i>Oecophylla longinoda</i> (major)	0.0065	16
<i>Messor regina</i> (media)	0.0121	8
<i>Camponotus maculatus</i>	0.0104	8
<i>Odontomachus troglydytes</i>	0.0109	8
<i>Tetramorium termitobium</i>	0.0025	40

extract was first reduced to 300 μ l by passing oxygen free nitrogen over it. 0.1 μ l of a 0.1% solution of pentadecane standard solution was added and the extract injected onto the column. The fractions eluting before and after pentadecane were collected in 500 μ l of dichloromethane using the micropreparative method of Baker et.al. (1976; 8.1.3a).

Fractionation on an E 301 column; which separates essentially on a molecular weight basis, using a temperature programmed run to emphasise differences in boiling point, was carried out in order to separate 'volatile' from 'non-volatile' (cuticular) compounds. The volatile compounds from glands are often used by ants as pheromones, and Wilson (1973) postulates that they will usually contain less than 14 - 15 carbon atoms because of volatility considerations. Most of these compounds will elute before n-pentadecane on an E 301 GC column. The cuticular compounds of ants are usually of an higher molecular weight (eg Lok et.al. 1975) of more than 18 carbon atoms, and would elute after pentadecane. The fractionation scheme used should thus give a crude separation into volatile (glandular) and involatile (cuticular) components. Bioassay was then used to ascertain which fraction gave the greatest repellent response.

iv) Extracts of glands. The bioassay of crude fractions from iii above (Table 5.8) indicated that in repellent ants most of the activity was contained in the 'volatile' fraction from either the head or the gaster. Studies on Myrmicaria eumenoides indicated that glandular components from the poison gland in the gaster could also be detected on the head (Fig. 5.11). As it was possible that exocrine chemicals are commonly found in small quantities all over the bodies of ants (5.3.1c), tests were carried out on excised glands from ants found to have repellent head or gaster volatile fractions

The Dufour's/poison gland complex in myrmecine and ponerine ants was removed by gripping the sting of a dry ice immobilized ant with fine forceps and pulling. The gland complex was removed with the sting

and placed in 300 µl of dichloromethane. Two gland complexes from M.foetens (minor workers) and M.eumenoides, five from Bothroponera, Anochetus and Crematogaster sp.E and ten complexes from Crematogaster spp.C, Tetramorium spp. and Decamorium were used in the extractions. Mandibular glands were excised by grasping one of the mandibles with forceps and pulling it away from the head with the glandular material attached, this was repeated with the other mandible. Throughout the text 'one mandibular gland' refers to the pair of glands from one ant. Ten glands were used from Megaponera, Myrmicaria, Bothroponera, Anochetus and Crematogaster spp. and 20 glands from Tetramorium and Decamorium were used in each extraction. The glandular extracts were presented to the termites in 1 µl aliquots on filter papers.

v) Bioassay of single compounds extracted from ants and synthetic samples. Compounds from ants which were not obtainable as synthetic samples were assayed after preparation from the ants. The compounds for bioassay were fractionated by preparative GC (8.1.3a) and collected in 300 µl of dichloromethane. The compounds were diluted with solvent to give a 0.01% solution.

Synthetic samples were prepared as 0.01% solutions in dichloromethane and presented to termites in 1 µl aliquots on filter papers as described in 5.1.3b.

5.1.4 Bioassay Results

The reactions of major worker termites to whole ants revealed a wide range of responses (Table 5.5). Most ant species did release some level of repellency in termites, the notable exceptions being Aenictus sp.A, Carebara sp.A, Monomorium bicolor, Monomorium sp.D (? scabriceps), Crematogaster sp.C, Messor regina, Decamorium uelense, Tetramorium termitobium, Camponotus maculatus and C.sericeus.

Repellency did not reflect any straightforward taxonomic or predatory relationships. In the tribe Tetramoriini two species were non-repellent and four repellent. In the genus Crematogaster four species were repellent, one slightly repellent and one non-repellent. The specialised termite predators D.uelense and Carebara sp.A were not repellent, nor were the opportunist camponotine species or the

non-predatory Messor spp.

Comparison of head and gaster volatile secretions, and cuticular non-volatile secretions (5.1.3d.iii) revealed (Table 5.8) a wide range of responses. In general, in most ants excepting Myrmica eumenoides and Tapinoma melanocephalum, the head volatiles or extracts were more repellent than those from the gaster. Both head and gaster volatiles were more repellent overall than cuticular components. In M.eumenoides and T.melanocephalum gastral volatiles were more repellent than cephalic, the repellents in M.eumenoides originating in the poison gland (Table 5.9). If it is assumed that small quantities of volatile glandular compounds are always present on the cuticle (5.3.1c.ii), extractions of whole parts of ants (Table 5.7) may have confused the localisation of the source of repellents. Tests were therefore carried out on extracts of glands (Table 5.9), which proved to be more conclusive. Except for M.eumenoides and Megaponera foetens, mandibular glands were much more repellent than the combined Dufour's/poison gland, suggesting that some of the repellency of gaster volatile fraction extracts (Table 5.8) may have been due to contamination with cephalic chemicals.

The significance of the response of termites to pure chemicals and fractions of single components (Tables 5.10 & 5.11) from ant glands will be examined in the discussion later (5.3).

Tables 5.5 - 5.11 Test method used (p. 122) as follows. All bioassays in chambers described in 5.1.3a.ii and 5.1.3a.iii. Either a. or b. (p.122) used:

Table 5.5 - a, Table 5.6 - b, Table 5.7. - b, Table 5.8 - b,
Table 5.9 - a, Table 5.10 - a, Table 5.11 - all a except monoterpene hydrocarbons and alkyl pyrazines, b.

Table 5.5 Repellent response (Repellency Rating) of Macrotermitinae major workers to live anaesthetised ants

	Macrotermes bellicosus	Macrotermes subhyalinus	Odontotermes pauperans	Odontotermes smeathmani	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Brachyponera sennaarensis	+	o	+	+	+	+	+
Bothroponera soror	++	+	++		++	++	+
Anochetus sedilloti	+	+	++		++	+	++
Megaponera foetens	++	+	++	++	++	++	+
Paltothyreus tarsatus	+	+	+	+	+	+	+
Dorylus (Rhogmus) sp.A	++	+		+	+	+	+
Dorylus (Anomma) sp.	o		o		o	+	o
Aenictus sp.A	+		o		o	o	o
Tapinoma melanocephalum	+++	++	+		++	+	+
Camponotus maculatus	o	o	o	o	o	o	o
Camponotus sericeus	o	o	o	o	o	o	o
Acantholepis sp.A	+	+		o	o	o	o
Polyrachis viscosa	o			+	o	o	o
Oecophylla longinoda	++	+	++	++	+	+	+

contd. over/

Table 5.5 continued

	Macrotermes bellicosus	Macrotermes subhyalinus	Odontotermes pauperans	Odontotermes smeathmani	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Carebara sp.A	o	o	o		o	o	o
Monomorium bicolor	o	o	o		o	+	
Monomorium pharaonis	+	+	o		+	+	
Monomorium scabriceps	o	o			o	o	
Monomorium sp.	++		+			+	
Crematogaster sp.B	+	+	+	+	+	o	+
Crematogaster sp.C	o	o	o		o	o	o
Crematogaster sp.A	+	++	++	+	++	++	++
Crematogaster sp.E	++	+	++	++	+	++	++
Crematogaster sp.F	+++				++	++	
Messor regina	o	o	o		o	o	o
Messor sp.B	o	o	o	o	o	o	
Pheidole sp.A	++	++	++		++	++	++
Pheidole sp.B	+	++				+	
Pheidole sp.C	+	+	o		+	+	+
Decamorium uelense	o		o	o	o	o	o
Tetramorium sericeiventris	+	+	++		+	++	++
Tetramorium guineense	+	o	+		+	+	+
Tetramorium termitobium	o	o	o		o	o	o
Tetramorium angulinode	++	++	++			+++	++
Tetramorium sp.	++				++		
Myrmicaria eumenoides	+	o	++		++	+	+

all results represent the average repellency rating
of at least three separate presentations

Table 5.6 Repellent responses (Repellency Rating) of Macrotermitinae minor workers to live anaesthetised ants

	Macrotermes bellicosus	Macrotermes subhyalinus	Ancistrotermes cavithorax	Microtermes sp.A
Brachyponera sennaarensis	+	+	+	+
Megaponera foetens	++	++	+	++
Paltothyreus tarsatus	+			++
Dorylus (Rhogmus) sp.A		+	+	++
Carebara sp.A	o	o	o	o
Monomorium bicolor	o	o	o	+
Monomorium scabriceps				+
Crematogaster sp. D	+	+	+	
Myrmicaria eumenoides	+	o	++	+
Messor regina	o	o		o
Camponotus maculatus	o	o	o	o
Camponotus sericeus	o	o	o	o

Table 5.7 Repellent response (Repellency Rating) of Macrotermitinae major workers to extracts of whole ants

	Macrotermes bellicosus	Macrotermes subhyalinus	Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Brachyponera sennaarensis	++	+	+	+	++	+
Bothroponera soror	+	+	+	++	++	++
Anochetus sedilloti	+	+	++	+	++	+
Megaponera foetens	++	+	++	+	+	+
Dorylus (Rhogmus) sp.A	++	++	+	+	+	+
Carebara sp.A	o	o	o	o	o	o
Crematogaster sp.D	+	+	++	+		+
Crematogaster sp.C	o			o	o	
Crematogaster sp.A	+				++	+
Crematogaster sp.E	++		+++	++	+	+
Myrmicaria eumenoides	+	o	+++		++	
Pheidole sp.A	++		++	++	++	++
Decamorium uelense	o		o	o	o	o
Tetramorium sericeiventris	+		++	+		++
Tetramorium termitobium	o			o	o	o
Tetramorium angulinode	++		++	++	++	+
Tapinoma melanocephalum	++		++	++	++	
Oecophylla longinoda	++			++	++	o

Table 5.8 Repellent responses (Repellency Rating) of Macrotermitinae major workers to extracts of heads and gasters and broad spectrum GC fractions from ants

		Macrotermes bellicosus	Macrotermes subhyalinus	Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Brachyponera sennaarensis	H	++	+	+	++	+	++
	G	+	o	+	+	+	o
	C	o	o	+	+	o	o
Bothroponera soror	H	+		++	++	++	++
	G	+		o	+	o	o
	C	o		o	o	o	o
Anochetus sedilloti	HC			++	++	+	++
	GC			o	o	o	o
Megaponera foetens	H	++	+	+	+	++	+
	G	+	o	+	+	+	o
	C	+	o	o	+	o	+
Paltothyreus tarsatus	HC	++	+			+	
	GC	+	o			o	
Dorylus sp.A	HC	+	+	++	+	++	+
	GC	+	+	o	+	+	+
Dorylus sp.	HC	o	o	o	o	+	o
	GC	o	o	o	o	+	o
Tapinoma melanocephalum	HC	+	+		o		o
	GC	+++	++		+++		+
Camponotus sericeus	H	o	o		o	+	
	G	o	o		o	o	
	C	o	o		o	o	
Camponotus maculatus	H	o			o	o	
	G	o			o	o	
	C	o			o	o	
Oecophylla longinoda	HC	++	+	++		+	+
	GC	+	o	o		o	o

contd. over/

Table 5.8 continued

		Macrotermes bellicosus	Macrotermes subhyalinus	Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Crematogaster sp.A	H	+	+	++	+	++	++
	G	+	+	+	+	+	+
	C	o	o	o	o	o	o
Crematogaster sp.C	HC	o	o		o	o	
	GC	o	o		o	o	
Crematogaster sp.E	HC	++	+	+	++	++	+
	GC	+	+	+	o	o	+
Crematogaster sp.F	HC	++			++	+++	
	GC	+			o	o	
Pheidole sp.A	HC	++	+	+	++	++	+
	GC	+	o	o	o	o	o
Tetramorium sericeiventris	HC	+	++	++	+	+	++
	GC	o	o	o	o	o	o
Tetramorium angulinode	HC	++			++	++	
	GC	o			o	o	
Decamorium uelense	HC	o			o	o	
	GC	o			o	o	
Myrmecaria eumenoides	H	+	o	o	+	o	o
	G	++	o	++	++	+	++
	C	o	o	o	o	o	o
Messor regina	H	+		+	o		
	G	o		o	o		
	C	o		o	o		

H - head, volatile fraction G - gaster, volatile fraction

C - non volatile "cuticular" fraction

HC, GC head and gaster volatile/nonvolatile not fractionated
by gas chromatography

Table 5.9 Repellent responses (Repellency Rating) of Macrotermitinae major workers to extracts of glands from ants

		Macrotermes bellicosus	Ancistrotermes cavithorax	Microtermes sp.A
Bothroponera soror	M	++	++	++
	PD	o	o	o
Anochetus sedilloti	M	++	++	+
	PD	o	+	o
Megaponera foetens	M	++	+	++
	PD	+	+	+
Myrmicaria eumenoides	M	+	+	+
	PD	+	++	++
Crematogaster sp.C	M	o	o	o
	PD	o	o	o
Crematogaster sp.E	M	++	++	+++
	PD	o	o	o
Decamorium uelense	M	o	o	o
	PD	o	o	o
Tetramorium sericeiventre	M	+	+	++
	PD	o	o	o
Tetramorium angulinode	M		++	++
	PD		o	o

M- excised mandibular gland

PD- excised poison/Dufour's gland complex on sting

Table 5.10 Repellent responses (Repellency Rating) of Macrotermitinae major workers to broad fractions and single components from ants

		Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
Bothroponera soror^a					
MG Fraction	1	o	o	o	o
	2	++	++	+	++
	3	o	o	o	o
	4	+	o	++	++
Component	7	++	++	+	
	13	o	o	o	
	21	++	+	++	
Anochetus sedilloti^b					
MG Fraction	1	+	+	o	o
	2	+	+	o	o
	3	+	+	o	+
	4	++	++	+	++
	5	o	o	o	o
Component	8	+	+	+	
Megaponera foetens^c					
MG Fraction	1	o	o	+	
	2	o	o	o	
	3	o	o	o	
	4	+	+	+	
	5	o	o	o	
DG Fraction	1	++	+	o	
	2	o	o	o	
	3	o	o	+	
	4	o	o	o	
	5	o	o	o	

For identity of fractions and components refer to a Fig. 5.5
b Fig. 5.9
c Fig. 5.6

contd. over/

Table 5.10 continued

		Macrotermes bellicosus	Ancistrotermes cavithorax	Microtermes sp.A
Crematogaster sp.C ^a				
MG Fraction	1	+	o	o
	2	o	o	+
	3	o	o	o
Component	11	o	o	o
Crematogaster sp.E ^b				
MG Fraction	1	++	++	++
	2	+	+	+
	3	o	o	o
Tetramorium sericeiventris ^c				
MG Fraction	1	++	++	++
	2	+	+	o
	3	o	o	o
Decamorium uelense ^d				
MG Fraction	1	o	o	o
	2	o	o	o
	3	o	o	o
Component	1	o	o	o

MG - mandibular gland; DG - Dufour's gland

For identity of fractions and components refer to a Fig.5.22

b Fig.5.24

c Fig.5.14

d Fig.5.19

Table 5.11 Repellent responses (Repellency Rating) of Macrotermitinae major workers to pure compounds

	Macrotermes bellicosus	Ancistrotermes cavithorax	Microtermes sp.A
2-hexanone	+++	++	++
2-octonone	++	++	++
2-nonanone	++	++	+
2-decanone		++	+
2-undecanone	+	++	+
2-dodecanone	+	+	+
3-octanone	++	++	++
3-decanone	++	++	++
4-heptanone	++	+	+
octanal	++	++	++
pentanal	++	++	
1-heptanol		o	o
1-octanol	o	o	o
1-pentanol		o	
1-nonanol	o	o	
2-undecanol	o	o	o
2-dodecanol	o	o	o
3-heptanol	o	o	o
3-octanol	o	o	
4-methyl-3-heptanol	o	o	o

contd. over/

Table 5.11 continued

	Macrotermes bellicosus	Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
β -myrcene	o	o	o	o	o
camphene	o	+	+	o	o
sabinene	o	+	o	o	o
(+)- α -pinene	o	++	++	++	+
(-)- α -pinene	o	++		++	
(-)- β -pinene	o	++	++	o	o
(+)- β -phellendrene	o	o	o	+	o
(+)- α -phellendrene	o	++	++	+++	+
terpinolene	++	+++	++	+++	+++
(+)-limonene	+	++	+++	++	+++
dimethyl disulphide ^a	o	o	+	o	o
dimethyl trisulphide	o	o	o	o	o
benzyl methyl sulphide	o	o	o	o	o
5-methyl-methyl salicylate			+	++	
1-undecane	o	o	o	o	o
1-pentadecane	o	o	o	o	o

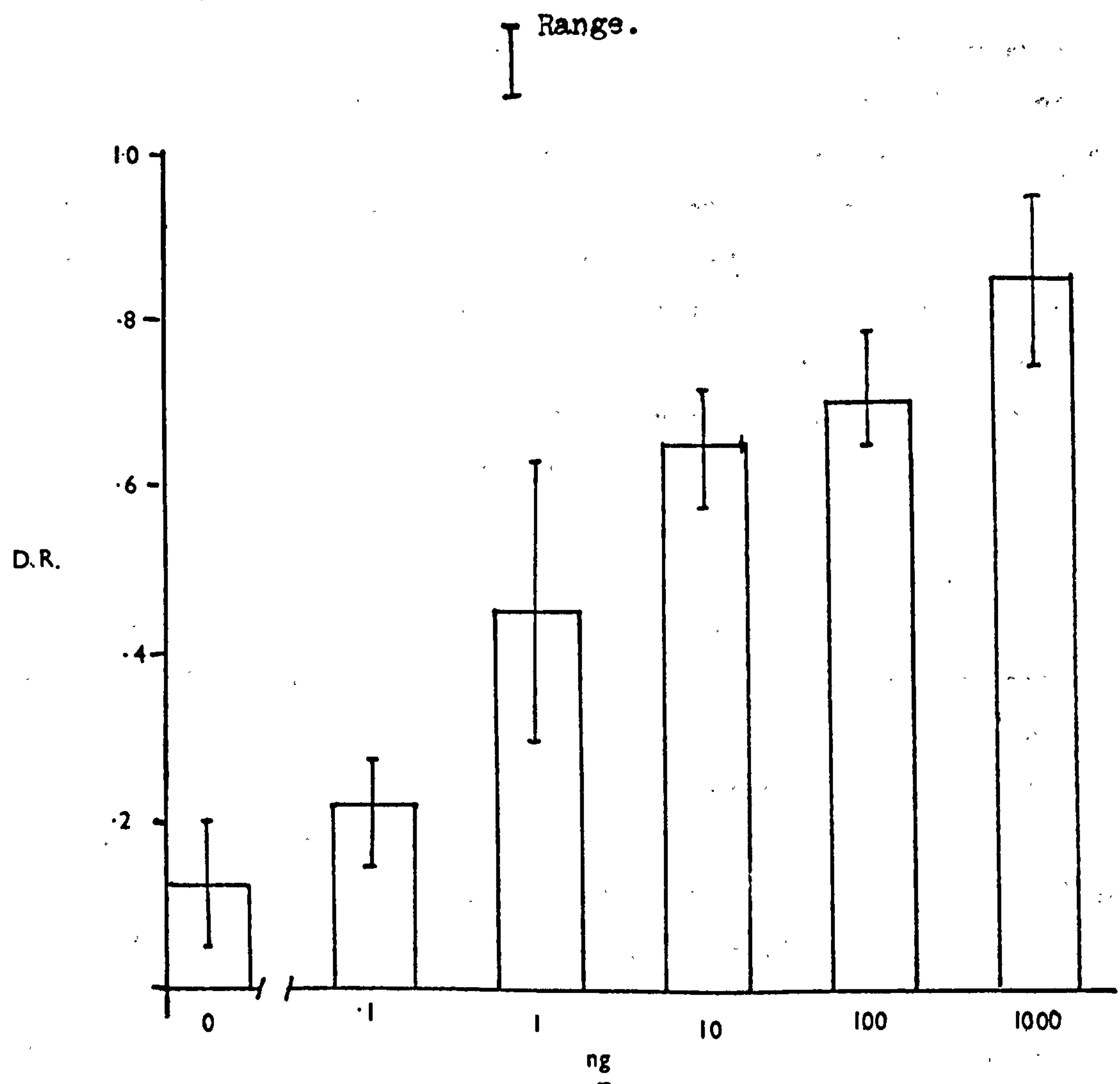
contd. over/

^a Initial trials on dimethyl disulphide and trisulphide suggested that they were repellent, but replicates on repurified samples did not elicit repellency.

Table 5.11 continued

Alkyl Pyrazines	Macrotermes bellicosus	Odontotermes pauperans	Ancistrotermes cavithorax	Microtermes sp.A	Microtermes sp.B
2,3-dimethyl pyrazine			+		††
2,3-dimethyl-5- <u>iso</u> -propyl		+	+	o	o
2-methyl-5-propyl			+		††
2,5-dimethyl-3-ethyl	+	o	+	o	+
2,5-dimethyl-3- <u>n</u> -propyl		+	†††	+	†††
2,5-dimethyl-3- <u>n</u> -butyl	o	o	††	o	+
2-methyl-6- <u>n</u> -propyl	+	o	††	o	††
2-ethyl-6- <u>n</u> -propyl	+	o	†††	o	††
2,6-dimethyl	o	o	o	o	o
2,6-dimethyl-3-ethyl			††		+
2,6-dimethyl-3- <u>n</u> -butyl	††	o		o	†††

Figure 5.4 Histogram showing the degree of repellency (DR) of Microtermes sp.A to 3-octanone at various concentrations.



5.2 Glandular Secretions.

5.2.1 Introduction.

The bioassay results (5.1) indicated that the strongest repellent compounds in most ants were found in the volatile fractions (Table 5.8). Breakdown of the ant into various sections revealed that the head was the major source of repellency in the majority of ants (Table 5.8), and that compounds in the mandibular glands of the head were very important in eliciting repellency (Table 5.9). In an attempt to elucidate some of the general principles governing the repellency of ants to termites the mandibular gland secretions of various ponerines and myrmecines were examined, and the contents of the glands, either as authentic samples or pure fractions, were tested for their effect on termites (Tables 5.10 - 5.11). The main exceptions to mandibular gland repellency was Myrmicaria eumenoides which has a poison gland containing repellent substances, and whose head was a very poor source of repellents. The head of this ant contains very little volatile secretion compared with the gaster (<1% of total volatiles, Fig. 5.11).

The majority of ant secretions from the mandibular glands are pheromones found at levels of less than one microgram per ant (eg Wilson 1971, Blum 1973), and in many of the ants examined in this present study the major compounds were at levels of less than 100 nanograms per ant. When such ants live in small colonies (2.3.1) sufficient quantities of live material cannot be obtained to provide FT-NMR studies on chemicals from most species. Fortunately linked GC-MS spectra can be obtained on quantities of less than one microgram, and coupling this with co-elution studies (McNair and Bonelli 1968), syringe reactions (Fredericks and Taylor 1966) and post-loop analysis (Bierl et.al. 1969) the identity of small quantities of natural products can be elucidated. Unfortunately no information on chirality could be obtained from these methods, a factor which can have important considerations in the responses of insects to compounds (Tamaki 1977).

5.2.2 Materials and Methods^a.

Ants were investigated using a variety of methods, depending on the availability of live material. SSGC (8.1.2) on excised glands and whole heads was the preferred method and was used whenever ants were available in culture. In other cases solvent extracts (8.3.2) of whole heads were used. Although this does not identify the compounds as glandular in origin, by correlation with other studies (eg, Crewe et.al. 1973, Wheeler and Blum 1973), it can be deduced that the material came from the mandibular glands in the head. The methods utilized in the identification is indicated in the results section (5.2.3) for each ant species.

The methods of identification used were GC-MS (8.2.1), co-elution with authentic samples (8.1.6), post-loop analysis (8.1.4) and sodium borohydride syringe reductions (8.1.5). The methods used varied with the ant species studied, depending on the availability of material, and are indicated for each species in 5.2.3.

5.2.3 Glandular Secretions - Results.

Bothroponera soror (Chromatogram, Fig. 5.5).

SSGC of Bothroponera heads and excised mandibular glands indicated a medley of over twenty compounds. SSGC of gasters and thoraces confirmed the origin of these compounds in the head. Repellency studies (Table 5.10) indicated that fractions 2 and 4 (Fig. 5.5) contained active compounds, but that the major cephalic component (in fraction 3) was not repellent. The majority of the compounds were in quantities of less than 20 ng per ant, component 13 being the most abundant (209 ng). SSGC-MS on the heads of fifteen ants gave data on the major components, which were subsequently tested for repellency as pure compounds or pure fractions (Tables 5.10- 5.11).

^a See also Section 8. Compounds referred to (eg. Compound 23) are listed in 8.5

Mass spectral data, co-elution studies, sodium borohydride syringe reaction and the use of boric acid and ϕ -dianisidine post loops revealed the following information on the most abundant compounds. Co-elution studies were carried out on columns A,B and C and GC-MS studies on columns A and B (8.1.1).

Component 7 - The base peak at m/e 58 corresponded to the McLafferty re-arrangement product $\text{CH}_2=\text{C}(\text{OH})\text{CH}_3^+$ from a methyl ketone. The molecular ion at m/e 170 suggests the formula $\text{C}_n\text{H}_{22}\text{O}$; a synthetic sample of 2-undecanone (compound 44) co-eluted with and gave a mass spectrum congruent with component 7. The natural product was removed by sodium borohydride reduction, but was not abstracted by the boric acid or ϕ -dianisidine post-loops.

Component 10 - The mass spectrum corresponded to that of a methyl ester, although the full identity of the compound could not be determined from the information available. The compound was removed by sodium borohydride reduction, was 15% abstracted by the ϕ -dianisidine post-loop but was unaffected by the boric acid post-loop.

Component 13 - The mass spectrum of this compound, with a very weak M^+ at m/e 172 was congruent with that of published spectra of 2-undecanol (Friedel et. al. 1956), the fragment at m/e 154 corresponding to the loss of water from this secondary alcohol. Synthetic 2-undecanol (compound 53) gave an identical mass spectrum to the natural product and co-eluted with component 13 on columns B,C and E. The compound was unaffected by the borohydride reduction but was 80% abstracted by the boric acid post-loop and 25% abstracted by the ϕ -dianisidine post-loop. There was not a sufficient quantity of this compound to determine its optical rotation.

Components 14 and 15 - The mass spectra of these two compounds were very similar, and corresponded to those of 2-undecenols. The double-bond position was not easily ascribed from spectral data.

It is probable that these two compounds differ only in the position of the double bond. On a non-polar (Column A) G.C column, being of a lower molecular weight than 2-undecanol, the components eluted before the major component (13) as a single peak. These two components were unaffected by borohydride reduction, but component 14 was 75% and component 15 80% abstracted by a boric acid post-loop, and both components 20% abstracted by the α -dianisidine post-loop.

Component 18 - The mass spectrum of this component corresponded with that of a synthetic sample of 2-dodecanol (Compound 54) with which it co-eluted on columns B and C.

Component 20 - The mass spectrum of this compound indicated that it was a 2-alcohol with a molecular weight of 200. No synthetic standards were available for co-elution studies, but comparison with the retention times of components 13 and 18 suggested that this compound was 2-tridecanol.

Component 21 - This compound exhibits a base peak at m/e 134, which corresponds to the loss of methanol from a methyl ester (Williams and Fleming 1968) as does the fragment at m/e 106 (loss of $HCOOCH_3$). There is a strong molecular ion at m/e 166. Fragments at m/e 77 and 78 suggest that the compound is aromatic, and the spectrum corresponds with published spectra of a methyl substituted methyl salicylate. The position of the methyl substitute on the ring cannot be determined from mass spectral evidence. A synthetic sample of methyl-5-methyl salicylate gave a mass spectrum congruent with the natural product but did not co-elute with it. Methyl-6-methyl salicylate has previously been found as a natural product in the ponerine Gnamptogenys pleurodon (Duffield and Blum 1975) and Camponotus spp. males (Brand et.al. 1973) and until further evidence is available the compound in Bothroponera is tentatively identified as the methyl-6 substituted methyl salicylate.

Anochetus sedilloti (Chromatogram, Fig.5.9)

SSGC of heads and excised mandibular glands of this ponerine ant

revealed at least ten compounds, at a level of 10 ng per ant or greater, which were not present in the gasters or thoraces.

SSGC-MS of twenty worker ants heads on column B indicated that all the compounds had a base peak at m/e 122, indicative of either 2,5 or 2,6-dimethyl-3-alkyl pyrazines (Wheeler and Blum 1973), with the substituent on the third carbon being greater than $-C_2H_5$. 2,5 and 2,6 isomers cannot be distinguished from mass spectral evidence but an attempt was made to distinguish between the two isomers by the use of a microscale reaction. Alkyl pyrazines can be quarternized with methyl iodide and then reduced to the piperizine with sodium borohydride; the 2,5 and 2,6-dimethyl-3-alkyl piperizines giving distinctive and different mass spectra (Wheeler and Blum 1973). Attempts to carry out this reaction on 500 ng samples of pyrazines (8.3.3) in sealed tubes suitable for SSGC-MS were not successful, consequently the 2,5 and 2,6-dimethyl-3-alkyl pyrazines could only be characterized by differences in GC retention times. The 2,5 dimethyl isomers eluted before the 2,6 dimethyl isomers on GC columns B, C and D, although they could not be resolved on a non-polar OV101 column.

Components 1 and 3 (Compounds 30 & 35) gave identical mass spectra, corresponding with either 2,5 or 2,6-dimethyl-3-isobutyl pyrazine; the differences in retention time on GC column B identified component 1 as the 2,5-dimethyl isomer and component 3 as the 2,6-isomer. Component 2, and the major component 4, gave identical mass spectra. The side chain of these compounds was identified as being secbutyl, the differences in retention time identifying component 2 as the 2,5 dimethyl isomer and component 4 as 2,6-dimethyl-3-secbutyl pyrazine. The second most abundant compound (component 8) gave a mass spectrum congruent with that of 2,5- or 2,6-dimethyl-3-npentyl pyrazine; comparison with the relative retention times of synthetic pyrazines and co-elution studies on columns B and C identified component 8 as 2,5-dimethyl-3-npentyl pyrazine (compound 32). Component 5 shared an identical retention time and mass spectrum with 2,6-dimethyl-3-nbutyl pyrazine (compound 37), and co-eluted with a synthetic sample on column B.

The mass spectra of components 6,7 and 9 were too contaminated by background impurities to draw any definite conclusions on their identity: they all exhibited a base peak at m/e 122, suggesting that they were 2,5 or 2,6-dimethyl-3-alkyl pyrazines. They all appeared to have a molecular ion at 178.

Brachyponera sennaarensis (Chromatogram, Fig. 5.8)

SSGC of the heads of this ant on GC column B revealed the presence of four major components. SSGC-MS revealed that they were 2,5 or 2,6-dimethyl-3-alkyl pyrazines (q.v. Anochetus sedilloti). There were not enough ants available for co-elution studies, but the relative retention indices indicated that components 2,3 and 4 were 2,6-dimethyl-3-n-butyl, n-pentyl and n-hexyl pyrazine respectively. The identity of component 1 is not known, co-elution was not found with 2,5 or 2,6-dimethyl-3-n-propyl pyrazine. The component could possibly have a branch on the 3-alkyl side group.

Odontomachus troglydytes (Chromatogram, Fig. 5.7)^a

SSGC of the heads of this ponerine revealed over twenty compounds at a level of 20 ng or greater, with four components predominating. GC-MS studies on the heads of eight ants, and co-elution studies on column B revealed that the four major components were all 2,6-dimethyl-3-n-alkyl pyrazines. Component 4 is the ethyl, 8 the n-butyl, 10 the n-pentyl and 13 the n-hexyl substituted compound.

Megaponera foetens (Chromatogram, Fig. 5.6)

Work on Megaponera foetens presented a number of difficulties. The smallest workers were too large for conventional SSGC, and pre-

^aThis work was carried out in collaboration with W.Speed, Chemical Entomology Unit.

column techniques (8.1.2c) gave inconsistent results, including poor resolution and probable thermal breakdown of some components. Solvent extraction did not prove useful; although whole colonies (400+ ants) were extracted into solvent at Mokwa. The loss, during preparation, of the important compounds found in small quantities, resulted in insubstantial quantities of material after work-up to give informative mass spectra. Other compounds appeared to degrade in the GC-MS link. A modified solid sample technique (8.1.2b) was developed which allowed comparison of castes on the GC columns used for standard analysis of other ant species.

Behavioural bioassay (5.1) revealed that fractions containing compounds repellent to termites were present in both the mandibular glands and the Dufour's/poison gland complex. Attention was concentrated on the cephalic secretions for both repellency studies and observations on the co-ordination of predation (6.3) revealed that mandibular gland components were important.

Modified SSGC (Fig. 8.1) studies revealed that at least thirteen compounds were resolvable, and that the castes did not differ significantly in the proportions or patterns of components present. In the major workers, the most abundant component (2) was present at the level of only 28 ng per ant. SSGC-MS and GC-MS studies with dichloromethane extracts revealed the following information about the mandibular gland components.

Component 2 - A molecular ion at m/e 94 (100%) with fragments at m/e 95 (3%) and m/e 96 (9%) suggested that the compound, of molecular weight 94, contained sulphur which has an isotopic ratio of ^{32}S (95.0%), ^{33}S (0.8%) and ^{34}S (4.0%). Comparison with published mass spectra (Crewe and Fletcher 1974) and an authentic sample of dimethyl disulphide (Compound 13) gave the identity of this compound. Co-elution studies on columns A, B and C confirmed the identity.

Component 3 - The mass spectrum of this compound revealed a base peak at m/e 103 (100%) which was also the molecular ion. Fragments at m/e 109 (4%) and m/e 110 (11%): suggested a sulphur containing

compound. A large fragment at m/e 80 (88%) is probably from the loss of C_2H_4 from the molecule. Although no published spectrum corresponding to this compound could be found, the evidence pointed to a compound with the empirical formula $C_3H_8S_2$; the loss of the ethyl radical suggesting $C_2H_5S_2CH_2$, ethyl methyl disulphide - to be a possible structure (compound 14).

Component 4 - The mass spectrum of this compound, with a base peak and molecular ion at m/e 126, and fragments at m/e 127 (4%) and 128 (12%) corresponded to the published spectrum of dimethyl trisulphide from the ponerine ant Paltothyreus tarsatus (Crewe and Fletcher 1974). A synthetic sample (compound 15) gave a mass spectrum congruent with, and coeluted with the natural product on columns A, B and C.

Component 8 - This compound exhibited a mass spectral molecular ion at m/e 138 (89%) with additional fragments at m/e 139 (2%) and 140 (8%), characteristic of the isotopic ratio of sulphur. The base peak at m/e 91 ($C_7H_7^+$) is characteristic of an aromatic ring with a methyl side chain, with further substituents on this methyl group ($C_6H_5CH_2X$). An aromatic ring is also suggested by the fragment at m/e 76, which is probably $C_6H_4^+$ from C_6H_5X . The loss of 47 from the molecular ion (m/e 138) to form the base peak suggests the loss of CH_3O_2 or CH_3S , the latter being the most probable because of the indication of the presence of sulphur from consideration of isotopic ratios. Fragments at m/e 61 and m/e 47 could be due to the CH_2SCH_3 and SCH_3 groups respectively. The overall spectrum is indicative of the aromatic compound, benzyl methyl sulphide ($C_6H_5CH_2SCH_3$).

A synthetic sample (compound 16) gave a mass spectrum congruent with the natural product and co-eluted on GC columns A and B.

Myrmicaria eumenoides (Chromatogram, Fig. 5.10).

In this myrmicine the poison gland/Dufour's gland complex proved

to be the strongest source of repellent compounds (5.1.4). SSGC of whole gasters and excised poison glands revealed the presence of 14 compounds. These proved to be monoterpene hydrocarbons similar to those found in Myrmicaria natalensis F.Smith from southern Africa (Brand et.al. 1974). Identifications were carried out on an hexane extract of 100 whole ants, the peaks which were specific to the poison gland were identified by GC-MS, co-elution studies and in the case of the four major components, FT-NMR provided additional confirmation of identity. Optical rotation measurements (8.2.3) were obtained for three of the optically active components of the secretion.

All components exhibited a molecular ion at m/e 136 and further information was gathered on each component from the following techniques.

Component	M.S.	Co-elution	FT-NMR	Optical Rotation
1. Camphene	+	+		
2. (+) α -Pinene	+	+	+	+
3. Sabinene	+	+		
4. (-) β -Pinene	+	+	+	+
5. β -Myrcene	+	+	+	not active
6. α -Phellendrene	+	+		
7. α -Terpinene	+			
8. (+) Limonene	+	+	+	+
10. Terpinolene	+	+		

Further details are given in 8.5.1, compounds 1 - 10. Mass spectra of authentic samples of all compounds except α -terpinene were obtained on the MS12 at Southampton University (8.2.1) and by comparison with published mass spectra of monoterpene hydrocarbons (Ryhage and von Sydow 1963).

Table 5.12 Quantities of monoterpene hydrocarbons in the
the poison gland of Myrmicaria eumenoides

Peak No.	Identification	ug per gland ^a
1	camphene	0.04
2	(+) α -pinene	1.66
3	sabinene	0.08
4	(-) β -pinene	6.60
5	β -myrcene	1.24
6	α -phellendrene	0.16
7	α -terpinene	0.24
8	(+)-limonene	5.34
9	?	0.04
10	terpinolene	0.34
11	?	0.06
12	?	0.06
13	?	0.06
14	?	0.06

^a mean of 10 workers from colony C; determined by
SSGC of whole gasters.

Tetramorium guineense (Chromatogram, Fig. 5.13).

The major source of repellency in this ant was located in the mandibular glands (Table 5.9). SSGC of whole heads and excised glands revealed the presence of two main compounds, the second constituting 87% of the volatile secretion of 260 ng per ant. Component 2 was examined by SSGC-MS of 15 ants heads. The mass spectrum revealed the presence of an ethyl alkyl ketone of molecular weight 128. The spectrum was congruent with that of an authentic sample of 3-octanone (compound 46). The identification was confirmed by SSGC co-injection with authentic 3-octanone which co-eluted with component 2. Components 1 and 2 were both removed by borohydride reduction to give two different components, one of which co-eluted with an authentic sample of 3-octanol. Neither component 1 or 2 were affected by boric acid or α -dianisidine post-loops.

Tetramorium caespitum (Chromatogram, Fig. 5.12).

Although this species of Tetramorium was not found at Mokwa, the mandibular gland secretion was examined for comparison with other Tetramorium species. Ants were obtained from Furzebrook, Dorset, U.K. SSGC of excised mandibular glands revealed the presence of five components, the major (2) constituting 71% of the volatile secretion and amounting to 315 ng per worker ant. This major component gave a mass spectrum congruent with and co-eluted with 3-octanone on columns B and C. Sodium borohydride reduction eliminated components 2 and 3 and one major new component, which eluted before component 4 and had the same retention time as 3-octanol. Components 4, 5 and 7 were nearly completely abstracted by the boric acid post-loop, and component 7 was 16% abstracted by the α -dianisidine post-loop, which also abstracted component 1. Component 1 could be an aldehyde and 4, 5 and 7 alcohols.

Tetramorium sericeiventris (Chromatogram, Fig. 5.14)

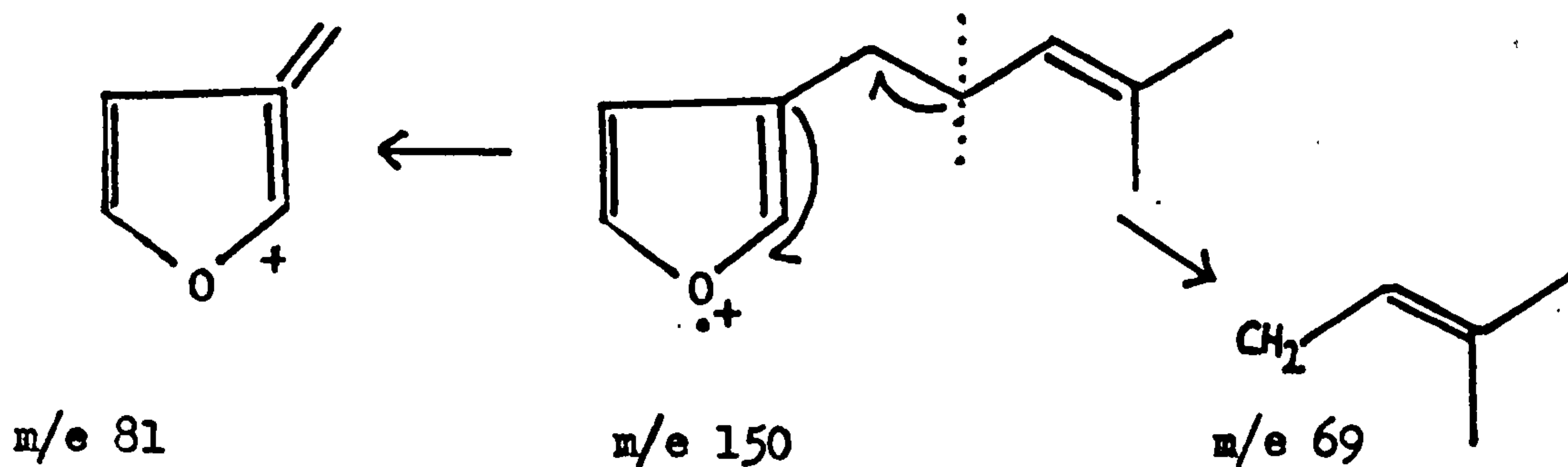
The major mandibular gland components of this ant constitutes 70%

of the secretion, at 170 ng per worker ant. This compound (3) gives a mass spectrum congruent with an authentic sample of 3-octanone, and also co-elutes with the ethyl ketone. Sodium borohydride reduction results in the disappearance of components 1,3 and 4 and the appearance of a new large peak with the same retention time as component 5, which co-elutes with 3-octanol. A second small component is formed after borohydride reduction which elutes after component 5. A boric acid post-loop abstracts components 1 (100%), 5 (95%) and 6 (80%) and the α -dianisidine loop abstracts component 1 (53%) and component 6 (31%).

Tetramorium angulinode (Chromatogram, Fig. 5.16)

The mandibular glands of this ant, which is repellent to termites, contain 7 components, the major component (2) constituting 91% (3.783 μ g) of the secretion. The seven peaks were not present in the gaster or the thorax.

Component 2 - The major component has a base peak at m/e 69 and a molecular ion at m/e 150. Methyl radical loss gives a fragment at m/e 135. The spectrum corresponds to published spectra of perillen (3-(4'-methyl-pent-3'-ene) furan (Bernaldi et al. 1967)). The fragments at m/e 69 and m/e 81 are explained by (m/e 69), the loss of the terminal isoprenic group by allylic cleavage and (m/e 81) from cleavage of the bond β to the furan ring as in the following scheme:



The large fragment at m/e 41 ($\text{CH}_2=\text{CH}-\text{CH}_2^+$) is probably derived from the fragmentation of the side chain, and the fragments corresponding to the cyclopropenyl cation and its higher homologues (m/e 39, 53 and 67) from the fission of the furan ring (Bernaldi *et.al.* 1967).

Component 3 - Mass spectral evidence indicated a compound of molecular weight 144, there being a small (0.2%) molecular ion at 144. A fragment at m/e 126 would correspond to the loss of water and the overall spectrum was congruent with that of the published spectra of 2-nonanol. The compound was not affected by sodium borohydride reduction, but was 85% abstracted by a boric acid post-loop but was unaffected by the σ -dianisidine loop.

Component 5 - A base peak at m/e 58 corresponds to the McLafferty re-arrangement product from a methyl ketone, and the molecular ion at m/e 170 suggests the formula $\text{C}_{11}\text{H}_{22}\text{O}$. A synthetic sample of 2-undecanone (compound 44) gave an identical spectrum to the natural product, and co-eluted with it on columns B and C. Sodium borohydride reduction caused a 90% loss of this component and component 6 (2-undecanol) was increased in intensity by ca. 15%. Component 5 was unaffected by boric acid and σ -dianisidine post-loops.

Component 6 - This compound gave a mass spectrum similar to that of component 3, differing in a small (0.1%) molecular ion at m/e 172 and a fragment at m/e 154, corresponding to the loss of water from the molecule. The mass spectrum corresponded to that of a synthetic sample of (+) 2-undecanol (compound 53) which also co-eluted with the natural product on GC columns B and C. Component 6 was unaffected by sodium borohydride reduction and was not abstracted by a σ -dianisidine post-loop. It was abstracted (92%) by a boric acid post-loop.

Tetramorium sp.D (Chromatogram, Fig. 5.17)

The mandibular gland secretion of this Tetramorium sp. was essentially

the same as that of T.angulinode (Fig. 5.16), differing in the presence of an extra component (1A) eluting before component 1. and in the greatly reduced size of components 3, 5 and 6. Mass spectra could not be obtained on these components in this species, but components 5 and 6 co-eluted with synthetic samples of 2-undecanone and 2-undecanol respectively. Perillen (component 2), the major component, comprised 98% of the mandibular gland secretion at 2.700 µg per ant.

Tetramorium termitobium (Chromatogram, Fig. 5.18)

SSGC of excised mandibular glands from this non-repellent ant revealed the presence of at least nine components, with component 8 accounting for 80% of the secretion at the level of 150 ng per ant. SSGC-MS was carried out on ten heads; useful information was obtained only on the major component. A small molecular ion (0.2%) at m/e 172 and the fragment at m/e 154 was congruent to that of a synthetic sample of 2-undecanol, which co-eluted with the natural product on GC column B. No further ants were available for co-elution studies on other GC columns or for syringe reactions or loop abstraction studies.

Decamorium uelense (Chromatogram, Fig. 5.19)

SSGC of this myrmicine revealed the presence of one major component comprising 96% of the cephalic volatiles at 370 ng per ant. GC-MS revealed a compound with the molecular formula $C_{18}H_{38}O$, the base peak at m/e 59 suggested a secondary alcohol with the -OH on carbon 3 (Friedal et.al. 1956). Comparison with published mass spectra suggested either 3-octanol or 4-methyl-3-heptanol, the 2,5 and 6-methyl branched heptanols having distinctive and different mass spectra. Mass spectra of authentic 3-octanol and 4-methyl-3-heptanol were obtained under identical conditions, and the spectrum obtained from 3-octanol was congruent with the major component of D.uelense; the identity was confirmed by co-elution studies. Not enough of the alcohol was available to ascertain its optical rotation.

Crematogaster species.

The cephalic volatiles of a number of Crematogaster species have previously been examined by Crewe et.al. (1972). They found that in the subgenera Eucrema, Nematacrema, Sphaerocrema and Orthocrema, 3-octanone, 3-nonanone, 3-octanol, 6-methyl-3-octanone and 6-methyl-3-octanol were usually present, with 3-octanone being the dominant component in the majority of species in these subgenera. In the carton-nesting subgenus Atopogyne (given generic status by Leston 1973) trans hex-2-enal and trans hex-2-en-1-ol were present, with the saturated ketones and alcohols present at only low levels.

Examination of five species of Crematogaster from Mokwa revealed that one species (Crematogaster sp.C = ?bequaerti) had 6-methyl-3-octanol as the major component. Species A,B,D and E had alkyl ketones and alcohols in similar proportions to those investigated by Crewe et.al. (1972). Crematogaster sp.F (subgenus Atopogyne) contained hex-2-enal as the major component.

Crematogaster sp.B (Chromatogram, Fig. 5.21)

This ant was only slightly repellent to termites (Table 5.5), and the fractions containing repellent compounds were in the head. SSGC of the excised mandibular glands of this species revealed the presence of ten compounds which were not present in the gaster or thorax. SSGC-MS of twenty worker heads revealed that components 1 and 5 had mass spectra congruent with authentic samples of 3-octanone and 3-octanol respectively. Co-elution studies on GC columns B and C confirmed the mass spectral evidence. Components 3 and 6 co-eluted with synthetic samples of 6-methyl-3-octanone and 6-methyl-3-octanol respectively.

The major components, 3-octanone and 3-octanol, accounted for 23% and 62% of the mandibular gland secretion at a level of 25 and 65 ng per ant.

Crematogaster sp.A (Chromatogram, Fig. 5.20)

The cephalic secretion of this repellent ant (Table 5.5) was examined by conventional GC. Ants were not available for SSGC, but head and gaster extracts were prepared for GC examination. Workers were taken with the minimum of disturbance. A large section of bark with ants on it, from a tree on which foraging was in progress, was removed. The ants were cooled to -13°C in a deep freeze and the heads and gasters of 50 ants were removed and placed separately in 750 μl of dichloromethane. The biological material was crushed with a glass rod and GC and GC-MS studies were carried out on the solvent extracts.

On GC column B 14 components, not present in the gaster, could be resolved. Mass spectra were obtained on components 1, 3, 4 and 7. Components 3 and 7 gave mass spectra identical to authentic samples of 3-octanone and 3-octanol respectively and both of these compounds co-eluted with their natural analogues on GC columns B, C and E. Co-elution studies with authentic samples indicated that components 5 and 9 were 6-methyl-3-octanone and 6-methyl-3-octanol. The identity of components 1 and 4 could not be determined from mass spectral data. The effects of post-loops and sodium borohydride reduction on the compounds in Mokwa Crematogaster species are summarised in Table 5.14.

The major component, 3-octanone, accounted for 77% of the cephalic volatiles, and a worker ant contained approximately 340 ng of this alkyl ketone.

Crematogaster sp.C (Chromatogram, Fig. 5.22)

The mandibular gland secretion was analysed from ants collected and transported back to the laboratory inside a termite bait block. SSGC of excised mandibular glands and whole heads revealed the presence of at least 17 components, with one component (11) predominating. SSGC-MS on the heads of twelve ants revealed that

component 3 had a mass spectrum congruent with that of an authentic sample of 3-octanone, this identification was confirmed by co-elution studies on column B. The major component, which comprised 80% of the secretion at a level of 354 ng per ant, gave a mass spectrum identical to that of a synthetic sample of 6-methyl-3-octanol (compound 60). A small (0.1%) molecular ion at m/e 144, with a fragment at m/e 126, corresponding to the loss of water were present. The base peak at m/e 59 corresponded with that of a secondary alcohol with the hydroxyl group on carbon 3 (Friedel et. al. 1956). Components 6 and 9 were identified as 6-methyl-3-octanone and 3-octanol by co-elution and microscale reaction (Table 5.14).

Crematogaster sp.D (Chromatogram, Fig. 5.23)

Analysis of the cephalic secretion of this ant was carried out on solvent extracts of ants collected from under bark on a tree. The ants were frozen and the heads and gasters of 50 ants were placed separately in 1 ml of dichloromethane. GC and GC-MS studies were carried out on these solvent extracts. Eleven components were resolved from the heads of this species. GC-MS studies revealed that components 3 and 7 gave mass spectra congruent with those of authentic samples of 3-octanone and 3-octanol respectively. Co-elution studies on columns B and C confirmed their identity and also indicated that components 5 and 9 co-eluted with 6-methyl-3-octanone and 6-methyl-3-octanol. The major component, 3-octanone (3), comprised 77% of the cephalic volatiles at approximately 80 ng per ant.

Crematogaster sp.E (Chromatogram, Fig. 5.24)

SSGC and SSGC-MS studies on this ant, which was repellent to termites, revealed that the mass spectrum of component 1 was identical with an authentic sample of 3-octanone. The other components were identified by co-elution studies and microscale reactions (Table 5.14) as 6-methyl-3-octanone (component 3), 3-octanol (component 5)

Table 5.13 Quantities of mandibular gland volatiles in Crematogaster spp. and Pheidole sp. (ng)

	CREMATOGASTER					PHEIDOLE
	A	B	C	D	E	A
3-octanone	340	25	23	80	710	248
6-methyl-3-octanone	1	1	1	1	17	8
3-nonanone (?)	2	1	2	1	1	5
3-octanol	43	65	23	15	42	37
6-methyl-3-octanol	13	1	1770	1	14	10

Table 5.14 Components from Crematogaster and Pheidole spp.
 reacting with sodium borohydride and abstracting
 with boric acid and σ -dianisidine post-loops

	<u>Crematogaster</u>						<u>Pheidole</u>
	B	A	C	D	E	F	A
Boric Acid post-loop ^a	5,6, 7,9	7,8, 9	9,10, 11,13, 17	7,9, 10,11	5,6, 8	5,6, 7,9	4,5, 6,7
σ -dianisidine post-loop ^b		1,2	1	1,2, 2a		1,2, 3	
sodium boro- hydride syringe reaction ^c	1,2, 3,4	1,2, 3,5, 6	1,2, 3,4, 5,6, 8	1,2, 3,4, 5,6	1,2, 3,4	1,2, 3	1,2, 3

^a Abstracts primary and secondary alcohols; occasionally terpene aldehydes and ketones (Higgs 1976)

^b Abstracts aldehydes and some ketones

^c Reduces the carbonyl group to the corresponding alcohol

For identity of components refer to gas chromatogram for the species

and 6-methyl-3-octanol (component 6). The major component was 89% of the secretion at a level of 710 ng per ant.

Crematogaster sp.F (Chromatogram, Fig. 5.25)

A section of carton nest containing ants was brought back to Southampton intact. SSGC studies revealed two major components. Component 1 gave a mass spectrum congruent with and co-eluted with an authentic sample of trans hex-2-enal and component 6 gave a spectrum congruent with published spectra of hex-2-en-1-ol. This compound co-eluted with the borohydride reduction product of hex-2-enal. None of the components present co-eluted with any of the aliphatic 3-ketones or 3-alcohols found in other Crematogaster species.

Pheidole sp.A (Chromatogram, Fig. 5.15)

SSGC of excised mandibular glands and whole heads of minor workers of the dimorphic Pheidole sp.A revealed that it had a pattern of cephalic volatiles similar to that of Crematogaster spp. A,B,D and E and Tetramorium sericeiventre. SSGC-MS studies on the heads of 20 minor workers revealed that components 1 and 4 gave mass spectra identical to authentic samples of 3-octanone and 3-octanol, and co-eluted with these samples on GC column B. Component 2 co-eluted with 6-methyl-3-octanone and component 5 with 6-methyl-3-octanol. 3-Octanone, the major component, accounted for 76% of the cephalic volatiles at a level of approximately 248 ng per ant.

Chromatograms, Figures 5.5 - 5.25.

Unless otherwise stated the biological material was run on a Carbowax 20M column (GC Column B).

Column conditions: 80 - 180°C @ 6°C/min. with an initial hold of 3 minutes at 80°C

Horizontal axis, scale 10 mm = 3 minutes

Figure 5.5 Chromatogram of the mandibular gland secretion of Bothroponera soror.

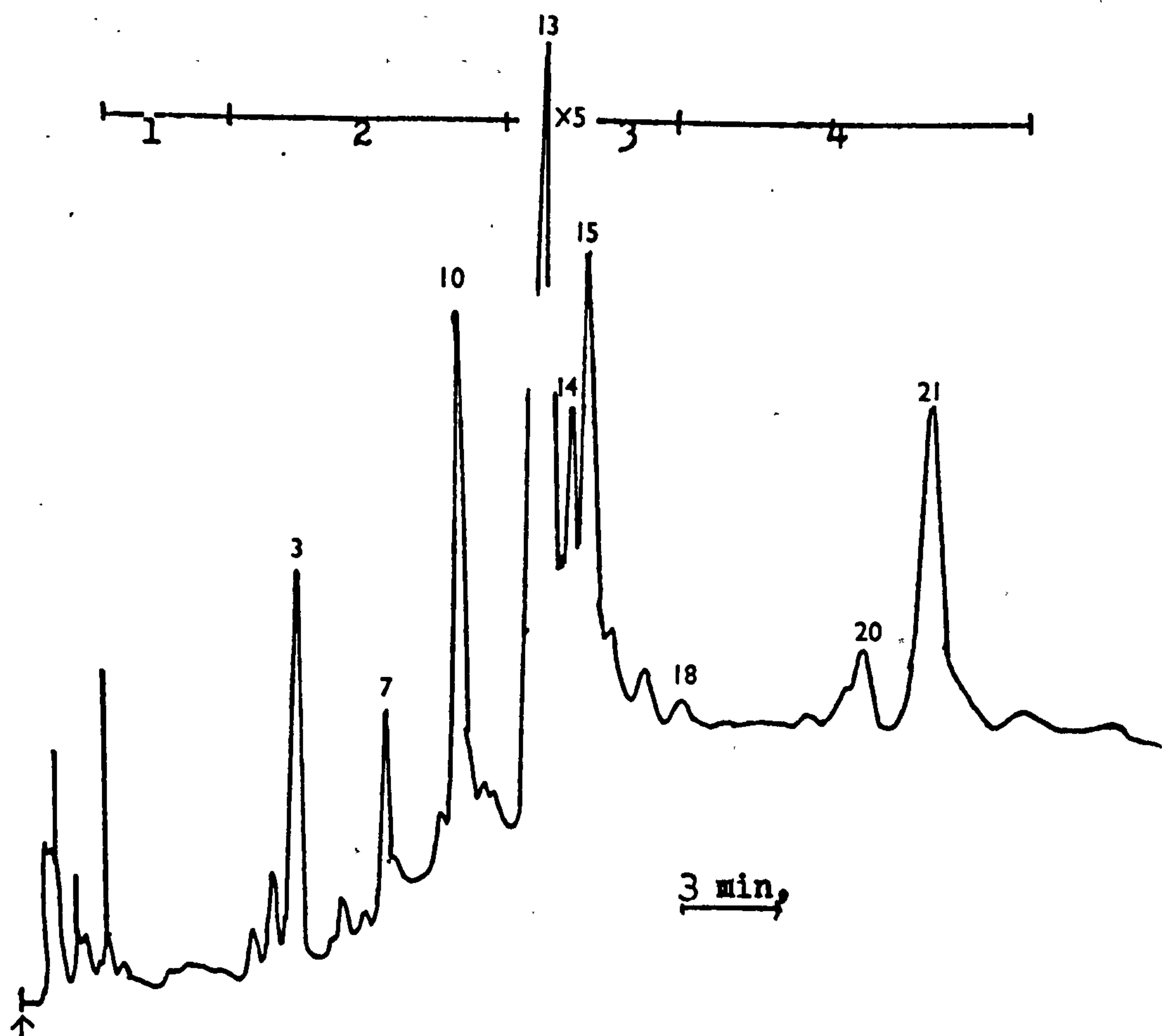


Figure 5.6 Chromatogram of the mandibular gland secretion of a major worker of Megaponera foetens.

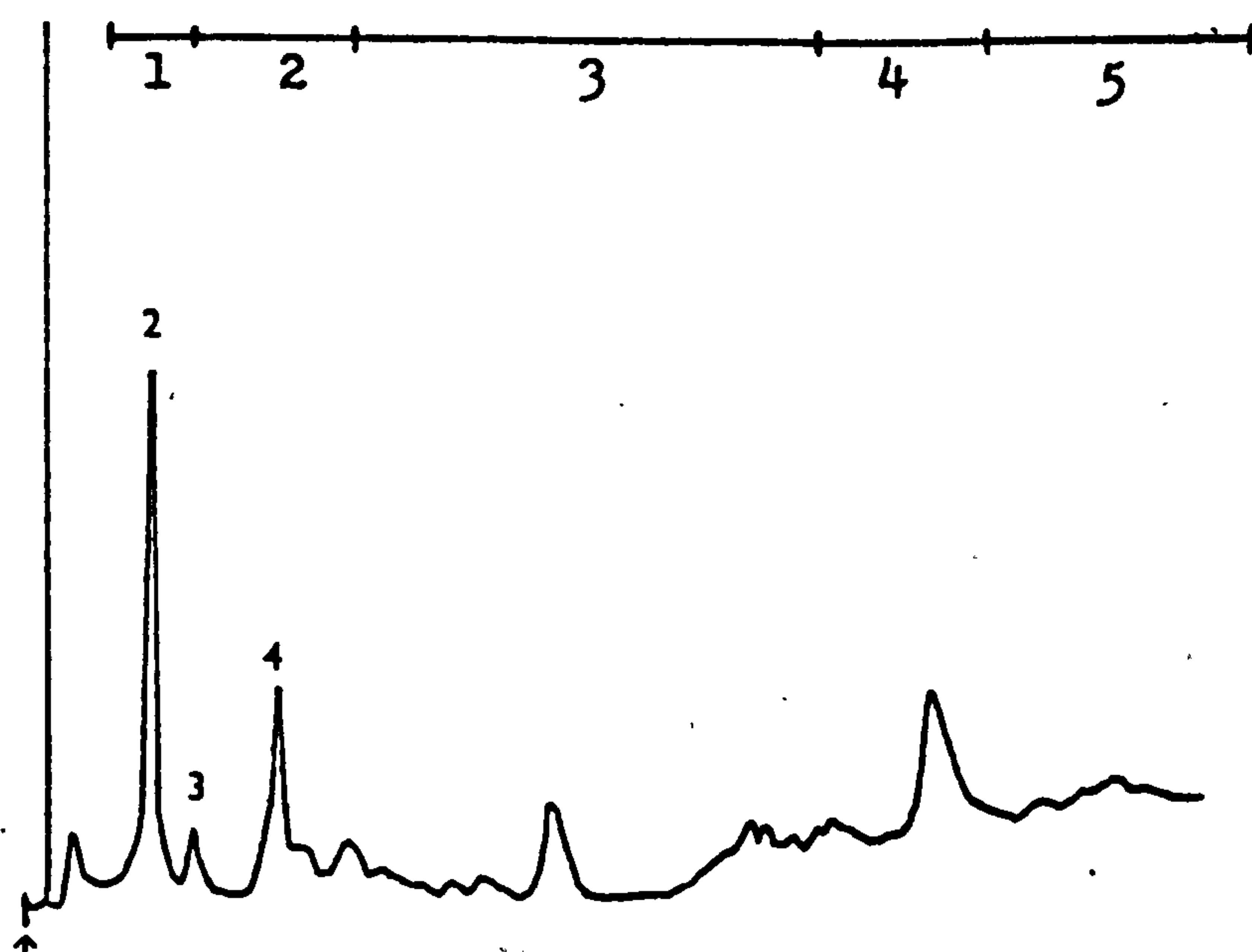


Figure 5.7 Chromatogram of the
cephalic secretion of
Odontomachus troglydytes.

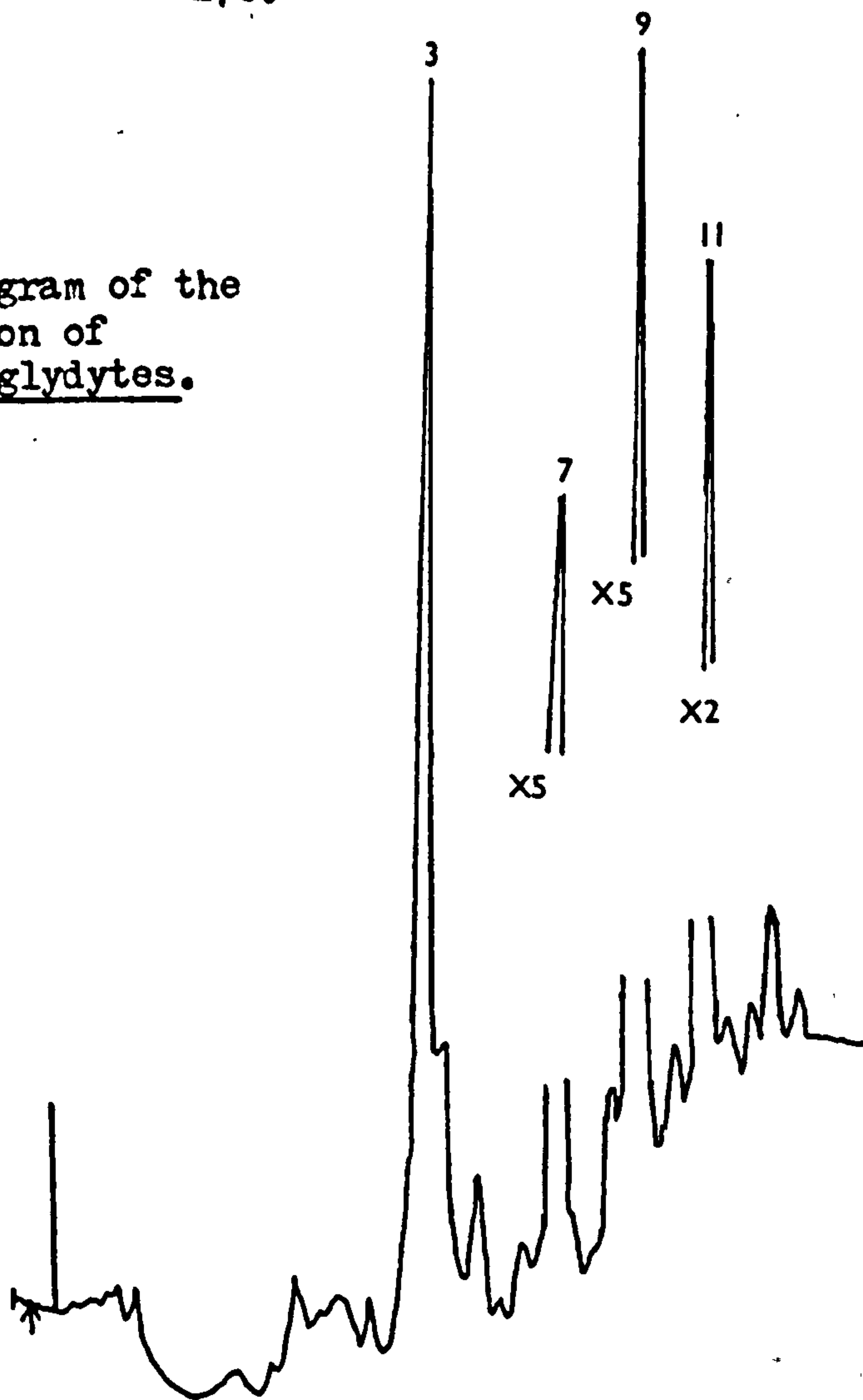
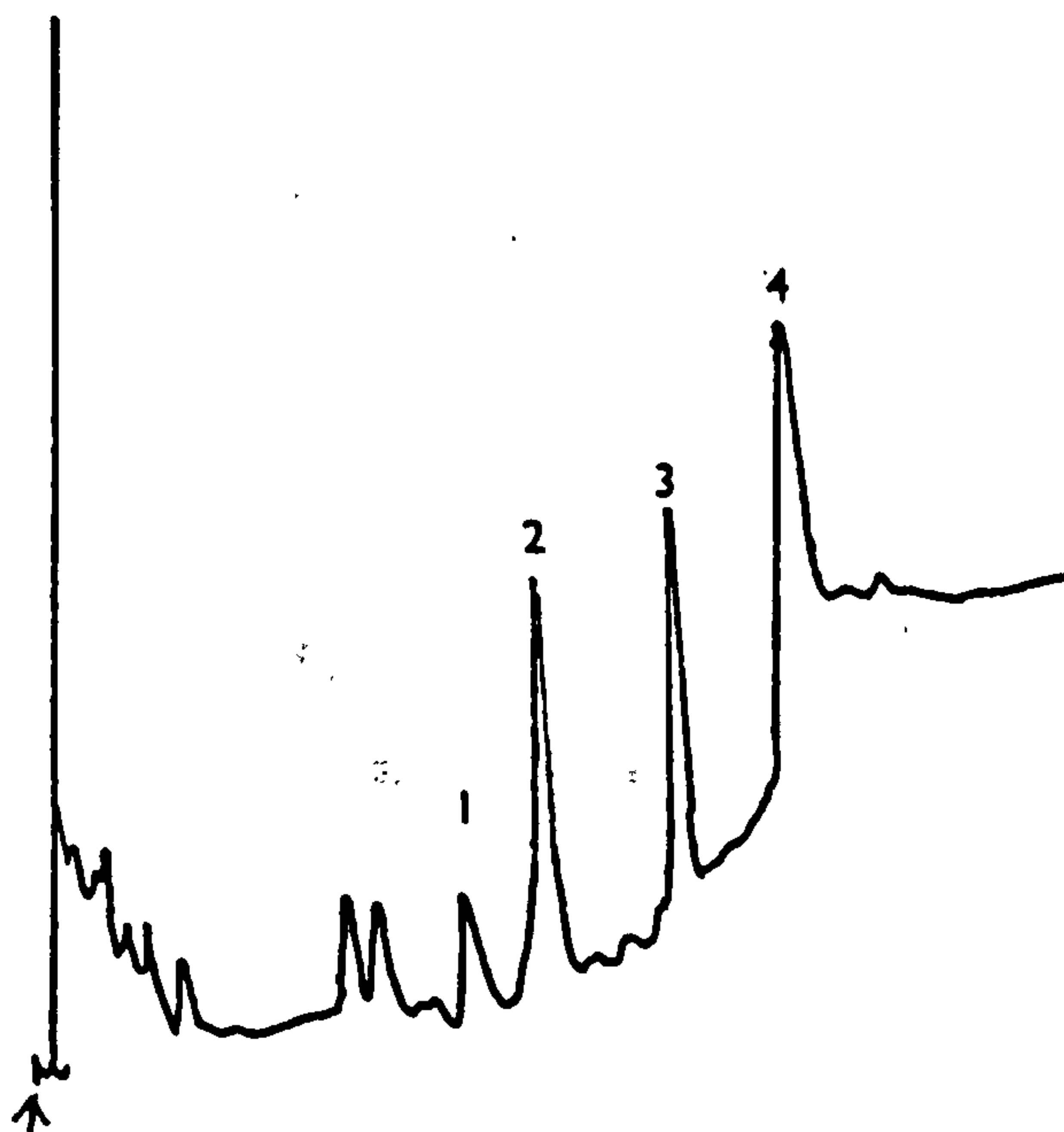


Figure 5.8 Chromatogram of the cephalic secretion of
Brachyponera sennaarensis.
Programmed from 100°C @ 6°C/min.



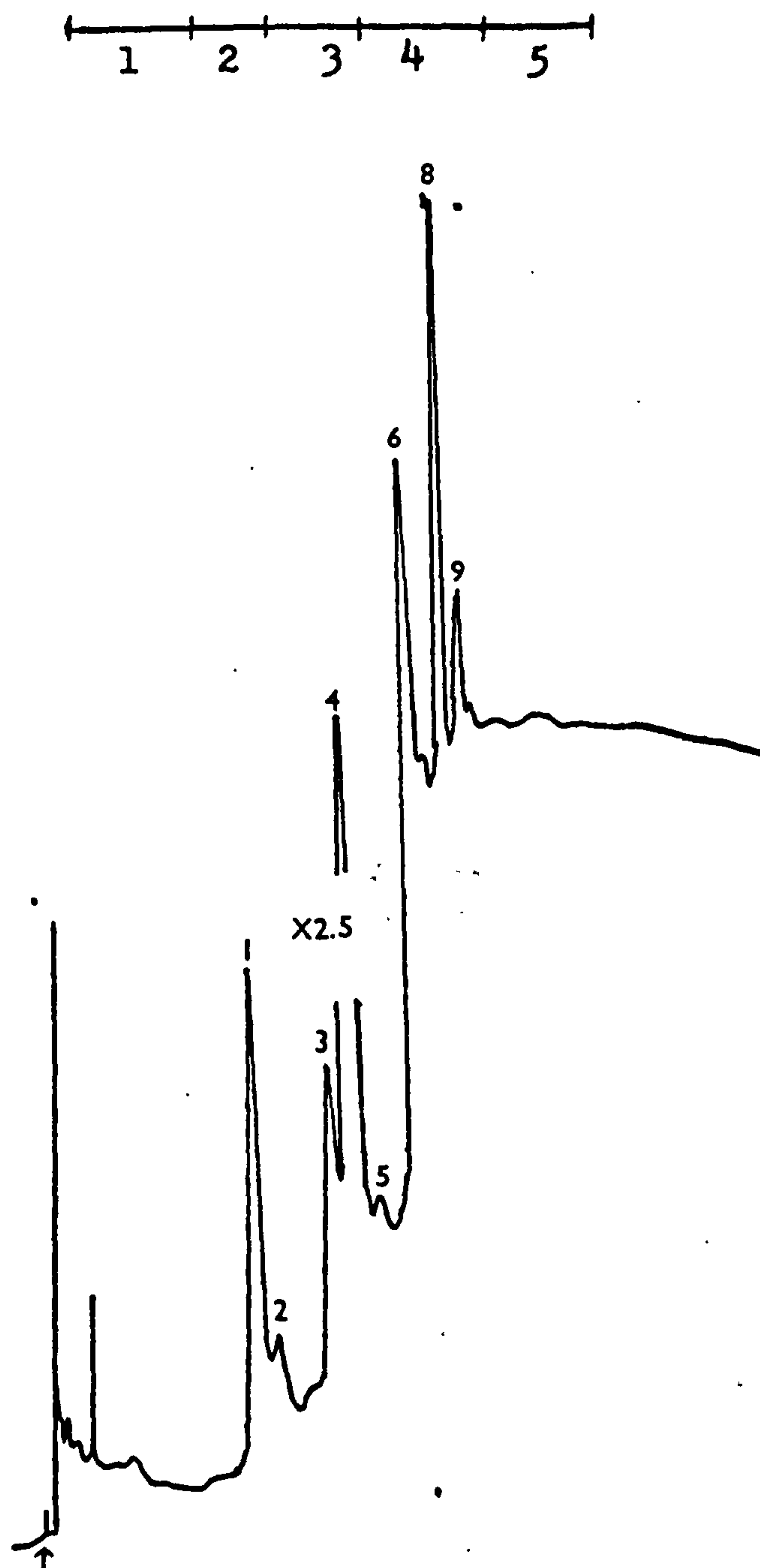


Figure 5.9 Chromatogram of the cephalic secretion of Anochetus sedilloti.
Programmed from 100°C @ 6°C/min.

Figure 5.10 Chromatogram of
of the poison gland
secretion of Myrmicaria
eumenoides.

5% E30; 50 - 150° @ 3°C/min.

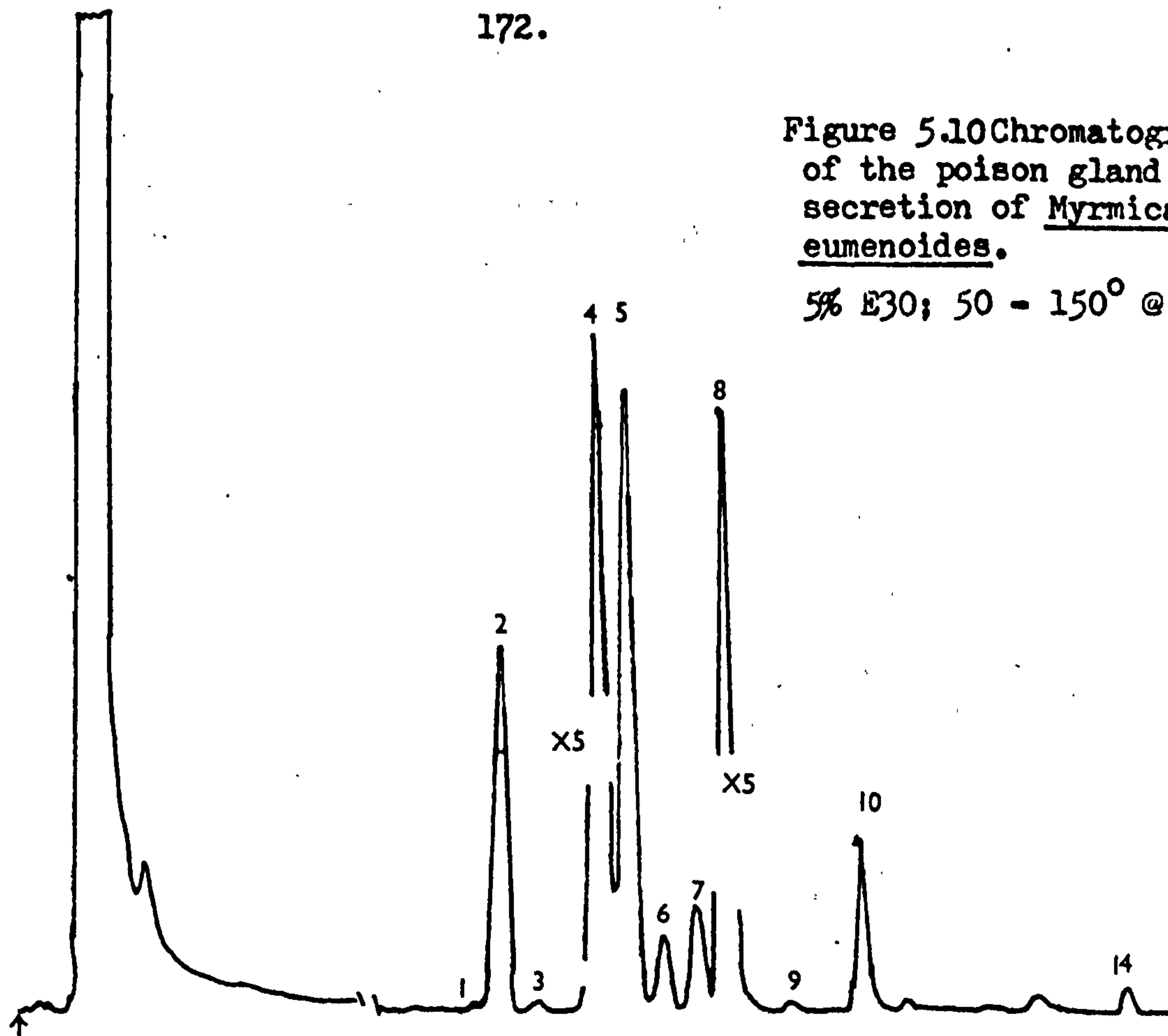
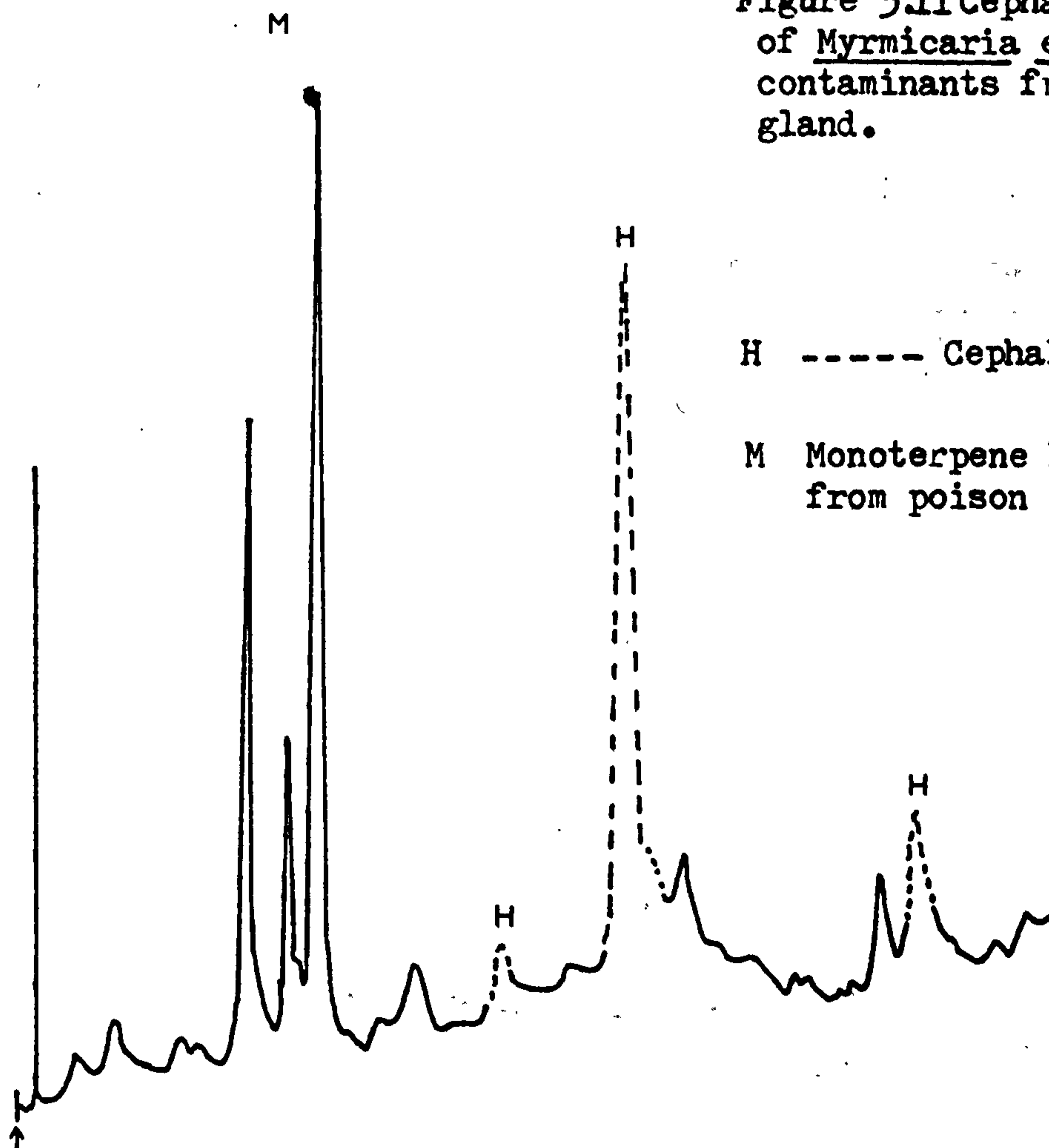


Figure 5.11 Cephalic secretion
of Myrmicaria eumenoides, with
contaminants from the poison
gland.



H ----- Cephalic components

M Monoterpene hydrocarbons
from poison gland.

Figure 5.12 Mandibular gland secretion
of Tetramorium caespitum.

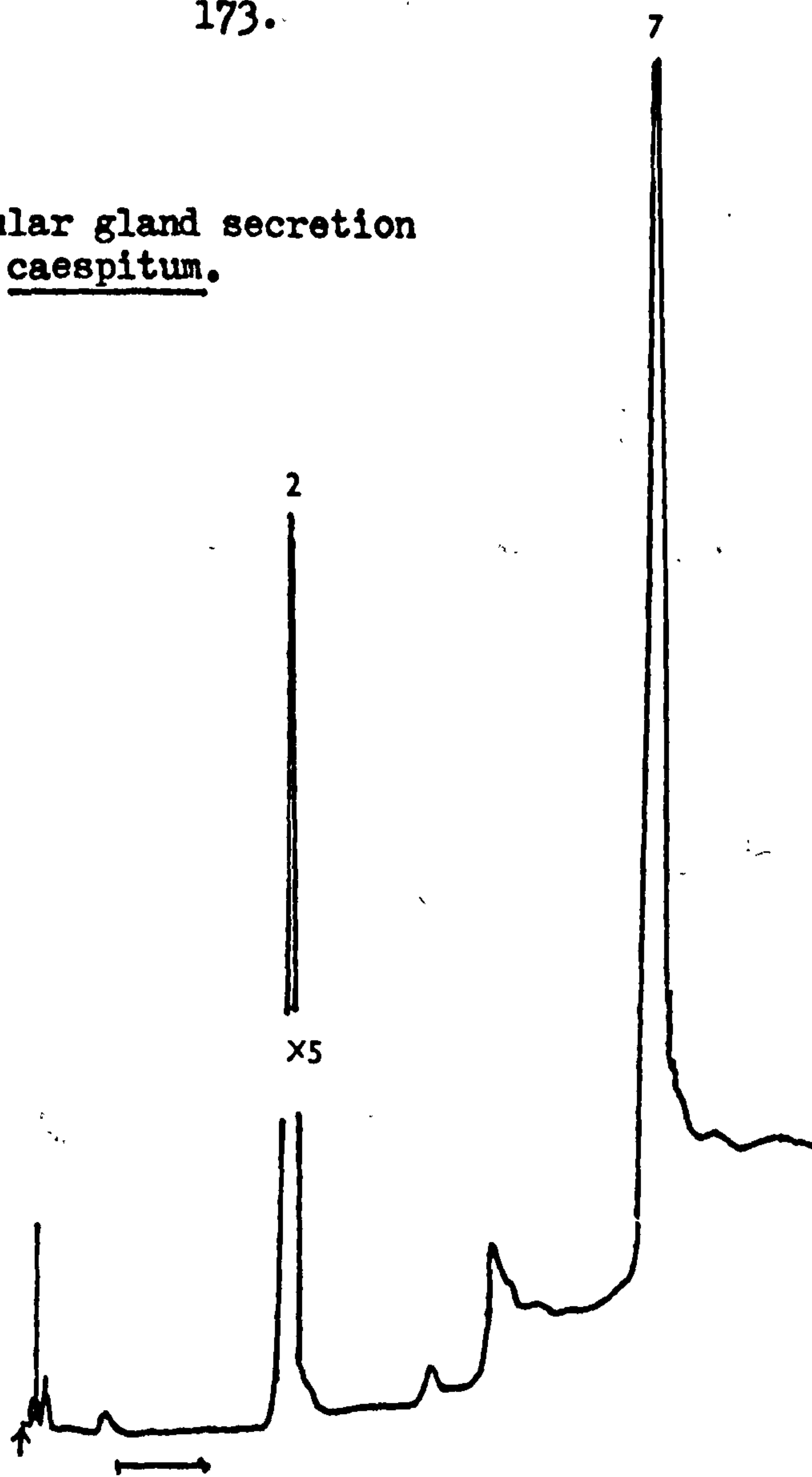


Figure 5.13 Cephalic secretion
of Tetramorium guineense.

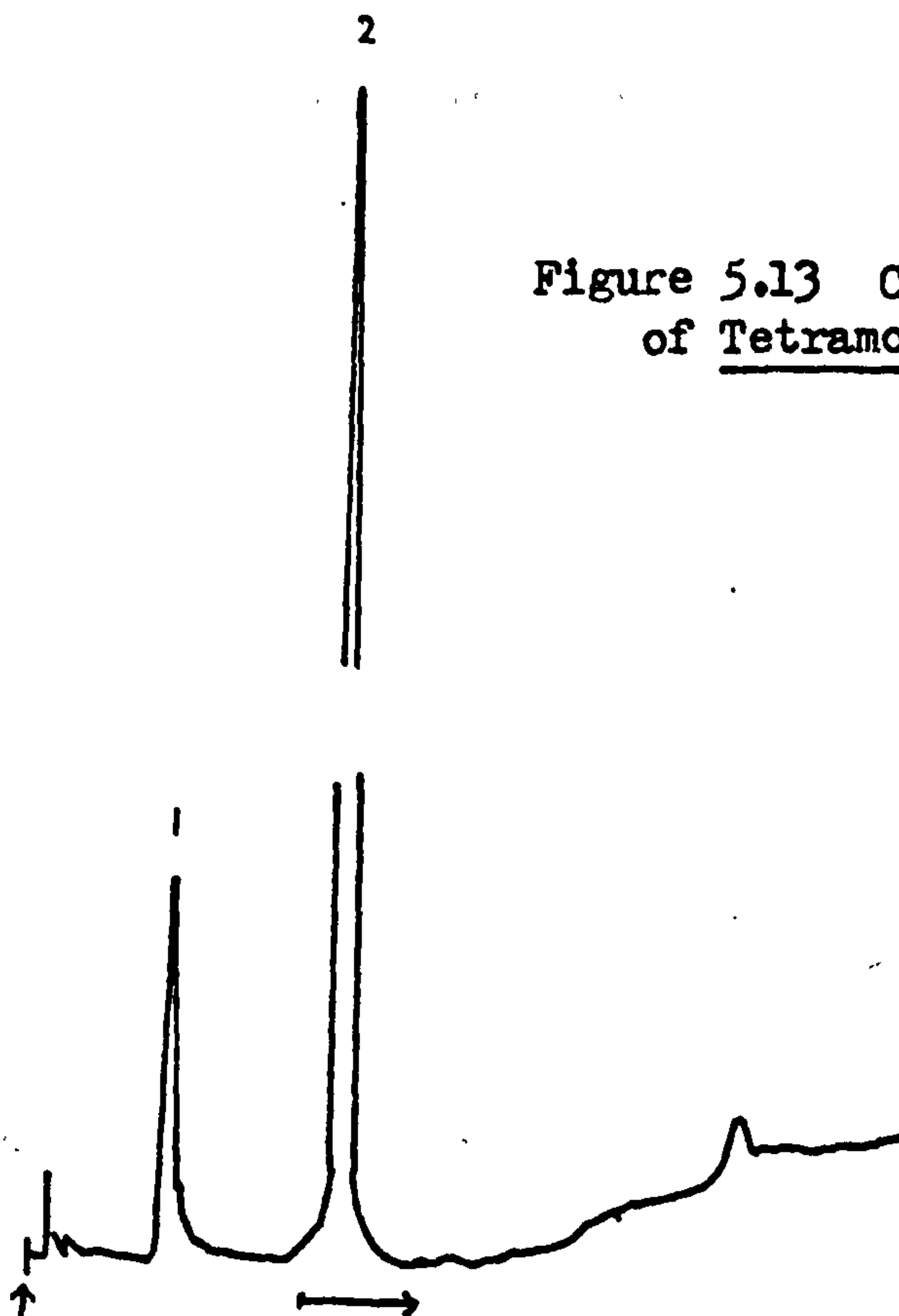


Figure 5.14 Cephalic secretion of Tetramorium sericeiventris.

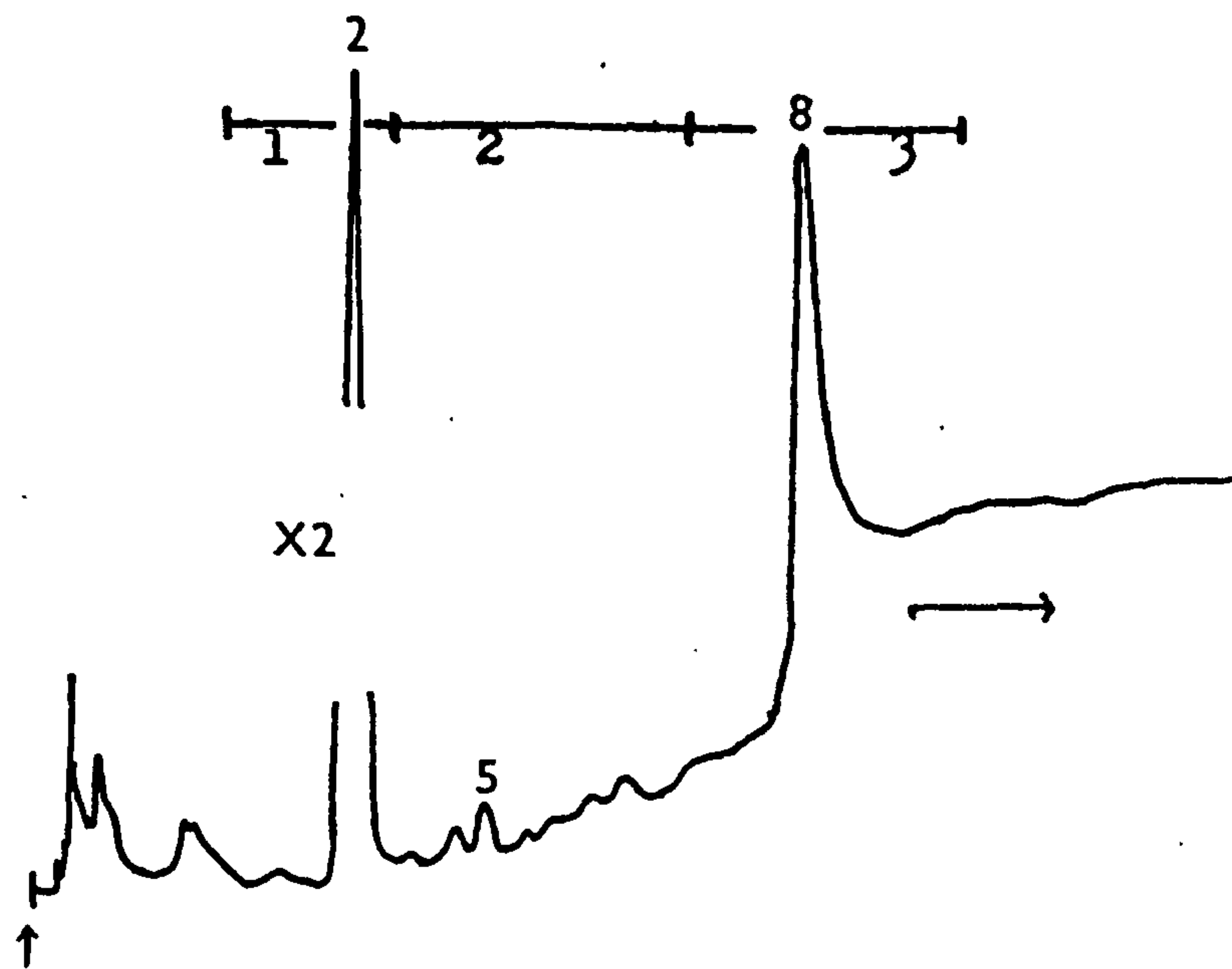


Figure 5.15 Cephalic secretion of Pheidole sp.A.

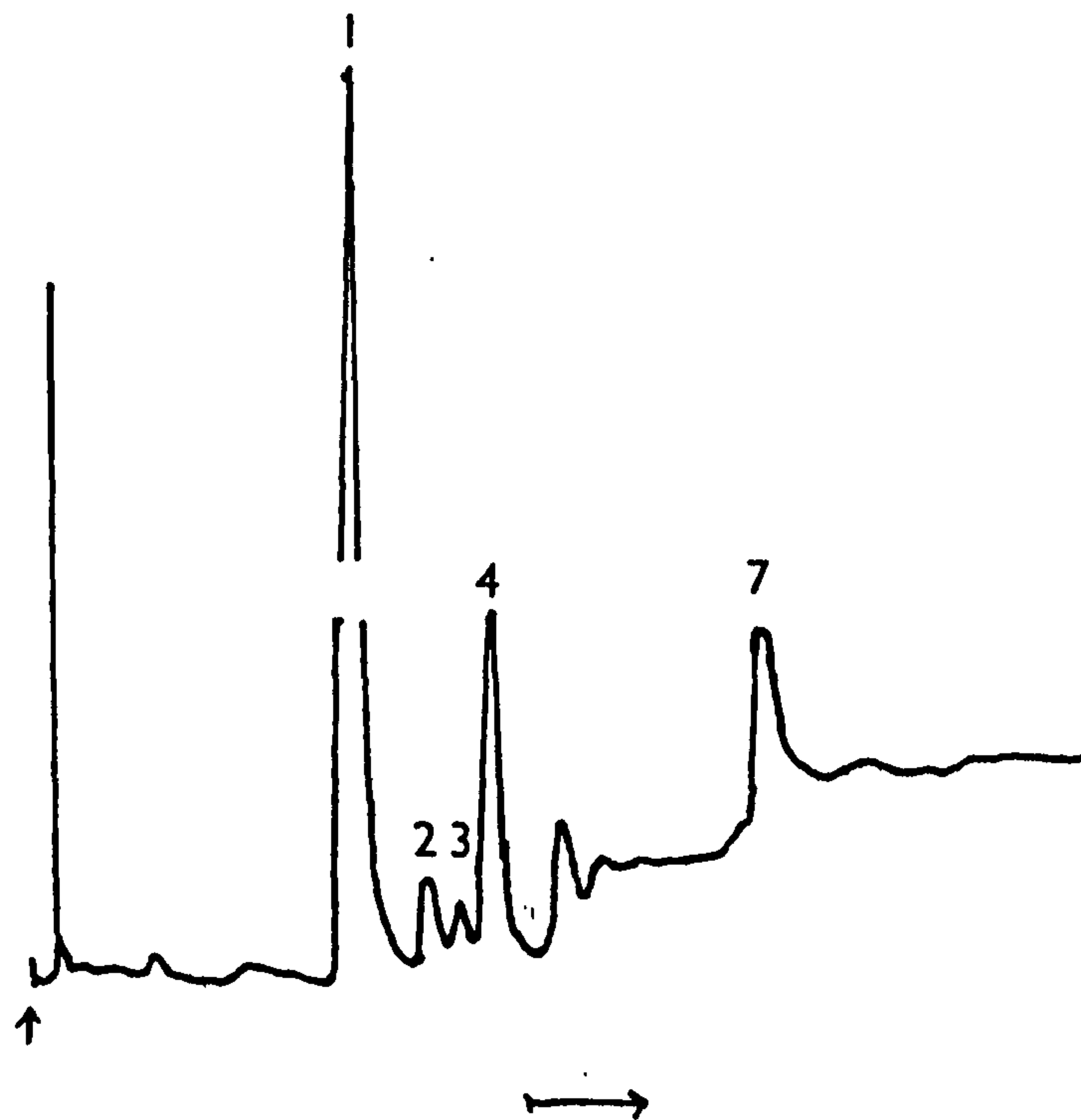


Figure 5.16 Cephalic secretion
of Tetramorium angulinode.

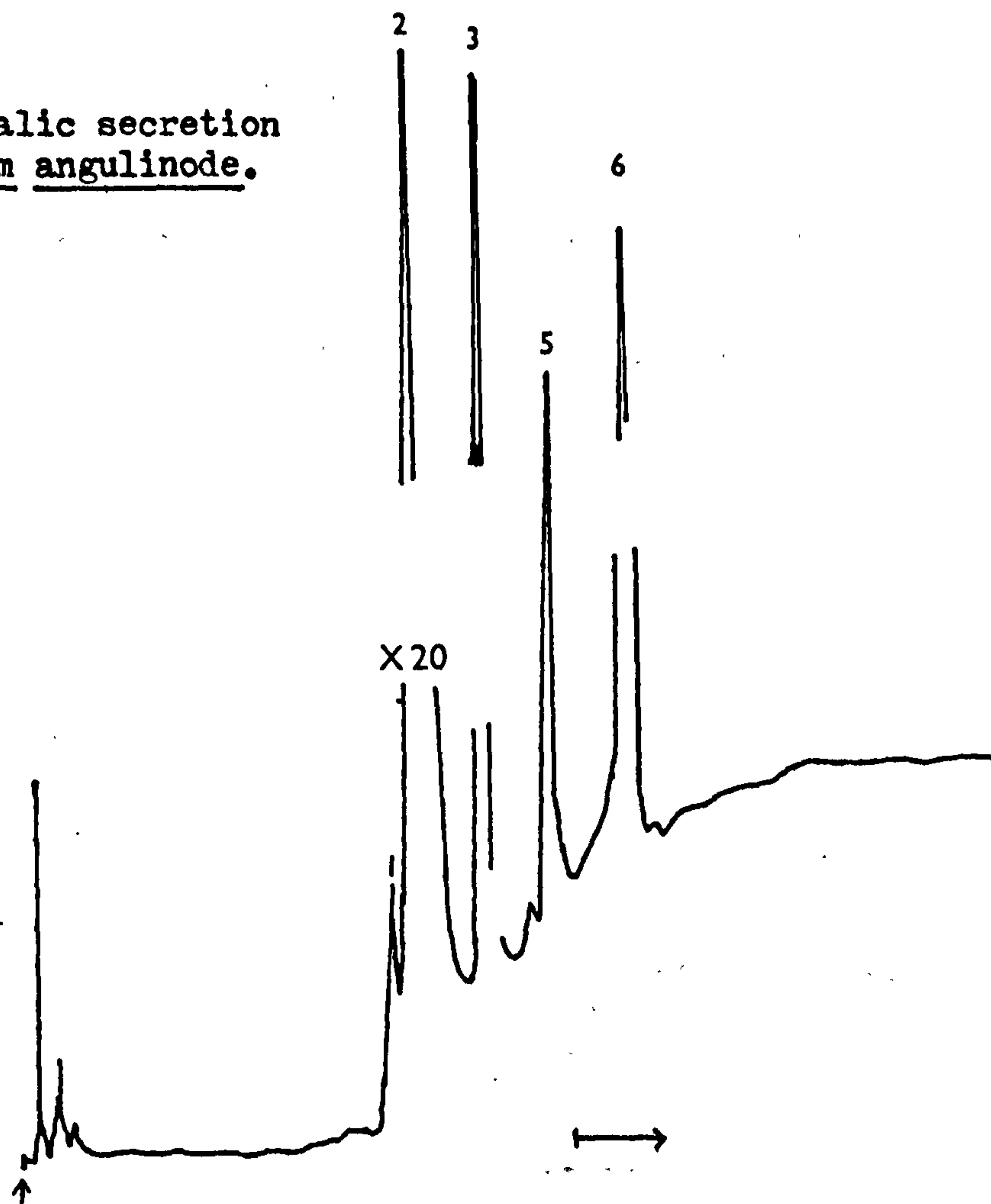


Figure 5.17 Cephalic secretion
of Tetramorium sp.D.

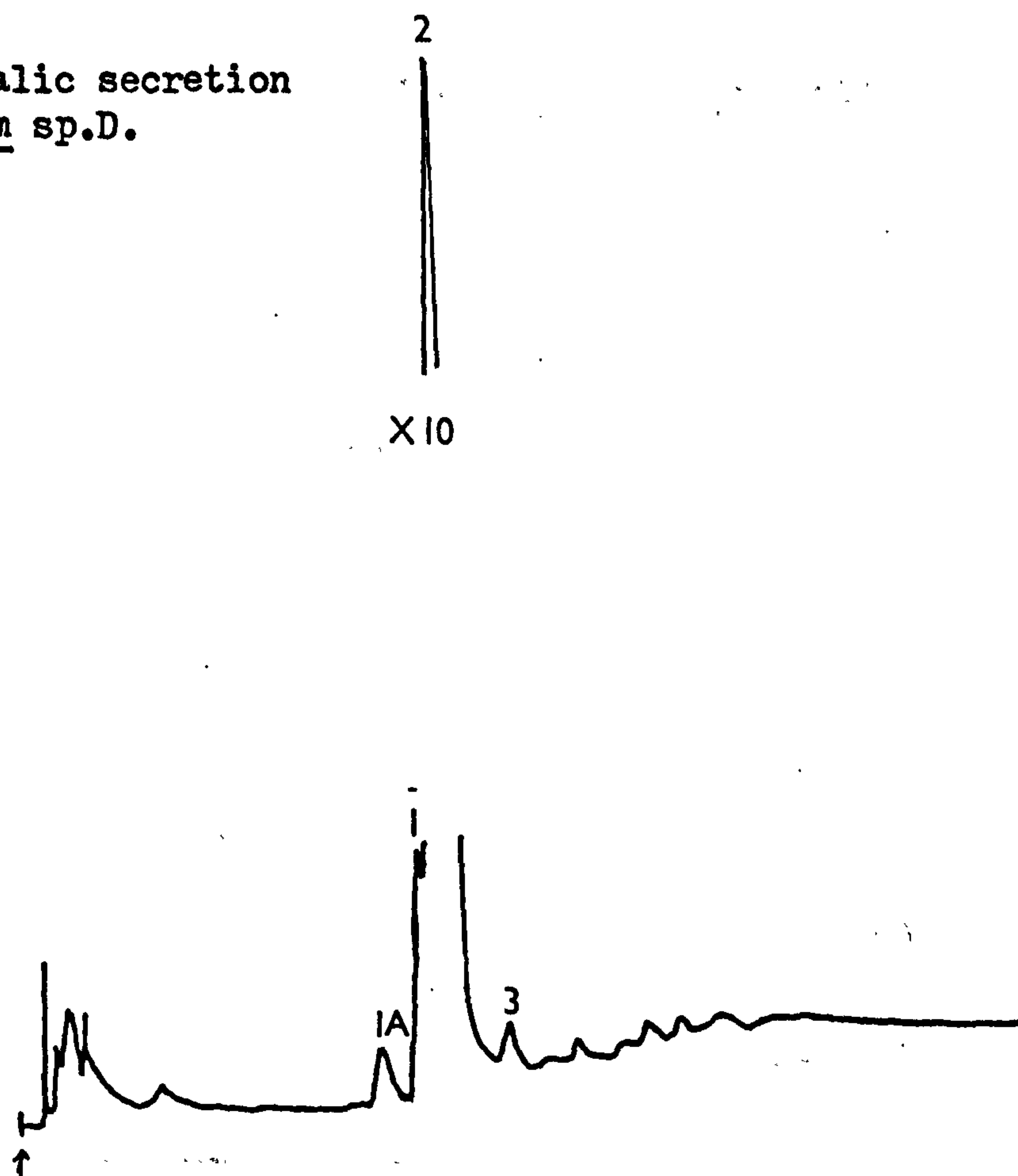


Figure 5.18 Cephalic secretion of Tetramorium termitobium

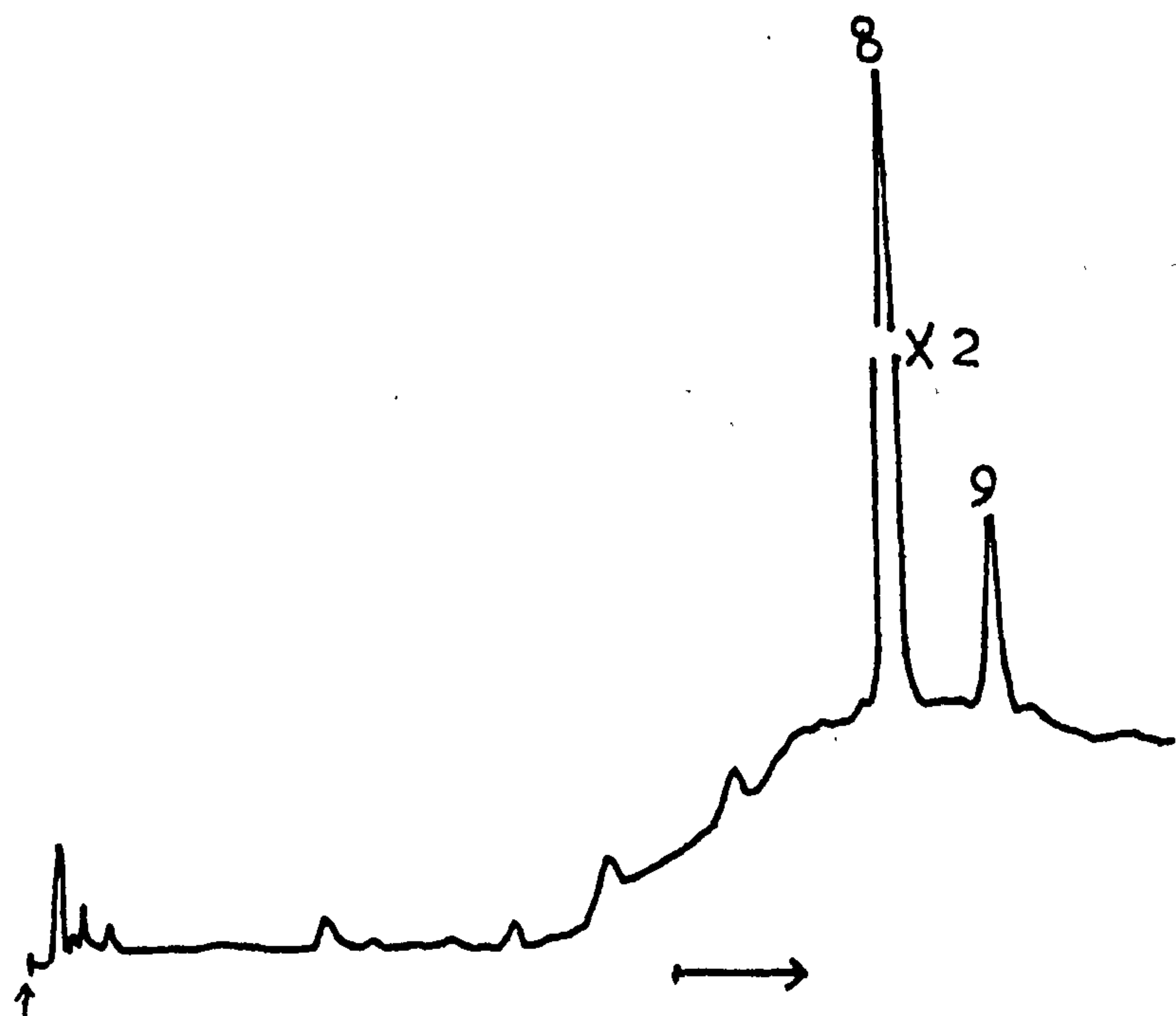


Figure 5.19 Mandibular gland secretion of Decamorium uelense

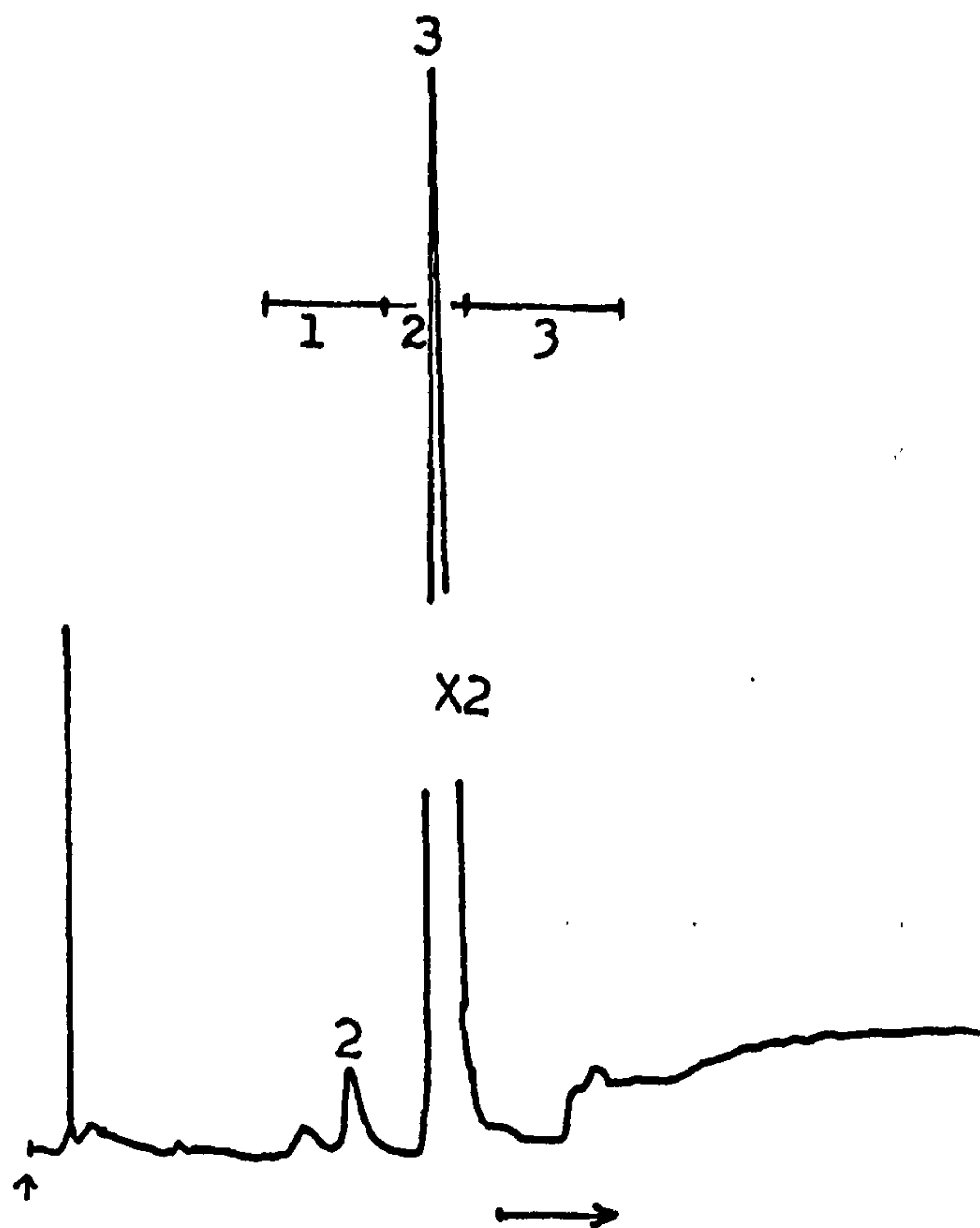


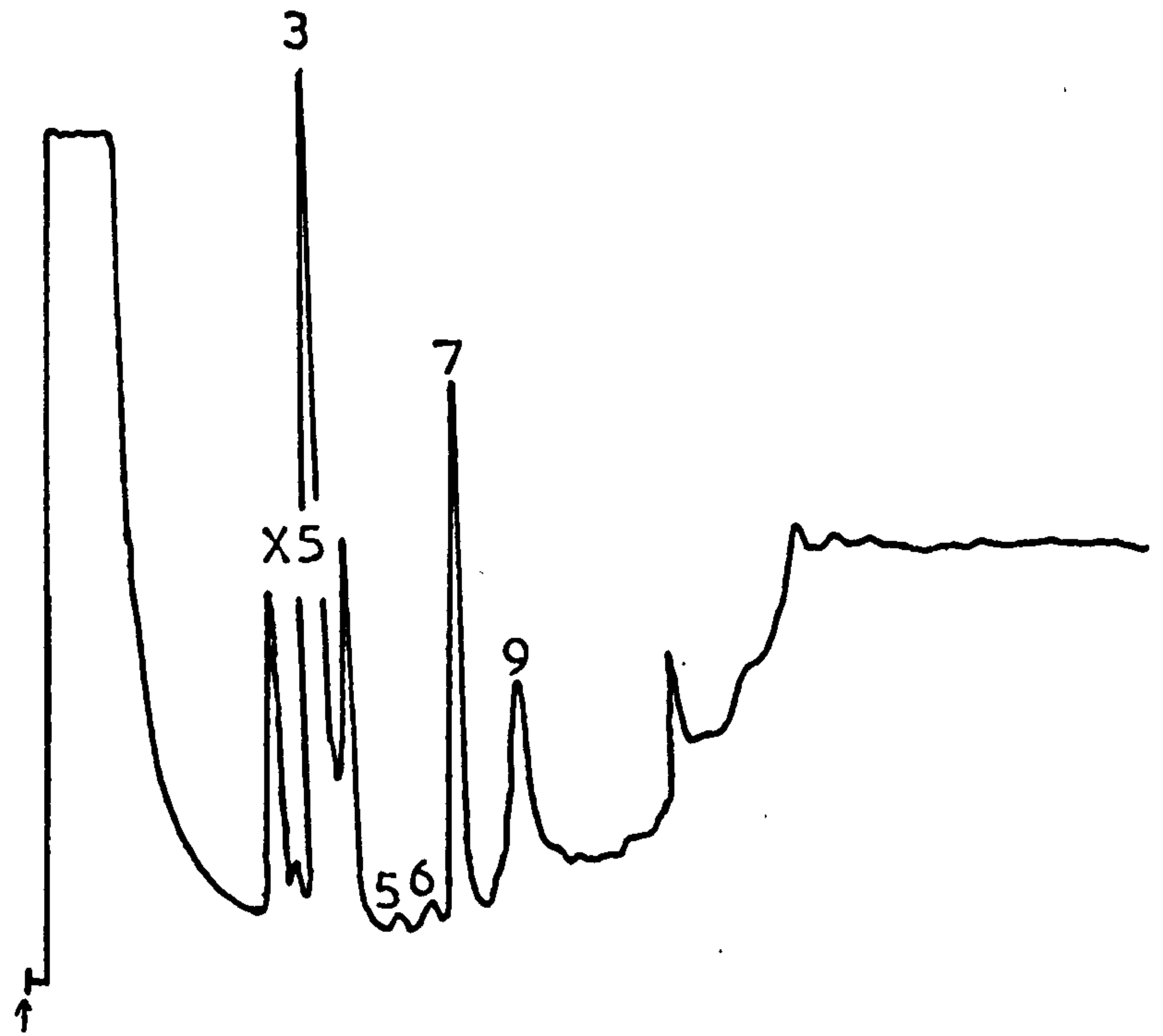
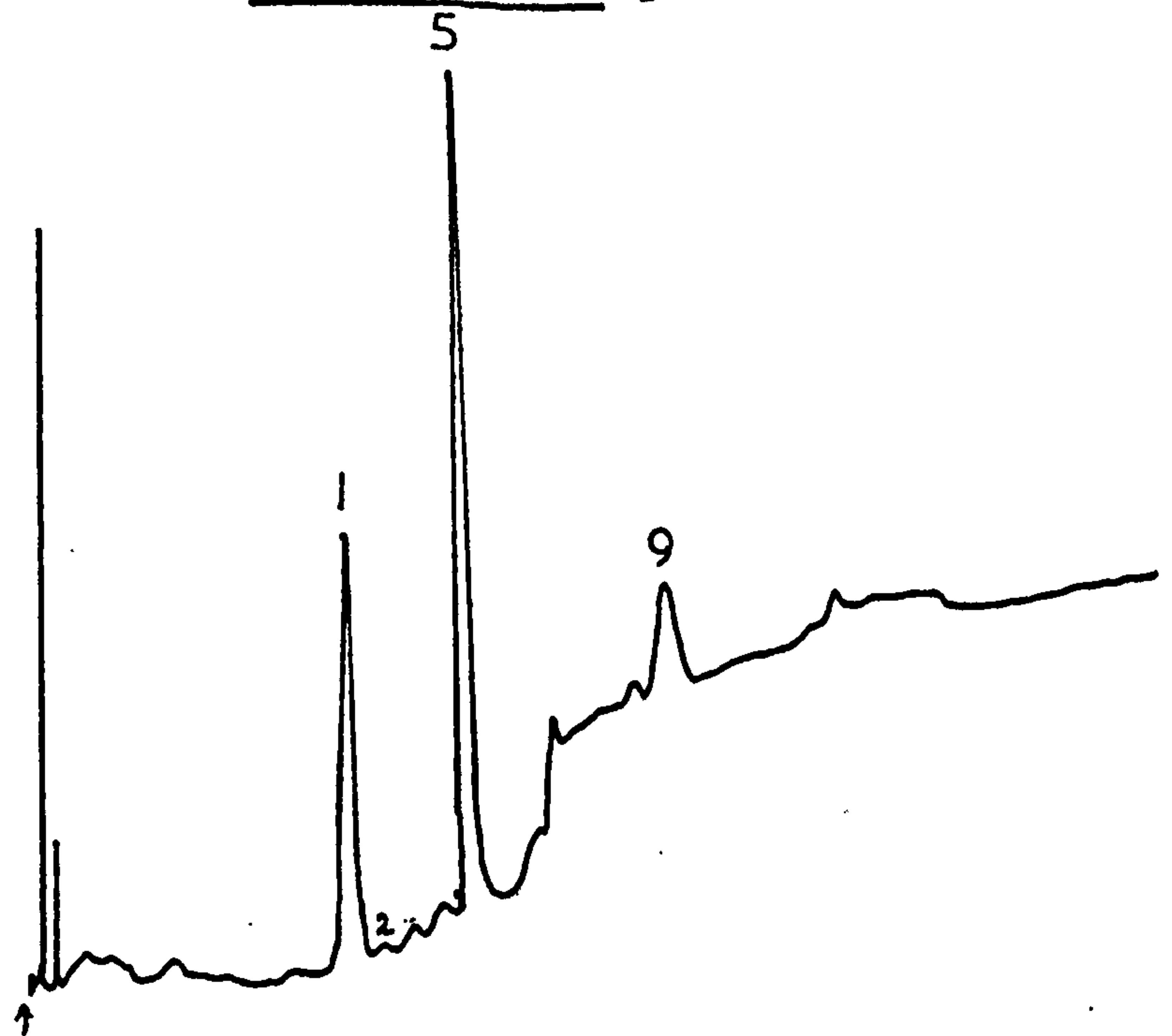
Figure 5.20 Cephalic secretion of Crematogaster sp.A.Figure 5.21 Cephalic secretion of Crematogaster sp.B.

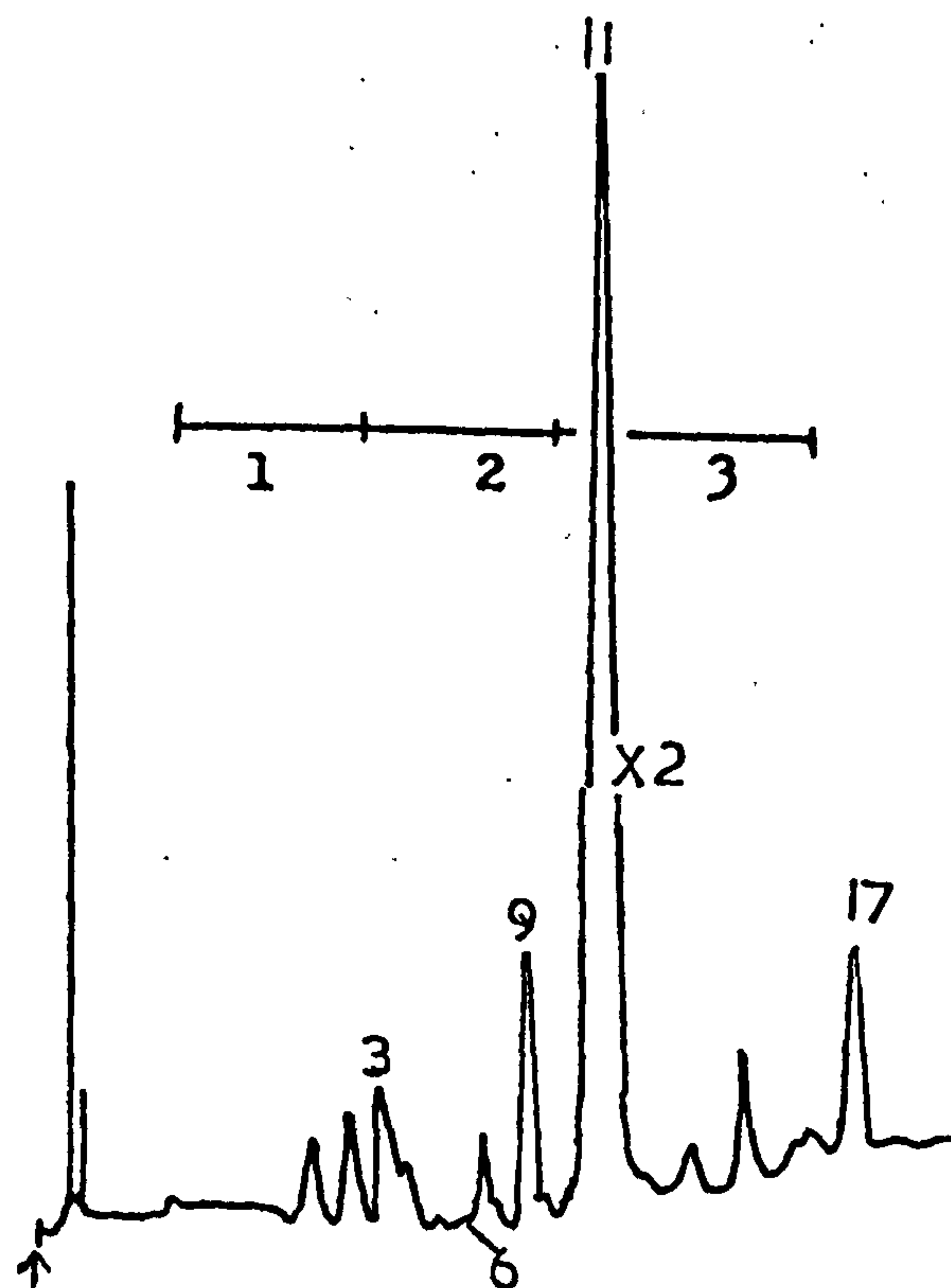
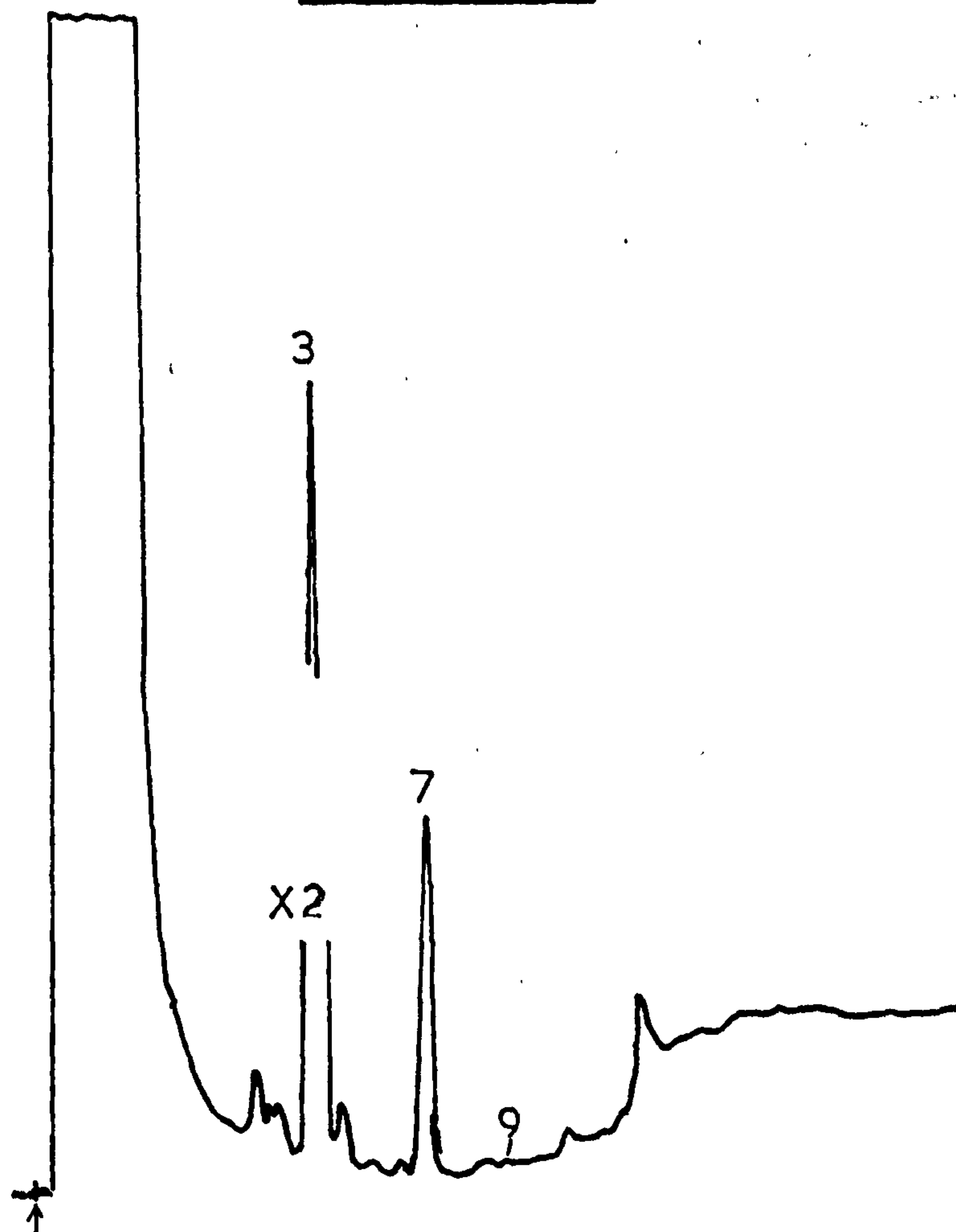
Figure 5.22 Cephalic secretion of Crematogaster sp.C.Figure 5.23 Cephalic secretion of Crematogaster sp.D.

Figure 5.24 Cephalic
secretion of Cremato-
gaster sp.E.

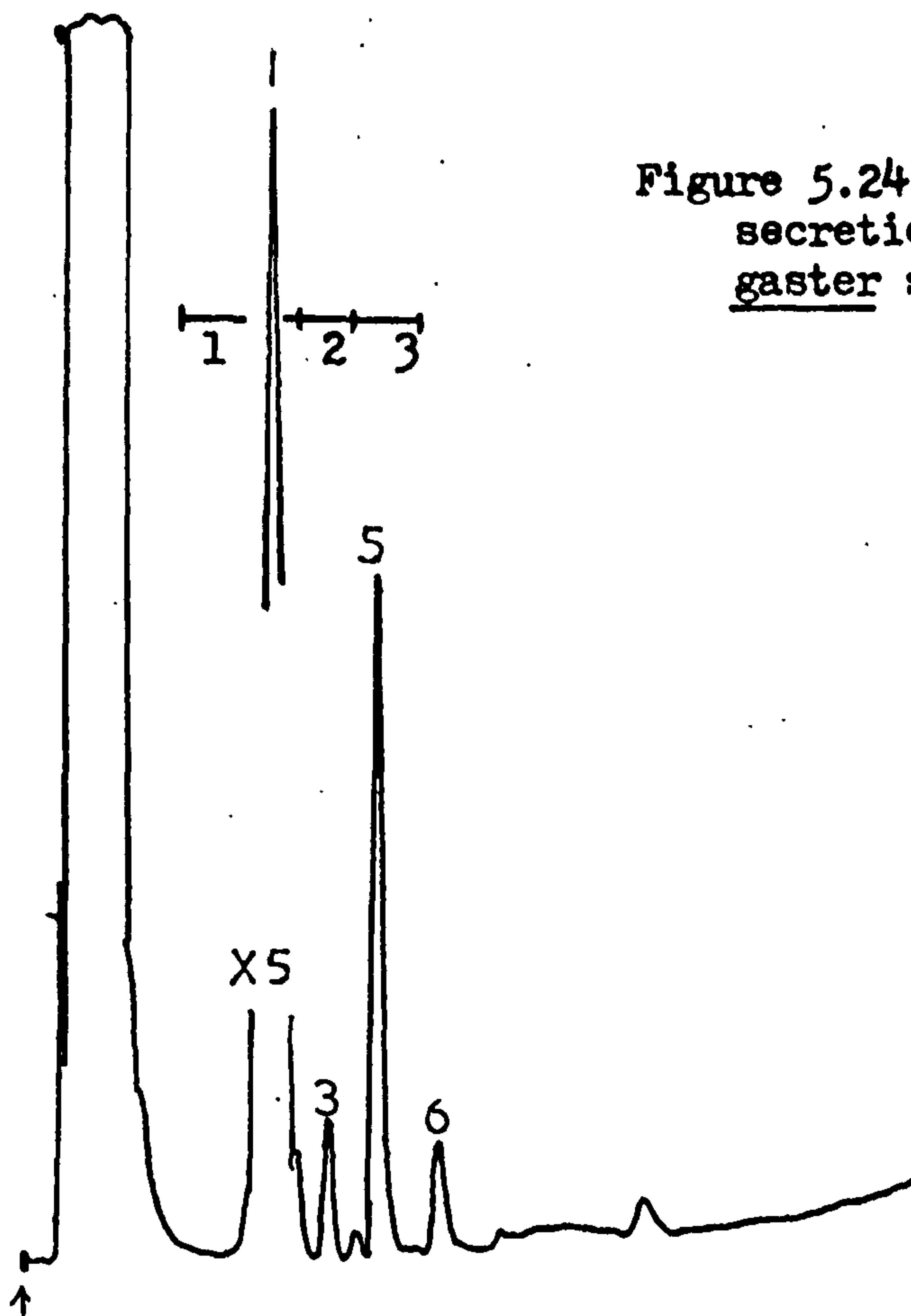
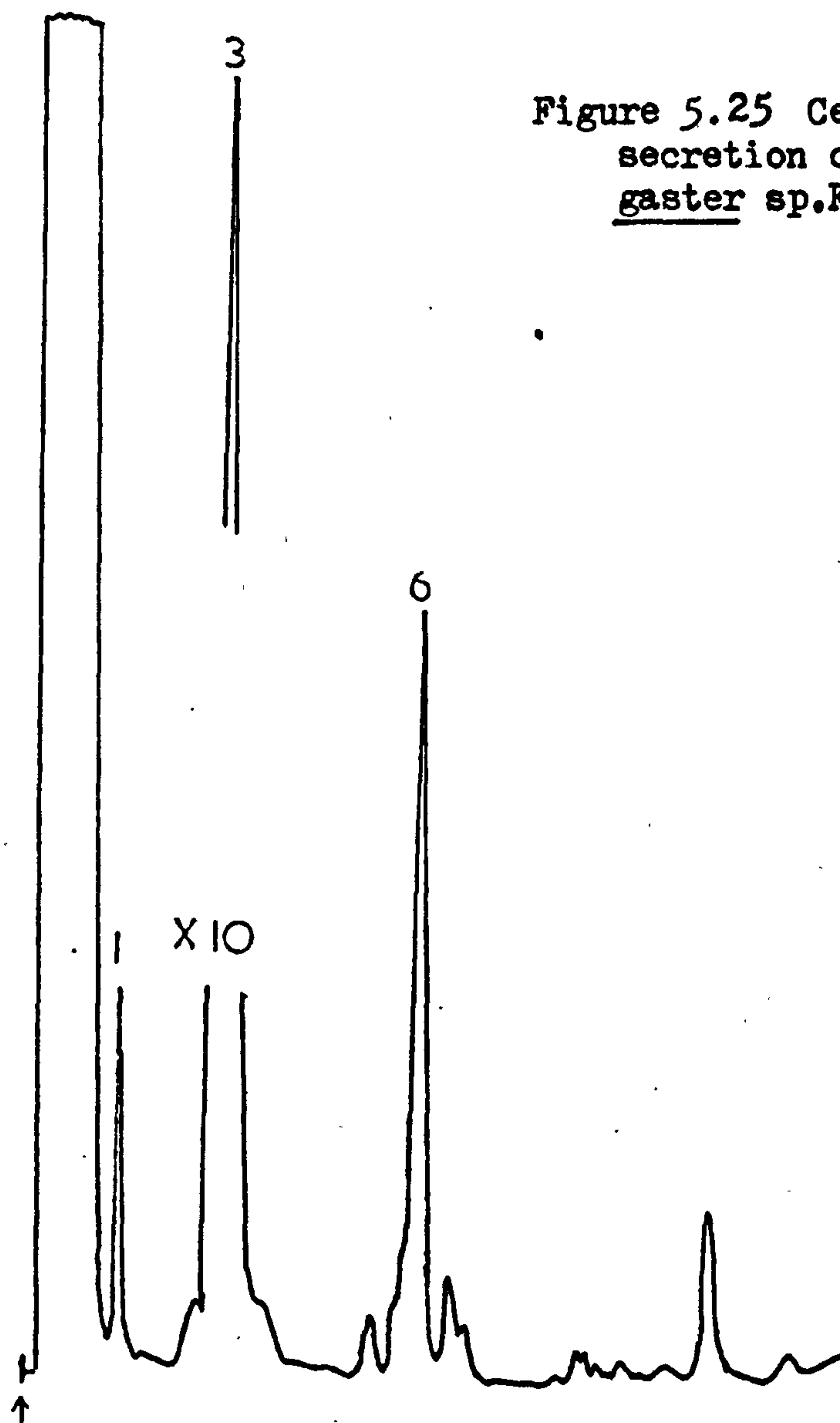


Figure 5.25 Cephalic
secretion of Cremato-
gaster sp.F



5.3 Discussion.

5.3.1 Repellency of ants to termites.

a. Survey of Responses.

The savanna ants of Mokwa elicit a wide range of responses from the Macrotermitinae which do not reflect any straightforward predatory or taxonomic relationships. The predatory Decamorium uelense, Crematogaster sp.C, Carebara sp.A, Camponotus maculatus and C.sericeus are not repellent, nor are the non-predatory Messor regina and Messor sp.B. A wide range of predatory ants from five subfamilies examined are repellent. Because both repellent and non-repellent ants in the same taxonomic groups are predatory, the secretions of both behavioural classes were examined in greater detail. Extracts of volatile and non-volatile head and gaster fractions (Table 5.8) revealed that although the cephalic volatiles from the majority of repellent whole ants were active, the isolated gasters often showed some activity also. Indirect evidence suggests that ant pheromones are found in small quantities on the cuticle(eg Fig.5.11, 5.3.1c.iii), and contamination of the gaster might be expected by cephalic glandular substances.

It was therefore necessary to examine excised gland extracts, and the results of these assays proved to be more clearcut, with excised mandibular glands eliciting greater repellency than the Dufour's/ poison gland complex (Table 5.9). The major exception was Myrmecaria eumenoides which has an hypertrophied poison gland producing monoterpene hydrocarbons, some of which are repellent (Table 5.11).

Analysis of repellent and non-repellent ants in the tribes Crematogasterini and Tetramoriini revealed that in general, non-repellent ants (D.uelense, Tetramorium termitobium, Crematogaster sp.C) contained aliphatic alcohols rather than the usual (Blum 1973) alkanones, in their mandibular glands. This was reflected in the responses of Macrotermitinae to these compounds (Table 5.11). Alkyl ketones and aldehydes were repellent but alkyl alcohols were not. In this context it is interesting to note that fractionation of the mandibular gland secretion of Bothroponera soror revealed that fractions 2 and 4,

containing alkyl ketones and methyl-6-methyl salicylate respectively, were repellent to most Macrotermitinae. The major component, 2-undecanol, in fraction 3, was not repellent.

Monoterpene hydrocarbons (Table 5.11), found in the poison gland secretion of M. eumenes, proved to be generally repellent to Odontotermes, Ancistrotermes and Microtermes, but not repellent to Macrotermes. The results quoted for terpinolene should be treated with some caution, re-examination of the sample approximately 18 months after the bioassays had been performed, showed that it had changed from 98% to <50% GC purity, despite being stored in the dark at -13°C. The acyclic β -myrcene was not repellent to any termite. The bicyclic compounds, camphene, sabinene and β -pinene showed some variability, as did the monocyclic β -phellendrene. α -Phellendrene, α -pinene (+ & -) and limonene released repellency in all four genera. Floyd (1975) and Floyd et.al. (1976) found that myrcene, α -phellendrene and β -phellendrene were repellent to the primitive termite Reticulitermes lucifugus santonensis at a level of 10 ng per termite. Floyd suggested that there was some evidence of a correlation between the three-dimensional molecular structure and biological activity, with an isopropyl or isopropylidene group locating the molecule on the receptor. No evidence for this was found in the Macrotermitinae where differing inter-generic responses to the same compound were found.

Alkyl pyrazines were found in the mandibular glands of Anochetus sedilloti, Odontomachus troglydytes and Brachyponera sennaarensis; a number of these heterocyclic compounds were found to be repellent (Table 5.11). Inter-generic differences in response are more noticeable than correlations between activity and structure. Ancistrotermes and Microtermes sp.B are strongly repelled by a wide range of alkyl pyrazines, including 2,5 and 2,6-dimethyl-3-n-alkyl compounds. Odontotermes and Microtermes sp.A are relatively unaffected by pyrazines, only responding strongly to 2,5-dimethyl-3-n-propyl pyrazine and the fraction from A. sedilloti containing 2,5 dimethyl-3-n-pentyl pyrazine. There does seem to be a trend for pyrazines with longer n-alkyl side-chains to be more repellent than those with shorter side chains, but the trend is not clearcut. Bioassays with compounds of up to C₁₀ alkyl might be revealing.

b. Predatory Systems of Ants.

A better understanding of the differing responses of termites, and of differences in the presence of glandular chemicals, might be obtained through a consideration of the predatory strategies of the various ants. Wheeler (1936) recognised five types of reaction between ants and termites:-

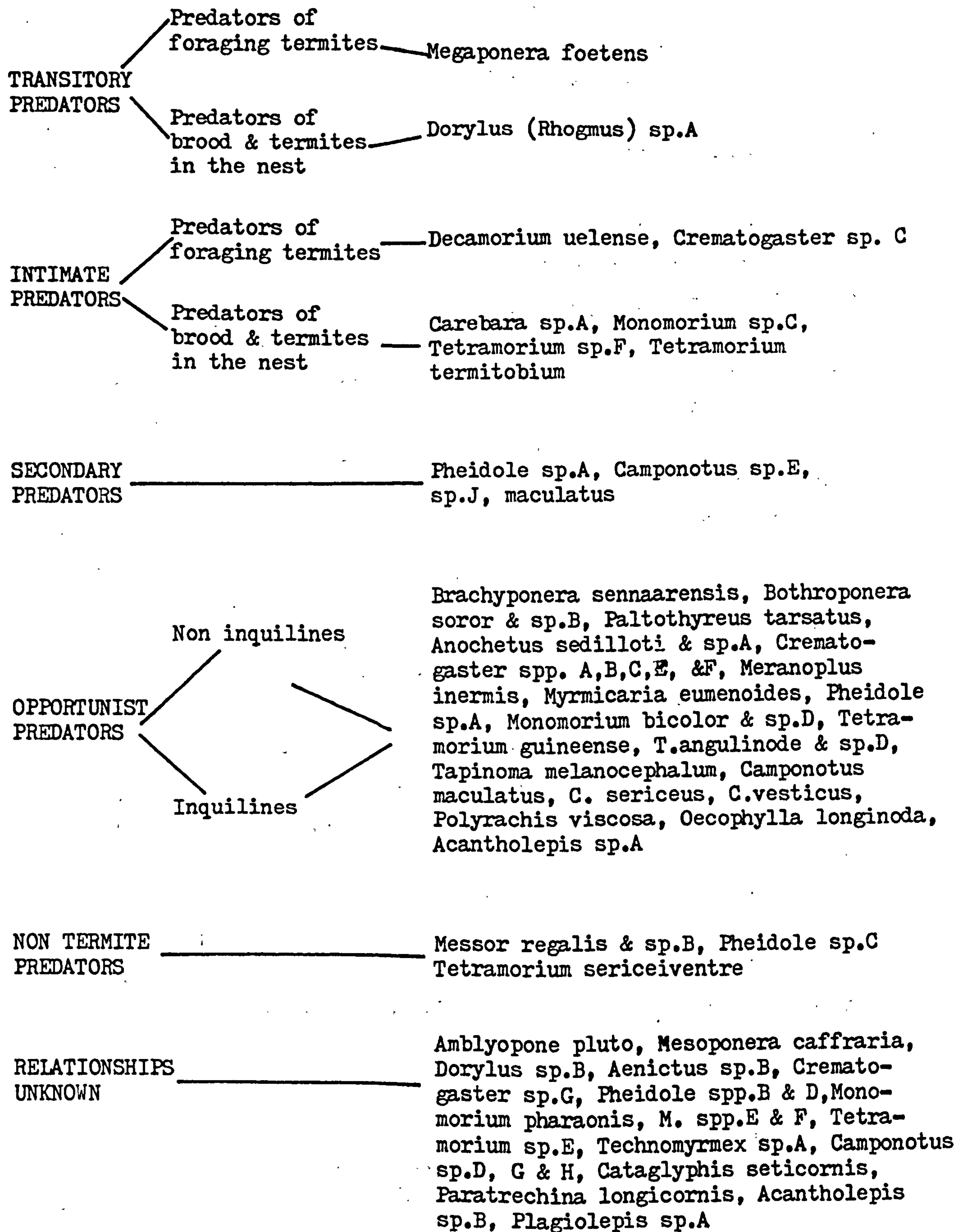
- 1) "Termitarpactic ants, or frankly hostile raiders. The Dorylinae, some Cerapachyinae and the dulotic Formicinae and Myrmicinae exhibit very similar behaviour towards other ants as the ponerines to termites.
- 2) Cleptobiotic, or footpad ants which wrest the termite prey from the raiders. Here the significant relations are really between different species of ants.
- 3) Lestobiotic, or thief ants - minute species that prey on the termite brood or disabled termites.
- 4) Inquiline ants, which either prey occasionally on the termites or in many cases, perhaps, merely inhabit portions of termitaries as indifferent neighbors.
- 5) Termitoxenic, or guest-ants which may actually live among the termites on a friendly footing.."

The evidence for ants of group 5) is poor and they can probably be placed in group 3). Even if they are fed by trophallaxis, by diverting energy from the brood they can be considered as acting as predators. Group 4) is not a useful category as many of the predatory ants in this group occur also as free-living predators. The term inquiline implies an almost neutral relationship, although many inquiline ants may be predators.

In consideration of both ecological and behavioural/chemical interactions observed at Mokwa, the system of Wheeler (1936, above) has been reclassified. Four main categories of termite predators are recognised, with three of these being sub-divided. It is also recognised that some ants have no predatory impact on termites and that the relationships of some ants to termites are unknown. The four categories of ant predators (Fig. 5.26) are :-

- 1) Transitory Predators. These ants are recognised by their rapid raids on termites, those of Megaponera foetens taking ca. 540 seconds (3.3.2). Two sub-groupings are apparent; predators of foraging termites (M.foetens, occasionally Paltothyreus tarsatus) and nomadic predators of termites and brood in the nest (Dorylus sp.A).

Figure 5.26 Classification of ant predators of termites
in southern Guinea Savanna at Mokwa



- 2) "Intimate Predators". This grouping includes Wheeler's (1936) lestobiotic predators along with myrmicines such as Decamorium uelense and Crematogaster sp.C, which prey on foraging termites. Carebara sp.A, Monomorium spp. C and D, Tetramorium sp.F and T. termitobium live in the interstices of termitaria and probably attack brood and termites in the nest. This predatory group is recognised by the fact that they do not release antagonisms in their prey, and consequently can attack foraging and nest-tending termites over longer periods of time and in smaller numbers than group 1.
3. Secondary Predators. This group is equivalent to Wheeler's cleptobiotic ants. Secondary predators are represented at Mokwa by Pheidole sp.A, Camponotus sp.E and Camponotus maculatus which attack M.foetens columns returning from raids on Macrotermitinae and relieve the ponerines of their prey. It is interesting to note that Pheidole sp.A release high intensity alarm (Appendix B) in M.foetens. Camponotus sp.J have been recorded taking termites from M.eumenoides, and may be specialised as mimics (2.3.2.5) to increase their efficiency as predators. Pheidole sp.A and Crematogaster sp.E have both been observed as secondary predators of D.uelense
- 4 Opportunist Predators. This group includes the majority of ants at Mokwa whose relationships with termites are known (56%). Wheeler (1936) included some of the ants in this category with the more specialised transitory predators, as termitharpactic ants, and recognised inquilines as a separate grouping. Many ants which could be classed as inquilines are also found free living; thus inquilines and non-inquilines are included together as opportunist predators. The percentage of termites in the prey of these opportunists probably extends from a few percent in some formicines and myrmicines which have localised alternative food resources in the form of honey-dew, homopterous commensals and seeds; to over 50% in some of the ponerines (4.3.3.).

Non-predators, such as Messor spp. and Pheidole sp.C are essentially "seed predators"; utilizing seeds as a convenient concentrated protein, fat and carbohydrate source. They have never been observed taking termites, nor have termite remains been found in their rubbish piles.

c. Relation of Repellency Response to Predatory Systems of Ants.

i) Tetramoriini. Decamorium uelense is closely related to the genus Tetramorium. Decamorium are characterised by ten antennal segments, whereas Tetramorium have eleven or twelve. Tetramorium (sensu lato) were previously divided into the eleven antennal segmented genus Xiphomyrmex and the twelve segmented genus Tetramorium (sensu stricto) (Bolton 1973), but have been recently combined by Bolton (1976), who also thought the grounds for keeping Decamorium as a separate genus were poor.

Three mandibular gland chemical groupings can be found in this tribe:

- a) Those with a complement of aliphatic 3-alcohols and 3-ketones similar to those in Pheidole, Crematogaster (Crewe et.al. 1972) and myrmicines in general (Blum 1973) - Tetramorium caespitum, T. sericeiventris, T. guineense.
- b) Those with perillen as the major component, with additional aliphatic 2-alcohols and 2-ketones - Tetramorium sp.D, T. angulinode.
- c) Those with a high (> 90%) proportion of aliphatic alcohols compared with ketones - Tetramorium termitobium, Decamorium uelense.

The two specialised termite predators in this tribe both fall into group c), with non-repellent 2- and 3- alcohols dominating the glandular secretion (Figs. 5.18 & 5.19, Table 5.11). The predatory behaviour of T. termitobium has not been observed, but its habit of nesting in termitaria probably indicates that it is an "intimate" predator of brood and termites, and would then benefit from a neutral interaction with its termite prey. The specialised predatory behaviour of D. uelense has been described in 3.4, and during the immobilization phase (3.4:3) this ant moves among its prey without alarming or repelling it. If, like many other myrmicines (Blum 1973, Crewe et.al 1972), D. uelense produced 3-octanone as its major mandibular gland component it would not be able to move among its prey (3.4.3).

ii) Crematogastrini. The repellent Crematogaster spp. A and E both produce 3-octanone as the major mandibular gland component. Crematogaster (Atopogyne) sp.F produces hex-2-enal as the major component and this ant is very repellent. Crematogaster sp.C is not repellent and does show some specialised behaviour as a termite predator (2.3. : 2.3); the major component in this ants mandibular glands is 6-methyl-

octanol. This compound was not available for bioassay, but tests on the pure fraction (Table 5.10) containing this compound, revealed it to be non-repellent, as are other aliphatic alcohols (Table 5.11). Crematogaster sp.B is anomalous in producing 3-octanol as the major component (61%), but with 3-octanone as a large second component (23%). This ant is repellent to some species of Macrotermitinae (Table 5.5) but not to others; this may represent differing threshold responses of different termite species to the ketone.

The dose-response relationship of 3-octanone as a repellent was only obtained against one termite species, Microtermes sp.A (Fig. 5.4). 3-Octanone is not repellent in presentations of <1 ng, and from this figure it is possible to estimate the theoretical quantities of compound present on the cuticles of ants (see iii below), which may influence predatory interactions.

Crematogaster sp.B and sp.C, which are not repellent to Microtermes sp.A, have a glandular secretion containing 28 and 25 ng of 3-octanone per ant. Crematogaster spp. A, D and E contain 340, 80 and 710 ng per ant respectively, and are repellent. If the whole of the glandular contents were released at once, sp.B and sp.C would also be repellent (threshold ca <1ng), but as they are not, less than 4% of the secretion must be present on the cuticle. In species A, D and E this would represent 14, 3 and 23 ng per ant, which is well into the repellency range of 3-octanone for Microtermes sp.A; thus the ants are repellent.

iii) Evidence for the presence of glandular secretions on the cuticle.

The presence of glandular secretions on the cuticle has been suggested in a number of contexts; the evidence for such a system is poor, but there are a number of items of indirect evidence. Leuthold and Schlunegger (1973) recorded that 3-octanone and 3-octanol could always be detected in small quantities from the nest atmosphere of Crematogaster scutellaris. In this species these two compounds were assumed to be released in the 'alarm on the trail' behaviour, when workers concentrated at an alarm centre on recruitment trails. The function of the general low level of compounds in the nest atmosphere is unknown, they could perhaps be connected with colony odour, 3-Octanone,

3-octanol and other minor compounds could be present at a level below the threshold alarm concentration for the species. The chemicals in the atmosphere are likely to be released from and re-absorbed onto the ants cuticle, and these compounds would form an active space around the ants which could be detected by termites during antagonistic interactions. The cuticles of ants are composed of branched hydrocarbons (LoK et al. 1975) and would form a layer for slow release of more volatile compounds.

In Crematogaster sp.C an high level of 6-methyl-3-octanol in the "ant active space" could serve any hypothetical colony odour function, but, as a non-repellent alcohol, would not repel its termite prey. A similar effect might be found with the non-repellent alcohols in Decamorium uelense and Tetramorium termitobium.

No direct evidence for the leakage of pheromones onto the cuticle is available from ants; but in Apis mellifera (Hymenoptera) Butler and his fellow workers (Butler et.al. 1974), using radioactive tracers, found that the queen bee pheromone (trans-9-keto-2-decenoic acid) could be found all over the cuticle. Tagawa (1977) found that the sex pheromone producing glands of Apanteles glomeratus (Hymenoptera: Braconidae) were located at the base of the second valvifer, but that the whole body of the adult female was attractive to males. Tagawa concluded that the female pheromone diffused over all parts of the body, including the wings and legs, and that pores through the cuticle at the base of the valvifer aided the diffusion of the pheromone onto the body cuticle. Bruinsma and Leuthold (1977) concluded that a "building pheromone" from queens of Macrotermes subhyalinus was released onto the cuticle, from the fat body, through the stigmata.

Lewis (1962, 1963) tested the diffusion of oil films containing di-iodo-octadecane (labelled with ^{131}I) and radiolabelled insecticides over insect cuticles. He found that the compounds advanced over the epicuticle of living insects through the small elastic movements of cuticular surfaces, with a flow rate over the wings of 1.5×10^{-8} ml per minute. A similar mechanism may account for the flow of small amounts of chemicals originating from exocrine glands, over the cuticles of insects. His findings may illustrate a general principle

of glandular secretions being spread over the cuticle, which could then act as a slow release system to maintain (for example) the colony odour of the ants

The secretions of glands in the gasters of ants may also leak onto the cuticle. In many species alkanes and alkenes are the most abundant compounds present (eg Bergström and Löfqvist 1971, 1973) in the Dufour's gland. n-Undecane and n-pentadecane have been shown to be non-repellent to Macrotermitinae (Table 5.11). In M.eumenoides, with large quantities of monoterpene hydrocarbons in the gastral poison gland, small quantities of these compounds can be detected on other parts of the body (Fig. 5.11). These are probably present in the cuticle as they are in similar proportions to monoterpenes in the poison gland.

5.3.2 Taxonomic and Functional Considerations of Ant Secretions

The wide variety of chemicals already discovered in the glands of the Formicidae (Blum 1973) makes the interpretation of any taxonomic patterns difficult. For instance the presence of methyl-6-methyl salicylate in the males of Camponotus species (formicinae, Brand et al. 1973), the workers of Gnamptogenys pleurodon (ponerinae, Duffield and Blum 1975) and Bothroponera soror (ponerinae, 5.2.3) spans a wide taxonomic 'distance'. The ponerinae and formicinae are on different branches of the evolutionary dendrogram erected by Wilson et al. (1967), branches which diverged over 2×10^8 years ago.

Within the tribe Ponerini there is a diversity of chemical components in the mandibular glands. In Brachyponera sennaarensis (5.2.3), Hypoponera opacior and Ponera pennsylvanicus (Duffield et al. 1976) 2,5 and 2,6-dimethyl-3-alkyl pyrazines are found. In Bothroponera soror aliphatic ketones and alcohols along with methyl-6-methyl salicylate (5.2.3) predominate, whereas in Paltothyreus tarsatus and Megaponera foetens alkyl sulphides predominate in the mandibular gland.

Within the genus Crematogaster two main groupings can be found; producers of aliphatic 3-alcohols and ketones, and producers of hex-2-enal. These differences extend into the subgeneric level. Crewe and co-workers (Crewe et.al. 1972) found that in the subgenus Atopogyne, Crematogaster depressa, C.africana, C.jullieni and C.luciae were aldehyde producers, whereas C.clariventris and C.'africana' (different collection to above) were 3-ketone/alcohol producers.

In the tribe Tetramoriini, a pattern of 3-alcohols in Decamorium (10 antennal segments), perillen in the "subgenus" Xiphomyrmex (11 antennal segments) and 3-ketones in Tetramorium (12 antennal segments) is "spoilt" by the presence of 2-undecanol as the major component in T.termitobium. Thus straightforward taxonomic relationships do not explain the differing chemical constituents of the mandibular gland secretions in this tribe. The examination of functional considerations may then be more rewarding. The mandibular gland secretions can serve in a variety of roles, including alarm communication, defense and perhaps colony recognition.

Bradshaw (1976) has demonstrated that within Oecophylla longinoda, differences in the main components of the mandibular glands of major and minor workers have a functional significance. In the major workers hexanal, n-hexanol and 2-butyl-2-octenal produce a sequential message releasing alerting, attraction and attack/biting respectively. This chemical message rapidly communicates alarm whilst locating the attack to the object on which the glandular secretion has been deposited. The minor workers produce n-hexanol and nerol in their mandibular glands, and these compounds repel the minors and displace them from disturbances. The major workers have developed a system which is suited for use outside the nest, and the minor workers have retained the more "traditional" formicine compounds (ie nerol, geraniol - Blum 1973, Wilson and Regnier 1971) which are suited for their role in enclosed spaces.

In most cases the intra-specific function of ant glandular secretions is not known, as are the evolutionary pressures to elaborate some compounds at the expense of others. It is probable that pressures at levels higher than interspecific and inter-colony - intergeneric

or even inter-order - are important. In predator-prey reactions between ants and termites, the lack of repellency response of the Macrotermitinae to aliphatic alcohols (Table 5.11) may have allowed the termite predators D.uelense, Crematogaster sp.C and T.termitobium to become more specialised by emphasising alcohols at the expense of ketones in their mandibular glands. The role of these compounds in the ants is not yet known. In D.uelense observations in perspex chambers revealed that single ants did not usually sting termites, but groups did (3.4.3) ...It is suggested that 3-octanol may release attacking and stinging behaviour in the presence of termites, perhaps by releasing klino-kinesis and increasing the probability of contact between ants and termites

It may be possible for ants to utilize different compounds for the same role, thus allowing the emphasis of (eg) 3-octanol at the expense of 3-octanone. In the major workers of Oecophylla longinoda both 2-butyl-2-octenal and 3-undecanone can release biting, but the relative proportions of these two compounds can vary between colonies (Bradshaw 1976). In such a system the suppression of one of the compounds is possible if, because of other factors in the habitat, one of the components became maladaptive - for example if it was repellent in low concentrations to homoptera that the ant tended. In Crematogaster sp.C 6-methyl-3-octanol may have taken over the role played by 3-octanone in other species of Crematogaster.

The responses of different genera to the same compounds may also vary to suit the life system of the ant. Alkyl pyrazines are produced by both Hypoponera, a small cryptic ponerine (Duffield et. al. 1976) and Odontomachus, a more aggressive surface foraging ant with powerful mandibles (Wheeler and Blum 1973). Hypoponera workers retreat in the presence of alkyl pyrazines, but Odontomachus workers are attracted to these compounds, which they attack vigorously.

It therefore appears that the glandular secretions and behavioural responses of ants are a labile adaptive system which has evolved in response to intra- and inter-specific pressures - including predation of termites - to maximise the efficiency of the ants life system.

5.3.3. Economic Usefulness of Ant Compounds as Termite Repellents

The most effective repellents against Macrotermitinae were 2-undecanone, (+) limonene, 2,5-dimethyl-3-n-propyl and 2,6-dimethyl-3-n-butyl pyrazine. The monoterpene, limonene, might not be generally useful. Macrotermes bellicosus is not repelled by it, and can be persuaded to feed on pine blocks rich in other monoterpene hydrocarbons. It is possible that termites may be able to develop detoxification mechanisms so that rich food sources containing repellents no longer need be avoided.

Alkyl pyrazines are found as natural products in a number of plants, 2,5-dimethyl-3-n-propyl pyrazine is found, for example, in cocoa (Wheeler and Blum 1973). It may be possible to exploit some of these sources of compounds if alkyl pyrazines proved to be useful long term repellents, perhaps presented in slow release formulations.

SECTION 6. BEHAVIOURAL AND CHEMICAL STUDIES ON THE OBLIGATE TERMITE PREDATOR, MEGAPONERA FOETENS.

6.1 Reactions of Megaponera foetens to termite protective soil sheeting.

6.1.1. Introduction.

In 3.3.2 it was observed that recruitment of raiding columns of M.foetens was achieved through "scout ants" which located foraging termites, returned to the nest laying a scent trail and recruited sister workers to attack the termites. The termite prey of M.foetens (4.3.1) usually forage in protected environments, protection being needed against desiccation, smaller predatory ants and sunlight. Protection is invariably achieved by the utilization of particles of soil, bound together with salivary secretions, to form a soil sheeting under which the termites can forage (Plates 6.1 & 6.2).

The complete sequence of events involved in predation (Fig.3.1) can only be achieved if the scout ant can locate the foraging termites without disturbing them. The two most obvious stimuli for the ant to use are either chemical or mechanical cues. Mechanical cues could be in the form of vibration, perhaps caused by the feeding termites, being transmitted to the scout ants. M.foetens does have the capability to respond to stridulation from sister workers (Markl 1973 & pers.obs.), the same receptor organs might be able to pick up stimuli from feeding termites.

Chemical cues could be contained in the soil sheeting which surrounds the foraging termites. The sheeting is bound together by saliva, which could contain chemical cues, either from the salivary glands or admixed from the mandibular glands or crop. Certainly in the Macrotermitinae the workings are plastered with regurgitated soil, which emerges from the buccal cavity as a "very fluid paste containing a considerable amount of saliva" (Noirot 1970). The

Macrotermitinae appear exceptional in using saliva in building, other groups using faecal paste (Noirot 1970).

Field observations on foraging scout ants indicated that there was a change in behaviour when they came into contact with termite foraging sheeting. During the searching phase the ant moved slowly (ca $0.03 \text{ m}^{-1} \text{ s}^{-1}$), exploring among leaf-litter and under wood and making palpating movements with its antennae. If termites were located (Table 6.1), the scout ant altered its behaviour for the return journey to the nest. The gaster was bent downwards, with the sting partially extruded. The ant moved more rapidly ($0.045 \text{ m}^{-1} \text{ s}^{-1}$) with the antennae held upwards and away from the soil surface. This change in behaviour was used to assay the response of scout ants to soil sheeting.

6.1.2 Materials and Methods - General.

Cultures of M.foetens were kept in large perspex nest-boxes with plaster of Paris bases (9.4, Fig. 9.1). At Southampton University the relative humidity was 65 - 85%, temperature $24 - 29^{\circ}\text{C}$ and a 12 hours light; 12 hours dark cycle was maintained. At Mokwa experiments were carried out at ambient temperature and humidity, the natural light cycle was approximately 13 hours light; 11 hours dark. The ants had access from their nest boxes to foraging arenas, ca. $1 \text{ m} \times 0.35 \text{ m}$ at Southampton and 1.5 m^2 at Mokwa. The foraging arenas were covered with clean sand (Southampton) or sterilized topsoil (Mokwa). Bioassays were carried out at the beginning or end of the light regime, when scout ants were foraging. Statistical analysis was carried out using the chi-square test suitable for two independent samples which have achieved only a nominal level of measurement (Siegal 1956).

6.1.3 Responses to Macrotermes bellicosus Soil Sheeting.

A series of experiments were carried out at Southampton to determine the cues which determined the behavioural switch in foraging scout

ants. Fresh (< 4 hours old) or old (> 24 hours old) soil sheeting was placed in 50 mm diameter watch glasses which were flush with the sand surface in the foraging arenas. Fresh sheeting was collected from areas of sheeting being laid down by foraging termites over rough wood blocks. To obtain 'old' soil sheeting some of the fresh material was kept in open petri dishes for 24 hours in the culture room. The behaviour of the ants was observed when they came into contact with the soil sheeting. A positive response was scored when the ants behaviour changed as in the manner described in 6.1.1. 'Fresh and 'old' termite soil sheeting was presented with live termites under it, with ten anaethetised minor workers under it or with no termites under it. 'Old' sheeting was also presented with no termites after moistening it with distilled water.

The results of twenty trails each of six different experimental situations are presented in Table 6.2. The scout ant responded significantly more to fresh sheeting, irrespective of the presence or absence of live or anaethetised termites. The change in behaviour was not caused by the higher water content of fresh sheeting, as moistening old sheeting did not have any significant effect on response. The results indicated that the major cue causing a change in the behaviour of the scout ant was intrinsic to fresh termite sheeting, which was active in the absence of termites.

6.1.4 Responses to the Soil Sheeting of Various Termite Genera.

A series of experiments were carried out at Mokwa to determine the response of M.foetens scout ants to the soil sheetings of various termite genera. Fresh, moist sheeting was collected from areas of active termite foraging and either presented to the ants in watch glasses flush with the soil surface or stored for at least 24 hours in petri dishes at ambient temperature and humidity. The soil sheeting was presented without any termites under it and the responses of the scout ants were scored as in 6.1.3. Difficulty was found in handling some Odontotermes smeathmani sheeting when it was slightly powdery in texture and could not be easily picked

up from the ground. Some topsoil was invariably picked up with the soil sheeting when it was transferred to the watch glass with a wide spatula.

Table 6.3 indicates that scout ants responded strongly to the fresh soil sheeting of M.bellicosus, M.subhyalinus and Odontotermes smeathmani, but not to that of Microtermes sp.A, Ancistrotermes cavithorax or the non-Macrotermitinae, Coptotermes sp. The differences between fresh and old soil sheeting were significantly different at $p < 0.001$. The different scores for A.cavithorax and Microtermes sp.A were significantly different from that of M.bellicosus ($p < 0.001$), but those of M.subhyalinus and O.smeathmani were not. The scores for old sheeting of M.bellicosus were not significantly different from any of the other species assayed.

6.1.5 Responses to Extracts of Soil Sheeting and Termites.

The responses of ants to extracts of soil sheeting and termites were studied to ascertain the source of chemicals switching behaviour. M.bellicosus soil sheeting (1 g) was extracted in purified dichloromethane (1 ml); topsoil (1 g) collected from primary savanna was extracted similarly. The extracts were filtered through glass wool and aliquots (100 μ l) were presented to the ants on 130 mm diameter filter papers in watch glasses.

M.bellicosus minor workers, collected from the fungus gardens of an excavated nest, were killed by freezing. The abdomens and the heads/thoraces of 100 termites were added to dichloromethane (1 ml), crushed with a glass rod and filtered through glass wool. The extract was presented to the foraging scout ants in aliquots (50 μ l) on filter papers.

The responses of the scout ants to dichloromethane extracts are presented in Table 6.4. The results indicate that the cues involved in causing the behaviour change are extractable chemicals found in both fresh soil sheeting and the anterior region of minor

workers of M.bellicosus. The response to the head/thoraces of worker termites was significantly different to the response to abdomens at $p < 0.01$.

6.1.6 Discussion.

Studies on the stimuli that foraging scout ants of M.foetens use in the detection of termites indicate that chemical cues contained in the protective soil sheeting over foraging termites are important. Extraction of minor workers showed that the heads/thoraces were the most powerful releasers of the behavioural change in scout ants. It is possible that chemicals in the saliva which is used to bind together the soil particles (Noirot 1970) are the cues used by M.foetens. The lack of activity contained in soil sheeting >24 hours old may be due to either the volatilization of the chemicals, or their degradation by light or soil acids or alkalis.

The identity of chemicals in the salivary secretions of termites is not known, but proteinaceous materials are possible candidates (Bouillion 1970), in view of the nutritional use of worker saliva in tropholaxis. SSGC (8.1.2a) of the heads of minor workers of M.bellicosus did reveal the presence of a number of unidentified compounds; these were not fractionated, collected and assayed due to the lack of M.foetens in culture when required.

Bruinsma and Leuthold (1977), studied pheromones from Macrotermes subhyalinus queens used in building behaviour. In addition to these pheromones they found short-range (10 - 20 mm) chemical factors, which arose from already constructed structures, that modulated building behaviour. They implicated the alimentary system of worker termites as the source of these secondary pheromones, the compounds being added to the soil particles while they were being shaped with the mandibles and wetted. It is possible these compounds are the same short range chemical cues that M.foetens scout ants detect in soil sheeting.

The inactivity of Microtermes sp.A and A.cavithorax soil sheeting

may be due to the lack of the necessary chemical cues, or their presence in small quantities, below the threshold of detection by the foraging scout ants. The lack of cues in the sheeting of these two termite species could be one of the reasons for the low numbers of them recorded as prey (4.3.1, Table 4.2) of M.foetens. Predation on M.bellicosus (population 128 termites m^{-2}) was 142 termites per square metre per year, compared to a combined total of 10 termites per square metre per year for A.cavithorax and Microtermes spp. (total population 1,731 termites m^{-2}). The second most abundant prey genus, Odontotermes, also has attractive soil sheeting; as does M.subhyalinus. This latter species has a very low population density (37 m^{-2}) and effective soldier defences (6.3) which may account for the low predation on it.

A chemical which gives an advantage to the receiving organism to the detriment of the producer is termed a kairomone (Whittaker and Feeny 1971). The chemicals incorporated in to the soil sheeting of some of the Macrotermitinae act as kairomones to M.foetens scout ants, which are then able to recruit more worker ants to the foraging termites without disturbing the prey. The soil sheeting chemicals are acting in a manner similar to the kairomones which attract the braconid parasitoid, Microplitis croceipes (Cresson) to the frass of its host Heliothis zea (F.) (Jones et al. 1971) or n-heptanoic acid from Phthorimea opeculla (Zell.) which attracts the parasitoid Orgilus lepidus Muesebeck (Hendry et al. 1973).

Table 6.1 Reactions of Megaponera foetens to termite soil sheeting in the field.

Behavioural Reaction	No. of observations	No. of cases with termites	Termite species
Moved away from the soil sheeting at ca. 0.03 m s^{-1} , still searching.	21	3	Coptotermes sp. Microtermes sp.A
Moved away from the area at ca. 0.045 m s^{-1} with gaster downcurved and sting extruded	8	7	Macrotermes bellicosus (6), O.pauperans (1)
Moved away from the area at ca. 0.045 m s^{-1} , but resumed searching within 14 s	2	1	Ancistrotermes cavithorax

Table 6.2 Number of Megaponera foetens scout ants (out of 20) responding to Macrotermes bellicosus soil sheeting

Age of sheeting	Termites (minor workers)	Number of scout ants responding
Fresh	-	15
Fresh	10 live	18
Fresh	10 anaesthetised	14
> 24 hours	-	2
> 24 hours	10 live	5
> 24 hours	10 anaesthetised	3
> 24 hours ^w	-	3
> 24 hours ^w	10 live	7
> 24 hours ^w	10 anaesthetised	0

^w moistened with distilled water.

Table 6.3 Number of Megaponera foetens scout ants (out of 20) responding to the soil sheeting of different termite genera (in the absence of termites).

Termite species	Age of sheeting	Number of scout ants responding
Macrotermes bellicosus	Fresh	14
	> 24 hours	2
Macrotermes subhyalinus	Fresh	18
	>24 hours	0
Odontotermes smeathmani	Fresh	18
	>24 hours	2
Microtermes sp.A	Fresh	0
	> 24 hours	0
Ancistrotermes cavithorax	Fresh	5
	> 24 hours	3
Coptotermes sp. (out of 10)	Fresh	0
	> 24 hours	0

Table 6.4 Number of Megaponera foetens scout ants (out of 10) responding to extracts of Macrotermes bellicosus soil sheeting and minor workers.

Extract	Number of ants responding
100 µl of fresh soil sheeting	7
100 µl of 24 hour old soil sheeting	0
100 µl of topsoil extract	1
50 µl of head + thorax extract	8
50 µl of abdomen extract	3
100 µl of dichloro- methane	0

PLATE 6.1 Soil sheeting of Odontotermes spp. over
grass-litter.

PLATE 6.2 Soil sheeting of Microtermes sp.A on wood.

PLATE 6.1



PLATE 6.2



6.2 Reactions of Megaponera foetens to Soldier Termites and and Soldier Specific Compounds

6.2.1 Introduction and Field Observations.

Field observations on M.foetens foraging to termites or attacking foraging termites revealed that some termite soldiers were able to disorganise the attacking ants. Chemicals have been recorded from the labial glands and the frontal glands of a number of termite soldier species which have been implicated as defence secretions, although in most cases the toxicity and behavioural effect of these compounds on potential predators has not been investigated.

Maschwitz et.al. (1970) and Maschwitz and Tho (1974) have recorded benzoquinone and toluquinone from the labial glands of Asian Macrotermitinae of the genera Macrotermes, Odontotermes and Microtermes. Macrotermes bellicosus from Mokwa produce quinones in a brown, watery defence secretion, although the secretion of Microtermes sp.A may differ as it is yellow and viscous. Macrotermes subhyalinus produces high molecular weight alkanes and alkenes (P.H.Briner, pers.comm.), and Ancistrotermes cavithorax from the same area produces a number of oxygenated sesquiterpenes (Evans et.al. 1977) in the labial glands, which are ejected onto predators.

Quennedy et.al. (1973) have recorded 1-tetradecen-3-one from Schedorhinotermes putorius which is smeared onto its attackers with a labral brush. The ethiopian Amitermes evuncifer was found to produce 4-11-epoxy-cis-eudesmane as the major component of the frontal gland secretion (Wadhams et.al. 1974, Baker et.al. 1977) which was implicated as a selective formicide (L.J.Wadhams, unpublished results). East African Trinervitermes spp., as well as producing monoterpene hydrocarbons (Prestwich, 1975, 1977), similar to those in Australian Nasutitermes (Moore 1968), also produce complex diterpenoids (Prestwich and Tanis 1976a, 1976b) from the frontal gland, the source of the sticky defence secretion thrown out through the nasus (Eisner 1970).

At Mokwa, the termites most frequently taken as prey (Table 4.4), M.bellicosus and Odontotermes spp., did not appear to give the attacking M.foetens any problems, and major and minor soldiers appeared in prey catches in abundance. Attacks on M.subhyalinus are rare in savanna at Mokwa, on those occasions when attacks on this species were observed it was noted that more ants were killed during the attack than usual, and that only a few termites were taken (4.3.1).

Records from predation studies revealed that there was invariably a loss of 3-4 ants per raid on M.bellicosus and Odontotermes spp., amounting to a loss of approximately 1%; some of this loss was probably due to ants losing the recruitment trail. In some cases, at the site of the attack, ants were observed to be decapitated, to lose legs and antennae or suffer other injuries from the mandibles of M.bellicosus and Odontotermes spp. soldiers.

In attacks on M.subhyalinus in primary savanna the observed mortality on three occasions was 6.4%, 3.9% and 7.6%, and only a few termites were brought back to the nest. Observations revealed that some of the dead ants did not appear to be suffering from any physical injury, which would implicate chemical defence toxins as killing the ants.

When M.foetens was observed attacking A.cavithorax, in conjunction with raids on M.bellicosus (Table 4.4), the ants were observed to exhibit behavioural patterns not observed when M.bellicosus alone was attacked. When ants came into contact with soldiers they exhibited alerting and low intensity alarm behaviour (Appendix B), circling a few centimetres from the soldiers with their mandibles open and their antennae porrect.

Trinervitermes spp. forage on the soil surface, mainly to grass, and their foraging forays may show a temporal overlap with those of M.foetens at certain times of the year (Ohiagu and Wood, 1976). They would appear to offer a suitable source of prey to M.foetens, but no record exists of them in prey catches. On five occasions M.foetens has been observed to encounter Trinervitermes spp. in the field (T.geminatus 3, T.togoensis 2). In these encounters the foraging

Trinervitermes spp. were foraging to grass across the recruitment trails laid by the ants. When M.foetens workers came into contact with these foraging termites, ants became entangled in the sticky defence secretions of the soldiers, other ants lost contact with their recruitment trail and disappeared into the surrounding vegetation. Counts (4 observations) on the numbers of ants returning to the nest up to one hour after the interaction with the termites revealed that a number of ants did not return (Table 6.5), although they may have returned to the nest later. Counts of the numbers of dead ants at the scene of the encounter revealed that a number of ants had died in the encounter (Table 6.5).

Grassé and Noirot (1951) reported that a colony of Trinervitermes sp. which was undergoing "sociotomy" (interpreted by Sands, pers. comm., as an escape response to subterranean Dorylus spp.) can defend itself against ant predators. The defence secretion thrown out by the soldiers "causes the ants to flee.... and kills them upon contact" (trans. in Bouillon 1970).

The field observations at Mokwa suggested that soldier defence secretions of Trinervitermes spp., A.cavithorax and M.subhyalinus were obnoxious to the ants, leading to the disruption of normal predation patterns. A series of tests were carried out to define the behaviour caused by odours from termite soldiers, and, in the case of T.geminatus, to investigate the effects of some of the identified compounds on the ants.

6.2.3 Materials and Methods.

6.2.3a General

Colonies of M.foetens, collected from primary savanna, were kept in culture as described in 9.4, with access to foraging areas. Ants in culture were presented with M.bellicosus (Mokwa) or M.bellicosus and R.lucifugus (Southampton) in petri dishes. Exploring scout ants would recruit ants to the termites. Because the distance between the termites and the nest entrance was <1 metre, a single discrete column of ants (3.3.2) was not observed, but a continuous column of

Table 6.5 Fate of Megaponera foetens foraging columns after encountering foraging Trinervitermes spp.

<u>Trinervitermes</u> species	Time of day	No. of ants in outward column	Amount of time nest observed after encounter	No. of dead ants at site of encounter	% dead ants at site of encounter	No. of ants returning to the nest	No. of ants missing	% of ants missing
<u>T. geminatus</u>	a.m.	357	1 hour	21	5.9%	287	170	19.6%
<u>T. geminatus</u>	p.m.	296	38 min	5	1.7%	109	187	63.2%
<u>T. geminatus</u>	p.m.	471	30 min	42	8.9%	254	217	46.1%
<u>T. togoensis</u>	p.m.	428	1 hour	11	2.5%	323	105	24.5%

ants moving in both directions formed. Some ants would leave the two-way column and forage in the arena, it was these ants who encountered the stimuli. Major workers were observed as they contacted the stimuli (6.2.3b) and their behaviour noted. At least ten presentations of each species of termite major soldier was made to ensure that an accurate description of the behaviour was compiled. Distances were judged from 1 mm diameter metal stakes pushed into the soil (Mokwa) or marks on the sand (Southampton) of the foraging arenas.

6.2.3b Presentation of Macrotermitinae Soldiers to Foraging Ants.

Whole major soldiers of M. bellicosus, M. subhyalinus, O. smeathmani, A. cavithorax, and whole soldiers of Microtermes sp.A were crushed onto 130 mm diameter filter papers with a clean spatula. The filter paper was immediately placed in the foraging arena at least 250 mm from the edge of the foraging column of ants. The reactions of single foragers were then observed as they approached the stimulus. Clean filter papers were presented as controls.

6.2.3c Presentation of Trinervitermes spp. soldiers and soldier defence secretions to foraging ants.

Crushed heads of major soldiers were presented to the ants in the manner described in 6.2.3b.

The heads of T. geminatus were analysed by GC on columns A and D. GC traces revealed that major and minor soldiers contained essentially the same medley of volatile components. The secretion was fractionated into 6 fractions (Fig. 6.1). Major soldiers (100) were placed in dichloromethane (2 ml), crushed with a glass rod and filtered through glass wool. The extract was reduced to 200 μ l by passing oxygen-free nitrogen over it. The extract was then fractionated on GC column H, using the method of Baker et.al. (1976, 8.1.3a), the fractions being collected in 200 μ l of dichloromethane.

The fraction extracts were presented in 25 μ l aliquots on 130 mm diameter filter papers, as described in 6.2.3b, after the solvent had been allowed to evaporate off. The reactions of the ants were observed. Each fraction was presented five times.

Figure 6.1. Chromatogram of the heads of
Trinervitermes geminatus.
10% E30, 80 - 250 @ 8°C/min.

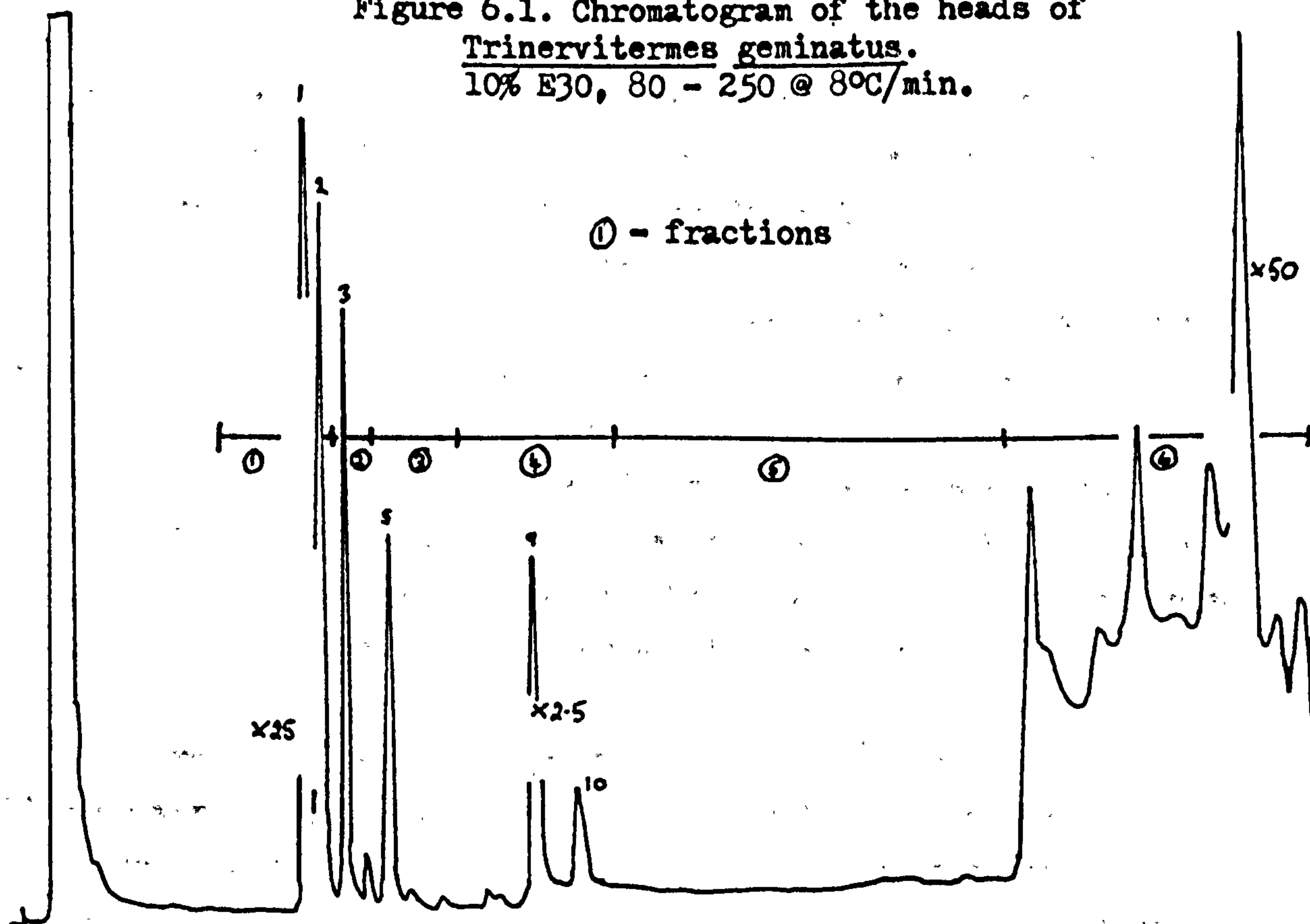
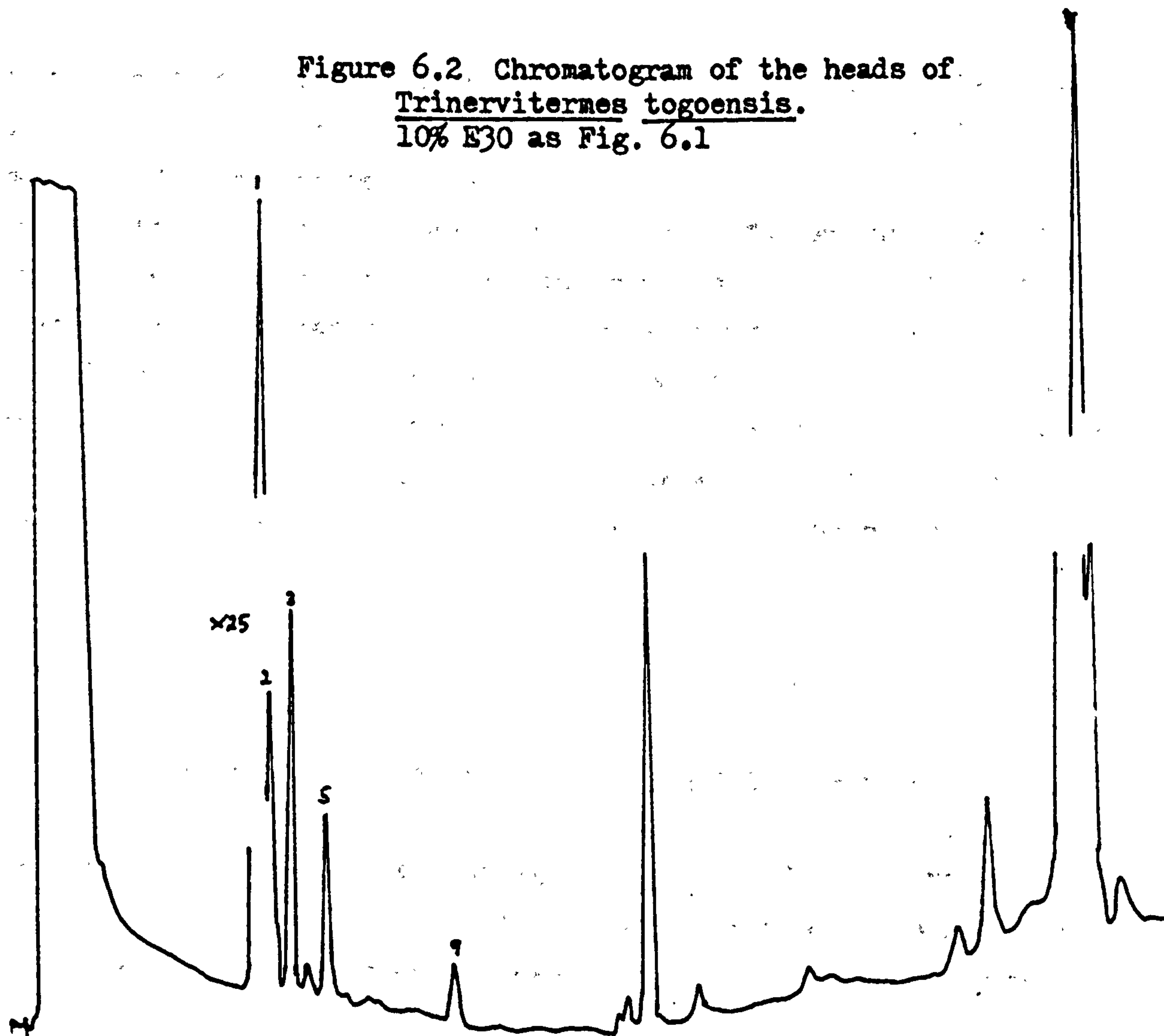


Figure 6.2. Chromatogram of the heads of
Trinervitermes togoensis.
10% E30 as Fig. 6.1



GC-MS studies on the compounds were carried out on a solvent extract of 40 whole termites in 1 ml of dichloromethane, reduced in volume to 50 μ l. The GC-MS studies revealed the following identities of components (Fig. 6.1): 1 - α -pinene; 2 - sabinene; 3 - β -pinene; 4 - myrcene; 5 - limonene; 9 & 10 - monoterpene alcohols. Commercial samples of the monoterpene hydrocarbons were available and were tested as the most commonly occurring natural optical isomer or the racemic mixture, as the chirality of the termite compounds was not known. The identity of the major alcohol could not be confirmed by GC-MS studies, but the largest alcohol (component 9) was collected by preparative GC (8.1.3a) into 200 μ l of dichloromethane.

The monoterpene hydrocarbons (($\pm$) α -pinene, (-) β -pinene and ($\pm$) sabinene) were tested against the ants in culture at Southampton. The compounds were made up as 1% solutions in dichloromethane and 10 μ l aliquots were injected onto 130 mm filter papers. The solvent was allowed to evaporate and the filter paper was presented to the ants as in 6.2.3b. The dichloromethane extract of the monoterpene alcohol was presented to the ants in 20 μ l aliquots on filter papers.

The monoterpene hydrocarbons were also presented to naturally foraging columns of ants at Mokwa. The compounds (2 μ l) were placed in the open end of a melting point tube (30 mm long, 1 mm i.d.) which had been sealed at one end. Foraging columns of M.foetens were located and the trail to the termites was marked with wooden stakes (1 mm o.d.) after the ants had passed. The tubes containing the chemicals were pressed into the soil approximately 150 mm to the side of the trail, with the open end projecting 10 mm out of the soil. Blank tubes were also inserted in the soil. Sample tubes were at least 10 metres apart. The behaviour of the ants was observed as they passed the tubes.

6.2.4 Results.

6.2.4a Reactions of M.foetens to crushed soldier termites.

Macrotermes bellicosus. An ant approaching the filter paper first exhibited alerting behaviour 150-200 mm from the odour source. The ant stopped, with its antennae porrect (mandibles open in 3 cases) and

then orientated towards the odour source. This was immediately followed by rapid locomotion towards the filter paper. The ant was then arrested at the odour source and proceeded to bite at it whilst making stinging movements. This last stage of the behaviour continued for up to 20 seconds after the ant arrived, after which the ant moved away, sometimes carrying the filter paper in its mandibles.

Odontotermes smeathmani. The behaviour of ants to crushed major soldiers was similar to that described for M. bellicosus.

Macrotermes subhyalinus. An ant approaching the filter paper initially showed alerting and orientation towards the odour source (qv M. bellicosus). The ant then moved to ca. 50 mm from the odour source, but then retreated, walking backwards 50 - 70 mm, where it sometimes paused with its mandibles open and antennae porrect. The ant moved towards the odour source again but then retreated. This oscillatory behaviour continued for 40 seconds after presentation, when the ant moved towards the odour source, eventually biting and stinging at the filter paper.

Ancistrotermes cavithorax. There was initially alerting, orientation and low intensity alarm (Appendix B) 100 - 150 mm from the odour source. The ant stopped and faced the odour source with its mandibles open and antennae porrect. The ant then moved rapidly towards the odour source but halted 40 - 80 mm from it. The ant was held at this distance, but circled around the odour source. After about 120 seconds the ant either moved away from the stimulus or approached and investigated it, usually without biting or stinging at it.

Microtermes sp.A Initially the ant halted and orientated towards the stimulus, with antennae porrect and mandibles open. Locomotion towards the odour source then occurred, the ant halted at 30 - 50 mm and then backed away to 100 - 150 mm. The ant continued moving towards and away from the odour source, the mandibles were held open and occasional bursts of stridulation were heard. This behaviour continued for 35 - 45 seconds after the initial presentation, after which time the ant moved slowly towards the filter paper, where it was arrested, biting and stinging at the odour source.

In all the interactions described above, in some tests sister workers moved in towards the odour source after the first ant. No attempt was made to distinguish those i) attracted by mandibular gland pheromones (6.3.3a), ii) attracted by other ant pheromones or iii) foraging normally, as was the first ant when it encountered the stimulus.

Trinervitermes geminatus and T.togoensis (5 presentations).

Initially the ants halted about 200 mm from the odour source and orientated towards it. The ant started to move towards the odour source, but at a distance of approximately 150 mm the behaviour altered and the ant moved rapidly away from the odour source, stridulating and colliding with sister workers. These other ants became alarmed (Appendix B) and stridulation appeared to become more intense. Some workers approached the odour source, circling 150 - 200 mm from it. The level of alarm gradually subsided, and after ca 120 seconds the odour source was investigated with some ants biting at or near it.

The reactions of M.foetens to the two termite species was the same at this observational level of analysis.

Controls. Occasionally a worker ant would alert when a filter paper was placed in the arena, but no further behaviour to this (visual) stimulation was observed. Foragers encountering clean filter papers would explore them with their antennae and palps, but did not bite or sting at them.

6.2.4b Reactions of ants in foraging arenas to T.geminatus soldier secretions.

Fractions 1,2 and 4 (5 presentations each). The initial behaviour of ants to these fractions was the same as that observed when ants were presented with crushed whole Trinervitermes soldiers - high intensity alarm was released.

Fractions 3 and 6 (5 presentations). Ants did not respond to filter

papers treated with these fractions any differently than they did to control filter papers.

Fraction 6 (8 presentations). The ants did not appear to be attracted or repelled by this fraction, but on three occasions when foraging ants did encounter the filter paper they spent up to 40 seconds sensing it with their antennae and occasionally biting at it. No alerting or alarm behaviour was observed.

α -pinene, β -pinene, sabinene and monoterpene hydrocarbon fraction. The reactions of M.foetens to these compounds was the same as that observed to fractions 1,2 and 4.

6.2.4c Reactions of foraging ant columns to Monoterpene Hydrocarbons.

As the front of the column of ants approached the odour source the leading ants became alerted. They continued moving forwards with their antennae porrect instead of being close to the ground. As they approached to within 200 - 300 mm of the odour source they acted in a similar manner to ants in foraging arenas presented with monoterpene hydrocarbons. The ants moved in all directions, stridulation became intense and ants collided with one another. Some ants ran into the surrounding vegetation and the whole column of ants became agitated. The general direction of the column was still forwards, some ants who had left this column rejoined it 400 - 500 mm past the odour source. The level of unidirectional alarm and stridulation decreased as the column moved away from the odour source.

6.2.5 Discussion.

The reactions of M.foetens workers to crushed termite soldiers can be divided into three general classes:-

i) Those termite soldiers which are attacked by alerted ants without initial repellency - M.bellicosus, Odontotermes smeathmani.

ii) Those termite soldiers whose odours are able to arrest attacking ants a few centimetres away from the odour source, The ants also

show a low intensity alarm reaction (Appendix B) - M.subhyalinus, A.cavithorax, Microtermes sp.A. The finer details of the response differ to each species, but the overall reaction is initial avoidance of the odour source.

iii) Those termites which have an overall repellent effect on the ants, causing them to exhibit an high intensity alarm reaction (Appendix B) - T.geminatus, T.togoensis.

The reactions of ants to soldier termites in part reflects the predatory relationships between the two groups, although as discussed elsewhere (4.3.1) other factors are also important. M.bellicosus and Odontotermes, with innocuous soldiers are the two most common prey species (Table 4.4). Trinervitermes spp. with highly obnoxious soldiers have never been recorded as prey of M.foetens. If the soldier termites produce arrestants or repellents which interfere with the co-ordination of predation, it is likely that there would be selective pressure for the ants to only seek termites which had soldiers with innocuous defence secretions. This might explain the origin of the response to the termite soil sheeting (6.1) - that of Microtermes and A.cavithorax, with obnoxious soldiers, is not attractive to the ants.

The predatory ants must also influence the development of the defence secretions of the termites and the proportion of soldiers in the colony. The surface foraging Trinervitermes, constantly exposed to potential ant predators have a high proportion of soldiers in the colonies (C.E.Ohiagu, pers.comm.) and have developed a medley of chemicals to drive off attacking ants.

A.cavithorax has a complex mixture of chemicals in its defence secretion (P.H.Briner, pers.comm.). The defence secretion can arrest attacking ants, perhaps giving the workers time to escape.

The case of M.subhyalinus is interesting, the soldiers of termites collected at Mokwa can arrest and elicit low intensity alarm in the ants, and field observations in this area indicated that attacks on foraging termites were not very successful. Sheppe (1970) has

observed M.foetens taking M.subhyalinus in East Africa and observations in the riverain area at Rabba, 8 km south of Mokwa (Fig. 2.1) revealed that efficient raids were conducted on M.subhyalinus there; 345 ants took 132 termites including 22 major and 19 minor soldiers.

Briner (P.H.Briner, pers.comm.) compared the "Mokwa" and "Zugurma" (riverain, N. of Mokwa, Fig 2.1) M.subhyalinus major soldier defence secretions and found that they differed; the "Mokwa"soldiers containing a higher proportion of a C₁₆ diene hydrocarbon. M.subhyalinus from the "Rabba" riverain area gave GC traces similar to those of soldiers from "Zugurma" The ability of M.foetens to take M.subhyalinus at Rabba and Zugurma (R.A.Johnson, pers.comm.) could be due to the low concentrations of the Mokwa specific peaks in the soldiers from these two areas.

6.3 Co-ordination of predation with glandular secretions.

6.3.1 Introduction and field observations.

Glandular secretions from ants have been implicated in a number of behavioural repertoires (Wilson 1971). *n*-Undecane and other *n*-alkanes from the Dufour's gland of Acanthomyops claviger release attraction and excitement (Regnier and Wilson 1968). High concentrations of 4-methyl-3-heptanone from the mandibular glands of Pogonomyrmex spp. release alarm and digging (Wilson 1958). Methyl-4-methyl-pyrol-2-carboxylate from the poison gland of Atta texana is a trail substance used in recruitment (Moser and Blum 1963, Tumlinson et.al. 1972) and aliphatic acids from the rectal sac of Lasius fuliginosus act as trail recruitment substances (Huwyler et.al. 1975). The glands of some ants contain a medley of chemicals which provide a sequential message. In the mandibular glands of major workers of Oecophylla longinoda are hexanal, *n*-hexanol and 2-butyl-2-octenal which act as alerting, attracting and biting releasers in the "alarm" behaviour of this species (Bradshaw et.al. 1975).

In ponerines, alkyl pyrazines from the mandibular glands of neotropical and nearctic Odontomachus, Hypoponera and Ponera species have been implicated as alarm pheromones (Wheeler and Blum 1973, Duffield et.al. 1975), methyl-6-methyl salicylate from the mandibular glands of Gnamptogenys pleurodon has a similar role (Duffield and Blum 1975). Alkyl sulphides from the mandibular glands of Paltothyreus tarsatus release digging behaviour only when the pheromone is buried (Crewe and Fletcher 1974), this is possibly an important mechanism to release sister workers who have been trapped by earth falls in their subterranean nests.

The hind gut of Termitopone laevigata F. (Blum 1966) and the poison gland of Leptogenys spp. (Fletcher 1971) have been implicated as the sources of trail pheromones in these two genera related to Megaponera.

Field observations suggested that there were at least two instances in the co-ordination of raids on termites when chemical messages were

utilized. The first case was the laying of a scent trail by the scout ant to guide the ant column to the foraging termites, and the reinforcement of this trail to guide the ants back to the nest. The existence of these guide trails has been studied by Leveux (1966). He found that physical removal of the trails disorientated the ants, and that the trail could be obliterated with kerosene but not water. His use of kerosene was rather unfortunate as hydrocarbons are implicated in eliciting an high intensity alarm reaction in this species (Appendix B). Leveux also indicated that, in the Ivory Coast, where scout ants were not used (3.5.2), the trails were very long lasting and he suggests that that they are colony specific. When the trails of two colonies cross, the column only follows its own trail.

Observations on ants as the attack on the termites progressed indicated that the ants congregated around the exposed entrances to the termite galleries (Fig. 3.2, Plate 3.3), and it appeared that the ants were either following the trail of the ant first locating the termite gallery entrance, or that an aggregation pheromone was involved.

6.3.2 Materials and Methods.

6.3.2a Co-ordination of attacks on termites.

Ants were kept in culture at Southampton as described in 9.4, with access to termites presented in petri dishes in the foraging arena. Presentation of stimuli were made when the ants were actively foraging to termites (6.2.2b).

Ants were killed by plunging them into a bed of dry ice and breaking off the head and gaster from the thorax. The body sections were then crushed with a clean spatula onto 130 mm diameter filter papers, and presented to sister workers by placing the filter paper approximately 200 mm from the edge of the foraging column. Fractions (Fig. 5.6) prepared from ants by micro-preparative GC (8.13a), and pure chemicals were presented to the ants in a similar manner after allowing the dichloromethane solvent to evaporate. Fractions were

presented at a level of 1 gland equivalent (ge) of the components on the filter paper.

Pure chemicals were also presented to the ants foraging naturally in primary savanna at Mokwa. Chemicals were injected into melting point tubes (1 mm o.d. X 30 mm long, sealed at one end). In the case of dimethyl disulphide and dimethyl trisulphide 5 μ l were injected into each tube. For benzyl methyl sulphide a solventless preparative technique was used (8.1.3b). The compound was prepared from the GC into an open ended 1 mm o.d. tube surrounded by dry ice. The tube was sealed at both ends until needed for use, when one end was removed to leave a tube 30 mm long containing ca. 200 μ g of the synthetic compound. Columns of ants were followed on their outward journey, and the tube containing the chemicals, or control tubes, were sunk into the soil ca. 200 mm to the side of the foraging column and the reactions of the ants observed.

6.3.2b Trail following behaviour.

i) Field studies on the duration of trails.

Columns of ants foraging in the morning were observed and the trails marked with wooden stakes (1 mm diam.) at 0.2 m intervals. Major workers were captured from the returning column by trapping them separately in specimen tubes which were plugged with cotton wool. The captured ants were kept in the shade until required.

At recorded times the ants were released onto the trail at least 15 m from the nest. The tube was inverted over the trail and the plug removed. The ant was left covered by the tube for at least 60 seconds before it was released. It was then followed for at least 10 metres if it followed the trail or 300 seconds if it lost the trail and entered the surrounding vegetation. A number of ants were also released 2 metres to the side of the trail and observed.

ii) Laboratory studies on the source of the trail substances.

Twenty major and twenty minor workers from cultures at Southampton were placed in traps made from 100 X 50 mm polythene containers, with a sliding door at one end. Extracts of various parts of major workers,

killed by plunging into dry ice, were made in purified hexane at the following concentrations:-

Head + Thorax	1 unit in 500 μ l hexane
Gaster	1 unit in 500 μ l hexane
Dufour's/poison gland on sting	1 unit in 2 ml hexane
Hind gut, removed by dissection	1 unit in 1 ml hexane
Poison gland	1 unit in 2 ml hexane
Dufour's gland	1 unit in 1 ml hexane

The Dufour's gland and poison gland were separated by dissection under distilled water, any dissections in which one of the glands was ruptured were rejected. The hind gut was taken as a piece of gut ca. 5 mm long, starting from a point as close to the anus as possible.

100 μ l of the extract to be tested was taken up in a 'Drummond' microcapillary tube and streaked along a convoluted pencil line, ca. 250 mm long, on cartridge paper (300 X 200 mm). Presentations to sister workers were made so that any ant had to make a choice between two potentail trails. Presentations were paired as follows:-

Head+thorax	paired with	Gaster
Dufour's/poison gland	paired with	Solvent blank
Hind gut	paired with	Pencil blank
Poison gland	paired with	Hind gut
Dufour's gland	paired with	Poison gland

The start of the trail was made flush with the sliding door of the trap. Controls were run with purified hexane and pencil line alone. Ants were released from the trap and scored as responding positively if they followed a trail for ca. 200 cm from the trap door. A fresh piece of cartridge paper was used for each ant, as observations suggested that ants could be laying trails of their own on the paper.

In the comparison of natural trails with poison gland extract the experimental situation was different. Ants in culture were allowed to establish trails over cartridge paper to petri dishes of termites placed under the traps (as used above), at a distance of 0.5 metres from the nest entrance.

When the ants had finished foraging 20 major and 20 minor workers from the same colony were placed in the trap which was replaced in the foraging arena. The natural trail was fairly wide (ca 60 mm), but narrowed where it entered the trap, and was in a straight line between the trap and the nest entrance. The artificial trail was laid from the trap to the nest, to one side of the natural trail, and was slightly longer than the natural one. The ants were released from the trap and scored according to which trail they followed to the nest. No attempt was made to provide a new trail for each ant, but 10 trials were made with one natural trail against 0.1 or 1 ml of poison gland extract.

iii) Statistical Analysis.

The chi-squared test for one-sample cases achieving a nominal level of measurement could not be used as in some trials more than 20% of the expected frequencies were <5 . The binomial test was used, although it is probably a less powerful test than the chi-squared (Siegal 1956).

6.3.3 Results

6.3.3a Co-ordination of attacks on termites.

Reactions of ants to crushed body sections.

Worker ants presented with crushed heads orientated towards them and then moved rapidly towards the heads. The ants surrounded the odour source, making biting movements with their mandibles into the sand and the filter papers, and digging into the sand with their fore-legs. Two major behavioural components were thus distinguishable - attraction and digging. Digging was interpreted when biting movements occurred in conjunction with digging movements of the legs, as biting alone, or with stinging, occurred in 'attack' situations (6.2.3a). Excised, crushed mandibular glands elicited the same reaction as crushed whole heads (Table 6.6).

Presentation of crushed thoraces did not elicit any reaction. Ants presented with crushed gasters were initially alerted (appendix B), and then showed low intensity alarm with the mandibles open and the

antennae held porrect. There then followed a period of high intensity alarm - undirectional locomotion with ants colliding with sister workers and frequent bursts of stridulation. As this high intensity alarm subsided (30 - 50 seconds after presentation), a number of ants approached the odour source slowly and then stood around the crushed gaster, biting at it. Some of the ants remained with their mandibles fixed in the remains of the gaster or the filter paper for up to 60 seconds. The rapid biting and stinging seen in the reaction to some crushed termites soldiers was not observed. The overall reaction to crushed gasters was a complex one, probably because the contents of more than one gland were released when the gaster was crushed. The initial reaction is uncoordinated high intensity alarm, later followed by attraction towards the odour source, with the ants being eventually arrested at the odour source.

The behaviour elicited by crushed heads was congruent with the reactions of ants observed attacking termites. Ants were attracted to a sister worker digging into the soil at the entrance to termite galleries, these ants then also commenced to dig. In view of these observations the reactions of ants to GC prepared fractions from heads was observed.

Fractions 1 and 2 (Fig. 5.6).

Worker ants orientated towards the odour source and then moved rapidly towards it. They did not stop at the odour source, but usually overshot by 30 - 50 mm, and then turned about and re-orientated towards it. The ants explored the odour source, moved away, but were usually attracted back towards the odour source until 60 - 120 seconds after the initial orientation to it.

Fractions 3 and 5 (Fig. 5.6).

No reaction was observed to these fractions. Worker ants encountering the filter papers treated them in a similar manner to that observed for controls.

Fraction 4 (Fig. 5.6).

No initial reaction was observed to this fraction, but any ants encountering the filter paper in the course of their explorations

usually exhibited the following behaviour. The ants bit at the odour source and dug into the surrounding sand with their forelegs, in a similar manner to that observed to crushed heads.

Fractions 1 and 4 presented together.

The reaction of worker ants to these two fraction together was congruent with that described for the reactions of ants to crushed heads.

The identity of some of the mandibular gland components was described in 5.2.3, and three of these compounds were assayed. Dimethyl disulphide and dimethyl trisulphide were present as the main components in active fractions 1 and 2, and benzyl methyl sulphide in inactive fraction 4.

Reactions of ants to mandibular gland compounds on filter papers.

Dimethyl disulphide and dimethyl trisulphide: the reactions of ants to these two compounds, at a level of 100 ng per presentation, was the same as the reaction of ants to fractions 1 and 2 - attraction to the odour source.

Benzyl methyl sulphide: the ants did not react to this compound at a level of 50 ng per presentation

Dimethyl disulphide and Fraction 4 together: 20 ng of the sulphide and 1 ge of fraction 4 released the same behavioural repertoire as observed to crushed heads - attraction to the odour source followed by digging at and around the source.

Dimethyl disulphide (on filter paper) buried in the sand: the ants responded to presentation of the compound in this way in the same manner as they did to the disulphide presented on the surface.

The reactions of the ants to the various presentations are summarized in Table 6.6.

Dimethyl disulphide presented to foraging ants.

15 - 20 seconds after presentation of the stimulus ants left the foraging column, orientated towards the stimulus and then moved rapidly towards it. If the ants overshot the stimulus they usually reorientated towards it. This behaviour was continued for 60 - 120 seconds, after this the ants either re-located the trail and rejoined

Table 6.6 Reactions of Megaponera foetens to crushed body segments and mandibular gland fractions and compounds from sister workers.

Present- ation	Concent- ration	No. of present- ations	Behaviour observed	No of times behaviour observed
Crushed heads	1	10	Attraction, Digging.	10
Crushed thoraces	1	10	No reaction	
Crushed gasters	1	10	Undirected alarm, followed by attract- ion and arresting	10
Mandibular glands	1 pair	10	Attraction, Digging.	10
Fraction ^a 1	1 ge ^e	10	Attraction	10
2	1 ge	10	Attraction	8
3	1 ge	5	No reaction	
5	1 ge	5	No reaction	
4	1 ge	15	Digging	10
DMDS ^b	100 ng	10	Attraction	9
DMTS ^c	100 ng	5	Attraction	5
BMS ^d	50 ng	5	No reaction	
DMDS + Fraction 4	20 ng + $\frac{1}{2}$ ge	10	Attraction, Digging	7
DMDS buried in sand	100 ng	5	Attraction	5

^a Mandibular Gland Fractions (Fig 5.); ^b Dimethyl disulphide;

^c Dimethyl trisulphide; ^d Benzyl methyl sulphide; ^e Gland equivalent

the remainder of the column, or moved into the surrounding vegetation. In some cases, perhaps because of the large numbers of ants moving towards the odour source, occurrence of high intensity alarm, with bursts of stridulation were noted. The overall reaction to the disulphide was attraction, complicated by the large numbers of ants involved. Tests with benzyl methyl sulphide in similar conditions elicited no reaction, nor did control tubes.

6.3.3b Trail Following Behaviour

Duration of the trail in primary savanna.

The results of releasing major workers along existing trails of their own colony at different time intervals after its origin are presented in Table 6.7. Trails show some activity for up to 3 hours, but their efficacy was greatly reduced after 1.5 hours.

Source of the trail substances.

Major workers:- Although the differences between ants following gaster and head/thorax extracts (Table 6.8) was not significant ($p = 0.09$), because 14 ants followed the gaster extract trail and only 4 the head/thorax, further studies were carried out on extracts of gastral components. The attraction of the head/thorax may have been due to the presence of alkyl sulphides, which are also attractants (6.3.3a, Table 6.6).

The Dufour's poison gland complex proved to be more powerful than the hind gut ($p = 0.006$), and separate assays on these two glands showed that the poison gland was more active than the Dufour's gland ($p = 0.045$). The low probability value perhaps being due to accidental contamination of Dufour's gland with poison gland contents. Comparison of the poison gland extract with a natural trail revealed that at a level of 0.1 ge, 12 out of 20 ants followed the natural trail and 7 the poison gland extract ($p = 0.806$). At a presentation level of 1 ge 16 ants followed the extract and 3 the natural trail ($p = 0.011$).

Minor workers:- this caste appeared to be generally less competent at trail following than the major workers, When the Head/thorax

Table 6.7 Ability of major workers of Megaponera foetens to follow natural recruitment trails laid by columns of sister workers during morning raids on termites. Trails followed for at least 10 metres.

Hours elapsed since trail was laid	Number of major workers (out of 10) following the trail for at least 10 metres	Number of major workers (out of 5) which, after being released 2 metres to the side of the trail, moved in the direction of the trail for 10 m
0.25	9	0
0.5	8	
0.75	6	
1.0	7	1 ^a
1.5	6	
2.0	5	
2.5	5	
3.0	1	0
3.5	0	
4.0	0	
7.0	0	0

^a Trail relocated and followed to the nest

Table 6.8 Trail-following responses of Megaponera foetens major and minor workers to extracts of parts of the body of sister workers.

Extract	No. of major workers	No. of minor workers
Head/Thorax	4	2
Gaster	14	6
No trail followed	2	12
Dufour's/Poison gland	13	6
Solvent blank	1	0
No trail followed	6	14
Hind gut	4	3
Pencil blank	0	0
No trail followed	16	7
Poison gland	17	10
Hind gut	2	4
No trail followed	1	6
Dufour's gland	3	6
Poison gland	15	11
No trail followed	2	3
Natural trail	12	12
0.1 ge poison gland	7	5
No trail followed	1	3
Natural trail	3	
1 ge poison gland	16	
No trail followed	1	

For extract concentrations see page 218 .

extract was compared with the gaster only 6 ants followed the latter; 2 ants following the head/thorax extract and 12 not following any trail at all. Comparison of different parts of the gaster did reveal that major worker poison gland extract was more potent than the hind gut or Dufour's gland (Table 6.8), although in some cases the results were not significantly different because of the high numbers of ants not following any trail at all.

6.3.4 Discussion.

The obligate termite predator, M.foetens, shows at least two refinements of its chemical communication system to allow it to act efficiently as a predator of foraging termites. Previous authors (Fletcher 1973, Levieux 1966) have demonstrated the use of trails in Megaponera, and in this present study the trail substance has been shown to originate in the poison gland. No attempt has been made to identify the trail pheromone, although SSGC of excised poison glands did reveal the presence of more than 10 compounds, all at a level of a few nanograms per major worker.

In the related ponerines, Leptogenys attenuata and L.nitida, Fletcher (1971) has demonstrated that the source of the trail pheromone is also the poison gland. In both Leptogenys species the trail pheromone is used in the co-ordination of emigration, and in L.nitida is also used in the recruitment of ants to their isopod prey. In the termitophagous ponerine, Termitopone laevigata the glandular source of the trail pheromone is the hind gut (Blum 1966).

In a termite predator such as M.foetens where ants must arrive at the termites in a group, the odour trail allows the efficient guiding of ants to the foraging termites where other chemical cues can complete the co-ordination of predation.

The scent trails at Mokwa did not last more than 3 hours, which in an area where foraging occurs only in the morning and evening (3.3.1) may be useful in preventing confusion with the colonies trails from previous raids. In the Ivory Coast, where the details of

recruitment differ from Mokwa (3.5.2) and a scout ant is not used, Levieux (1966) claims that the trails last for up to 24 hours, from the observation that ants often use the same odour trails over a number of days. It is possible that differing foraging patterns, as observed by Levieux, has led to a lower threshold of detection of the trail odours. It is interesting to note in this context that the males of M.foetens (Appendix A) are able to follow trails at Mokwa for up to seven hours, reflecting the need for them to detect worker recruitment trails, from morning raids, in the afternoon.

The ability of glandular secretions to attract ants has been recorded in the alarm behaviour of a number of species, including Acanthomyops claviger (n-undecane, Regnier and Wilson 1968), Pogonomyrmex spp. (4-methyl-3-heptanone, Wilson 1958) and Oecophylla longinoda (n-hexanol, Bradshaw etal. 1975). Dimethyl disulphide and dimethyl trisulphide from M.foetens are also attractants. Without the benefit of field observations the rapid movements of the ants to the odour source might have been interpreted as 'alarm' behaviour. The lack of open mandibles (Appendix B), studies on crushed heads and field observations make their classification as attractants in a multi-component pheromone system more correct. When ants are attacking the foraging termites the distribution of the digging workers is noticable (Plate 3.3), and crushed heads and glands result in a similar distribution, with the ants digging in the area around the odour source. The spacing does suggest that the "digging pheromone" (Fraction 4) may be repellent at high concentrations, or that other undiscovered compounds may be involved in the regular spacing.

The development of this co-ordination system must increase the efficiency of predation. If each ant had to search out its prey, many termites might escape. If a number of ants can be concentrated at the entrances to the termite galleries more prey can be secured if the retreating termites can be cut off.

Some comment must be made on the different roles of dimethyl disulphide in M.foetens and P.tarsatus. In the latter species Crewe and Fletcher (1974) found that workers were "...indifferent to crushed worker heads and filter paper impregnated with (1µl of)

dimethyl disulphide" , but that buried whole ants and buried filter papers treated with 0.1 μ l of dimethyl disulphide elicited digging and rescue of ants or filter paper. The lack of response to unburied compound is difficult to explain; Crewe and Fletcher suggest that, as P.tarsatus, unlike M.foetens, lacks stimulatory organs (Markl 1973), the response is needed only to release sister workers trapped in nest falls. In M.foetens dimethyl disulphide has a completely different function, as an attractant in a multi-component system with another less volatile component which releases digging.

The amounts of dimethyl disulphide and dimethyl trisulphide also differ markedly in each species. P.tarsatus contains 4.6 μ g of the disulphide and 23.2 μ g of the trisulphide, whereas M.foetens major workers contain 28 ng and 10 ng respectively of the two compounds.

SECTION 7 SUMMARY OF FINDINGS.

The aim of this section is to collect and summarize the findings presented in Sections 1 - 6.

Evaluation of the ant fauna of the southern Guinea Savanna at Mokwa, Nigeria revealed the presence of at least 63 species of which 56% have been recorded as predators of termites. The majority of these ants are opportunist predators, with no obvious specific adaptations to secure termites. Two obligate termite predators were discovered at Mokwa.

The first of these, Megaponera foetens, is a large ponerine which mounts organised raids against foraging termites. The termites are first located by a scout ant which utilizes cues in the termites protective soil sheeting (6.1). The lack of cues in the sheeting of Ancistrotermes cavithorax and Microtermes spp. appears to be one of the factors responsible for the low levels of predation by M.foetens on these genera.

A successful scout ant returns to the nest and recruits a column of sister workers who set out to attack the foraging termites. The scout ant guides the workers to the termites along a scent trail which originates from the poison gland (6.3.2b). The attack on the foraging termites is coordinated by a multi-component pheromone system from the mandibular glands. An ant which locates groups of termites in their foraging area releases alkyl sulphides, which have been shown to attract sister workers to the area. An unidentified "digging pheromone" is also released which stimulates workers to dig into termite foraging galleries.

After the raid has been completed the ants gather up the termites and return to the nest. Counts of the numbers of termites carried by the ants at this stage in the raid (4.3.1) revealed that Macrotermes bellicosus and Odontotermes spp. are the most important prey

items at Mokwa. Predation on M. bellicosus ($141 \text{ termites m}^{-2} \text{a}^{-1}$) was so intense than Megaponera is able to turn over the termite worker/soldier population in less than a year.

Studies on the reactions of M. foetens to termite soldier defence secretions (6.2) revealed that the termite species which rarely or never appear as prey have defences which interfere the co-ordination of predation by the ant. Crushed soldiers of Macrotermes subhyalinus and Ancistrotermes cavithorax arrested attacking ants, which may give worker termites more time to escape into their foraging galleries. Monoterpene hydrocarbons from the frontal gland secretion of Trinervitermes spp., who never appear as prey, disrupt predatory columns of ants by releasing unidirectional alarm (Appendix B) in the ants.

A second specialised termite predator, Decanorium uelense, uses scout ants to search for its prey. A successful scout recruits a small column (10 - 30 ants) of ants which locate and immobilize the prey (3.4). A mass recruitment mass is then instigated to retrieve the prey. Only a small number of ants are required to immobilize the termites as the ants have a very potent sting and do not alarm their prey. This allows them to move among foraging parties of Microtermes in a way which many opportunistic predators cannot.

A survey of the responses elicited by ants and their secretions (5.1) to termites suggested that those ants which preyed, obligately or opportunistically, on termites and did not alarm or repel them had glandular secretions different from those ants which were repellent. Studies on the mandibular gland secretions of Crematogaster, Tetramorium and Decanorium revealed (5.2) that those ants which did not repel termites had developed glandular secretions predominating in aliphatic alcohols at the expense of aliphatic ketones and aldehydes. Decanorium, for example, has a mandibular gland secretion with 3-octanol as the principal component, whereas many Tetramorium and Crematogaster species produced 3-octanone as the major component.

It is suggested that int -specific influences, such as the need to be a successful predator, may have as much effect on the the development and evolution of the components of glandular secretions of insects as int -specific communicative and colony recognition factors.

SECTION 8. EXPERIMENTAL - CHEMICAL ANALYSIS AND SYNTHESIS

8.1 Gas Chromatography (GC).

8.1.1. Introduction and Column Conditions.

A Pye - Unicam 104 gas chromatograph, fitted with dual flame ionization detectors (FID), injection port heaters and a detector oven was used in all chromatographic studies. All-glass columns, with 6 mm outside diameter (o.d.) ground glass ends were used, connected to the detector with either a Pye - Unicam connector with a viton "O"- ring, or a modified column connector which allowed the use of graphite "O"- rings for high temperature work (Higgs 1976).

Gas chromatography columns were as follows; all packings and supports were supplied by J.J's. (Chromatography) Ltd., Kings Lynn, unless otherwise indicated.

Column A

2 mm internal diameter (i.d.) X 3m, 5% OV101 silicone gum on 100 - 120 mesh Diatomite CLQ, acid/alkali washed, DMCS treated (AAW/DMCS). Conditioned at 350° C.

Column B

2 mm i.d. X 3m, 5% Carbowax 20M on 100 120 mesh Diatomite CLQ, AAW/DMCS treated. Conditioned at 220° C.

Column C

2 mm i.d. X 3 m, 5% Polypropylene glycol adipate (PPGA) on 100-120 mesh Diatomite CLQ, AAW/DMCS treated. Conditioned at 200° C.

Column D

2 mm i.d. X 3 m, 5% OV275 on 100 - 120 mesh Diatomite CLQ, AAW/DMCS treated. Conditioned at 275° C.

Column E

2 mm i.d. X 3 m, E 30 silicone gum, probably 10%, support unknown. This column packing was supplied by Pye - Unicam and was coded 12428/2/10 E30/ T922. Conditioned at 300° C.

Column F

2 mm i.d. X 3 m, 5% F.F.A.P. on 100 - 120 mesh Diatomite C, AAW/DMCS treated. Conditioned at 200° C.

Column G

4 mm i.d. X 1.5 m, 10% Carbowax 20M on 100 - 120 mesh Diatomite C, AAW/DMCS treated. Conditioned at 200° C.

Column H

4 mm i.d. X 1.5 m, 10% E 301 silicone gum on 100 - 120 mesh Diatomite C, AAW/DMCS treated. Conditioned at 350° C.

Column I

7 mm i.d. X 1.5 m, 10% E 301, 60 -80 mesh Diatomite C, AAW/DMCS treated. Conditioned at 350° C.

"Bergström" column J

2mm i.d. X 1.5 m, 5% OV101 as column A.

"Bergström" column K

2 mm i.d. X 1.5 m, 5% Carbowax 20M as column B.

All columns were packed by applying reduced pressure at the exit and the packing was tamped down by tapping the column. Columns not containing hydroxyl bonding sites were regularly re-conditioned by the injection of 3 X 10 µl aliquots of Sily - 8 at 250° C.

The carrier gas was oxygen-free nitrogen (BOC, high purity) at 25 ml min for 2 mm i.d. columns and 50 ml min for 4 and 7 mm i.d. columns; the same flow rates were used in GC-MS studies where helium (BOC) was used as the carrier gas. The flame for the ionization detector was provided by air (BOC) and hydrogen (BOC, high purity).

8.1.2. Solid Sample Gas Chromatography (SSGC).

8.1.2a. Morgan and Wadhams Method

The solid-sample injector of Morgan and Wadhams (1972) is manufactured from a modified Hewlett - Packard injector. Ants for study were killed

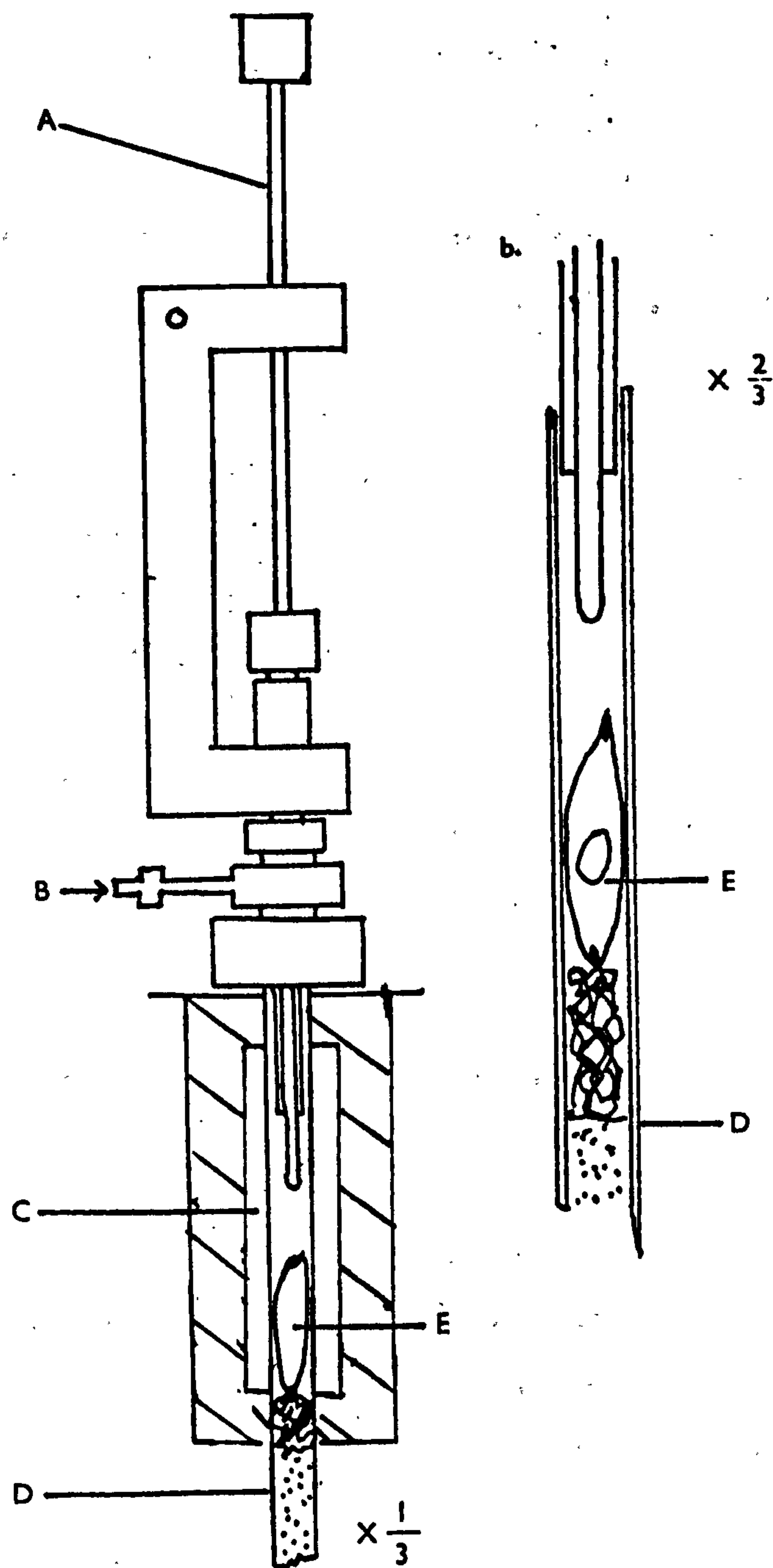


Figure 8.1. Modified Solid-Sampler (3.1.2b)

A - plunger; B - carrier gas inlet; C - injection port heater; D - GC column; E - biological material sealed in vial.

by plunging them into finely crushed dry-ice (Drikold, I.C.I.), the part to be examined was then snapped off with fine forceps and inserted into a vial made from the lower part of a 230 mm (9") Pasteur pipette, sealed at the bottom. The biological material was then pushed to the bottom of the vial with a clean, cold glass plunger and the vial sealed. Excised glands were first placed in fine-walled glass tubes before inserting them into the vial. The sealed vial was placed in the solid sample injector barrel and heated at approximately 150°C for 5 minutes. The vial was then crushed to allow any volatile chemicals to be flushed onto the GC column. The injection port heater was turned off after the GC oven had reached 100°C to prevent charring of the tissue. The tissue sample and glass fragments were removed at the end of the GC run.

8.1.2b. Modified Morgan and Wadhams Method.

The method described in 8.1.2a. cannot be used with samples of a diameter greater than 4 mm, because they will not fit into the glass sample vials. Although precolumn solid sample GC (PCGC) is a possible alternative, comparison with chromatograms obtained by SSGC is not easily facilitated because of the different column lengths and poorer resolution of PCGC. To allow SSGC of larger ants (e.g. Megaponera foetens) the method described in 8.1.2a. was modified by redesigning the solid sampler (Figure 8.1). Instead of inserting the sample into the barrel of the solid-sampler, the vial was placed on top of the glass wool plug holding the GC column packing in place, and the plunger used to crush the vial and release any volatile chemicals.

The vials used could be larger than those used in 8.1.2a.; they were manufactured from soda-glass tubing, drawn out into very thin walled lengths of ca. 3.75 mm o.d., and sealed at one end. The biological material to be analysed was placed in the vial which was sealed. The vial was then placed onto the top of the GC column, and the solid sampler fitted to the top of the column. The vial was heated at 150°C for five minutes and then broken with the plunger. The injection port heater was turned off when the oven temperature had reached 100°C , and the glass and tissue fragments removed from the column at the end of the GC run.

8.1.2c. Precolumn Solid Sample Gas Chromatography (PCGC).

The precolumn technique of Bergström (1973) was modified for use on a Pye - Unicam GC (Bradshaw 1976, Higgs 1976). A glass precolumn, 150 mm X 4 mm i.d. with ground glass ends and containing a glass wool plug at its lower end, was connected to a specially coiled GC column (columns J & K) with a Pye - Unicam connector. A beaker of finely powdered dry ice was placed around the U-bend of the GC column and the sample inserted into the precolumn, being pushed down onto the glass wool plug with a clean glass plunger. The carrier gas inlet was then re-coupled to the precolumn and the sample degassed at 150° C for ten minutes; the volatiles being condensed in the U-bend by the dry ice. The beaker of dry ice was then removed, the GC oven temperature programme commenced and the injection port heater switched off. After the GC run had been completed the precolumn was removed and replaced.

8.1.3 Preparative Gas Chromatography.

8.1.3a. Solvent Preparative Gas Chromatography.

An all glass-splitter, as described in Baker et al. (1976) was used in all preparative gas chromatography. The GC column was connected to the splitter with a 6 mm stainless steel Swagelok union, which had been bored out to 7 mm i.d. and fitted with Graphlok "O" rings (SGC, Melbourne).

The trapping system consisted of a removable angled capillary line manufactured from 1 mm i.d. electrode tubes (Jencons (Scientific) Ltd., Herts., U.K.) and a solvent trap. The capillary line was butted onto the splitter outlet and held in place with a 6 mm nut sealed with a viton "O" ring (Hewlett - Packard, Slough, U.K.). Traps were glass tubes, 100 mm long and 4 mm i.d. fitted with either B10 or B7 ground glass sockets (Quickfit, U.K.) at one end and sealed at the other. A Pye - Unicam injection port heater was used to maintain the splitter at least 15° C above that of the oven. Samples were collected by passing the column eluent through 200 - 500 µl of solvent cooled in dry ice/acetone (for subsequent bioassay) or uncooled to prevent the condensation of water (for FT.NMR studies). A clean capillary

line and trap was substituted for each fraction collected.

8.1.3b. Dry Preparative Gas Chromatography.

The presence of a solvent in chemicals trapped using method 8.1.3a. would prevent the testing of GC purified samples by bioassay in capillary tubes. It is therefore advantageous to prepare samples directly into capillary tubes. To collect samples in this manner the splitter is used as described in Baker et al. (1976) and 8.1.3a. above, but the solvent trap was dispensed with and the capillary line was a 150 mm X 1 mm i.d. electrode tube with one end pulled into a fine jet. This modified tube was placed in a dry ice boat (80 mm long, 35 mm wide and 35 mm deep) manufactured from aluminium. The unmodified end of the tube was placed in the splitter and the boat secured with a retort after being filled with finely powdered dry ice. The GC eluent passed into the capillary line where it condensed out. The capillary line and dry ice boat were changed for each fraction collected.

At the end of the collection period the capillary tubes were sealed in the following manner, to minimise the loss of volatiles. The capillary line, still in the filled dry ice boat, was adjusted so that the unmodified end was flush with the end of the boat. The modified end was sealed in a bunsen flame, any volatiles being driven by the heat into the cooled section, where they recondensed. The sealed end was then pushed through the dry ice boat until it was flush with the end, and the unmodified end of the capillary line was sealed.

8.1.4. Abstractor Post Loops.

The loops were constructed from single turns of 2 mm i.d. glass tube to a diameter of 60 mm and were fitted at both ends with 20 mm lengths of 6 mm o.d. ground glass in a configuration allowing an unstressed connection between the GC column and the FID connector. The loops were attached to the GC column with a 6 mm Swagelok union, bored out to 7 mm i.d. and fitted with graphite "O" rings. The post loop was attached to the FID with a standard column connector.

i) Boric Acid: Finely powdered boric acid (50 mg) was mixed with diatomite CLQ (450 mg) and packed into the loop under reduced pressure where it was held in place with glass wool plugs.

ii) σ -Dianisidine. The σ -dianisidine loop of Bradshaw (1976) was used. It was made by adding a chloroform solution of σ -dianisidine (25 mg) to diatomite CLQ (475 mg) and removing the solvent under reduced pressure.

Bradshaw (1976) and Higgs (1976) report that the boric acid post loop is capable of 100% abstraction of hexanol and citronellal and 30% abstraction of geraniol. These authors report that the σ -dianisidine loop gave efficient abstraction of hexanal, citronellal and neral, neither loop abstracting alkyl ketones, confirming the findings of Bierl et.al(1969).

8.1.5. Microscale Syringe Reactions.

Information on functional groups and the double bond saturation of a compound can be obtained by mixing reagents with solvent extracts in a syringe (Hoff and Feit 1964, Fredericks and Taylor 1966). 2 - 3 μ l of the solvent extract were taken up in a 10 μ l syringe, followed by the reagents:

i) Bromine in carbon disulphide (1:1 mixture) (2 μ l).

The plunger of the syringe was moved up the barrel to allow the reaction of the solution and the reagent on the syringe barrel wall, and the reaction mixture left for 5 minutes before expelling excess reagent and injecting the mixture onto the GC column. This reagent reacts across the double bonds of unsaturated compounds, and comparison with a standard GC trace reveals that the unsaturated compounds have been eliminated.(Fredericks and Taylor 1966)

ii) Fresh saturated 80% ethanolic sodium borohydride (2 μ l).

The reaction mixture was left for two minutes, after mixing, in the syringe, before injection onto the GC column. This reaction is reported to convert carbonyl compounds to the corresponding alcohol (Fredericks and Taylor 1966).

8.1.6. Co-elution studies.

Co-elution studies on solvent extracts were achieved by taking up an extract and authentic sample in a syringe and co-injecting the samples onto the GC column together. For SSGC co-elution studies a small quantity (0.1 μ l of solution of appropriate concentration) was injected into a solid sample tube; the biological material was placed in the same tube which was then sealed. The solid sample vial was placed in the solid sample injector and treated as in 8.1.2a.

8.2. Spectroscopy.

8.2.1. Combined Gas Chromatography - Mass Spectrometry (GCMS).

Mass spectra were obtained on an A.E.I. MS 12 spectrometer, and analysed by a V.G. Digispec 16 data system, calibrated with per-fluorokerosene. The spectrometer was tuned to m/e 28 at 70 eV, with a final tuning, to increase sensitivity on the ion monitor, at 20 eV. The spectrometer was interfaced with a GC column in a Pye - Unicam 104 system via a Watson Biemann glass-frit separator. All spectra were recorded at 70 eV. The carrier gas was helium in all cases, used at the same flow rates for nitrogen for the GC column used.

8.2.2. Nuclear Magnetic Resonance Spectroscopy (NMR).

Fourier Transform NMR (FT - NMR) spectra were recorded on a Varian XL 100 spectrometer on solutions in deuteriochloroform. The internal lock was made on deuterium .

8.2.3. Optical Rotation.

Optical rotations were determined on a ETL - NPL Type 143A Auto Polarimeter, with either dichloromethane or chloroform as the solvent. The compounds for determination were trapped into the solvent using the GC preparative system of 8.1.3.

8.3 Chemical Techniques.

8.3.1. Solvent Purification.

Dichloromethane (2.5 l) was washed with saturated aqueous sodium bicarbonate (100 ml) and water (50 ml), dried for at least 12 hours over magnesium sulphate and distilled through a 1 m X 50 mm fractionation column filled with Fenske's helices. The fraction boiling between 40 - 41° C was collected. Hexane (67 - 68° C fraction) was purified by colleagues in the Chemical Entomology Unit in the manner of Higgs (1976), as was deuteriochloroform (61 - 62° C fraction).

8.3.2. Treatment of Solvent Extracts.

Crude solvent extracts of ants or termites, in the concentrations stated in the text, were filtered through a glass wool plug and dried overnight with magnesium sulphate. The extracts were then refiltered through glass wool. Concentration of the solvent extract was achieved by passing oxygen free nitrogen over the sample until the required volume was obtained. Extracts were stored at either -22° (Southampton) or -13° C (Mokwa).

8.3.3. Attempted Reduction of Alkyl Pyrazines to Alkyl Piperazines.

Alkyl pyrazines can be quarternized with methyl iodide and then reduced to the piperazine with sodium borohydride (Wheeler and Blum 1973). 2,5 and 2,6-Alkyl pyrazines cannot be distinguished by mass spectroscopy, but their derived pyrazines give distinctive and different mass spectra. The heads of Anochetus sedilloti contain alkyl pyrazines at the levels of 30 - 70 ng per ant, and mass spectra can be obtained if ca. 12 ants heads are used. The reaction must therefore be capable of reducing a minimum of 350 ng of pyrazine to piperazine to give enough compound for mass spectroscopy. The reaction must also be capable of use in conjunction with SSGC on unextracted whole heads.

2,5-Dimethyl-3-n-butyl pyrazine (500 ng) in dichloromethane was injected into a 1 mm diameter X 20 mm fine walled capillary tube, and methyl iodide (1 µl) was added. The tube was sealed and left at 60°C for six hours. 80% Ethanolic sodium borohydride (2 µl) was added to a conventional SSGC vial (8.1.2a.) and the top broken off of the pyrazine/methyl iodide reaction vial which was then placed inverted into the second vial. The SSGC vial was sealed and shaken vigorously for 60 s⁻¹ to mix the contents, and left for 1 hour at 60° C. The

contents of the vial were then examined by SSGC (8.1.2a.). Only a 10% reduction of the pyrazine peak was observed, in some cases with a slight change in shape of the peak, but no additional components were observed. The reaction was repeated with more concentrated reagents, left to react over longer periods of time, but no improved reduction was observed. It was therefore concluded that the method would not be suitable for use in the identification of pyrazines from the mandibular glands of ants.

8.4. Synthesis.

8.4.1. (+) 2-Undecanol.

2-Undecanone (5 g, Koch Light) was stirred in 50% ethanolic sodium borohydride (15 ml). The reaction was monitored by GC and after 4 hours the product was extracted with ether after the addition of sodium chloride (1 g). The ether extract was dried with magnesium sulphate and the solvent removed by evaporation under reduced pressure.

8.4.2. (+) 2-Dodecanol.

2-Dodecanone (2 g, Koch Light) was stirred with 50% ethanolic sodium borohydride (10 ml). The reaction was monitored by GC and after 6 hours the product, after the addition of sodium chloride (0.5 g), was extracted in ether and treated as in 8.4.1.

8.4.3. Methyl-5-methyl salicylate.

Methyl-5 salicylic acid (10 g, Koch Light) was refluxed with concentrated sulphuric acid (10 drops) and methanol (50 ml) for 20 hours. The product was dried with magnesium sulphate, filtered through glass wool and excess methanol removed by evaporation under reduced pressure.

8.4.4. Benzyl methyl sulphide.

a) Preparation of sodium hydrogen sulphide reagent.

Disodium sulphide (15 g) was dissolved in distilled water (100 ml) and hydrogen sulphide from a Kipps apparatus was bubbled through the solution at 70° C for 5 hours (Khormandaryan and Brodovich 1930).

b) Preparation of benzyl sulphide.

Benzyl chloride (50 ml) was refluxed with 50% ethanolic sodium hydrogen sulphide (50 ml ethanol, 50 ml of aqueous NaHS solution from a.) for 4 hours.

c) The reaction mixture from b. (75 ml) was added to saturated sodium hydroxide solution (100 ml). Dimethyl disulphide (50 ml, Koch Light) was added to this and refluxed for three hours. The products were taken up in ether (50 ml) and left to separate out overnight. The ether extract was dried with magnesium sulphate and filtered through glass wool

GC examination of a concentrated extract on column B revealed the presence of at least ten products. GCMS revealed benzyl methyl sulphide (30%) as well as excess benzyl chloride and benzyl alcohol and benzyl methyl ether. The benzyl methyl sulphide was purified on GC column I, the product being trapped in cooled capillary tubes (8.1.3b.)

8.4.5. Dimethyl trisulphide.

Dimethyl disulphide (10 g) was refluxed with sodium hydrogen sulphide solution (30 ml) from 8.4.4. in ethanol (30 ml) with the addition of sulphur (5 g) and ethylamine (5 ml). The reaction mixture was refluxed overnight, the products taken up in ether and left to separate out for 24 hours. The ethereal solution was dried with magnesium sulphate and filtered through glass wool; excess ether was removed under reduced pressure. GC examination of the mixture on column A revealed the presence of six products, GCMS showed that dimethyl trisulphide was the largest of these (52%). The dimethyl trisulphide was purified by GC on column I by trapping into cooled capillary tubes (8.1.3b.)

8.5. Details of Natural Products, Synthetic and Commercial Samples.

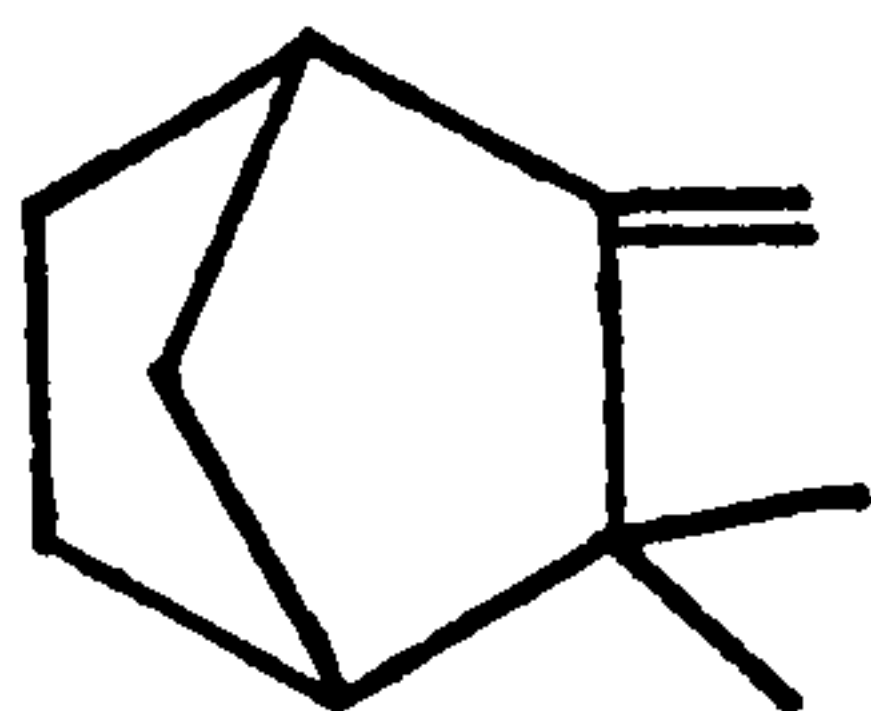
Mass spectral data are quoted as m/e with percentage intensity of the fragment in brackets, thus 59 (36).

8.5.1. Monoterpene Hydrocarbons.

Unless otherwise stated authentic samples of monoterpene hydrocarbons were obtained as purified samples from M.D.Higgs (Southampton University).

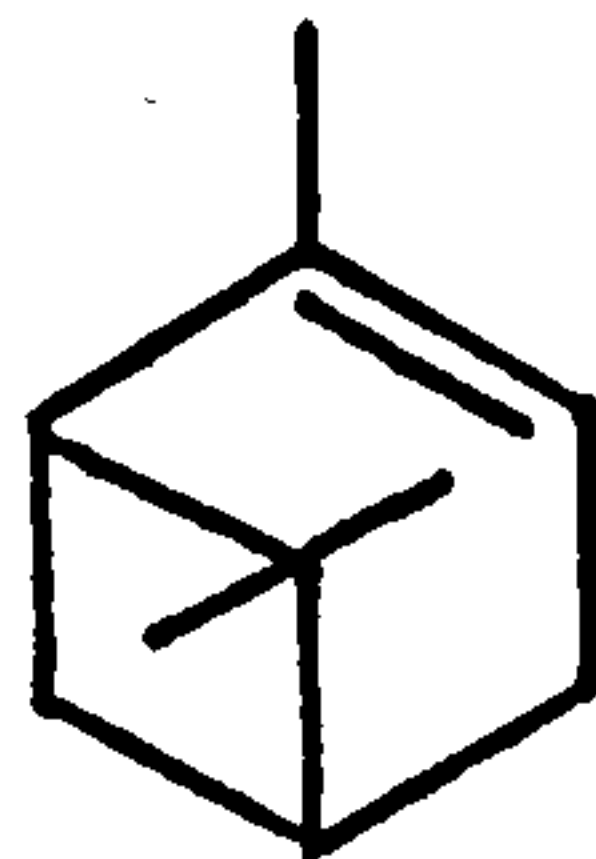
1. Camphene. Found as a natural product in Myrmicaria eumenoides.

Mass spectrum: M^+ 136 (28); 93 (100), 121 (91), 107 (35), 79 (34), 67 (30), 136 (28), 95 (24), 94 (20).



A authentic sample of camphene gave a similar mass spectrum to the natural product, differing only in the relative intensities of some of the fragments (Higgs 1976), and co-eluted with the natural product on GC columns A,B,D and E. The optical rotation of the natural product is not known.

2. α -Pinene. Found as a natural product in Myrmicaria eumenoides as the (+) optical isomer ($(\alpha)_D^{21} +43$). Also found in Trinervitermes geminatus and T.togoensis.

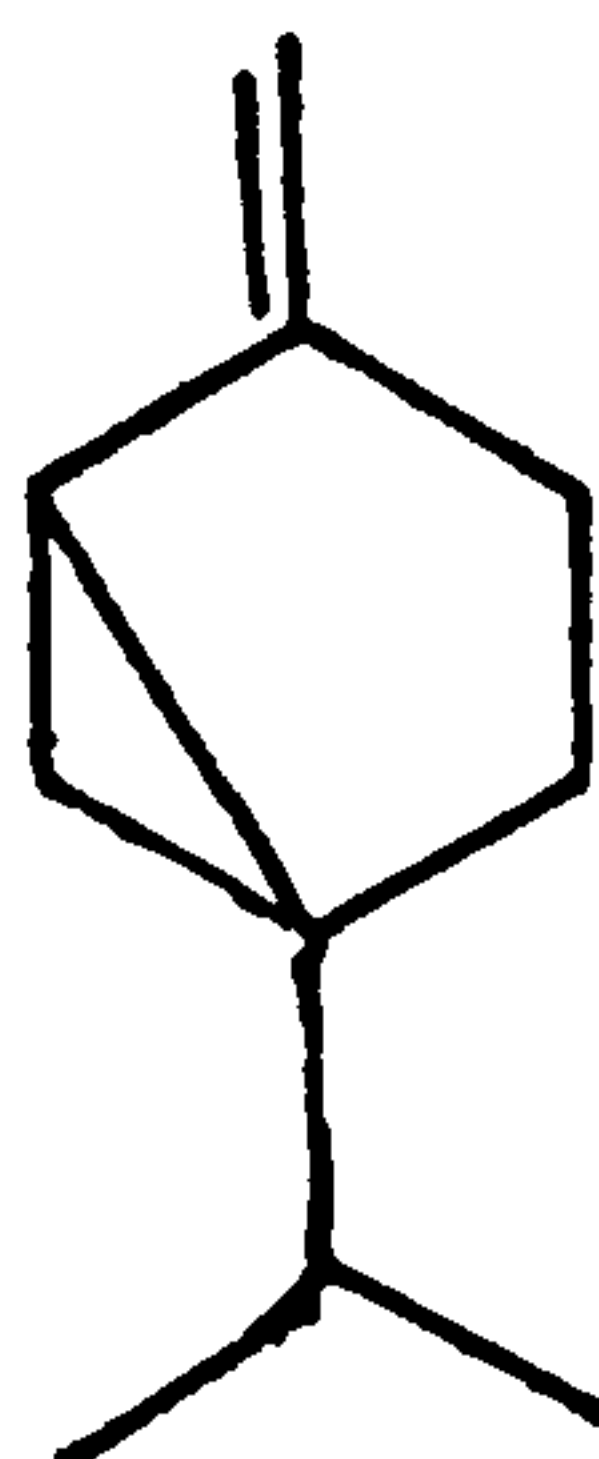


Mass spectrum: M^+ 136 (10); 93 (100), 92 (29), 91 (20), 77 (17), 79 (15), 121 (14), 136 (10), 41 (10).

100 MHz NMR (M.eumenoides): ^1H NMR ($CDCl_3$) δ 0.84 (3H, s, $-CH_3$); 1.26 (3H, s, $-CH_3$); 1.69 (3H, q, $-CH_3$); 5.18 (1H, m, CH-).

A authentic sample of α -pinene gave a mass spectrum congruent with that of the natural products, and co-eluted on columns A,B and E. The authentic sample had an optical rotation of $(\alpha)_D^{20} +50.3$.

3. Sabinene. Found as the natural product in Myrmicaria eumenoides, Trinervitermes geminatus and T.togoensis.

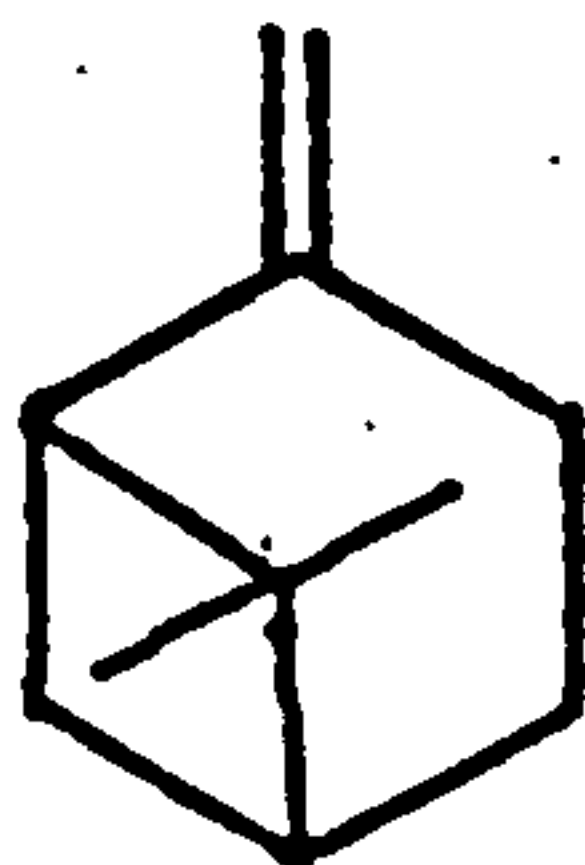


Mass spectrum: M^+ 136 (12); 93 (100), 77 (22), 91 (20), 79 (13), 136 (12), 41 (12), 94 (11), 69 (10).

An authentic sample of sabinene gave a mass spectrum congruent with the natural products, and co-eluted on

GC columns A,B,D and E.

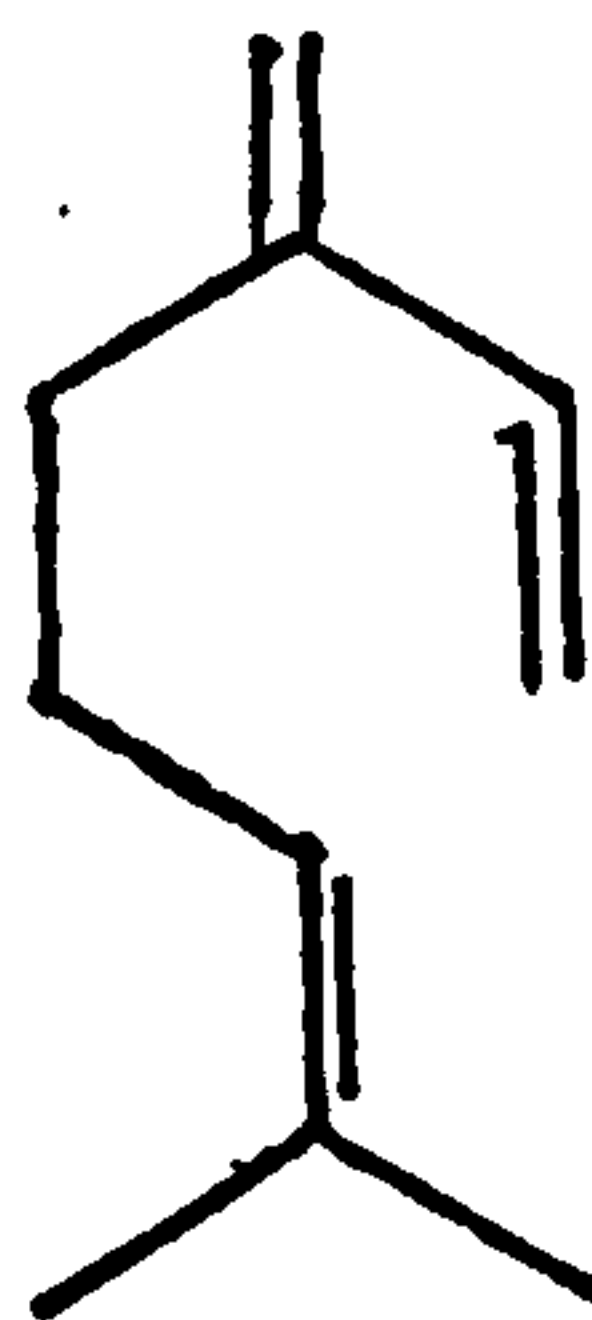
4. β -Pinene. Found as the natural product in Myrmicaria eumenoides with a optical rotation of $(\alpha)_D^{22}$ -22.5. Also isolated from Trinervitermes geminatus and T.togoensis.



Mass spectrum: M^+ 136 (11); 93 (100), 69 (39), 41 (38), 79 (17), 77 (16), 91 (16), 94 (14), 121 (11).
100 MHz NMR (M.eumenoides): ^1H NMR ($CDCl_3$) δ 0.71 (3H, s, $-CH_3$); 1.22 (3H, s, $-CH_3$); 4.60 (2H, m, $=CH_2$).

An authentic sample of β -pinene (Koch Light), with an optical rotation of $(\alpha)_D^{21}$ -24.5, gave a mass spectrum congruent with the natural products, and co-eluted on columns A,B and E.

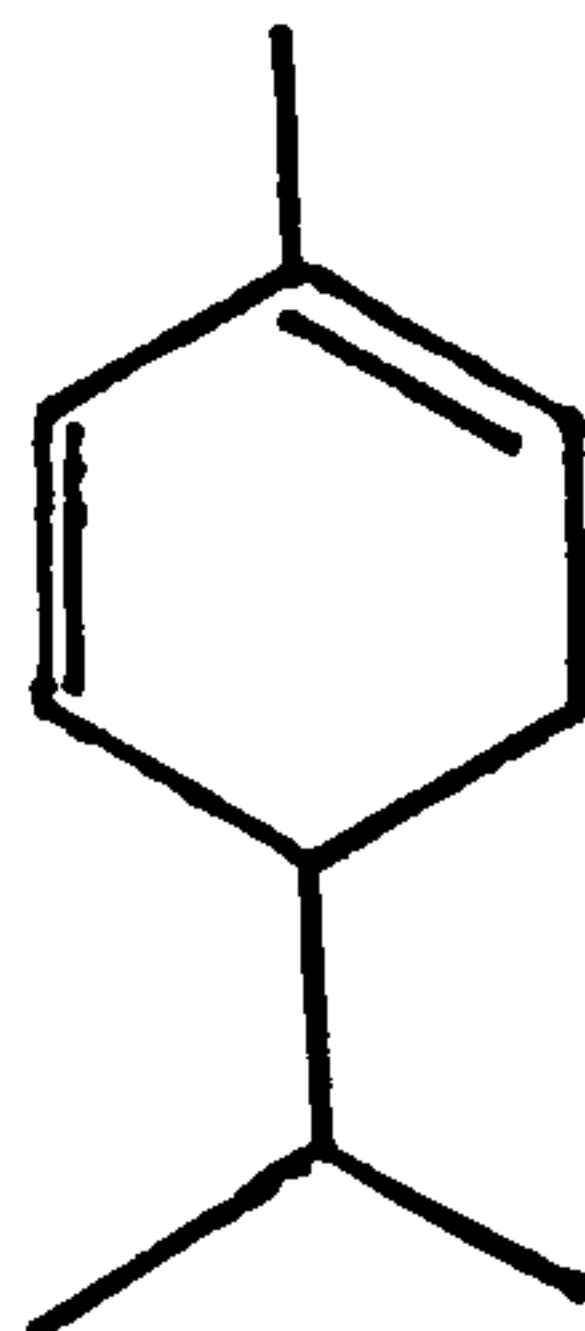
5. β -Myrcene. Found as the natural product in Myrmicaria eumenoides.



Mass spectrum: M^+ 136 (8); 93 (100), 69 (81), 41 (67), 79 (11), 94 (10), 77 (10), 136 (8), 91 (8).
100 MHz NMR (M.eumenoides): ^1H NMR ($CDCl_3$) δ 1.60 (3H, s, $-CH_3$); 1.70 (3H, s, $-CH_3$); 1.99 (4H, s, $-CH_2$); 5.01 (2H, s, $=CH_2$ superimposed on the doublet centred at 5.06); 5.06 (2H, d, $=CH_2$); 5.26 (1H, d, $=CH-$); 6.36 (1H, dd, $=CH$).

An authentic sample of β -myrcene (Koch Light) gave a mass spectrum congruent with that of the natural product, and co-eluted on GC columns A,B and E.

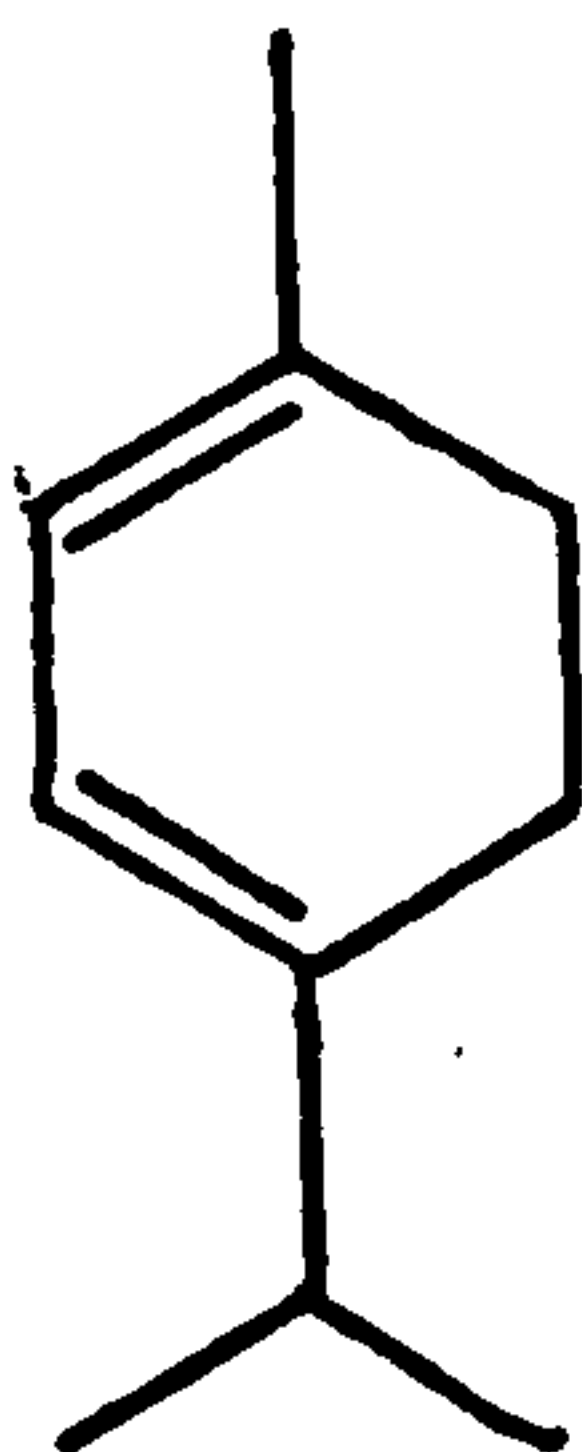
6. α -Phellendrene. Found as the natural product in Myrmicaria eumenoides, Trinervitermes geminatus and T. togoensis.



Mass spectrum: M^+ 136 (17); 93 (100), 91 (27), 92 (26), 77 (25), 136 (17), 94 (8), 41 (6), 119 (5).

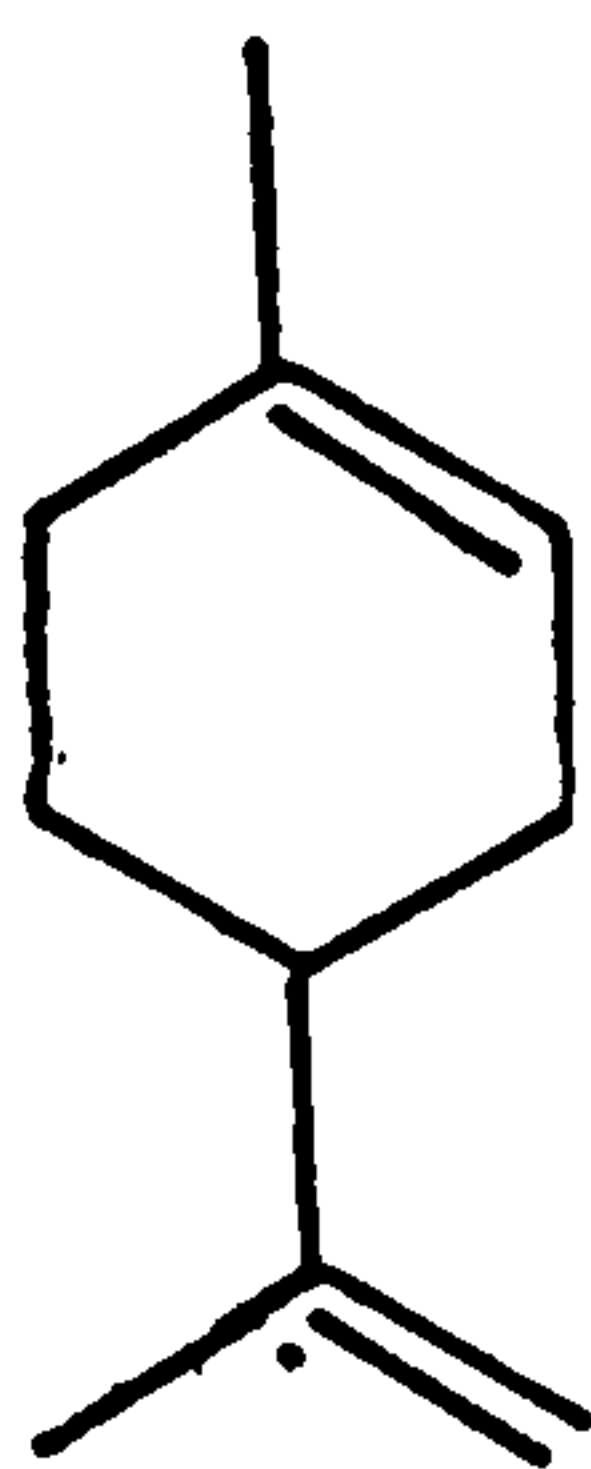
An authentic sample of α -phellendrene gave a mass spectrum congruent with the natural products, and co-eluted on columns A,B,D and E.

7. α -Terpinene. Found as the natural product in Myrmicaria eumenoides.



Mass spectrum: M^+ 136 (55); 121 (100), 93 (81), 136 (55), 119 (30), 105 (30), 79 (21), 91 (19), 134 (15). The mass spectrum of this natural product agreed with published mass spectra of α -terpinene (Rhyhage and von Sydow 1963).

8. Limonene. Found as the natural product in Myrmicaria eumenoides with an optical rotation of $(\alpha)_D^{22} + 118.5$. Found also as the natural product in Trinervitermes geminatus and T. togoensis.



Mass spectrum: M^+ 136 (20); 68 (100), 93 (50), 67 (36), 136 (20), 79 (19), 94 (17), 121 (17), 41 (16).

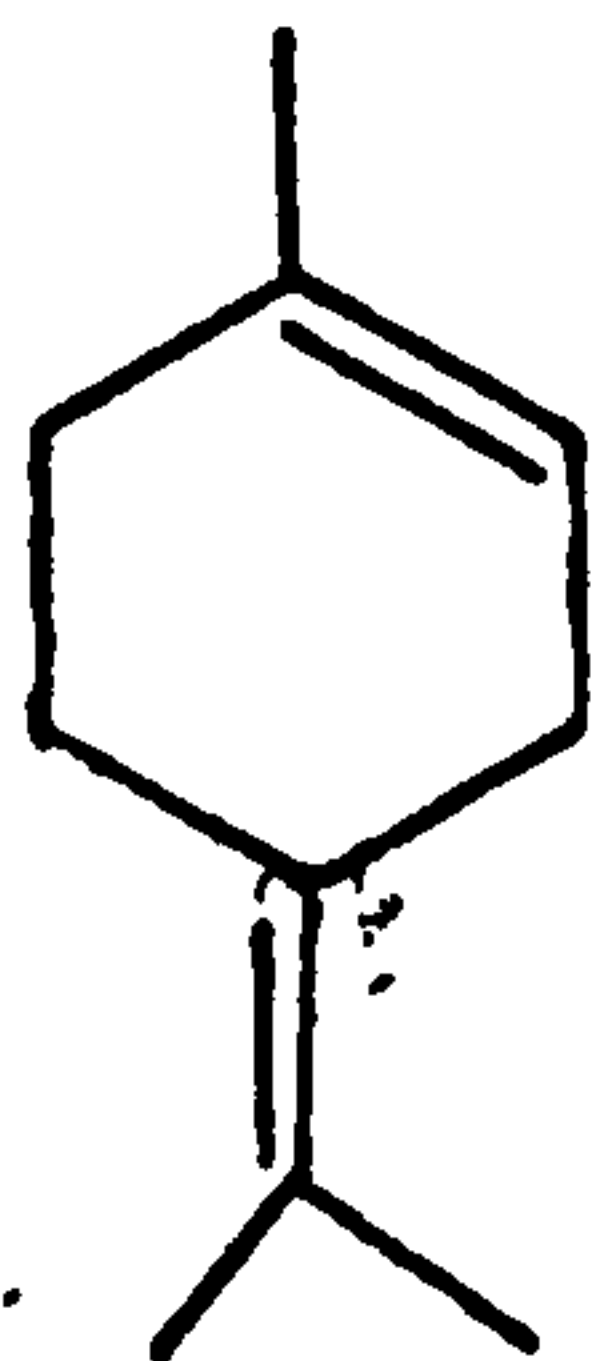
100 MHz NMR (M. eumenoides): ^1H NMR CDCl_3 ; δ 1.64 (3H, s, $-\text{CH}_3$); 1.75 (3H, s, $-\text{CH}_3$); ca. 1.90 (7H, methylene envelope); 4.73 (2H, s, $=\text{CH}_2$); 5.50 (1H, m, $-\text{CH}=$).

An authentic sample of limonene (Koch Light), with an optical rotation of $(\alpha)_D^{20} + 124$, gave a mass spectrum which was congruent with that of the natural products and co-eluted on GC columns A, B and E.

9. Terpinolene. Found as the natural product in Myrmicaria eumenoides.

Mass spectrum: M^+ 136 (60); 93 (100), 121 (87), 136 (60), 91 (56), 79 (43), 77 (41), 41 (40), 39 (40).

An authentic sample of terpinolene (May and Baker) gave a mass spectrum congruent with that of the natural product and co-eluted on columns A, B and E.



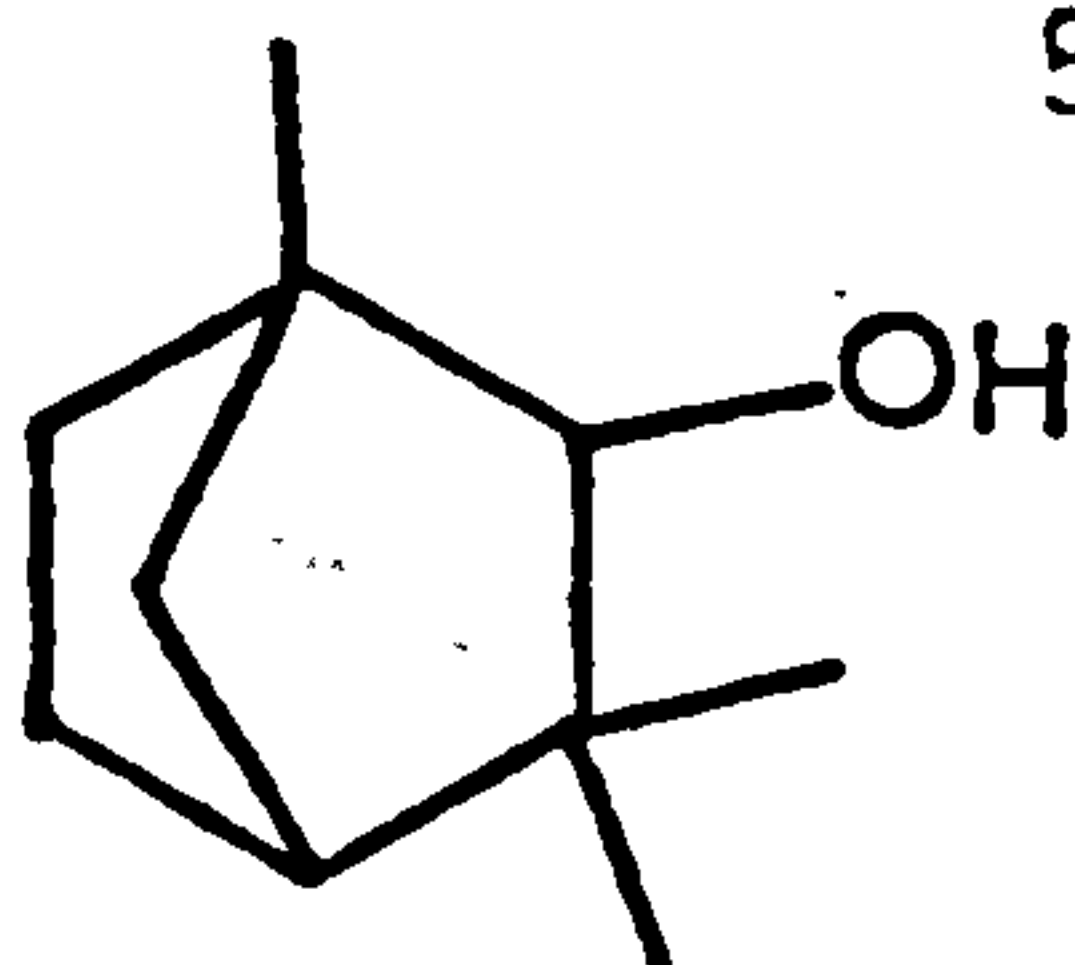
10. β -Phellendrene. Bioassayed as the pure compound, not found as the natural product

8.5.2. Monoterpene Alcohols.

Two monoterpene alcohols were found in Trinervitermes geminatus, full identity is not yet known.

11. Trinervitermes geminatus component 9 (Fig. 6.1)

Mass spectrum: M^+ 154 (3); 81 (100), 43 (64), 93 (46), 30 (45), 41 (35), 136 (32), 121 (29), 79 (22).

12. Trinervitermes geminatus, component 10 (Fig. 6.1).

Mass spectrum: M^+ 154 (0.1); 91 (100), 44 (99), 119 (57), 41 (56), 93 (56), 95 (49), 43 (49), 92 (43).

8.5.3. Organic Sulphur Compounds.

13. Dimethyl disulphide. Found as the natural product in Megaponera foetens.

Mass spectrum: M^+ 94 (100); 94 (100), 36 (91), 79 (50), 45 (46), 38 (29), 47 (23), 46 (27), 76 (24); 95 (3), 96 (9).

An authentic sample of dimethyl disulphide (Koch Light) gave a mass spectrum congruent with the natural product, and co-eluted on GC columns A, B and E.

14. Ethyl methyl disulphide. Found as the natural product in Megaponera foetens.

Mass spectrum: M^+ 108 (100); 108 (100), 80 (89), 45 (49), 47 (33), 76 (31), 94 (28), 79 (26), 46 (26); 109 (5), 110 (10).

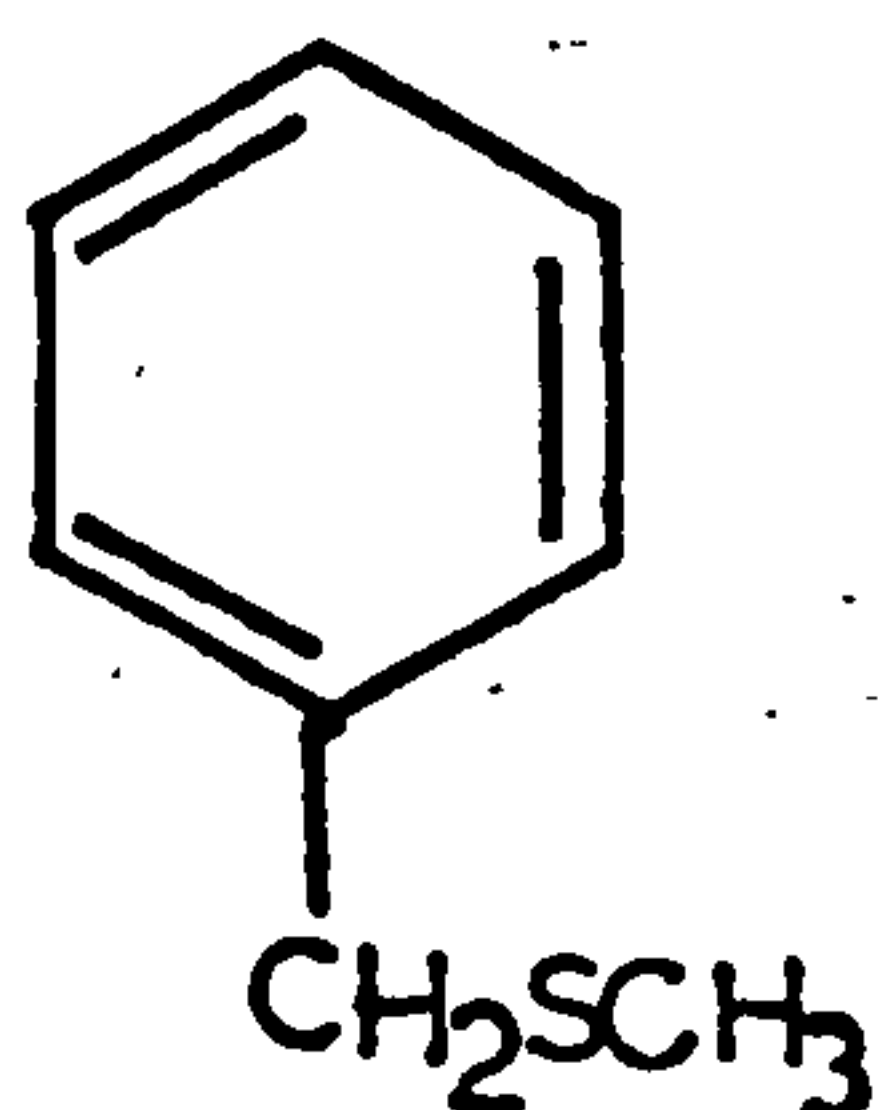
The mass spectrum of this natural product suggested that it was ethyl methyl disulphide, although no published mass spectra could be found for this compound.

15. Dimethyl trisulphide. Found as the natural product in Megaponera foetens.

Mass spectrum: M^+ 126 (100); 126 (100), 79 (40), 45 (36), 47 (31), 76 (20), 46 (16), 111 (14), 48 (14); 127 (4), 128 (12).

A synthetic sample of dimethyl trisulphide (8.4.5) gave a mass spectrum congruent with that of the natural product, and co-eluted on columns A,B and E.

16. Benzyl methyl sulphide. Found as the natural product in Megaponera foetens.



Mass spectrum: M^+ 138 (88); 91 (100), 138 (88), 76 (48), 45 (30), 47 (24), 36 (23), 44 (22), 46 (21); 139 (4), 140 (12).

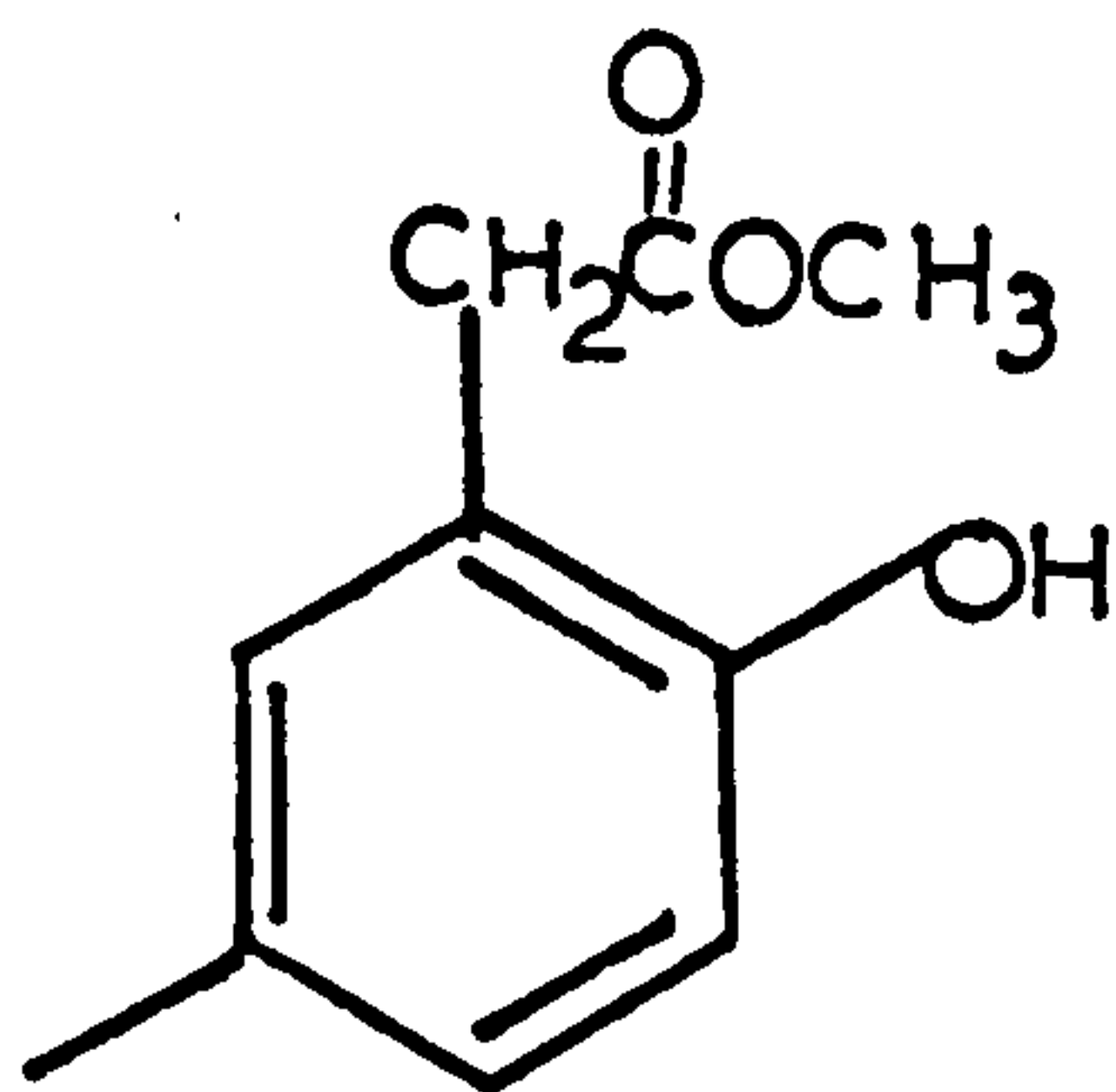
A synthetic sample (8.4.4.) of benzyl methyl sulphide gave a mass spectrum congruent with the natural product, and co-eluted on columns A,B,D and E.

17. "Digging Pheromone". Megaponera foetens, component 13.

Although there is no evidence that this is a sulphur containing compound, this is a convenient place to list it. No unambiguous mass spectrum was obtained; if the temperature in the line between the GC and the mass spectrometer was too high the compound appears to have degraded, as a number of differing spectra were obtained. At lower temperatures the compound stuck in the line and no spectra could be obtained.

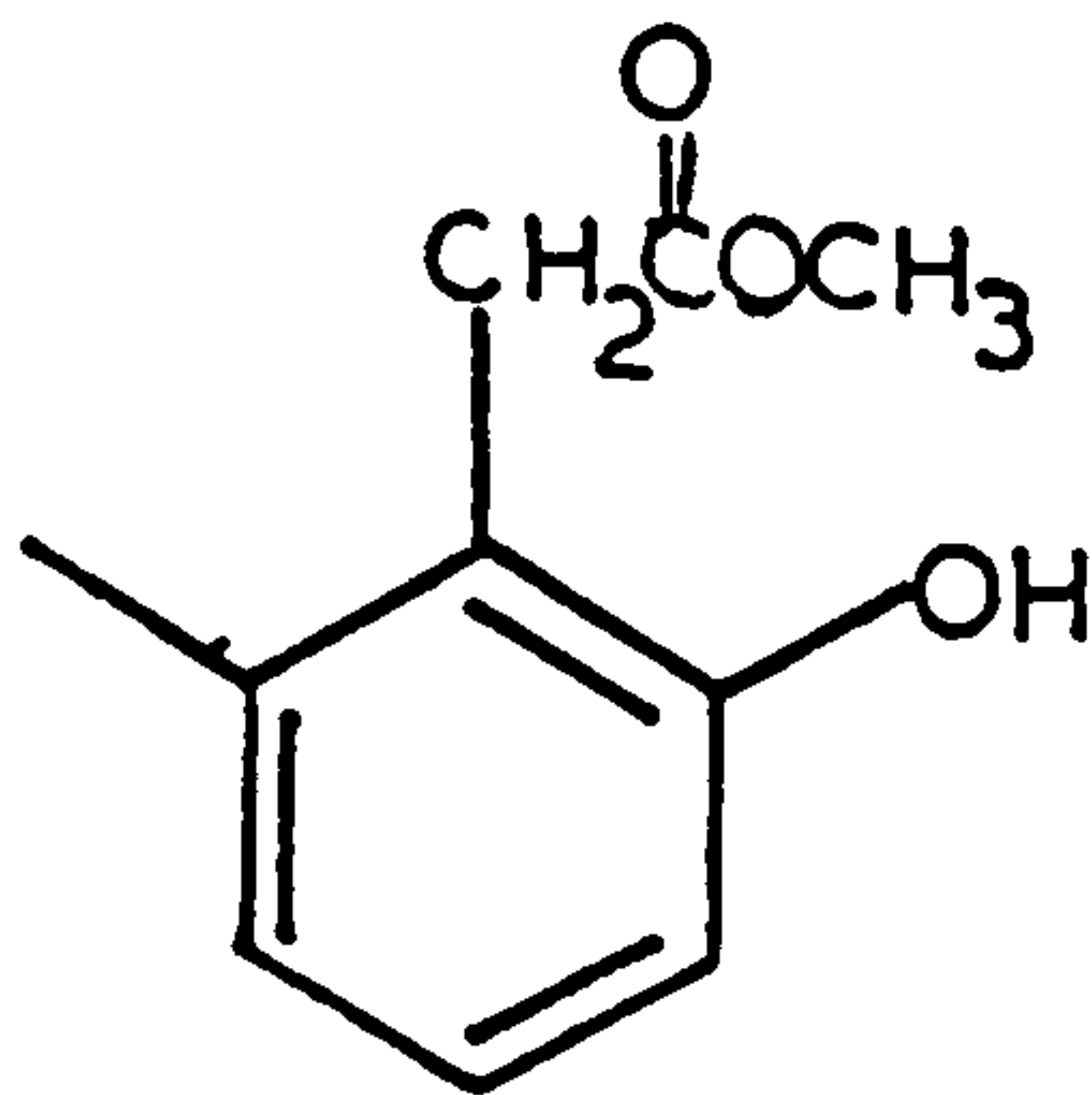
8.5.4. Methyl Salicylates.

18. Methyl-5-methyl salicylate. A synthetic sample of this compound (8.4.6.) gave a mass spectrum congruent with



component 21 of Bothroponera soror, but did not co-elute with the natural product on GC columns. Mass spectrum: M^+ 166 (38); 134 (100), 166 (38), 106 (28), 45 (26), 135 (20), 77 (18), 105 (14), 73 (9).

19. Methyl-6-methyl salicylate. Found as the natural product in Bothroponer soror.



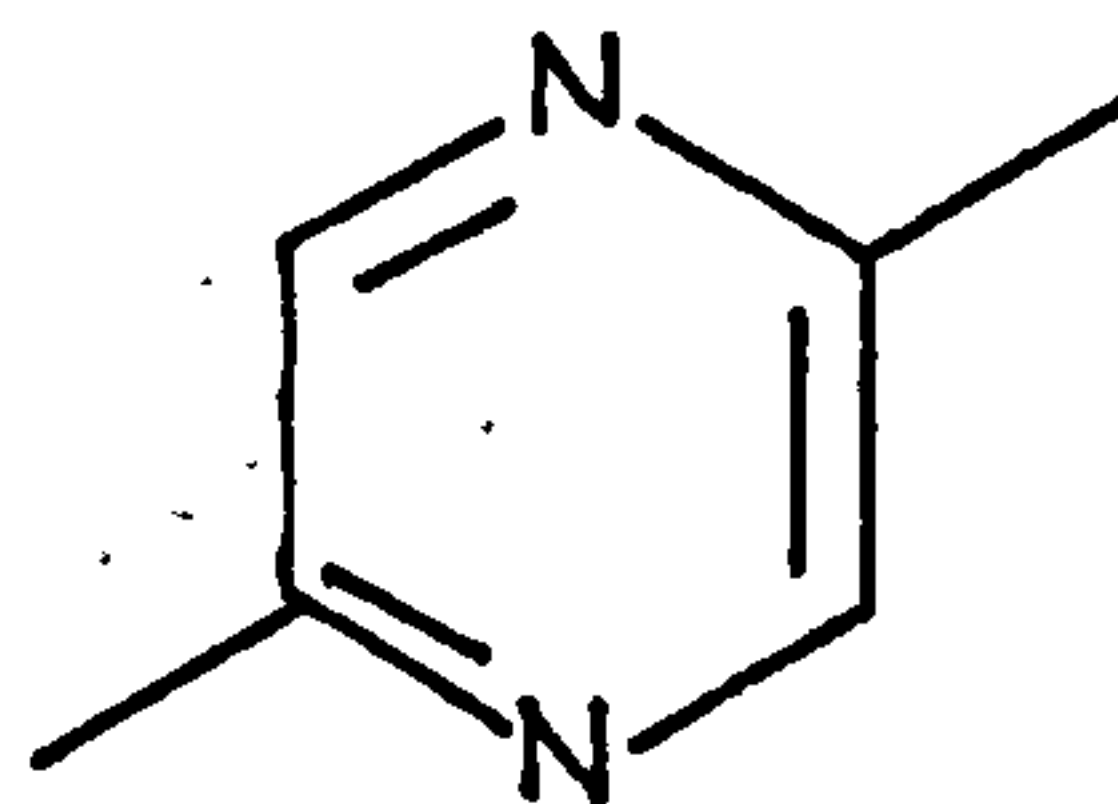
Mass spectrum: M^+ 166 (44); 134 (100), 166 (44), 106 (27), 45 (24), 135 (22), 78 (17), 77 (16), 105 (13).

It has not been possible to obtain an authentic sample of methyl-6-methyl salicylate, which has been found previously in the ponerine, Gnamptogenys pleurodon (Duffield and Blum 1975) and the males of formicine Camponotus spp. (Brand et al. 1973); in view of these findings the natural product in B. soror has been tentatively identified as the methyl-6 compound.

3.5.5. Alkyl Pyrazines.

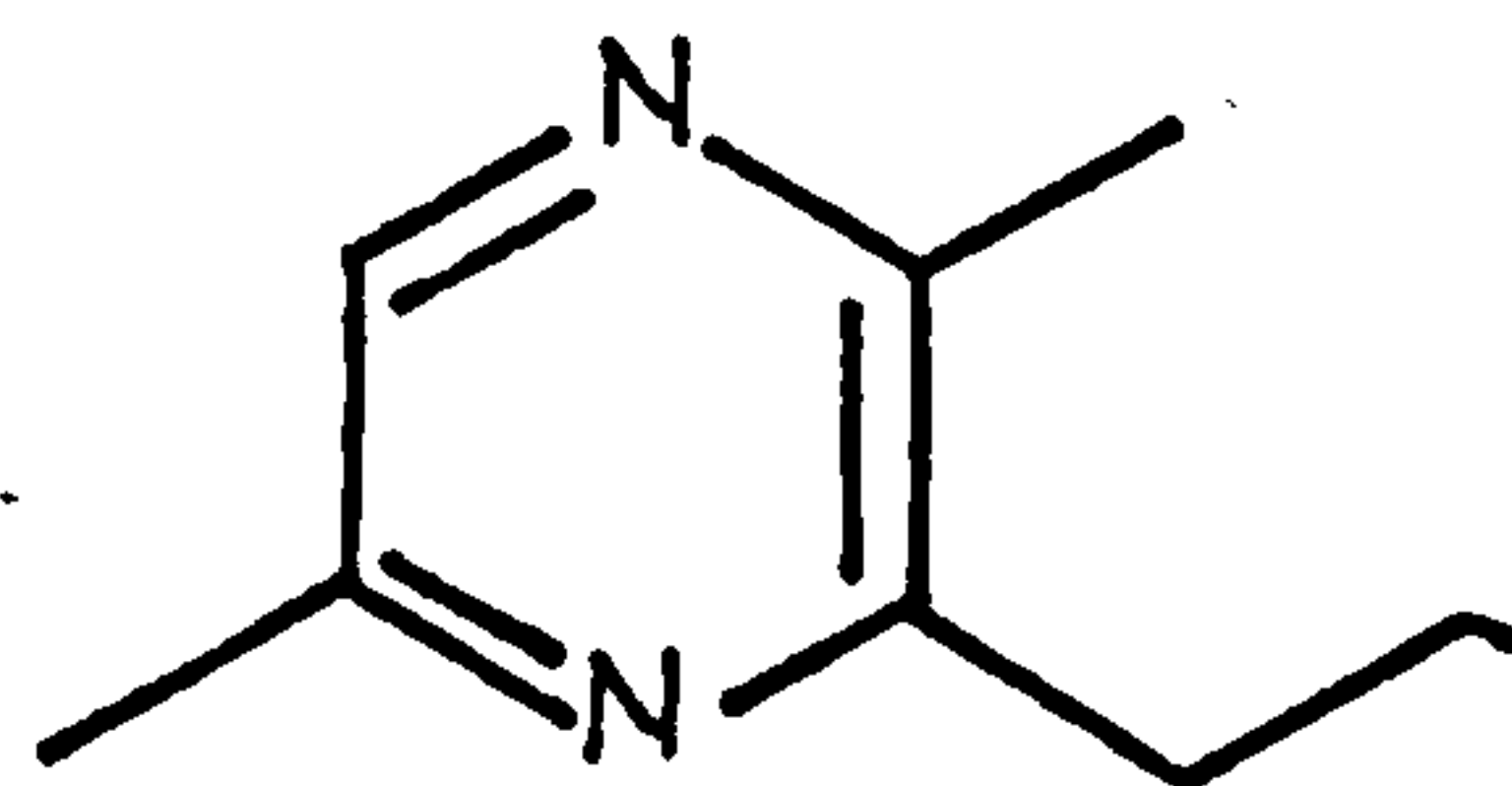
The following alkyl pyrazines were not found as natural products, but were assayed in repellency studies and used in co-elution identification studies.

20. 2,5 - Dimethyl pyrazine.

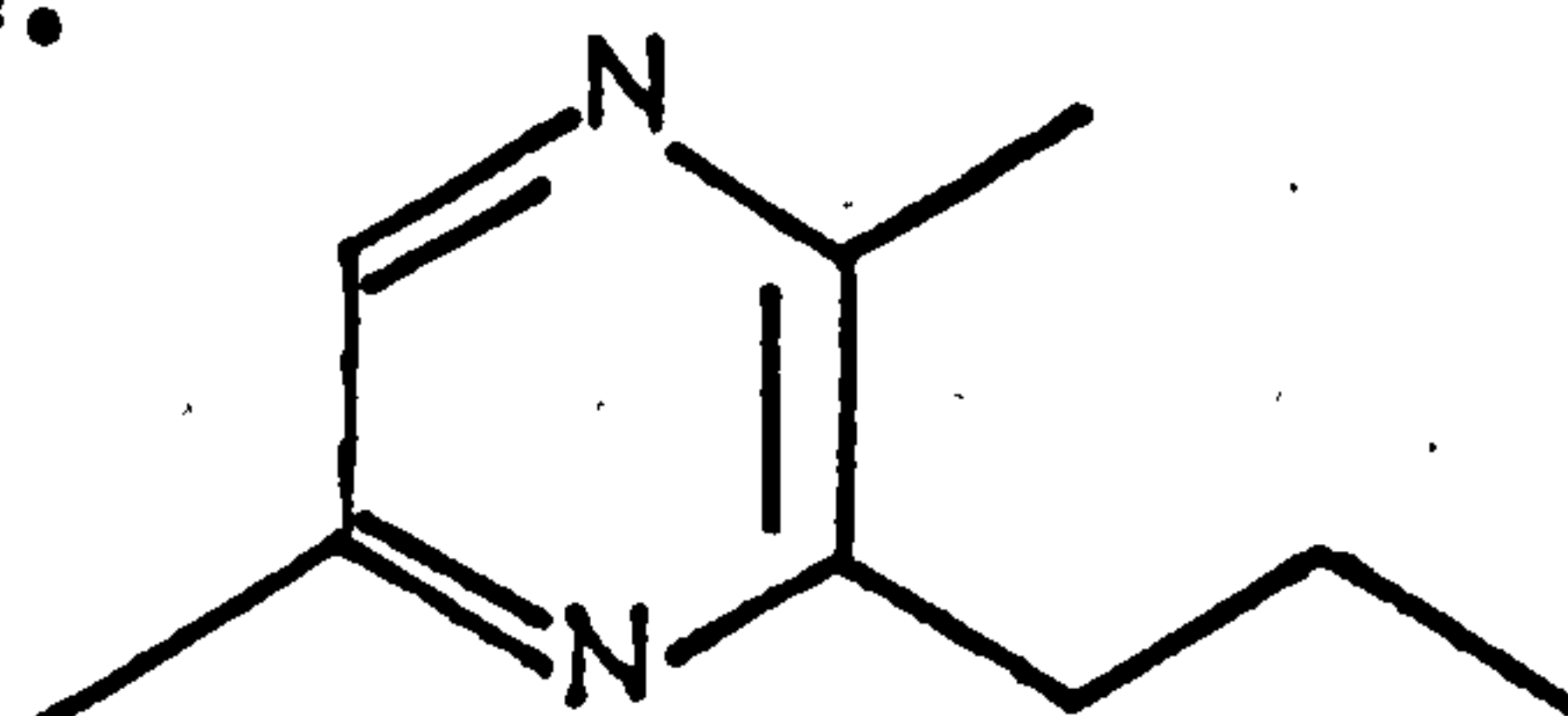


21. 2,5 -Dimethyl-3-ethyl pyrazine.

Synthetic sample supplied by W.A.Speed, Chemical Entomology Unit.

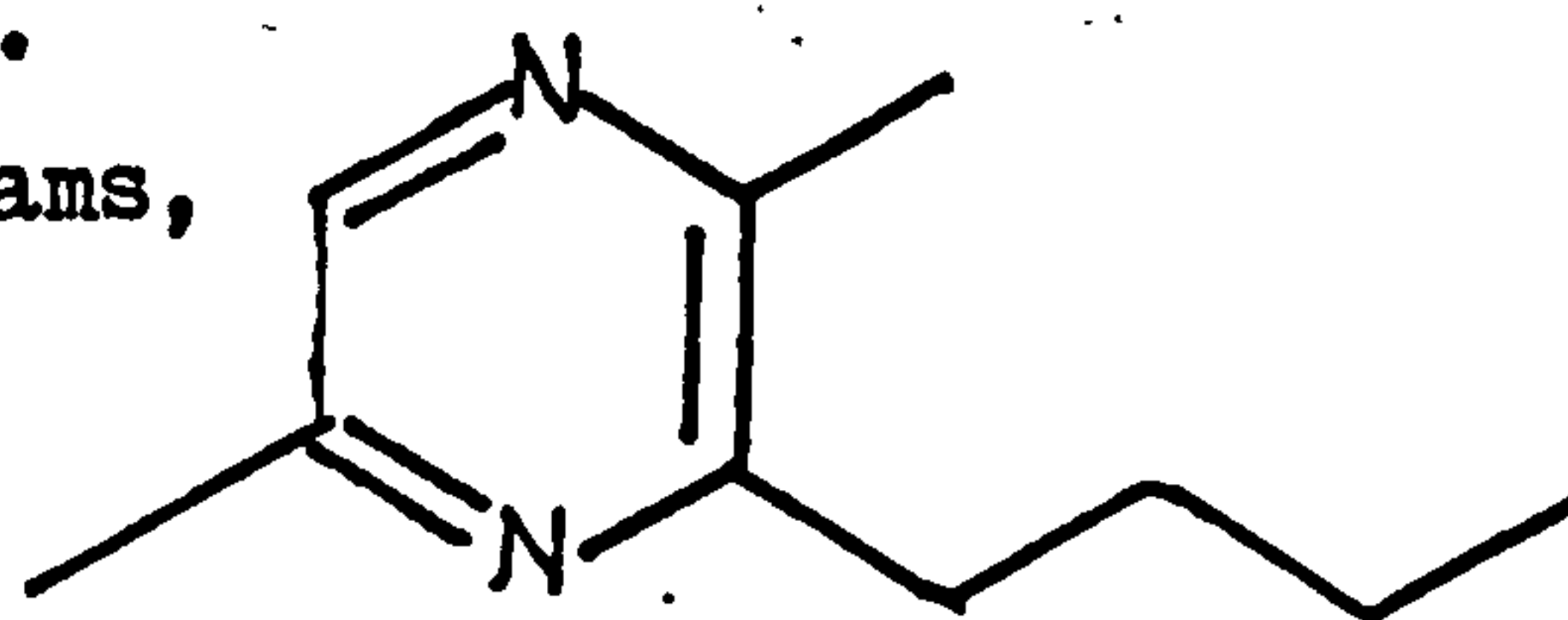


22. 2,5 -Dimethyl-3-n-propyl pyrazine.

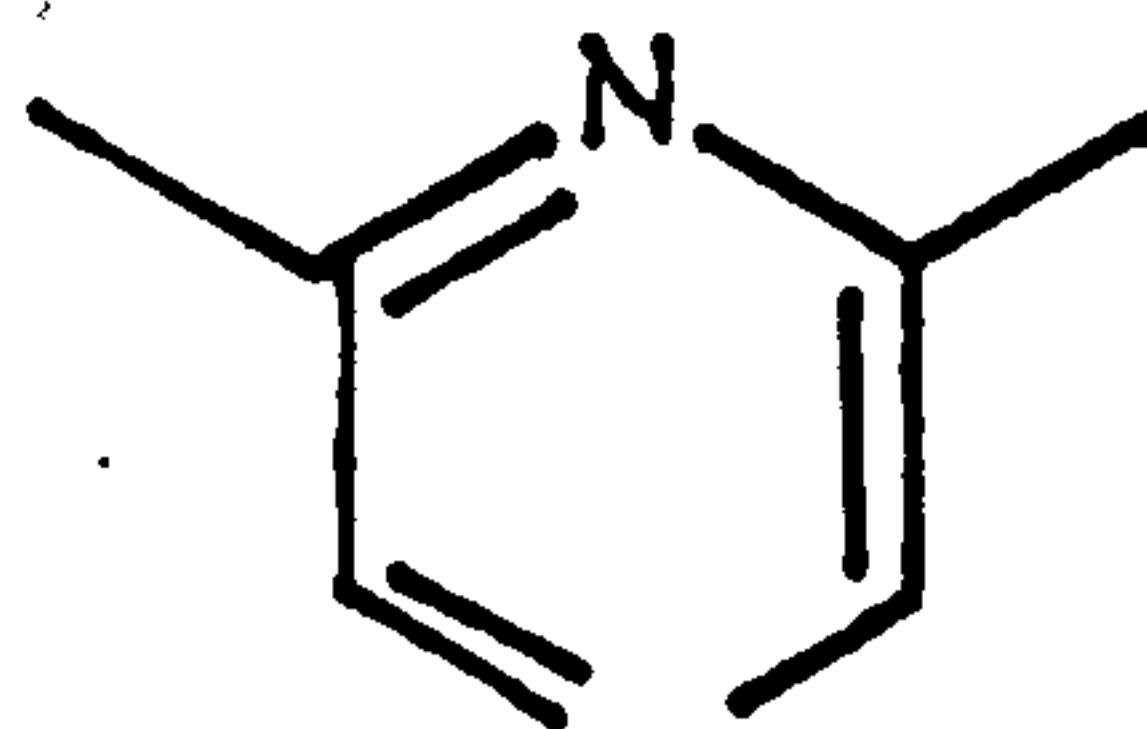


23. 2,5- Dimethyl-3-n-butyl pyrazine.

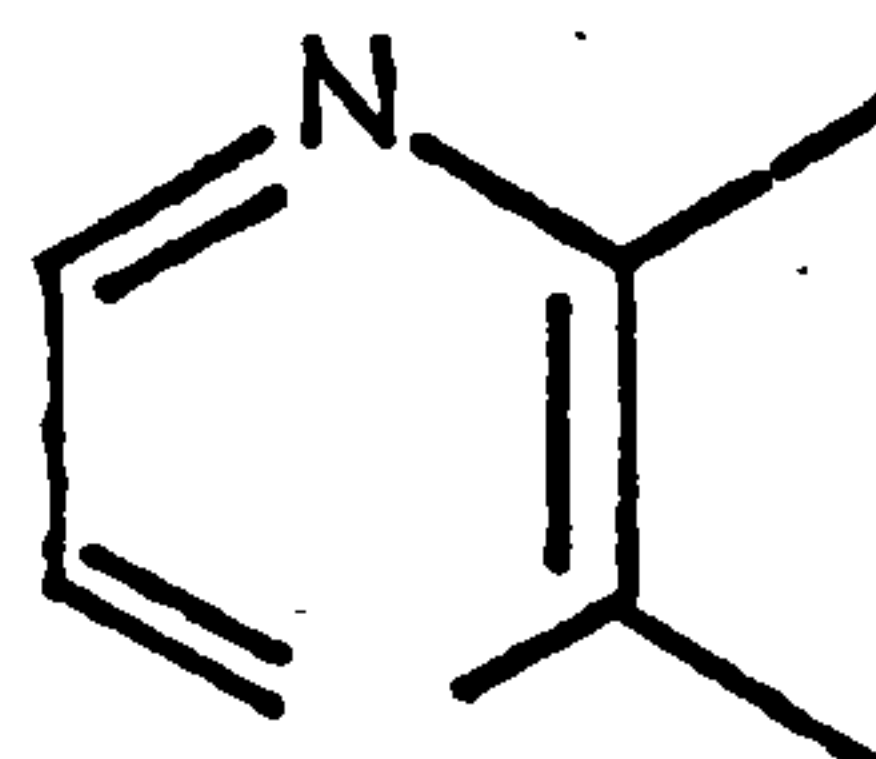
Synthetic sample supplied by L.J.Wadhams, Chemical Entomology Unit.



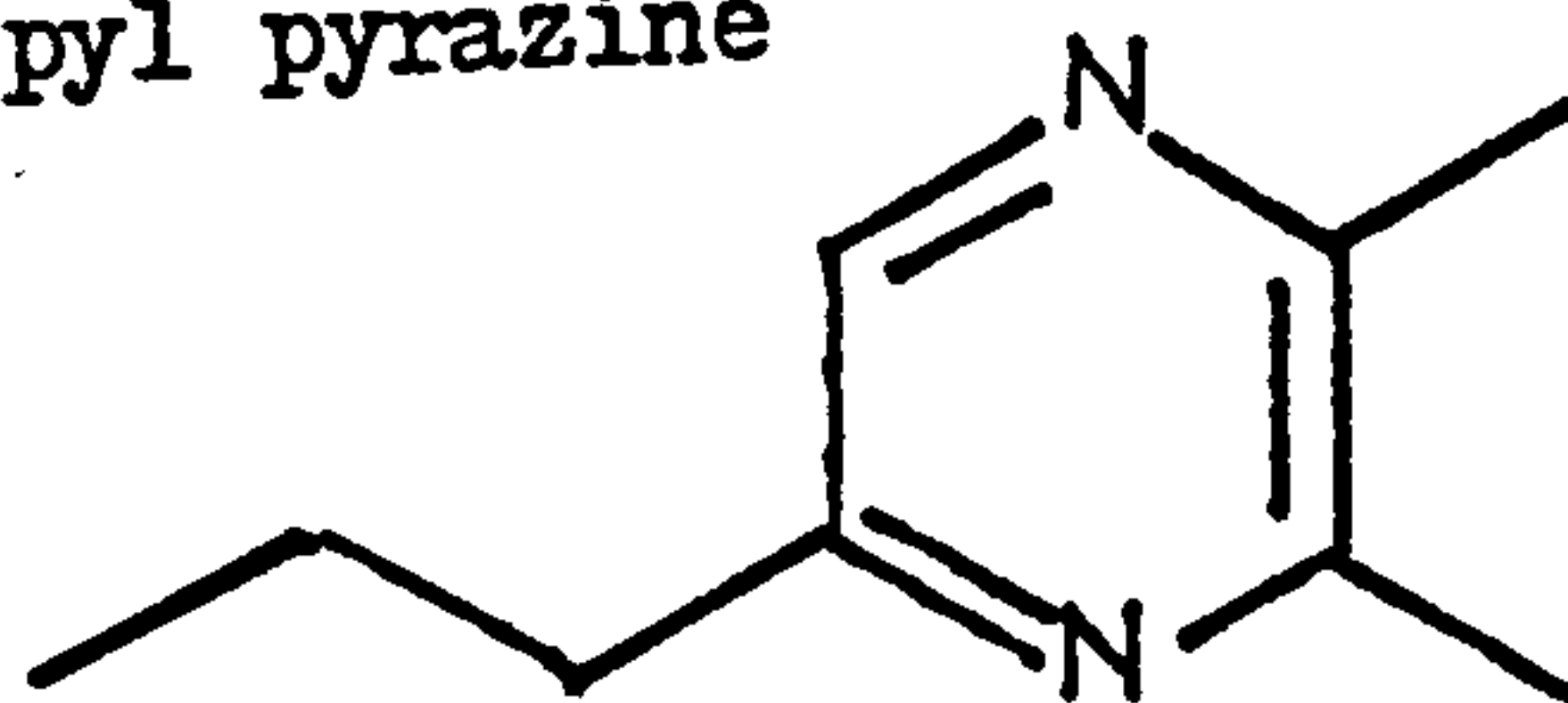
24. 2,6 -Dimethyl pyrazine.



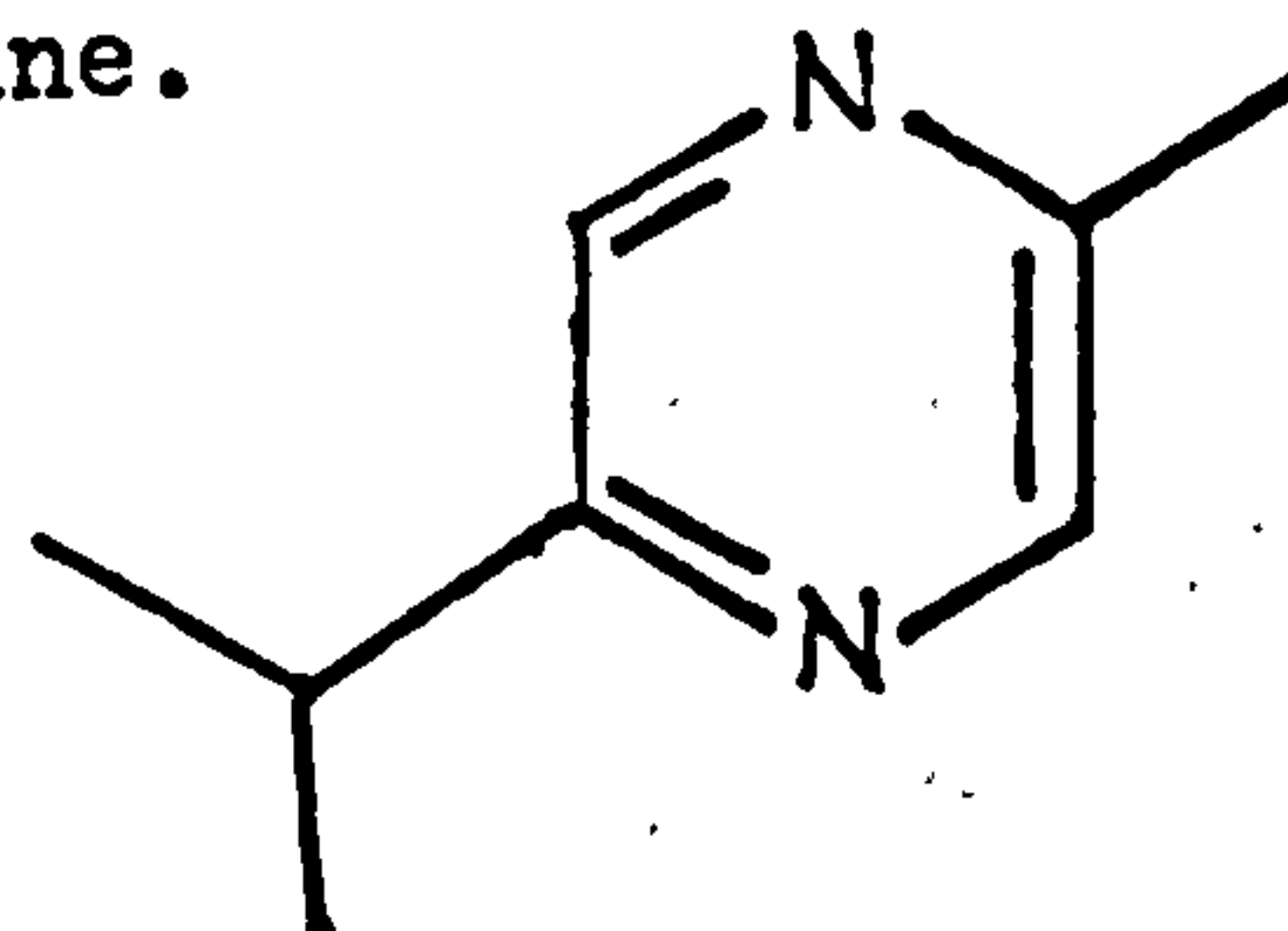
25. 2,3 -Dimethyl pyrazine.



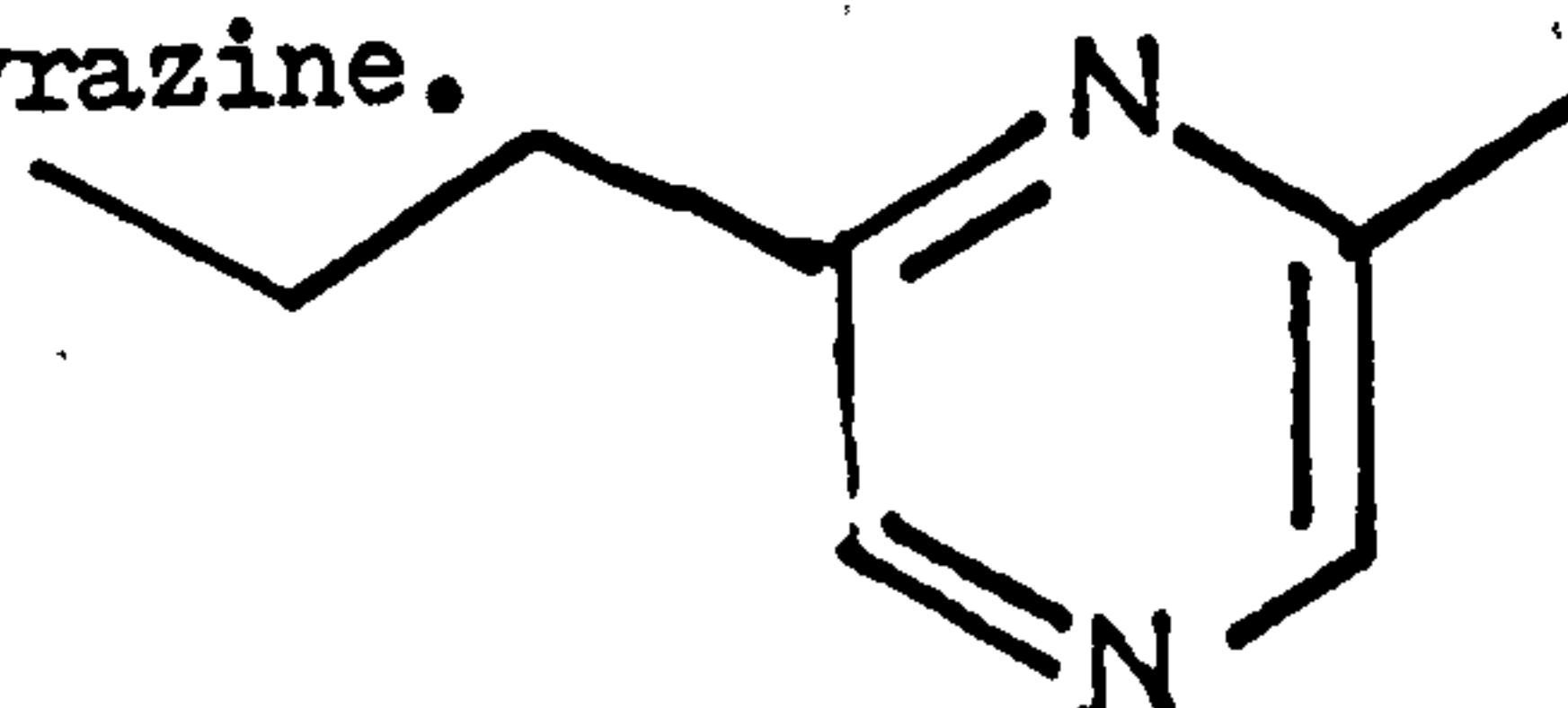
26. 2,3 -Dimethyl-5-iso-propyl pyrazine



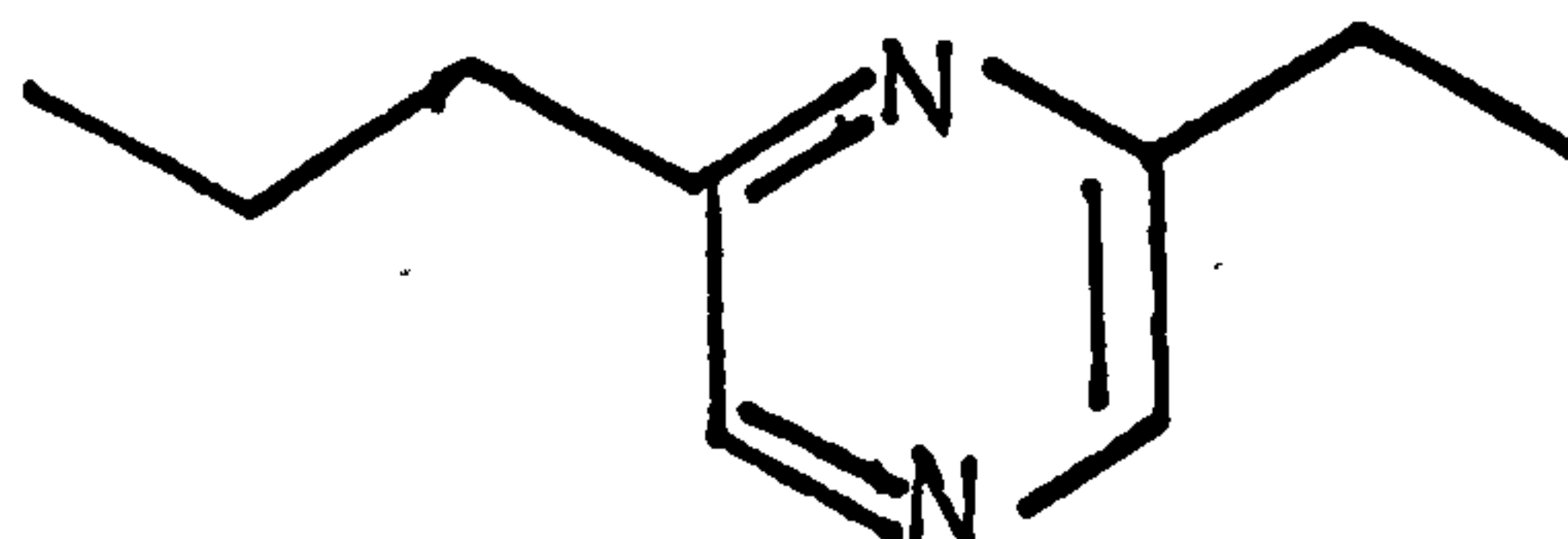
27. 2 -Methyl-5-n-propyl pyrazine.



28. 2 -Methyl-6-n-propyl pyrazine.



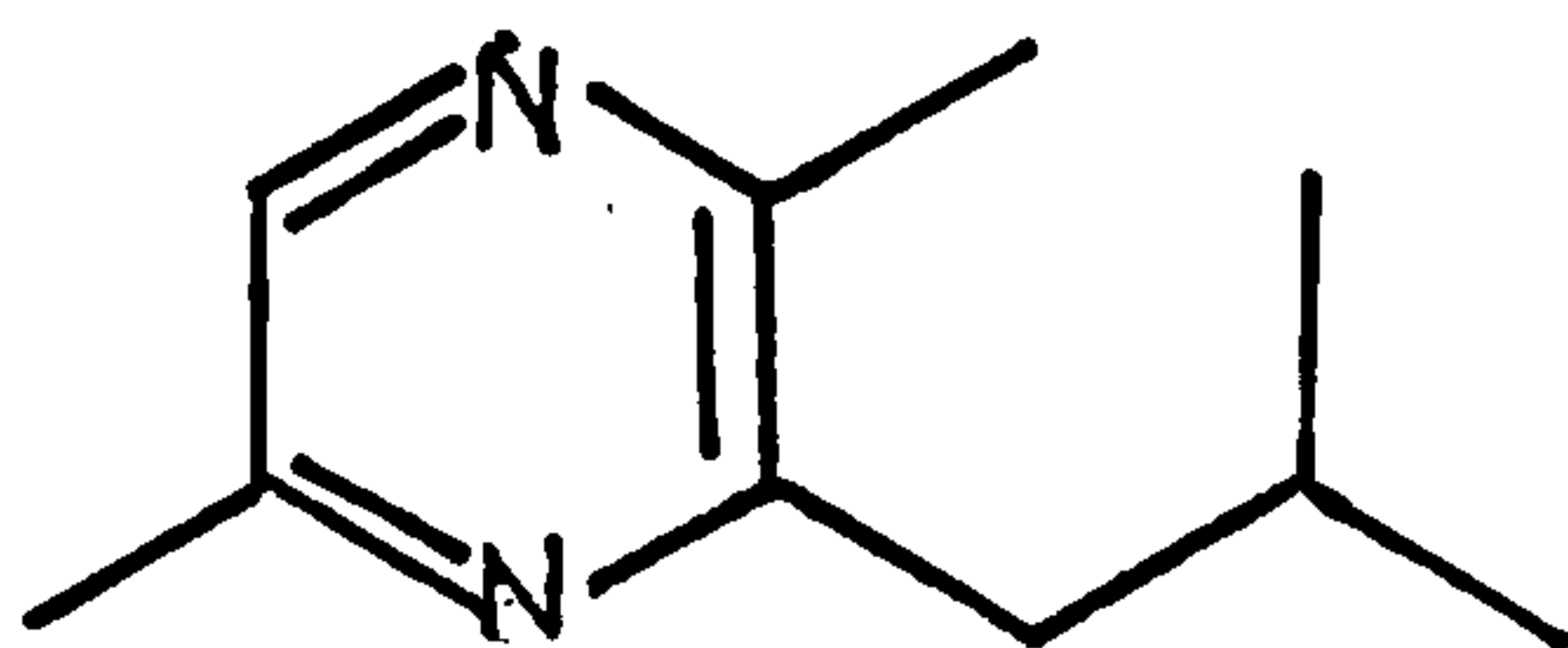
29. 2 -Ethyl-6-n-propyl pyrazine.



The following 2,5 and 2,6-dimethyl pyrazines were found as natural products in either Anochetus sedilloti, Odontomachus troglydytes or Brachyponera sennaarensis.

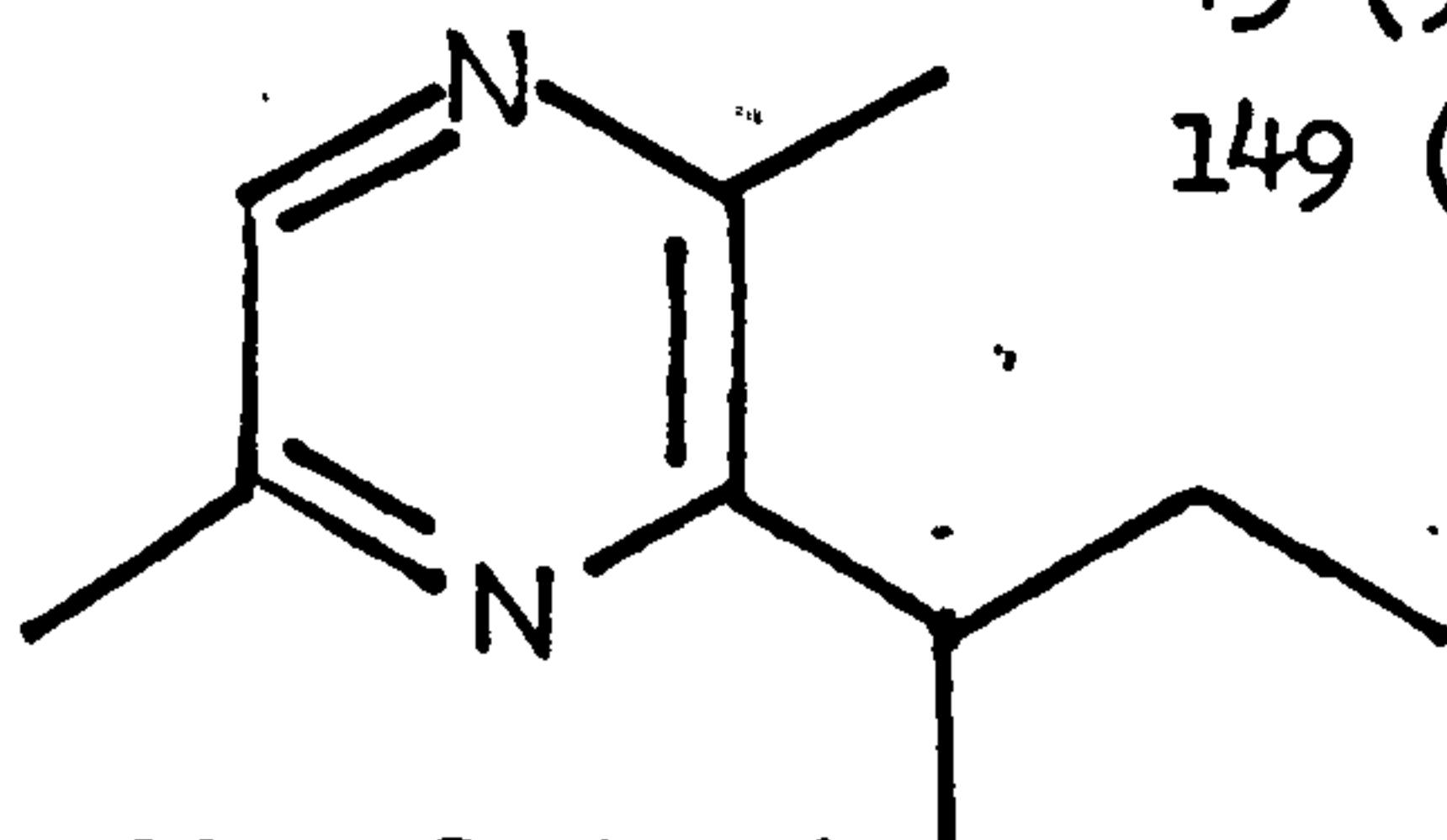
30. 2,5 -Dimethyl-3-iso-butyl pyrazine. Found as the natural product in Anochetus sedilloti.

Mass spectrum: M^+ 164 (4); 122 (100), 39 (17), 42 (16), 41 (15), 44 (13), 149 (10), 77 (10), 123 (7); 135 (1).



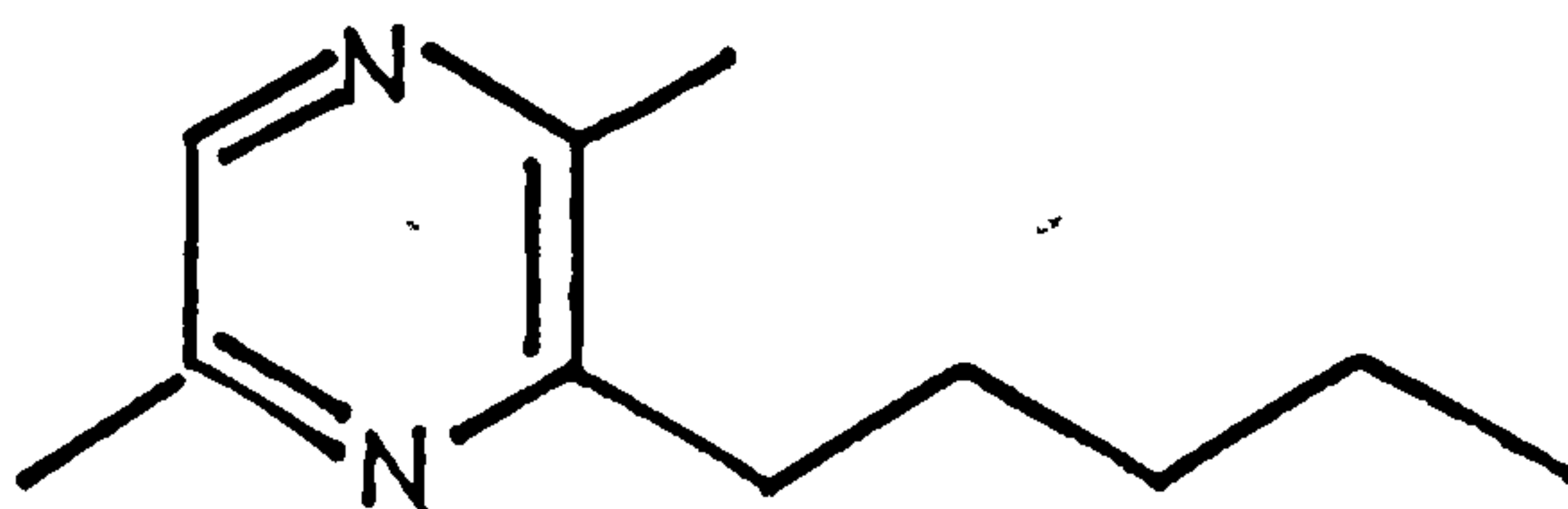
31. 2,5 -Dimethyl-3-sec-butyl pyrazine. Found as the natural product in Anochetus sedilloti.

Mass spectrum: M^+ 164 (6); 122 (100), 39 (10), 45 (9), 135 (8), 41 (8), 163 (7), 123 (7), 121 (6); 149 (2).



32. 2,5 -Dimethyl-3-n-pentyl pyrazine. Found as the natural product in Anochetus sedilloti.

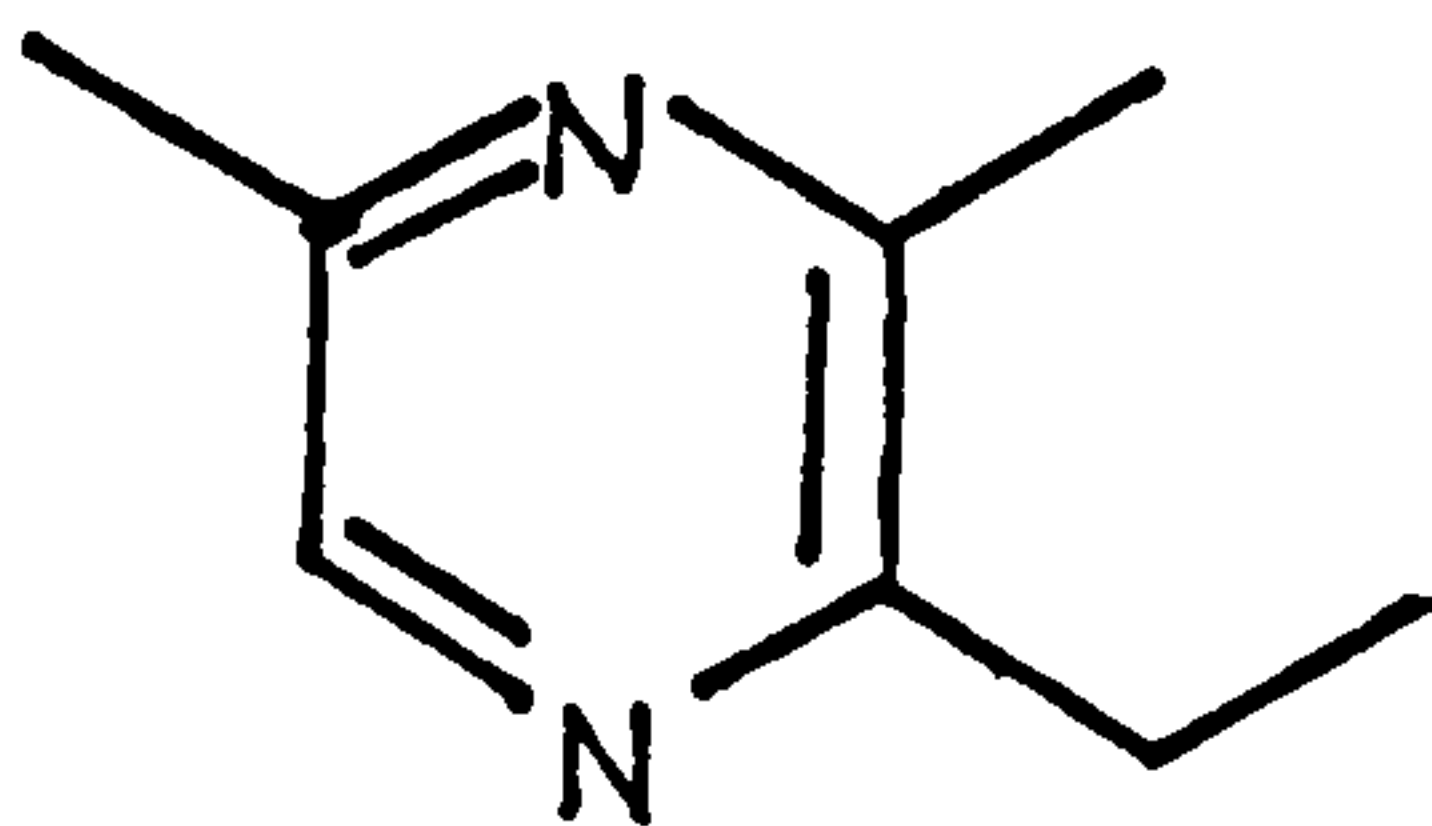
Mass spectrum: M^+ 178 (0.5); 122 (100), 41 (25), 44 (21), 39 (15), 69 (15), 69 (14), 135 (11), 123 (11); 149 (6), 164 (2).



A synthetic sample of this compound gave a mass spectrum congruent with the natural product and co-eluted on columns B,C and D.

33. 2,6 -Dimethyl-3-ethyl pyrazine. Found as the natural product in Odontomachus troglydytes.

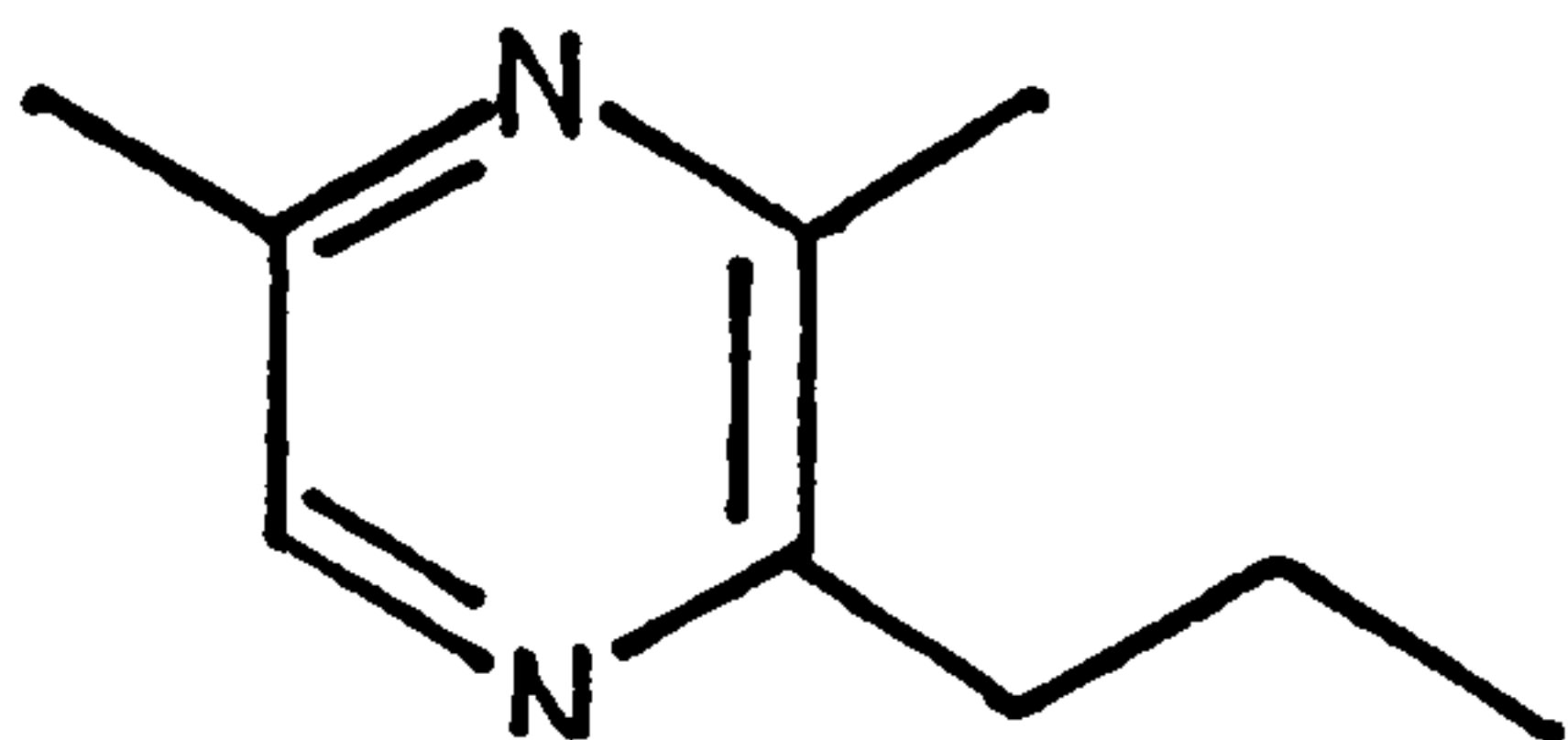
Mass Spectrum: M^+ 136 (79); 135 (100), 136 (79), 56 (50), 108 (43), 42 (30), 41 (18), 44 (17).



A synthetic sample (supplied by W.A.Speed, Chemical Entomology Unit) gave a mass spectrum congruent with the natural product, and co-eluted on GC columns B and C.

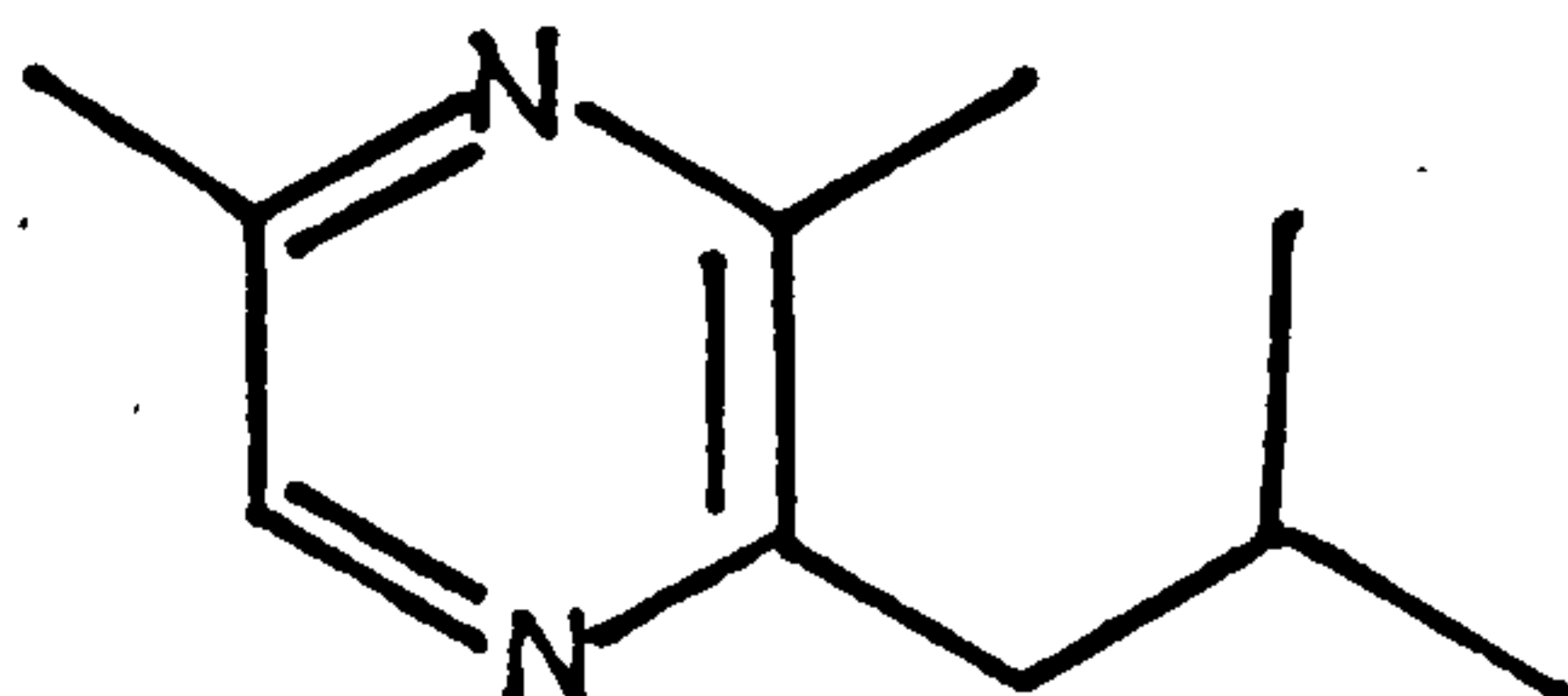
34. 2,6 -Dimethyl-3-n-propyl pyrazine. Found as the natural product in Odontomachus troglydytes.

Mass spectrum: M^+ 150 (9); 122 (100), 45 (68), 73 (31), 44 (29), 43 (27), 60 (23), 135 (14), 59 (14); 149 (4).



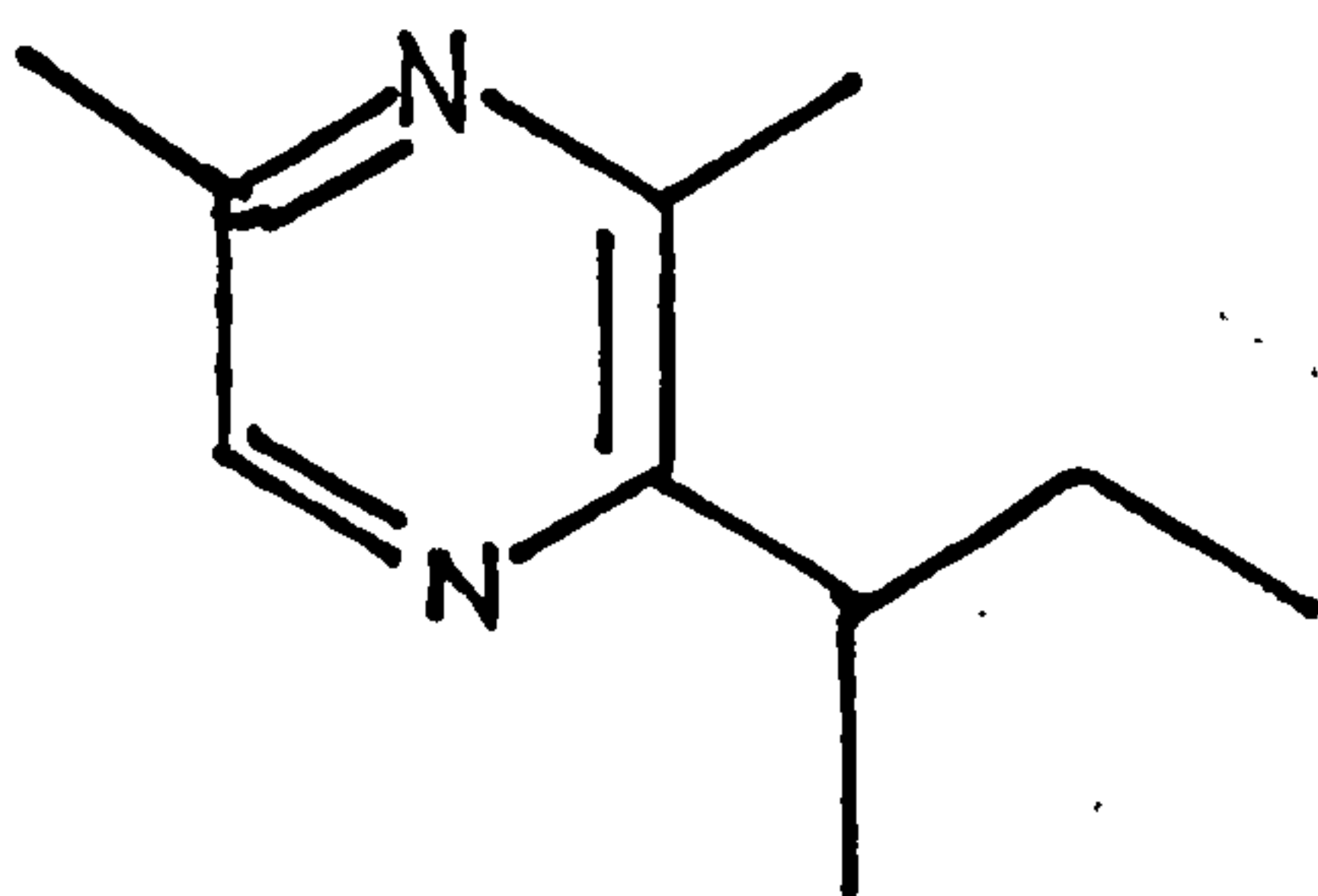
35. 2,6 -Dimethyl-3-iso-butyl pyrazine. Found as the natural product in Anochetus sedilloti.

Mass spectrum: M^+ 164 (2); 122 (100), 39 (18), 41 (16), 42 (14), 44 (14), 77 (7), 149 (6), 123 (6); 135 (1.5).



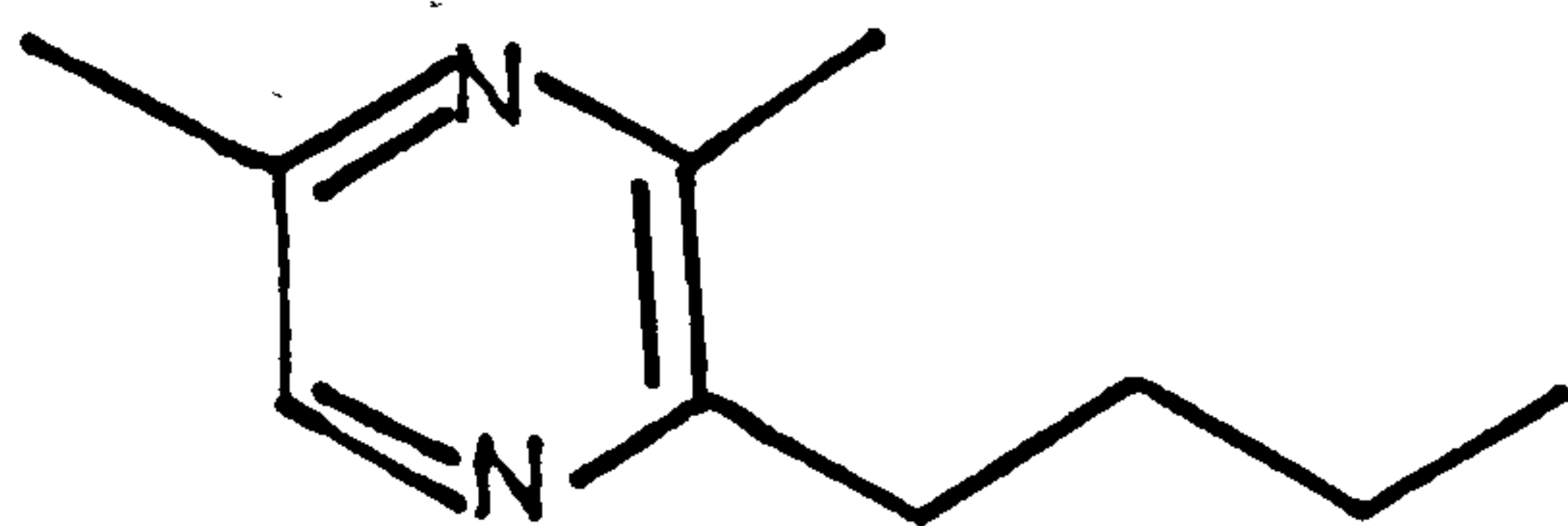
36. 2,6 -Dimethyl-3-sec-butyl pyrazine. Found as the natural product in Anochetus sedilloti.

Mass spectrum: M^+ 164(1); 122 (100), 39 (10), 42 (9), 135 (8), 41 (8), 123 (7), 121 (5), 53 (5).



37. 2,6 -Dimethyl-3-n-butyl pyrazine. Found as the natural product in Anochetus sedilloti, Odontomachus troglydytes and Brachyponera sennaarensis.

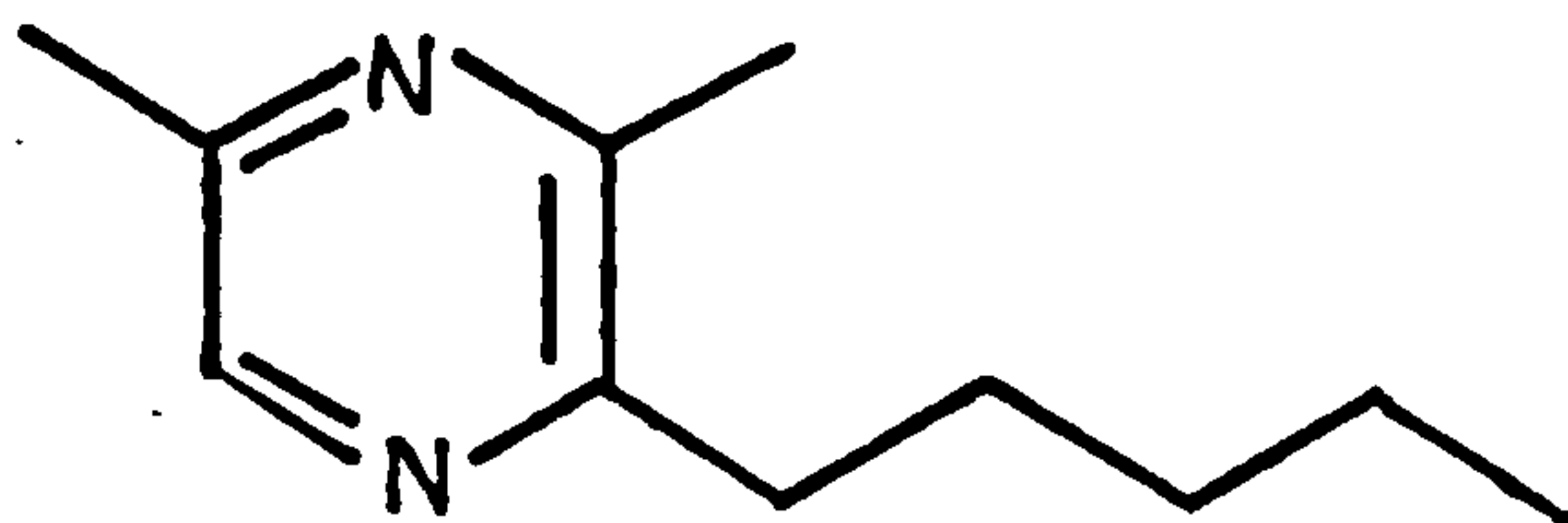
Mass spectrum: M^+ 164 (1); 122 (100), 39 (14), 42 (14), 135 (9), 121 (8), 148 (8), 41 (7), 53 (5).



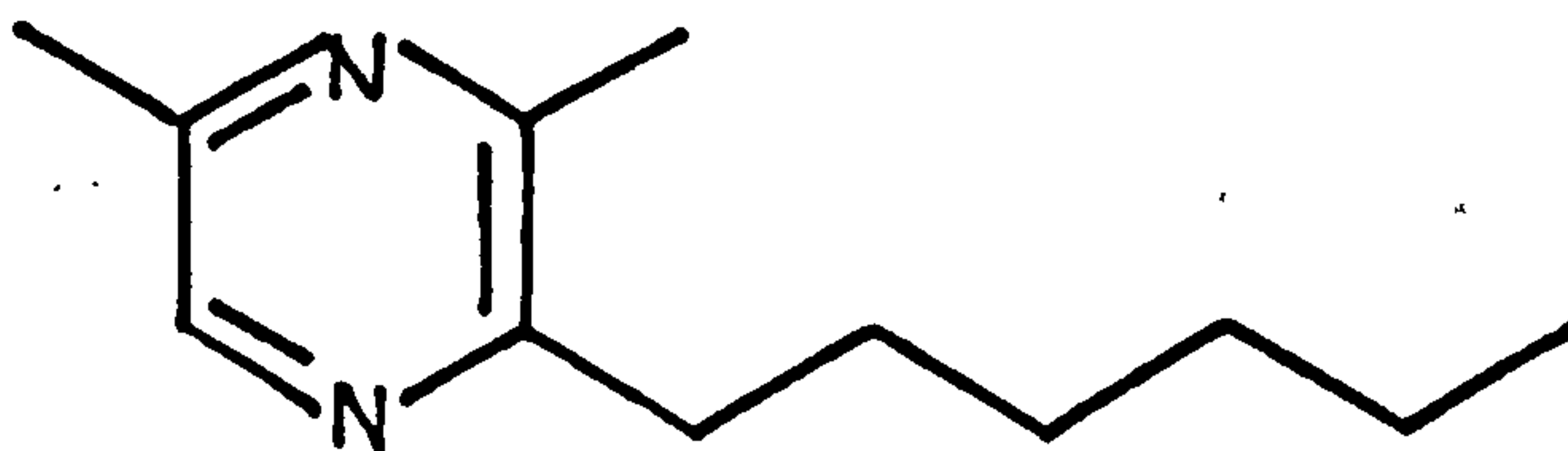
A synthetic sample (supplied by W.A.Speed, Chemical Entomolgy Unit) gave a mass spectrum congruent

with the natural products and co-eluted on columns B and C.

38. 2,6 -Dimethyl-3-n-pentyl pyrazine. Found as the natural product in Odontomachus troglydytes and Brachyponera sennaarensis. Mass spectrum: M^+ 178 (2); 122 (100), 45 (24), 39 (14), 44 (13), 43 (12), 134 (12), 41 (11), 52 (7).



39. 2,6 -Dimethyl-3-n-hexyl pyrazine. Found as the natural product in Odontomachus troglydytes and Brachyponera sennaarensis. Mass spectrum: M^+ 192 (0.7); 122 (100), 45 (81), 59 (75), 44 (62), 43 (45), 41 (43), 58 (41), 52 (11).



8.5.6. Aliphatic Ketones. (Figure 8.2)

40. 2-Hexanone. Used as a high purity commercial sample (Koch Light); GC > 99%.

41. 2-Octanone. Used as a high purity commercial sample (Koch Light); GC > 99%.

42. 2-Nonanone. The commercial sample (Koch Light) was only 80% pure (GC), the sample was purified by preparative GC on column G, and trapped in dichloromethane.

43. 2-Decanone. Used as a high purity commercial sample (Koch Light); GC > 99%

44. 2-Undecanone. Found as the natural product in Bothroponera soror, Tetramorium sp.D and T.angulinode.

Mass Spectrum: M^+ 170 (11); 58 (100), 43 (80), 45 (41), 59 (34), 71 (32), 41 (25), 44 (13), 57 (11).

An authentic sample (Koch Light) gave a mass spectrum congruent with that of the natural product, and co-eluted on columns B,C and D.

45. 2-Dodecanone. Used as a high purity commercial sample (Koch Light); GC > 99%.

46. 3-Octanone. Found as the natural product in Crematogaster spp. A,B,C,D, and E, Pheidole sp.A, Tetramorium sericeiventre, T. guineense and T.caespitum.

Mass spectrum: M^+ 128 (8); 43 (100), 57 (90), 72 (55), 99 (52), 71 (52), 41 (31), 55 (11), 44 (9).

An authentic sample of 3-octanone (Koch Light) gave a mass spectrum congruent with the natural products and co-eluted on columns B and C.

47. 3-Decanone. Used as a high purity commercial sample (Koch Light); GC > 99%.

48. 6-Methyl-3-octanone. Found as the natural product in Crematogaster spp. A,B,C,D and E. and Pheidole sp.A.

Mass spectrum: M^+ 142 (5); 57 (100), 43 (60), 72 (48), 95 (43), 41 (38), 70 (22), 113 (17), 55 (15).

A synthetic sample (supplied by P.H.Briner, Chemical Entomology Unit) co-eluted with the natural products on columns B and C.

49. 4-Heptanone. Used as a high purity commercial sample (Koch Light); GC > 99%.

8.5.7. Aliphatic Alcohols. (Figure 8.3)

50. 1-Heptanol. A commercial sample (Koch Light) was only 91% pure (GC), and was purified by preparative GC on column G into dichloromethane.

51. 1-Octanol. Used as a high purity commercial sample (Koch Light); GC > 99%.

52. 1-Nonanol. Used as a high purity commercial sample (Koch Light); GC > 99%.

53. 2-Undecanol. Found as the natural product in Bothroponera soror, Tetramorium sp.D, T.angulinode and T.termitobium.

Mass spectrum: M^+ 172 (0.0); 45 (100), 43 (65), 41 (61), 55 (58), 57 (47), 84 (41), 69 (40), 56 (40); 154 (10).

A synthetic sample (8.4.1.) of racemic 2-undecanol gave a mass spectrum congruent with that of the natural products, and co-eluted on columns B, C and D.

54. 2-Undecenol (?). Two related compounds were found in Bothroponera soror whose mass spectra implied that they were 2-undecenols, differing in the position of the double bond.

Bothroponera soror:

Component 14, M^+ 170 (11); 45 (100), 41 (65), 55 (58), 67 (52), 82 (52), 95 (45), 81 (40), 69 (41).

Component 15, M^+ 170 (5); 45 (100), 41 (59), 55 (53), 67 (51), 82 (48), 95 (45), 81 (40), 69 (37).

55. 2-Dodecanol. Found as the natural product in Bothroponera soror. Mass spectrum: M^+ 186 (0.0); 45 (100), 43 (49), 41 (39), 55 (35), 57 (32), 69 (29), 83 (27), 56 (27); 168 (12).

A synthetic sample (8.4.2.) gave a mass spectrum congruent with that of the natural product, and co-eluted on columns B and C.

56. 2-Tridecanol. Found as the natural product in Bothroponera soror. Mass spectrum: M^+ 200 (0.2); 45 (100), 43 (43), 41 (37), 55 (33), 69 (24), 57 (22), 56 (20), 70 (18); 182 (10).

57. 3-Heptanol. Used as a commercial sample (Koch light); GC > 99%.

58. 3-Octanol. Found as the natural product in Crematogaster spp.

A,B,C,D and E, Pheidole sp.A. and Tetramorium serceiventre.

Mass spectrum: M^+ 130 (0.0); 59 (100), 45 (29), 41 (26), 60 (25), 95 (23), 58 (22), 55 (21), 44 (21); 112 (1).

An authentic sample (purified by preparative GC) gave a mass spectrum congruent with the natural products, and co-eluted on columns B and C.

59. 4-Methyl-3-heptanol. Used as a commercial sample (Aldrich); GC > 98%.

60. 6-Methyl-3-octanol. Found as the natural product in Crematogaster spp. A,B,C,D and E and Pheidole sp.A.

Mass spectrum: M^+ 144 (0.5); 59(100), 55 (79), 97 (53), 41 (48), 43 (28), 69 (23), 57 (18), 115 (17), 70 (17); 126 (2).

A synthetic sample (supplied by P,H,Briner, Chemical Entomology Unit) gave a mass spectrum congruent with the natural products, and co-eluted on GC columns B and C.

61. Hex-2-en-1-ol. Found as the natural product in Crematogaster sp.F.

Mass spectrum: M^+ 100 (3); 57 (100), 41 (48), 39 (23), 67 (20), 82 (20), 44 (18), 43 (15), 56 (13).

A authentic sample of trans-hex-2-enal, reduced by syringe reaction with sodium borohydride (8.1.5.) gave a component which co-eluted on the GC (column B) with the natural product, and gave a congruent mass spectrum.

8.5.8. Aliphatic Aldehydes. (Figure 8.2)

62. Octanal. Used as a commercial sample (Koch Light); GC > 99%.

63. Decanal. Used as a commercial sample (Koch Light), purified by preparative GC and collected in dichloromethane.

64. Hex-2-enal. Found as the natural product in Crematogaster sp.F.

Mass spectrum: M^+ 98 (29); 41 (100), 39 (68), 42 (53), 55 (42), 83 (41), 69 (41), 57 (33), 98 (29).

An authentic sample (Aldrich) gave a mass spectrum congruent with the natural product, and co-eluted on GC columns B and C.

8.5.9. Sesquiterpene Furans.

65. Perillen (3-(4'-methyl-pent-3'-ene)furan). Found as the natural product in Tetramorium sp.D and Tetramorium angulinode.

Mass spectrum: M^+ 150 (49); 69 (100), 41 (74), 81 (55), 150 (49), 82 (27), 53 (21), 39 (18), 51 (8).

The mass spectrum of the natural product was congruent with published spectra of perillen (Bernaldi et.al.1967).

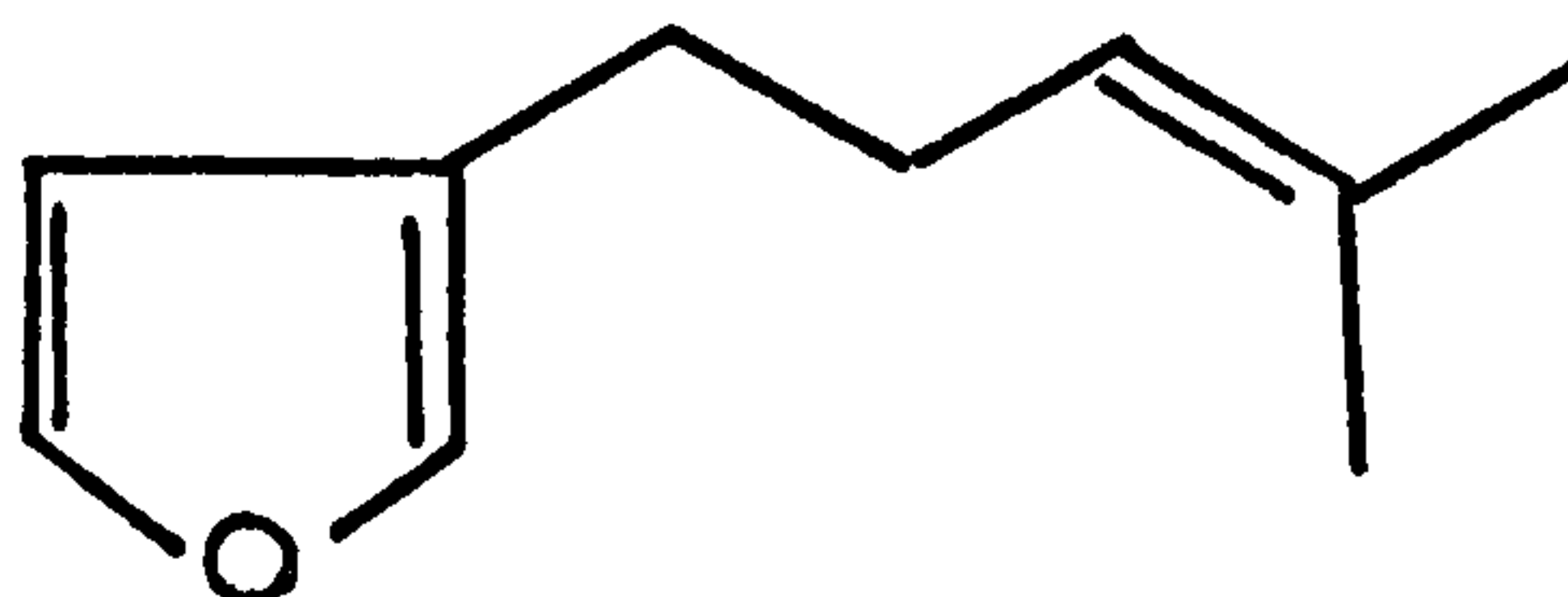
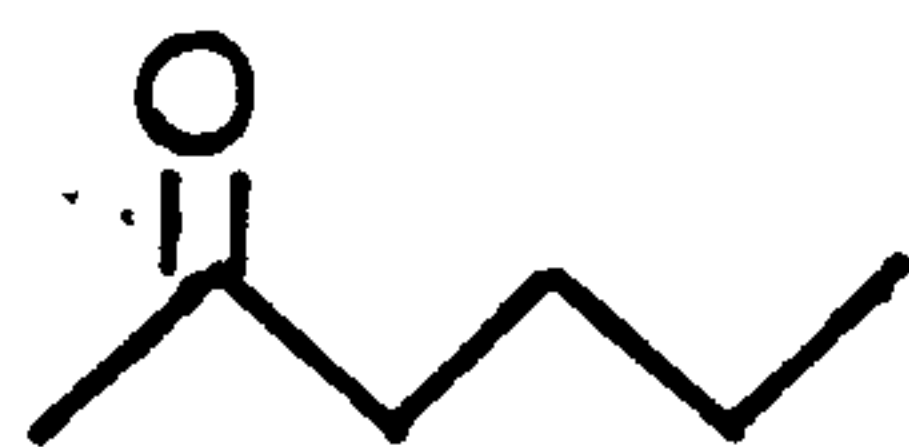


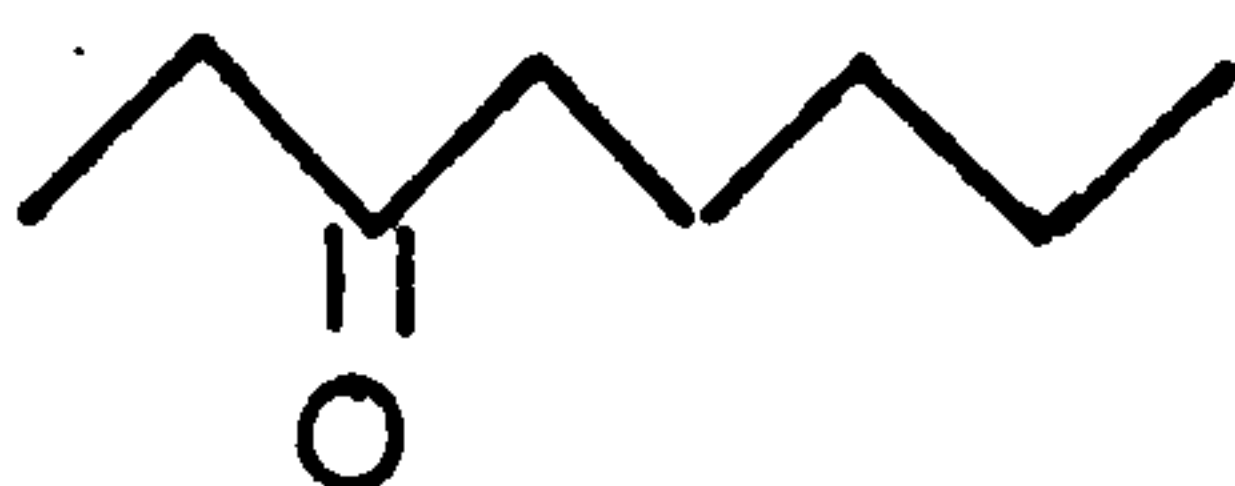
Figure 3.2. Aliphatic Ketones and Aldehydes.

2-Hexanone
(40)2-Octanone
(41)2-Nonanone
(42)

2-Undecanone (44)



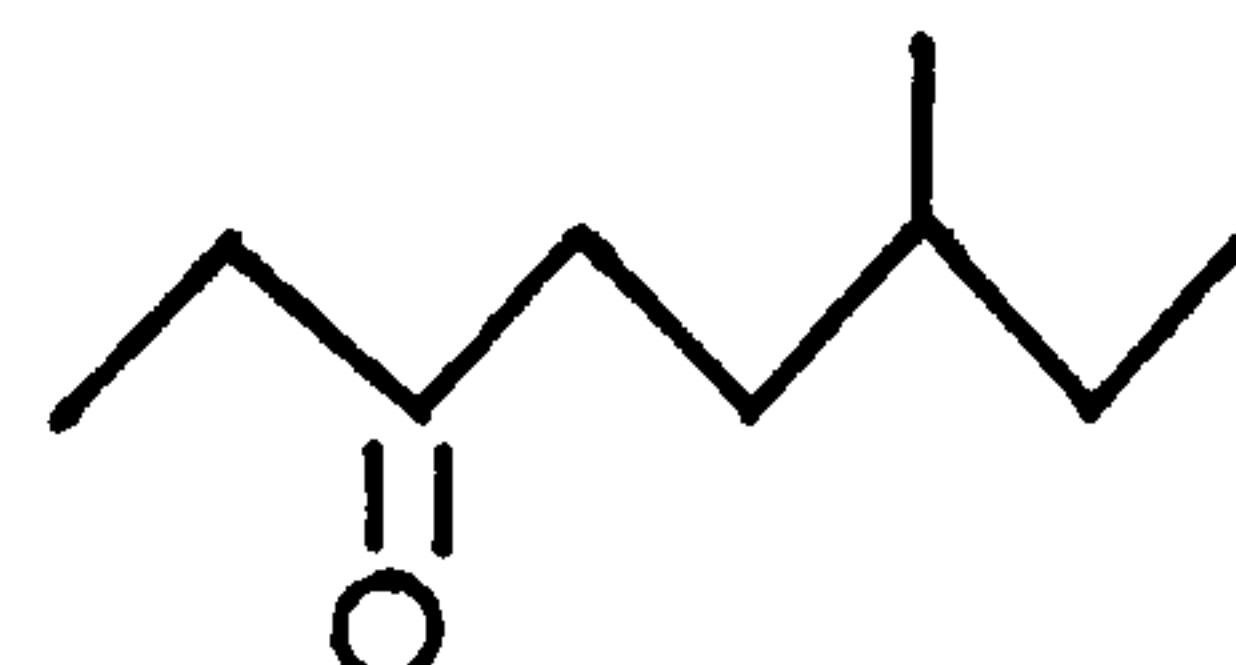
2-Dodecanone (45)



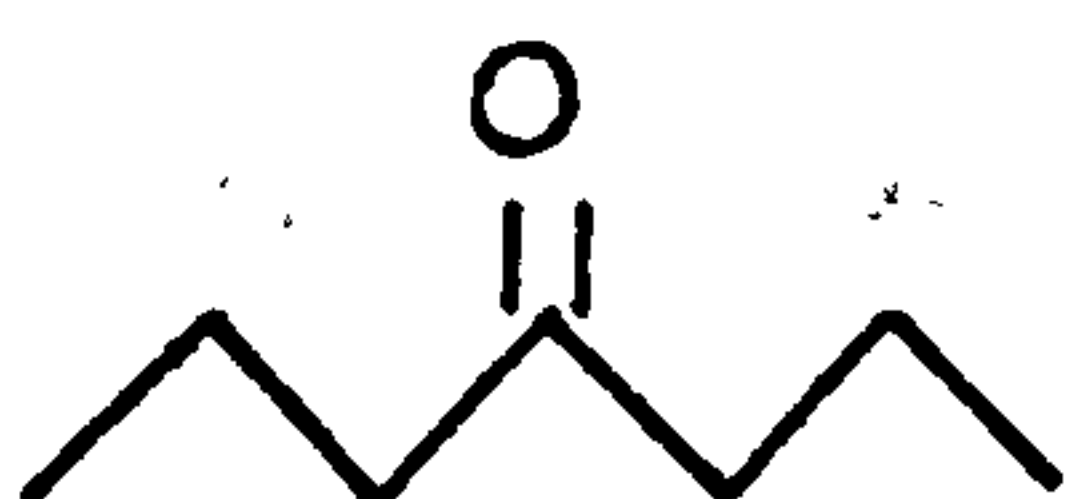
3-Octanone (46)



3-Decanone (47)



6-Methyl-3-octanone (48)

4-Heptanone
(49)

Octanal (62)



Decanal (63)



Hex-2-enal (64)

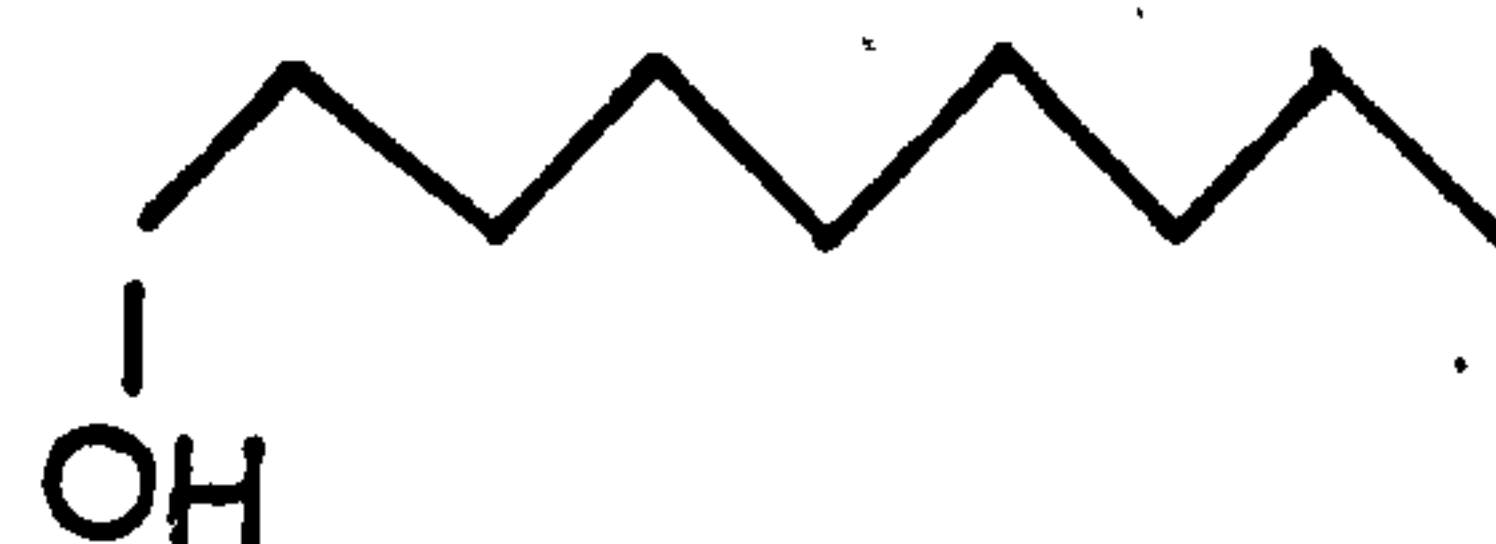
Figure 8.3. Aliphatic Alcohols.



1-Heptanol (50)



1-Octanol (51)



1-Nonanol (52)



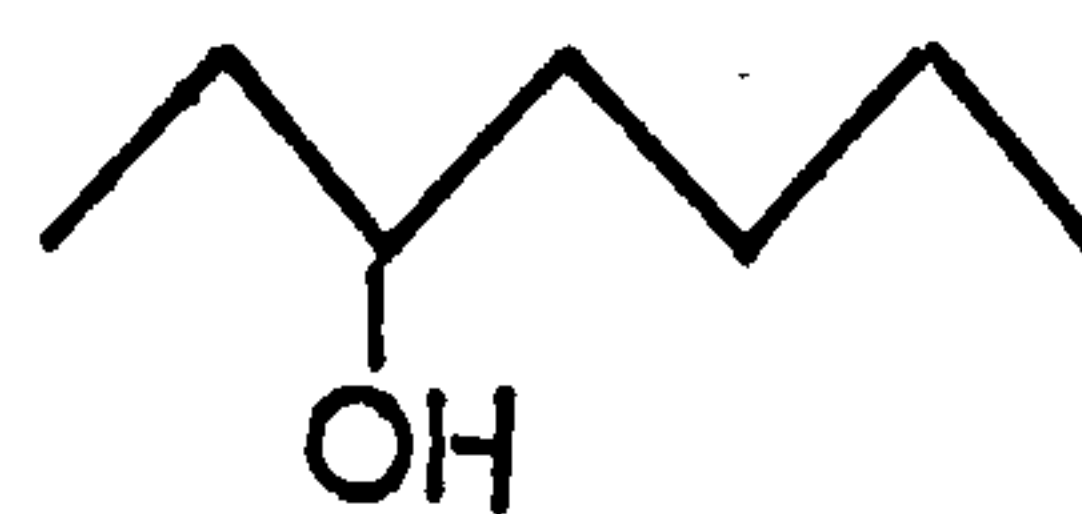
2-Undecanol (53)



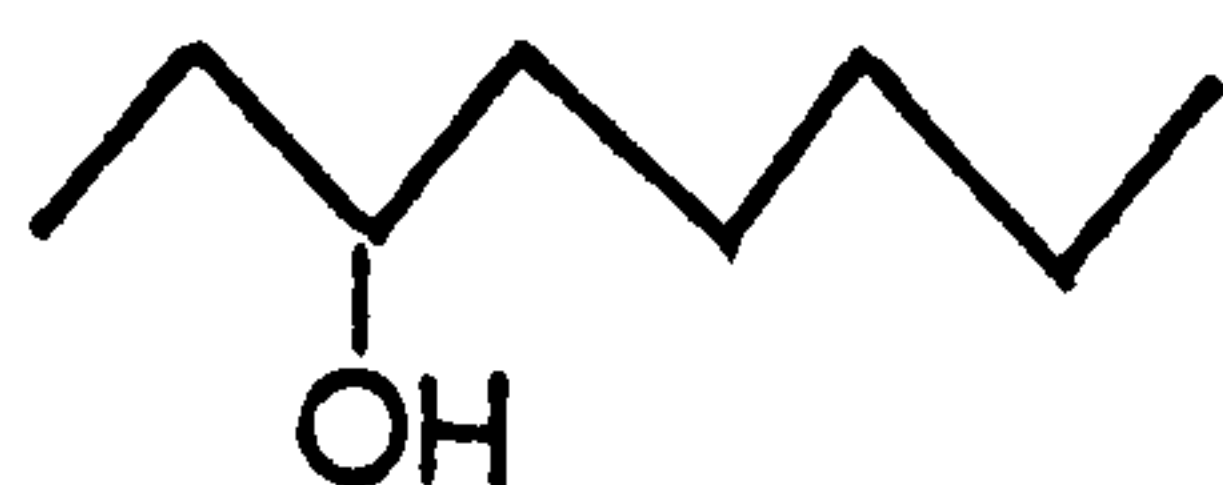
2-Dodecanol (55)



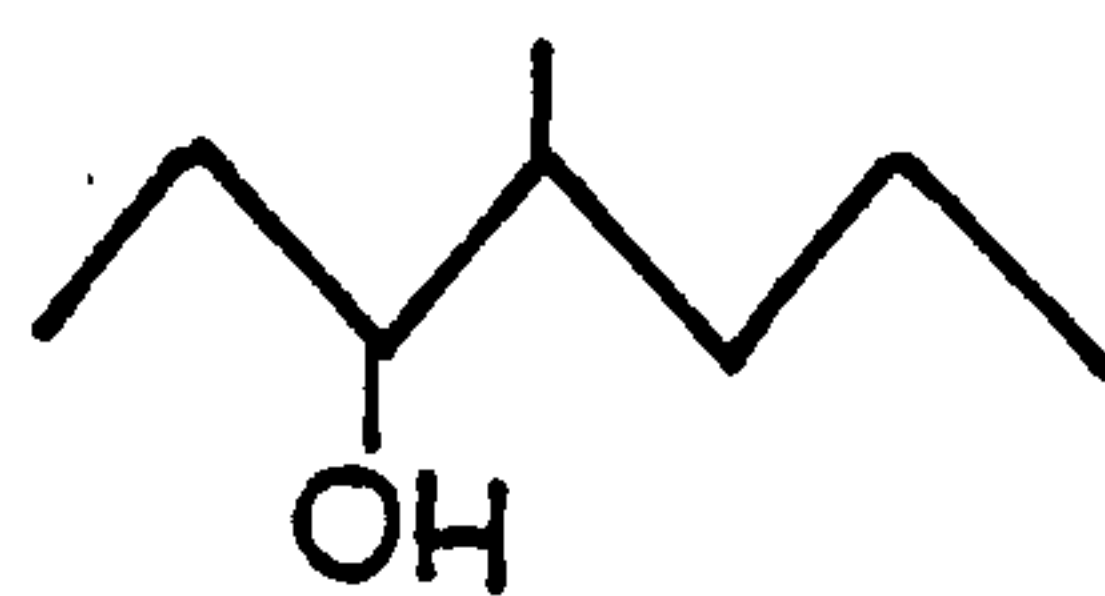
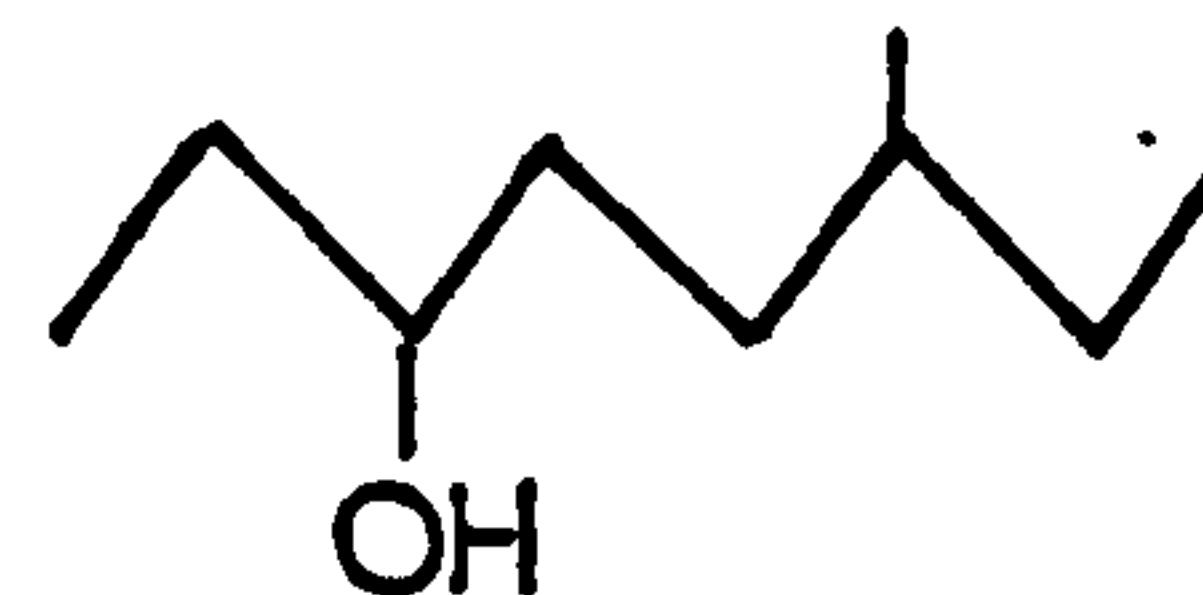
2-Tridecanol (56)



3-Heptanol (57)



3-Octanol (58)

4-Methyl-3-heptanol
(59)

6-Methyl-3-octanol (60)



Hex-2-en-1-ol (61)

SECTION 9. EXPERIMENTAL: COLLECTION AND CULTURE OF ANTS AND TERMITES.

9.1. Collection of Ant Colonies.

The majority of ants collected were ground nesters, and three main digging regimes were used.

9.1.1. Megaponera foetens, Anochetus spp., Myrmicaria eumenoides and Decamorium uelense.

A pit was dug in front of the nest ca. 1 X 0.6 X 0.5 m deep. The nest was then entered through the pit wall and the ants collected as they fell into the pit. The use of a straight sided pit was especially important when digging the aggressive M.foetens which has a painful sting. The ants were taken from the pit with suitably sized specimen tubes and transferred to poly thene containers.

9.1.2. Camponotus sericeus, C.maculatus.

The nest entrance was located and, leaving a cylinder of earth 15 cm in diameter around this entrance, an annulus of earth, ca. 0.5 m in cross section was removed to a depth of ca. 0.6 m. The central cylinder of earth was collected, taken to the laboratory and cooled in a freezer. The immobile ants and brood were then transferred to containers with a pooter or fine forceps.

9.1.3. Bothroponera soror, Tetramorium spp., Pheidole sp. and Crematogaster spp.

The nest was excavated in its entirety, soil and ants were taken to the laboratory and spread out in large plastic trays which were placed in a freezer. The immobilized ants and brood were transferred to suitable containers with a pooter or fine forceps.

9.1.4. Non soil nesters

Pieces of wood or carton containing ants and brood were placed directly into suitable containers.

9.2 Transport and Maintenance of Cultures.

9.2.1. Containers

Polythene and plastic containers of various sizes, with a ca. 10 mm depth of sterile soil on the bottom, and absorbant paper glued to the lid to soak up condensation, were used. Water was supplied on cotton wool pads in specimen tube lids, and food was supplied as termites (various species from baits) and sugar solution (20%) on cotton wool pads. The walls of the containers were painted with Fluon (I.C.I.) to prevent the escape of the ants.

9.2.2. Transport boxes

Hardboard boxes, lined internally with expanded polystyrene insulation were used to transport the containers of ants from Mokwa, Nigeria to Southampton University.

9.3. Culture Conditions (Southampton University).

The majority of ant species were kept in nest chamber type A (Fig. 9.2), manufactured from a plaster of Paris base and covered with glass to allow observation of the ants after removal of the outer tile cover. Various sizes and internal designs were used, the simplest type is illustrated in figure 9.2, and this was a successful design.

The nest was placed on a foraging table which had its legs placed in dishes of soapy water. The foraging table was successful for most myrmicines and formicines, but ponerines usually fell off of it. The ponerine nest chambers were therefore placed in glass aquarium tanks, with Fluon painted sides to prevent the escape of the ants. The plaster of Paris nests were damped on the underside every other day to maintain the humidity inside the

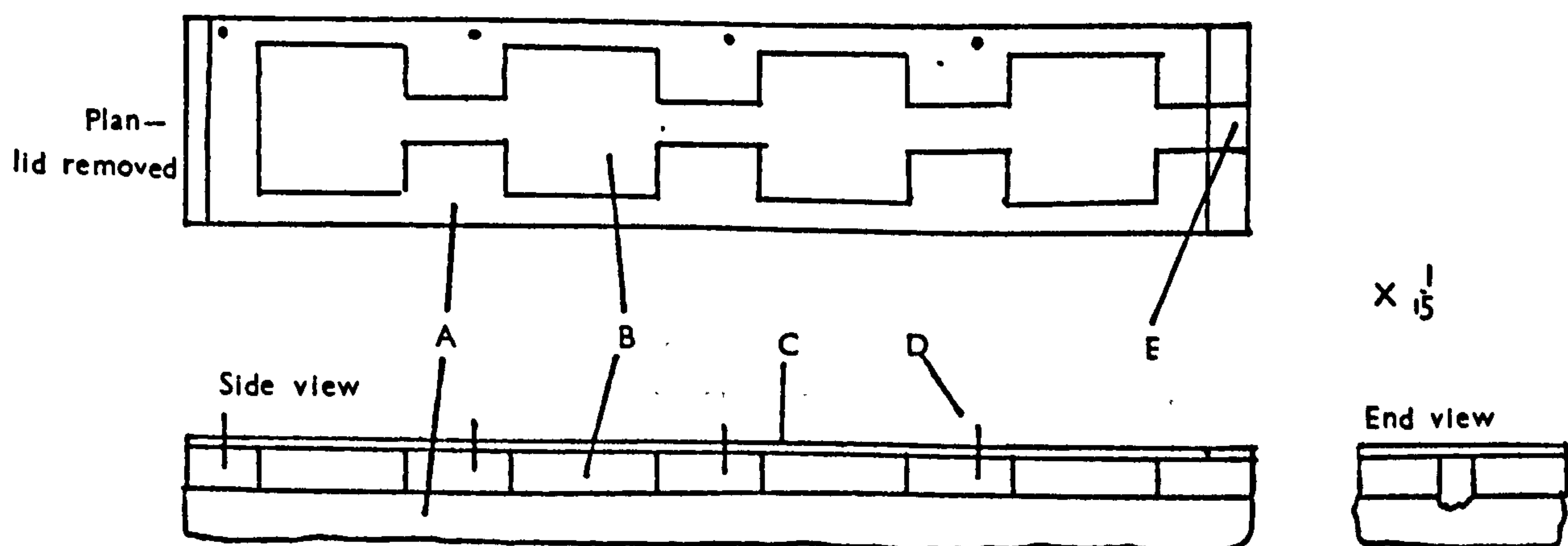
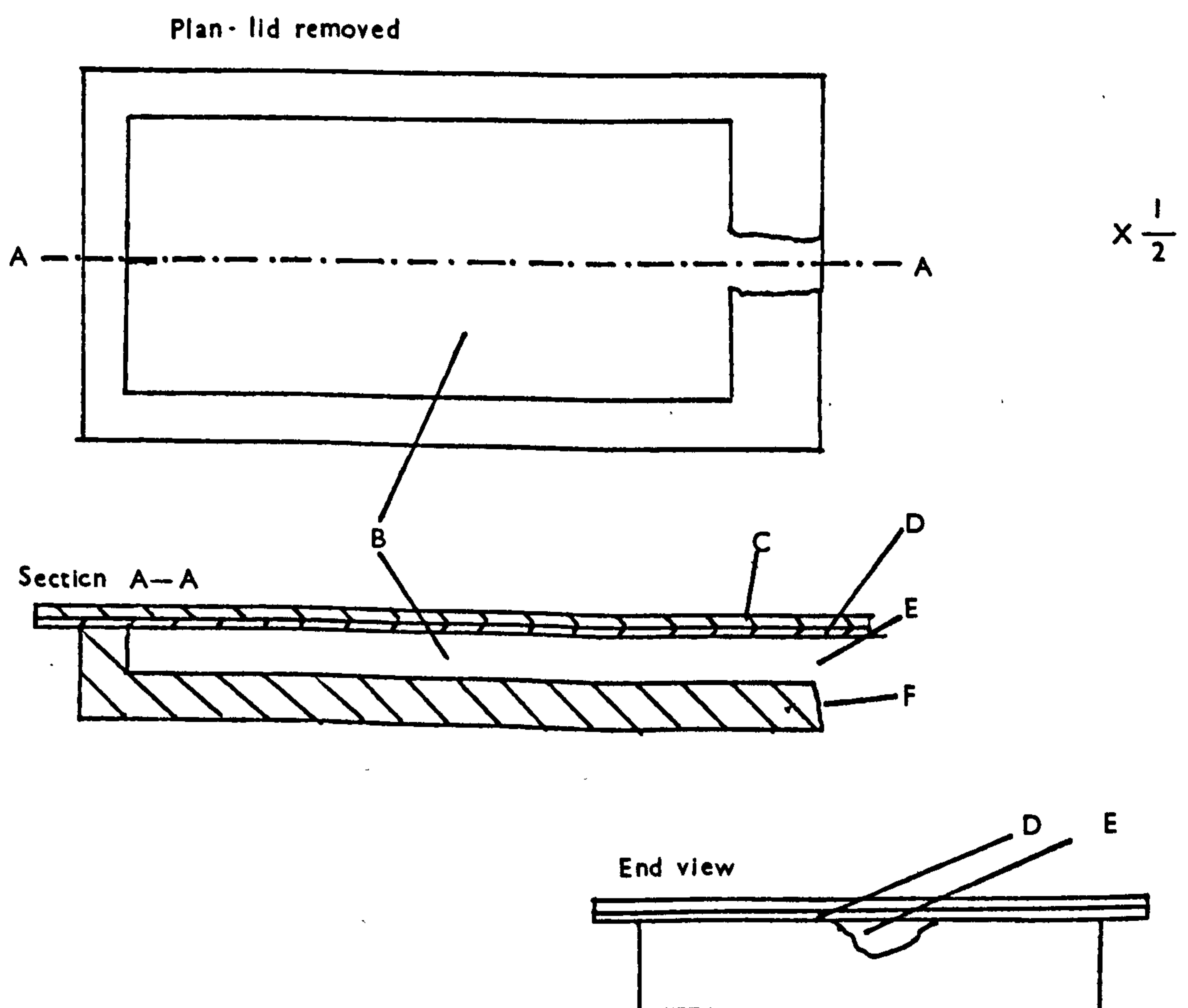


Figure 9.1 Perspex nest chamber for Megaponera foetens
 A - plaster of Paris base; B - nest chamber; C lid;
 D - securing screw; E - entrance.

Figure 9.2 Plaster of Paris nest chamber
 B - nest chamber; C - tile; D - glass; E - entrance;
 F - plaster of Paris base



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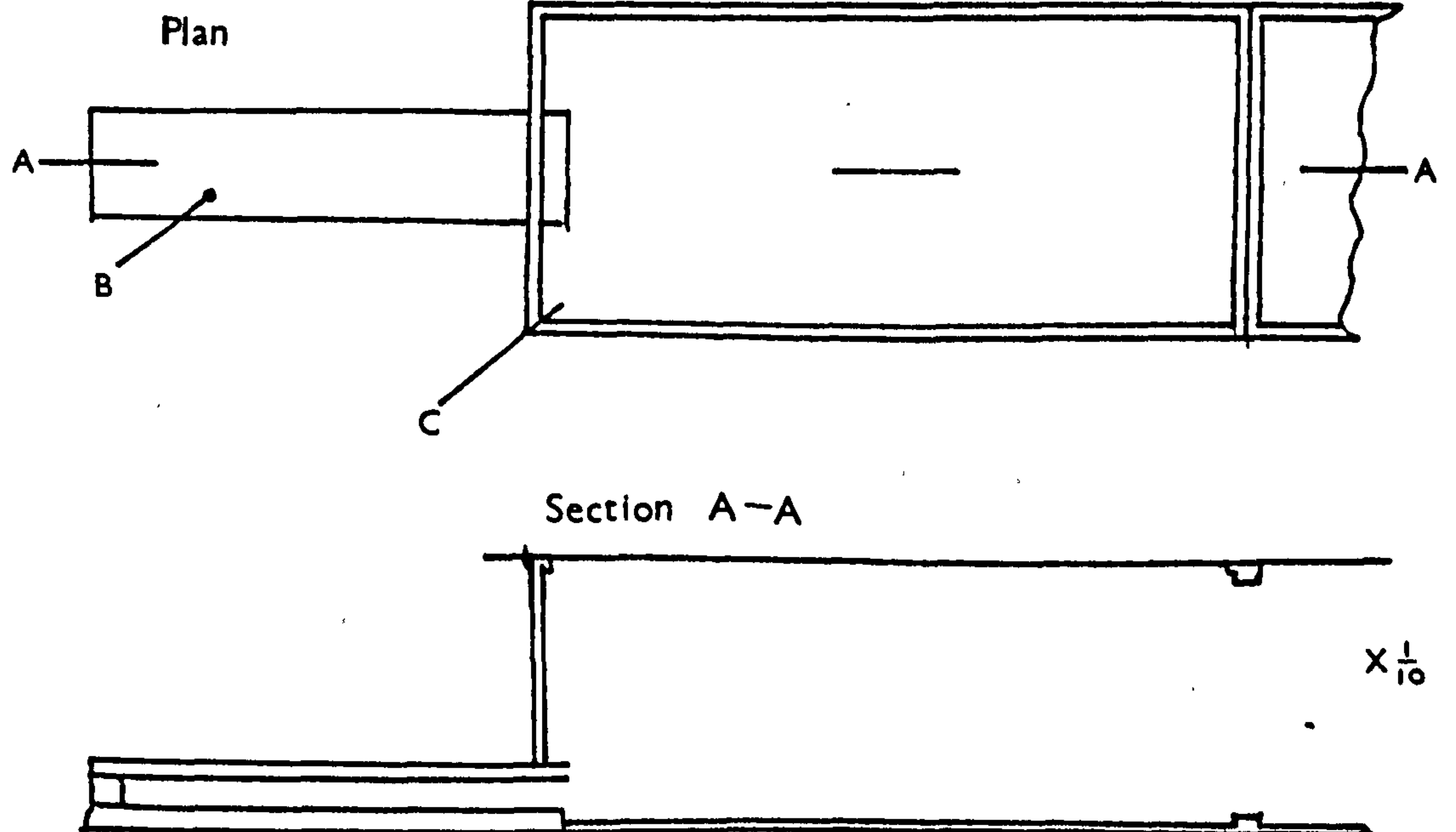


Figure 9.3 Modular foraging arena for Megaponera foetens
 B - perspex nest chamber (Fig. 9.1); C - foraging area

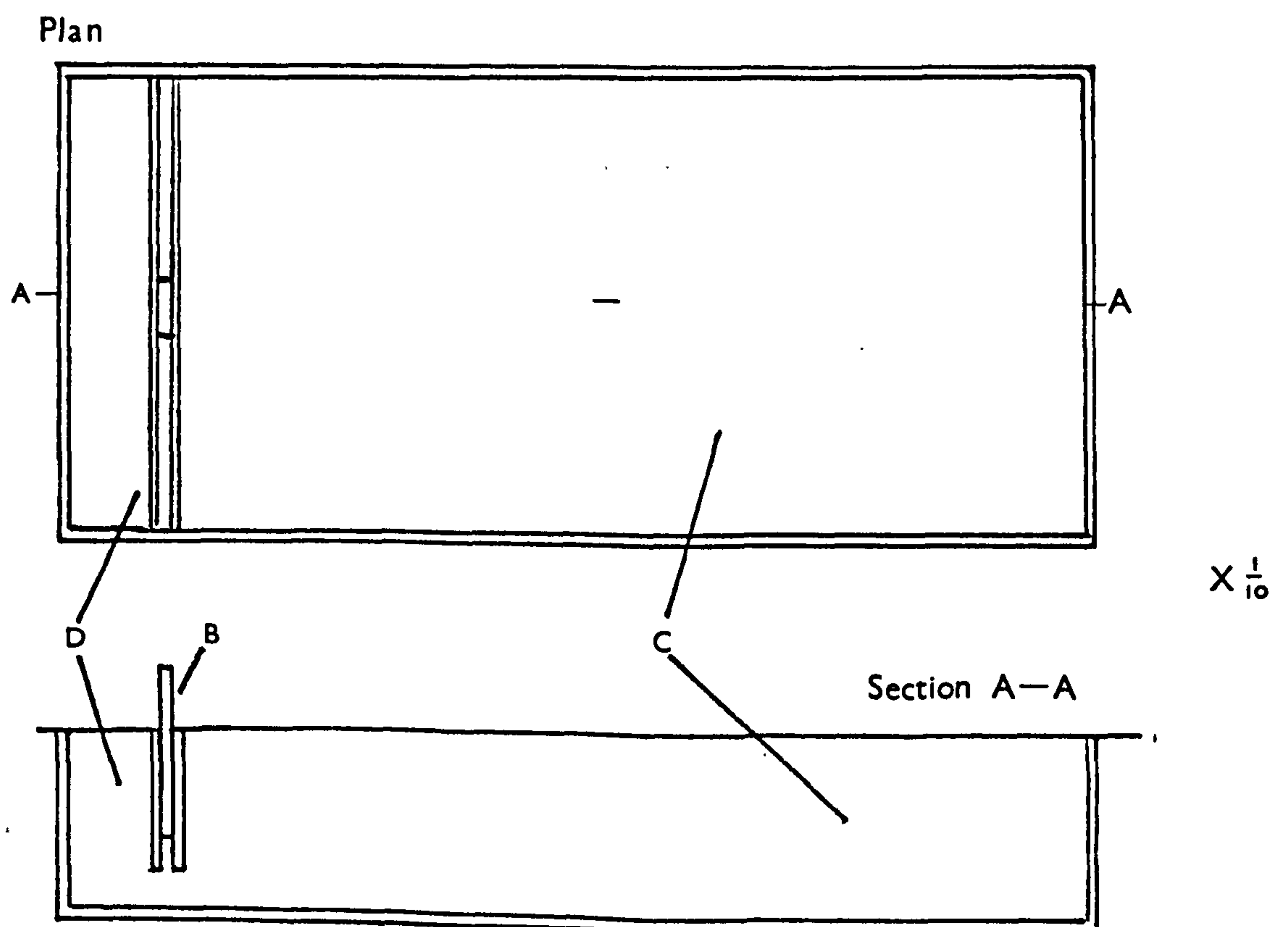


Figure 9.4 Foraging arena for Megaponera foetens
 B - sliding entrance, C - foraging area; D - nest chamber

nesting chamber.

All ants were supplied with cotton wool pads in small petri dishes, soaked in i) tap water and ii) 50:50 honey-water mixture. Most ants would take chopped locusts, blowfly larvae and crushed pupae and larvae of *Tenebrio* for food. The following, more specialised ants, were fed on Reticulitermes lucifugus and *Drosophila* larvae: Anochetus sedilloti, Bothroponera soror, Brachyponera sennaarensis, Paltothyreus tarsatus, Odontomachus troglydytes, Tetramorium spp. and Crematogaster spp. Decamorium uelense was fed on R.lucifugus only.

9.4. Culture Conditions for Megaponera foetens.

The specialised biology of M.foetens demanded special culture conditions, including a diet of termites and a large foraging arena.

Type I Foraging Arena: (Fig. 9.4) The ants were kept in large plaster of Paris nests (Fig. 9.2) in the enclosed end of a large hardwood arena (Fig. 9.4). The floor of the foraging arena was covered with clean sand and the arena then covered with glass. Termites were presented in clean petri dishes or watch glasses; Reticulitermes lucifugus was the usual food, although Macrotermes bellicosus were presented when available. Attempts to persuade M.foetens to take other insect food were not successful.

Type II Foraging Arena: (Fig. 9.3) A purpose built perspex nest chamber, with a plaster of Paris base (Fig. 9.1) was used. The same nest chamber was used to contain ants in larger foraging arenas at Mokwa. At Southampton the foraging arena was constructed from modular units 0.6 m long X 0.3 m wide X 0.3 m high; bolted together and covered with glass. The floor of the arena was covered with washed sand. The far-end of the arena was blocked off completely; the other received the perspex nest chamber. The arena was constructed from five units to give a 3 m X 0.3 m foraging area.

At Mokwa M.foetens was kept in the perspex nest chambers, with access to a 1.5 X 1.5 m foraging arena containing sterilized top soil.

9.5. Transport and Culture of Macrotermes bellicosus

The fungus gardens and base plate from a M.bellicosus colony were removed according to the method used by Collins (1977b) and placed in a plywood box, using polystyrene blocks as packing. In the culture room at Southampton the plywood box was placed in a 1.3 X 1.3 m metal tray containing sterilized top soil. 20 mm diameter holes were drilled around the base of the box and then filled with soil, to allow the termites access to the foraging arena. Termites were supplied with wood of various types, placed on the soil surface, to which they foraged. The fungus gardens were moistened daily with water.

9.6. Culture Room Conditions.

The culture room at Southampton was maintained at 24 - 29°C, RH 65 - 85% on a 12 hours light: 12 hours dark cycle.

9.7. Notes on Ants in Culture.

Megaponera foetens: this was a fairly difficult ant to keep in culture, the major problem was supplying enough termite food to prevent the workers eating any brood. There was usually a high initial mortality, followed by a stable period with 100 - 150 ants present. The longest living culture lasted for three months.

Bothroponera soror: this species was kept successfully in plaster of Paris nest chambers (Fig. 9.2), as well as the fully enclosed version of the perspex nest chamber (Fig. 9.1), with two chambers covered and two exposed, to form nesting and foraging areas. Colonies stabilized at 20 - 30 ants, and produced worker brood. A colony over nine months old is still in culture

Anochetus sedilloti: No attempt was made to keep this species for long periods as the ants were required for chemical studies.

Brachyponera sennaarensis: this ant was collected with only a small amount of brood, it did not culture successfully, most ants dying in transit.

Odontomachus troglydytes: a colony of this species was successfully established which produced both workers and males.

Paltothyreus tarsatus: this ant was collected without brood, workers survived for 2 - 3 months but did not establish a viable colony.

Myrmicaria eumenoides: queenless colonies of this species survived well and produced worker brood, one queenless colony surviving over 13 months in culture. Queen-right colonies of 100 - 200 ants established themselves well, producing both workers and alate sexuales. New nest chambers were supplied as the colony expanded, and these were soon occupied.

Tetramorium spp.: Tetramorium angulinode and Tetramorium sp.D survived well under culture conditions and produced worker and sexual brood. T.sericeiventre could not be established in culture, possibly because its main prey, Pheidole sp.A could not be supplied

Crematogaster spp., Pheidole spA: these genera did not survive for long in culture, showing a constant decline; colonies of 50 - 100 ants being extinct within one month of being set up.

Decamorium uelense: this species could be enticed to take Reticulitermes lucifugus in culture. A colony was kept for two months, producing worker brood, before it was eventually sacrificed for chemical studies.

Messor regina: a colony of this seed harvesting ant could not be established in culture. A selection of grass seeds were supplied but none were taken, the colony died within a month of its establishment in culture.

Camponotus spp.: camponotine ants were easily established in culture, producing workers and sexuales. A C. maculatus colony, set up from a de-alate queen captured in April 1975 is still surviving.

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APPENDIX A. SOME ASPECTS OF THE BIOLOGY OF THE MALES OF MEGAPONERA FOETENS.

A.1. Introduction.

The male of Megaponera foetens is winged, but the female is a wingless ergatoid (Arnold 1915), differing radically from the major workers only in the shape of the epinotum and petiole. In this appendix the dispersion and the method of location of new colonies by the males of this species is described.

A.2. Field Observations and Experiments.

Observations on the males of M.foetens were carried out in conjunction with studies on foraging and recruitment (3.3.) and predation (4.2.1.). Males were found from September - January (Table A.1) and could be seen entering and leaving established nests. There are probably always a few males present in the nests, but more in October/November than April/May (Table A.2, Fig. A.1).

Activity by males was most noticable during the afternoon and evening (1615 - 1830 GMT+1). On leaving its parent nest, a male M.foetens climbs any vertical surface, such as grass, shrubs or the pinnacles of termite mounds, for take-off. It is a weak flier, seldom rising above ca. 4 m, and sometimes landing and re-launching. Its maximum range appears to be approximately 100 m, but it may land sooner, usually on bare soil. It circles and searches, sometimes faintly stridulating, and may take off again or continue walking. If an established nest of M.foetens is encountered the male will enter. The sequence of events occurring in the movements of a M.foetens male from one colony to another are summarized in figure A.2.

Of 21 M.foetens males which were observed to continue walking

Table A . 1 Occurrence of male Megaponera foetens during observations on Megaponera nests.

Month	Leaving nest	Returning to nest	In vicinity of nest
Nov. 1974	3	5	2
April 1975	0	0	0
May 1975	0	0	0
Oct. 1975	28	9	6
Nov. 1975	21	12	4
Dec. 1975	8	4	0
Jan. 1976	2	1	1
Feb.-Aug. 1976	0	0	0
Sept. 1976	0	2	1
Oct. 1976	23	15	7

Table A.2 Numbers of worker, male and queen Megaponera foetens in excavated colonies.

Colony number	Month excavated	Workers (all castes)	Queens	Males	Percentage of males
2	Nov. 1974	728	1	27	3.71
5	May 1975	615	1	1	0.16
6	May 1975	589	1	2	0.33
12	Apr. 1975	357		0	0.00
10a	Oct. 1975	709		29	4.09
28	Oct. 1975	320	1	19	4.95
29	Oct. 1975	384	1	28	7.29
50a	Apr. 1976	663		6	0.90
54	May 1976	407	1	2	0.49
42	May 1976	688		1	0.15
44a	Oct. 1976	897		31	3.46
64	Oct. 1976	857	2	27	3.15
65	Oct. 1976	430	1	19	4.42
17b	Oct. 1976	567	1	18	3.17
72	Apr. 1977	532	1	3	0.56
74	Apr. 1977	481	1	1	0.20

Figure A.1 Histogram showing the percentage of males in Megaponera foetens colonies excavated at different times of the year

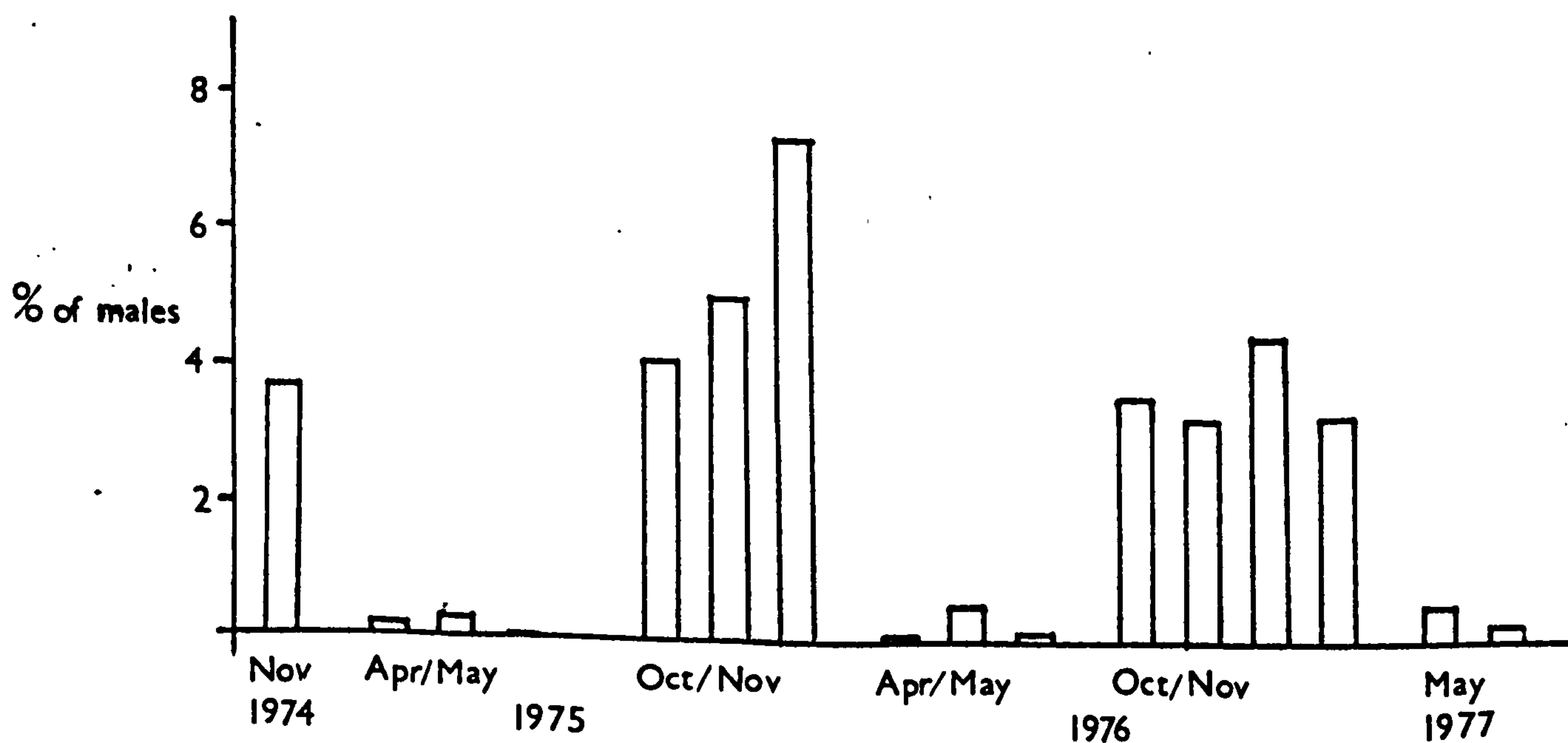
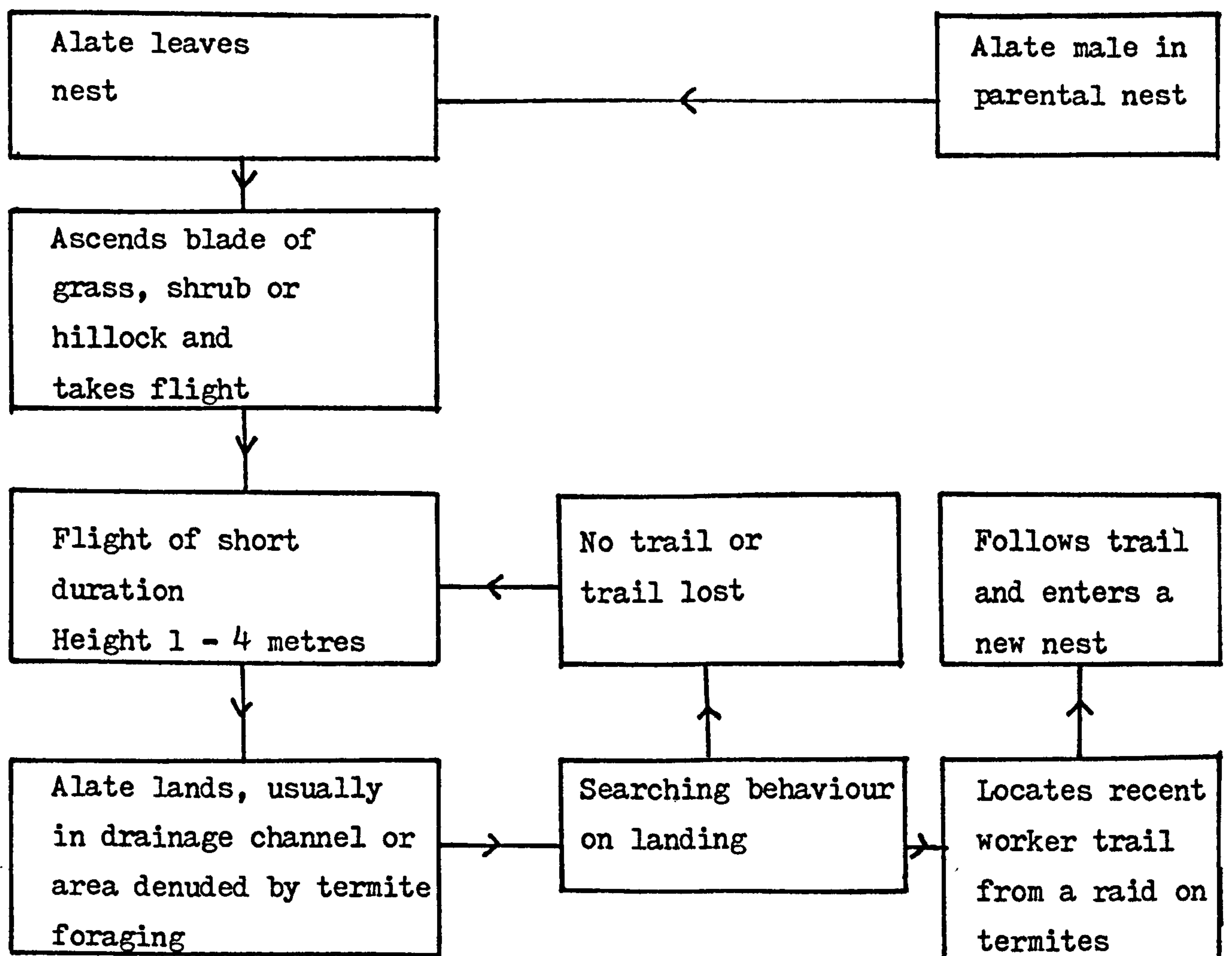


Figure A.2 Sequence of events in the location of new nests
by alate males of Megaponera foetens



on initially landing, 16 located a M.foetens nest, but 5 ants took flight after a period of searching. One male was seen to join a foraging column on a raid against Macrotermes bellicosus, afterwards returning with the worker ants to the nest.

A.3. Orientation of the Male Ants to the Nest.

The observations suggested that the males of M.foetens were following foraging trails (3.3., 6.3.2b.). If a male was removed from a presumed trail and released at a distance of two metres, 90° to the direction of locomotion, it became disorientated and exhibited typical searching behaviour. Of 20 ants removed from the trail 12 relocated the trail after a period of searching, 9 of these continuing in the direction they were initially moving; the other ants flew off, not necessarily in the direction they had been proceeding.

If, when the alate male was walking along a sandy drainage channel, the surface one metre in front of it was raked for a distance of two metres, the male exhibited searching behaviour; of 7 ants disturbed, 5 took flight and 2 relocated the trail after the period of searching. Alate males, obtained from established colonies, were released along trails left by worker ants during morning raids; the trails were marked as in Section 6.3.2. In the majority of cases the males could follow the worker trails for at least seven hours after they had been laid (Table A.3), although major workers could only follow these trails for up to three hours (6.3.2b).

A.4. Laboratory Observations and Experiments.

Ants were kept in culture at Southampton, with access to foraging arenas as described in Section 9.4. Worker ants were allowed to establish recruitment trails to dishes containing termites. Alate males were then placed at the end of the foraging arena away from the nest entrance, and the time it took them

Table A .3 Ability of major worker and alate male Megaponera foetens to follow natural recruitment trails laid by columns of worker ants during morning raids on termites. Trails followed for at least 10 metres.

Hours elapsed since the trail was laid:	Number of major workers (out of 10) following the trail for at least 10 metres.	Number of alate males (out of 5) following the trail for at least 10 metres.
0.5	8	5
1.0	7	5
1.5	6	-
2.0	5	5
2.5	5	-
3.0	1	4
3.5	0	-
4.0	0	5
6.0	-	3
7.0	0	4

to enter the nest chamber was noted. Controls were carried out by raking the sand between the termites and the nest entrance to destroy any odour trails. The time taken for males to enter the nest when a trail was available for them to follow was significantly shorter than in control situations when the trail had been destroyed (Table A.4) ($t = 12.46$; $p < 0.001$, Students 't' test for unequal variances).

A.5. The Acceptance of Males into Alien Colonies.

It was observed that alien colonies of M.foetens, when they encountered each other in the field, attacked one another, resulting in the disruption of foraging columns and the death of some of the workers. To enter an alien colony the male must be able to suppress the effects of its own colony-specific odours. An experiment was designed to determine whether the chemical signals from the non-volatile fraction of the cuticular waxes were responsible for inhibiting the aggression of alien workers.

Cuticular waxes from the gasters of alate males or major workers of M.foetens, which had been immobilised on a bed of dry ice, were removed with small glass splinters (5.1.3b) and spread onto model ants. The models were made by painting 150 mm lengths of 2 mm square pinewood, which had been washed in hexane for four days, with matt black paint ('Winifield' brand). The model was placed in the ants foraging arena, on a black entomological pin, 1300 seconds after applying the waxes. The reactions of workers to the models were noted and the duration of any attack timed (Table A.5). Observations were carried out under white light, untreated models being presented as controls.

Models treated with both worker and alate wax were attacked for significantly longer times in alien colonies than in their original colonies. Live alates in an alien colony were not attacked, but live workers from an alien colony were attacked. It therefore appears that the cues for repressing recognition

Table A.4 Time taken by alate males of Megaponera foetens to reach their own or alien nests in the presence or absence of worker foraging trails

	Time taken (s^{-1})	
	with trail	without trail
own colony	98 - 213	> 600
other colony	80 - 181	514 - >600

TableA .5 Reactions of major workers of Megaponera foetens to models treated with worker and alate cuticular wax from ants from their own and alien colonies

Caste	Colony ants collected from	Colony models placed in	Attack duration (s ⁻¹)	't' ^a probability of 't'
Worker wax	x	y	6.35	6.4 < 0.001
	x	x	0.75	
Alate wax	x	y	5.13	2.7 < 0.050
	x	x	1.25	
Live alates	x	y	Not attacked and accepted into the nest (5 observations)	
Live workers	x	y	Attacked and disabled (10 observations)	

^a 't'- test for unequal variances (Clarke 1969)

did not originate in the non-volatile part of the cuticular waxes remaining on the models after the evaporation of any volatile components.

A.6. Discussion.

The post-copulatory behaviour of male M.foetens, colony foundation and the behaviour of the ergatoid queen are still unknown factors in the reproductive biology of this ponerine.

The behaviour of the male, as observed in Nigeria, suggests that no swarming occurs, but that males enter new nests to fertilize virgin queens or re-fertilize existing queens. Orientation to the nest is mediated through scent trails laid by workers. This system may have the advantage of maximising the nest locating efficiency and minimising the need to produce males. Perhaps the production of males in the nests would be inversely correlated with nest density, more males being needed at low densities to increase the chances of locating nests. When the female is fertilized in the nest, production of queens can also be low, allowing greater worker production throughout the year.

Extra queens have only been found in one of the colonies examined, where two queens were present (Table A.2). Haskins (1970) suggests that, on theoretical grounds, M.foetens colonies must be founded in complete reliance on external food sources because of the ergatoid nature of the queen. The specialised methods of recruitment and foraging found in M.foetens (3.3) would appear to preclude non-claustral methods of foundation without the assistance of supplementary workers. Therefore, in view of the apparent low queen production, and the fact that no colony of less than 50 ants has been discovered in over 1800 hours of field observations, it is predicted that colony fission is the most likely form of colony foundation, probably occurring when the colony size approached 800 - 1000 workers. This would correspond to a Secondary Pleometrotic type of colony

foundation in Wheeler's (1933) classification of formicid methods of colony foundation.

The acceptance of males from alien colonies in ponerines has been remarked on by Wheeler (1900). A male of Pachycondyla harpax Fabre. was placed in a strange nest with workers and ergatoid females. The male was accepted and examined without any aggression, contrasting with the treatment given to alien workers, who were attacked. Wheeler also noted that the males of Leptogenys elongata Buckley are also accepted into alien colonies of workers. In Leptogenys ocellifera, Maschwitz and Mühlenberg (1975) observed that the males were found running along the permanent worker recruitment trails, and suggested that this "mating running" facilitated the fertilization of wingless ergatoid females. It seems likely that in many of the ponerines which utilize scent trails, male responses to them could be involved in reproductive strategies. Leptogenys spp. show similarities to M.foetens in their co-ordinated raids (Fletcher 1973) and the use of recruitment trails. It is possible that males are recruited to the new nests in a manner similar to that described for M.foetens.

The mechanisms involved in the acceptance of males into alien colonies and the suppression of any aggressive responses are poorly understood at present. Preliminary experiments reported in this appendix suggest that the cues are not cuticular. Volatile glandular components could form the basis for a system for the suppression of aggression, and would be worth investigating in greater detail.

(References in main body of thesis.)

APPENDIX B ALARM BEHAVIOUR IN MEGAPONERA FOETENS.

B.1 Introduction.

Although no detailed studies have been made on the alarm-defence system of Megaponera foetens, a number of observations have been made which are relevant to the behaviour of the ant as discussed in the main body of the thesis (eg. Section 6.2, 6.3).

B.2 Observations on Alarm Behaviour.

Observations on M.foetens, both in the laboratory and in the field, indicated that two states of alarm behaviour could be defined, preceded by an alerting stage. An alerted ant was arrested, with its antennae porrect, usually orientated towards the stimulus. Low-intensity (directional) alarm behaviour was defined as occurring when the ant stood with its antennae porrect and mandibles open. The ant sometimes approached the stimulus with its antennae and mandibles still in these positions. High-intensity (undirectional) alarm was characterised by the ants moving rapidly in all directions, often colliding with sister workers. Bursts of stridulation were often heard during this type of alarm.

B.3 Intraspecific Alarm Communication.

Crushed gasters, presented to the ants as in 6.3.2a (Table 6.), released undirectional alarm behaviour in Megaponera workers. Dufour's glands, dissected away from the poison gland, and presented crushed on 13 mm diameter filter papers, also released undirectional alarm behaviour. The poison gland, in contrast, was attractive to workers. Examination of the Dufour's gland (either solvent extracts or SSGC, 8.1.2a) revealed the presence of at least 45 components (Fig. B.1); major and minor workers had a similar compliment of components in the same relative proportions.

GC-MS (8.2.1) and relative retention time studies (McNair and Bonelli, 1969) revealed a mixture of alkanes and alkenes in the Dufour's

(Table B.1), with n-undecane, n-tridecane n-heptadecane and component 35 (Fig.B.1) predominating.

Alkanes have previously been implicated as alarm pheromones in the subfamily Formicinae. Examples are to be found in Acanthomyops claviger, Lasius alienus (Regnier and Wilson 1964) and Oecophylla (Bradshaw 1976). n-Undecane and n-tridecane were presented to M.foetens in culture and to raiding columns, as in 6.2.3c for Trinervitermes spp. defence secretions, and the behaviour of the ants observed. Both of these n-alkanes released unidirectional alarm in M.foetens workers.

Table B.1 Identification of some of the components in the Dufour's gland of Megaponera foetens (Fig. B.1).

Component No.	Identity	Method of Identification
A	n-decane	LP, RT
1	n-undecane	MS, LP, RT
4	n-dodecane	LP, RT
5	n-tridecane	MS, LP, RT
8	n-tetradecene	LP
9	n-tetradecane	MS, LP
12	n-pentadecene	MS, LP
13	n-pentadecane	MS, LP, RT
17	n-hexadecene	MS, LP
18	n-hexadecane	LP, RT
24	n-heptadecene	LP
27	n-heptadecane	MS, LP, RT
31	n-nonadecene	MS, LP
33	n-nonadecane	MS, LP
38	n-heneicosene	LP
39	n-heneicosane	MS, LP
41	n-docosane	MS, LP
43	n-tricosane	LP

MS - Mass Spectrometry, LP - Log. Plot, RT - Relative Retention Time with Standard.

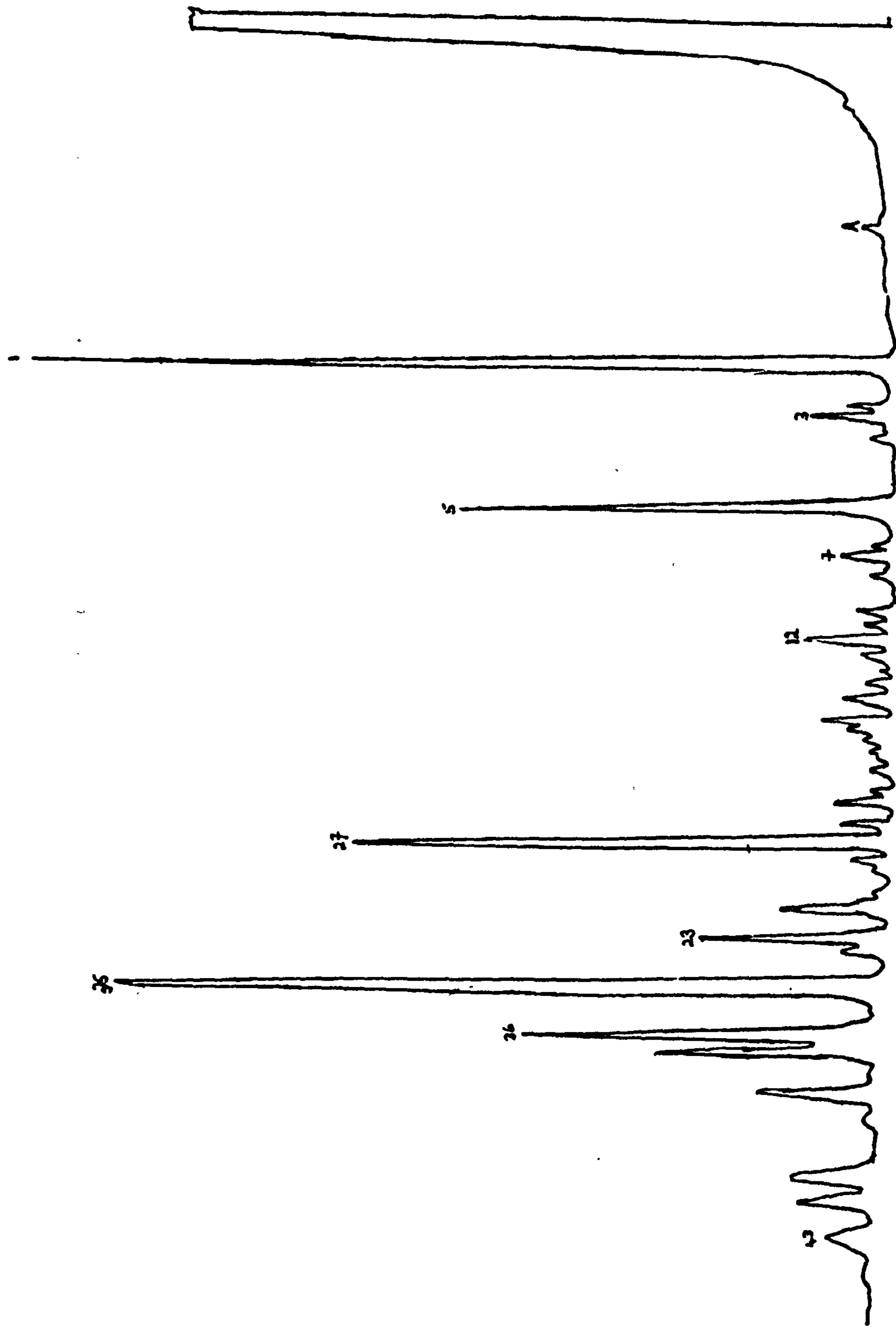


Figure B.1 Dufour's gland extract of *Megaponera foetens* (major workers).

5% OV101; 70 (3) - 250°C @ 8°C/min

APPENDIX C. A GAS CHROMATOGRAPHY - ELECTROANTENNOGRAPH (GC - EAG) LINK-UP TO MONITOR ANTENNAL RESPONSES TO MIXTURES OF COMPOUNDS.

C.1 Introduction.

In the initial stages of screening natural products for biological activity it is useful to have a closely linked system for fractionating mixtures of compounds and monitoring their activity. Such a system can be provided by a GC - EAG link-up (Moorehouse et al. 1969). The components of the mixture are separated on a GC and the eluent blown onto an insect antenna, between two electrodes in an EAG. The EAG measures the sum of receptor potentials generated simultaneously in antennal chemoreceptors in response to chemical stimuli. A fractionation system is especially useful and important if the test mixture contains components which could inhibit or mask the responses of components which normally give positive EAG responses. This appendix describes a prototype GC-Eag link-up which was assembled at Southampton University from existing components.

C.2 Materials and Methods.

A Pye - Unicam 104 GC oven system was used in conjunction with an "EAG - Splitter". The splitter (Fig. C.1) was adapted from a design of Wadhams (1972) and manufactured from glass. The oven system, with fitted splitter, was coupled with a conventional EAG system, as designed and built by Floyd (1975). The plan of the complete link-up is shown in Figure C.2 and the complete system shown in Plate C.1.

To test the system a mixture of nine compounds (1 μ l of each) was made up in 1 ml of dichloromethane. Column A (3.1.1.) was used to separate the compounds into the EAG splitter. The compounds were tested against Locusta migratoria (3rd instar) and

Reticulitermes lucifugus santonensis (worker nymphs). Excised antennae were mounted between two salt filled glass electrodes, according to the techniques of Floyd (1976). The compounds were flushed out of the splitter reservoir (Fig.C.1, B), with oxygen-free nitrogen, every thirty seconds and blown over the antenna for two seconds.

C.3. Results and Discussion.

Table C.1 shows the responses of the antennae to GC - EAG fractions, and to pure compounds (tests carried out by M.A.Floyd and P.G. McDowell, Chemical Entomology Unit). Figure C.3 shows a chromatogram of the test mixture from a conventional GC run, and Figure C.4 is a chromatogram of the same mixture with the EAG Splitter in place. The spikes in Figure C.4. are caused by chemicals being blown out onto the flame ionization detector of the GC when the puffer (Fig. C.2) is operating; they provide useful markers for the system.

C.3.1. Effects of the EAG Splitter on the GC System.

Resolution: The resolution of compounds was decreased by the presence of the splitter, peaks C and D (Fig.C.3) no longer being resolved with the splitter in place.

Retention Times: There was a slight increase in retention time when the splitter was in position.

Split Ratio: The EAG Splitter, as designed, has a split ratio of ca. 50 (EAG) : 1 (GC). There did appear to be some variation in this split ratio throughout the run; with a slightly greater ratio at lower temperatures.

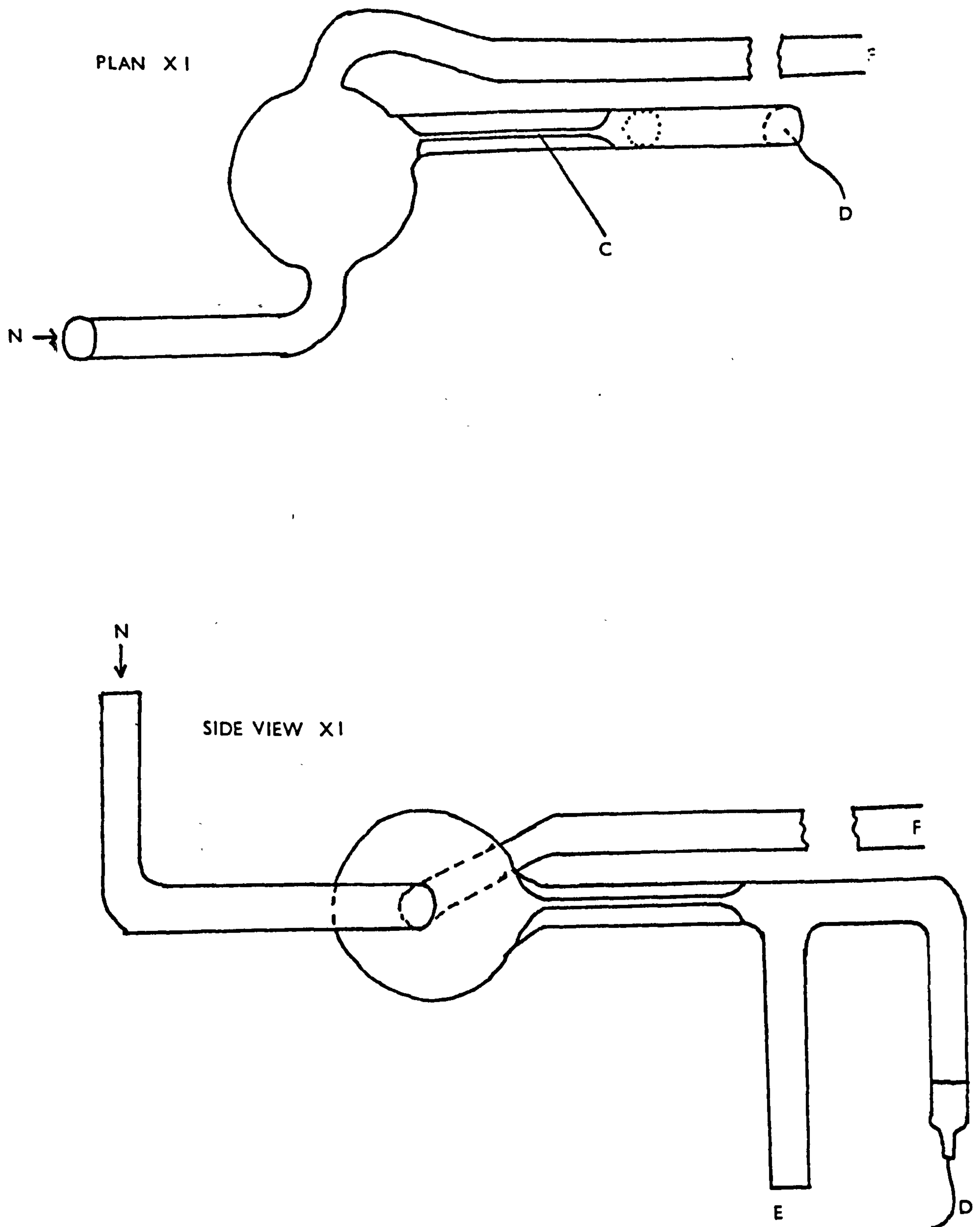
Viability of Antennae: The major drawback of the GC - EAG system, if long GC runs are intended, is the death or decline in response of the antennae during the run. It may be advant-

ageous to include a compound which gives a known positive response in with the mixture being tested. This standard should elute at the end of the test run so that the viability of the antenna can be checked.

Correlation of positive GC - EAG responses with responses from pure compounds: Reference to Table C.1. shows that there is a good degree of correlation between GC - EAG responses and responses obtained through conventional GC. It is interesting to note that although citral (neral + geranial) gives a positive conventional EAG response in Locusta, the GC - EAG shows that only one of the isomers, neral, is active.

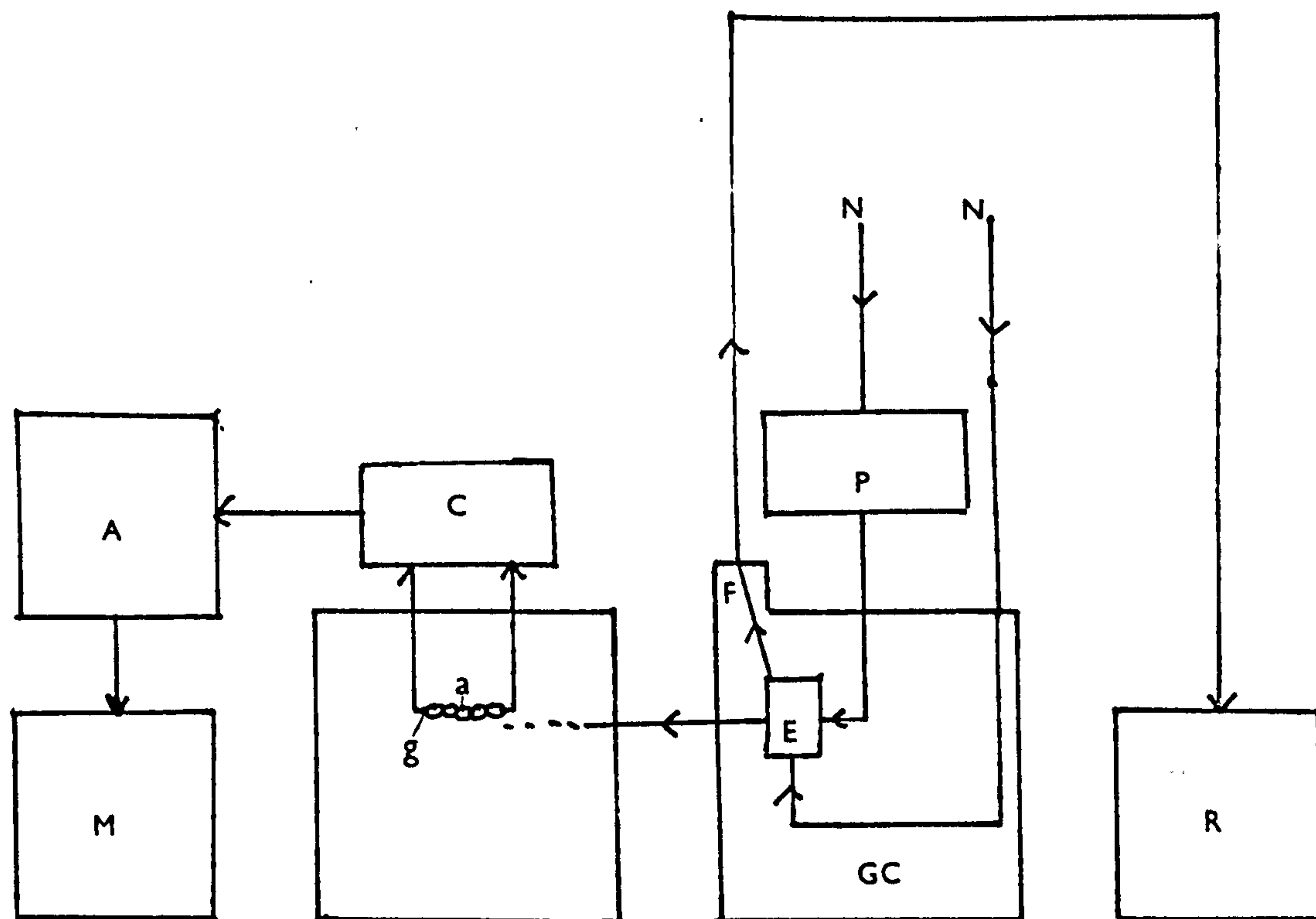
(References in main thesis)

Figure C.1. Scale diagrams of the GC - EAG Splitter.



B - reservoir; C - capillary tube; D - connection to
to GC flame ionization detector; E - input from GC column;
F - outlet to the EAG.

Figure C.2. Layout of the GC - EAG System.



- A - EAG amplifier
- C - EAG cathode follower
- E - EAG splitter
- F - Flame ionization detector
- GC - Gas Chromatograph
- M - EAG recorder
- P - Puffer
- N - Oxygen free nitrogen input
- R - GC recorder
- a - antenna
- g - saline filled glass electrodes

Figure C.3. Chromatogram showing resolution of the test mixture during a conventional GC run.

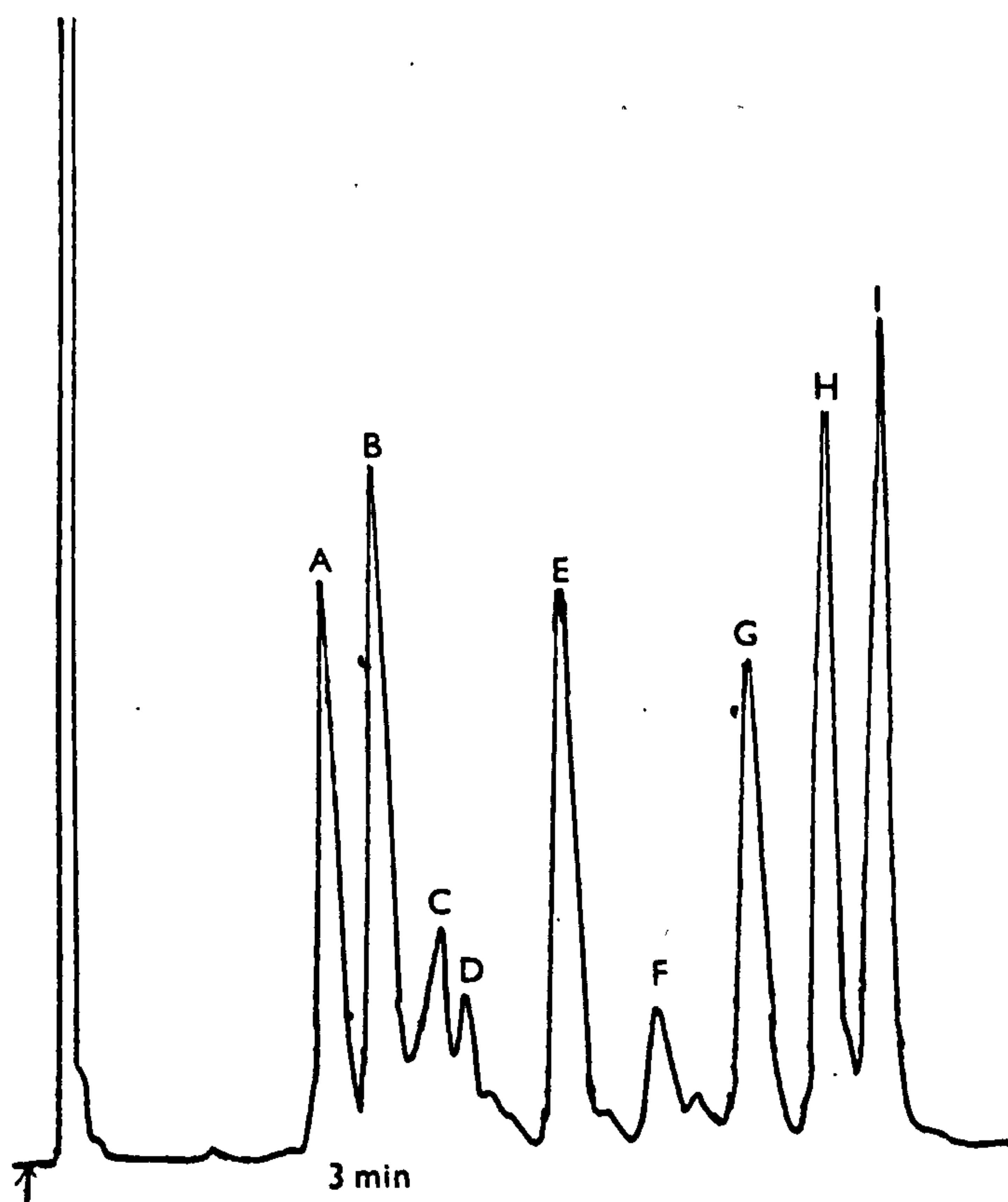


Figure C.4. Chromatogram showing the resolution of the test mixture with the EAG Splitter in place.

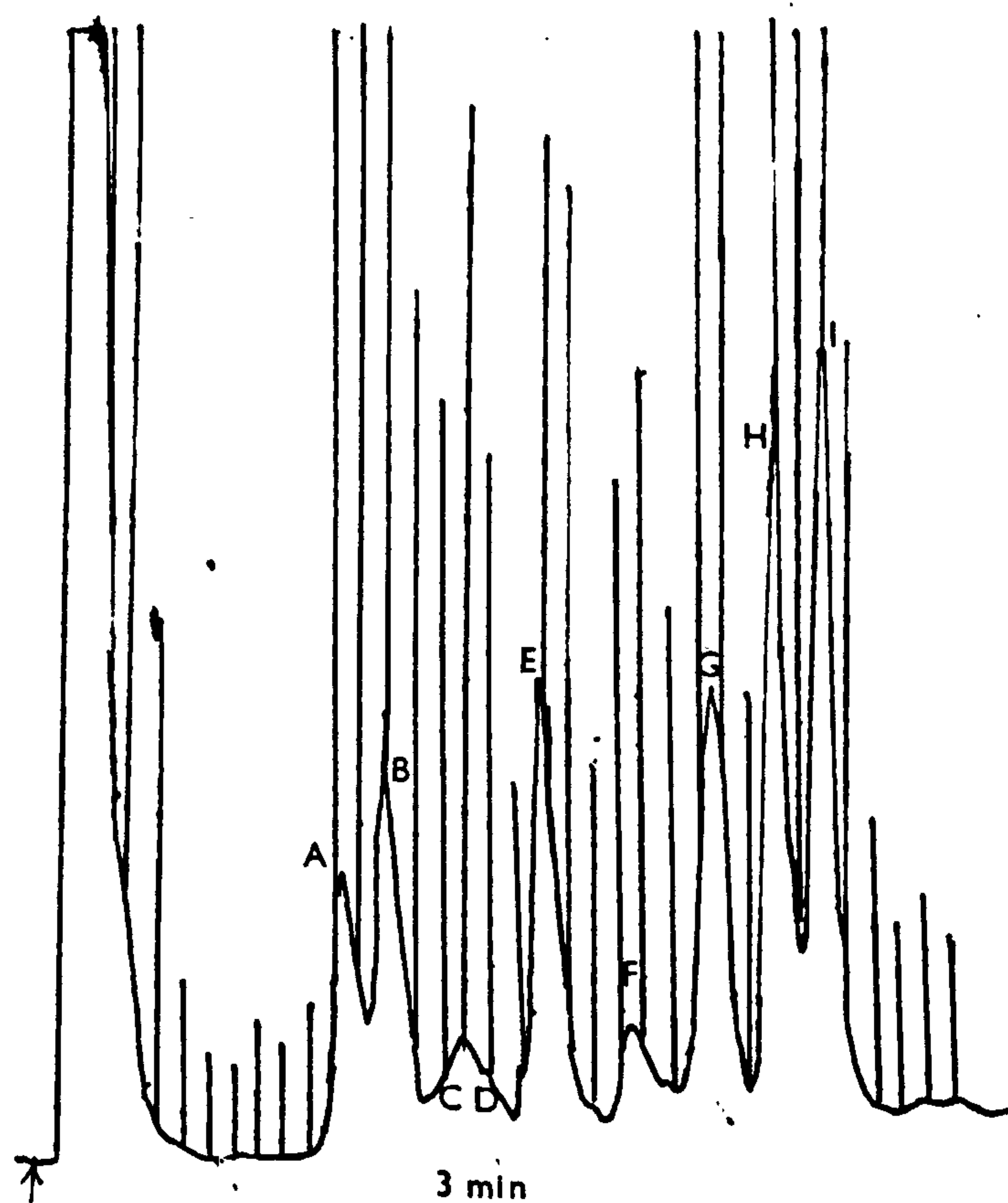


Table C.1. Antennal responses of locusts and termites to
GC - EAG fractions and pure compounds.

GC Peak	GC - EAG Result		EAG Result	
	LM ^a	RT ^b	LM ^a	RT ^b
A		.		
B (β -pinene)		+		+
C				
D				
E				
F				
G (terpineol)	+		+	
H (geranial)			c	+
I (neral)	+	+		+
Solvent	+	+	?	?

^a Locusta migratoria

^b Reticulitermes lucifugus santonensis

^c Citral (mixture of geranial and neral)

PLATE C.1. GC-EAG link-up in the laboratory
(GC-EAG Splitter arrowed)

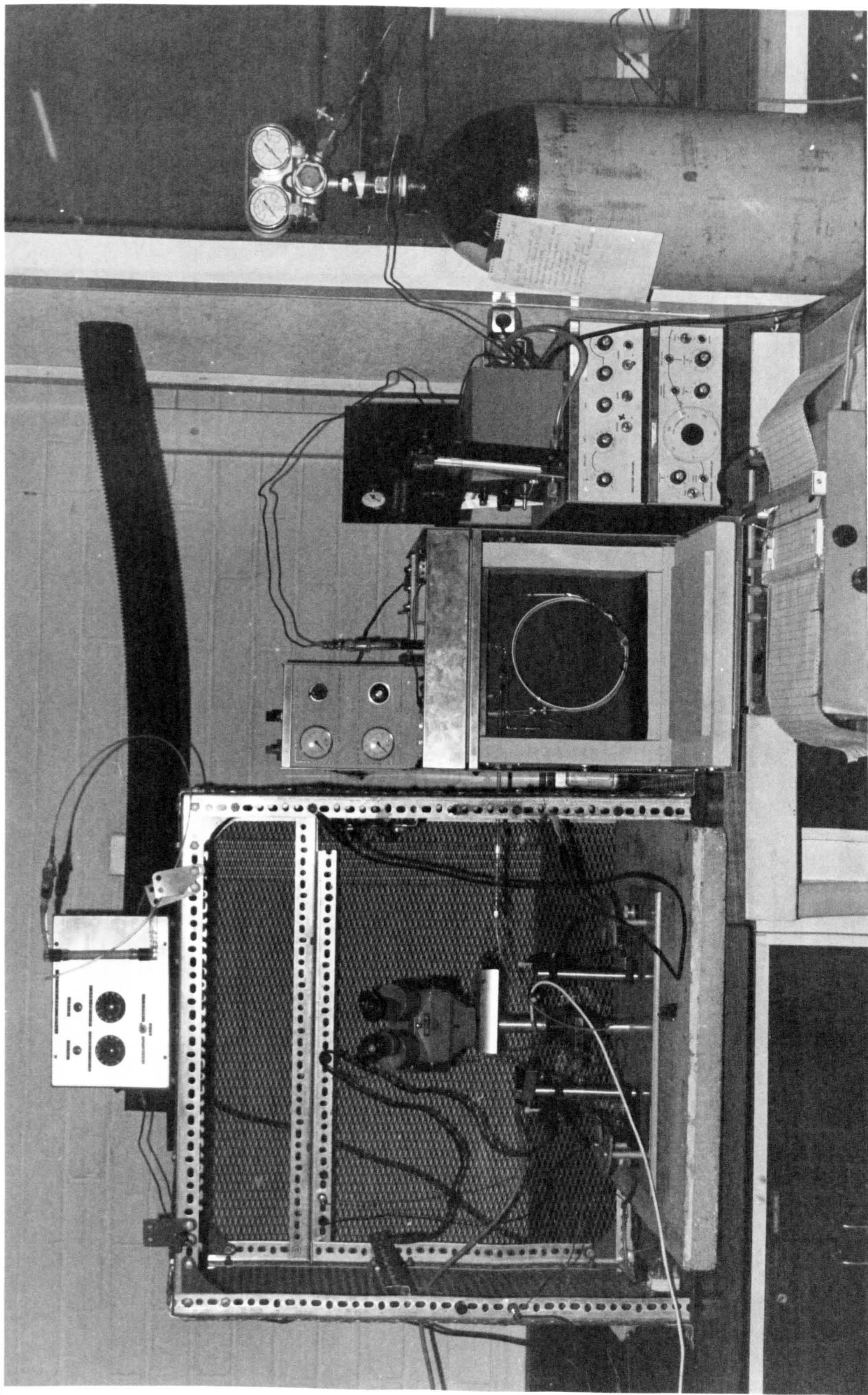


PLATE C.1

APPENDIX D TERMITE PREY OF ANTS AT MOKWA.

Any ants that were observed with termites were collected and their prey identified. The following table represents a qualitative list of the range of termites taken by ants.

	M.bellicosus	M.subhyalinus	Odontotermes spp.	A.cavithorax	Microtermes spp.	Trinervitermes spp.	Microcerotermes spp.	Amitermes sp.	Other species
Bothroponera soror	+	+	+	+	+	+	+	+	
Bothroponera sp.B	+	+	+	+	+	+		+	+
Brachyponera sennaarensis	+	+	+	+	+	+	+	+	
Megaponera foetens	+	+	+	+	+				
Paltothyreus tarsatus	+	+	+	+	+	+	+	+	
Anochetus sedilloti	+		+	+	+		+		
Anochetus sp.A			+	+	+		+		
Dorylus (Rhogmus) sp.A	+		+		+				
Aenictus sp.A					+				
Camponotus maculatus	+	+	+	+	+	+	+	+	
" sericeus	+	+	+		+		+		
" vesticus	+	+	+		+				
Polyrachis viscosa	+		+		+	+	+		
Oecophylla longinoda			+	+	+		+		+
Acantholepis sp.B	+	+					+		
Tapinoma melanocephalum.			+	+			+	+	+

M = Macrotermes

A = Ancistrotermes

contd. over/

Appendix D - continued

	M. bellicosus	M. subhyalinus	Odontotermes spp.	A. cavithorax	Microtermes spp.	Trinervitermes spp.	Microcerotermes spp.	Amitermes sp.	Other species
Crematogaster sp.A	+		+	+	+	+	+		
" sp.B				+	+		+		+
" sp.C			+	+	+	+			
" sp.D					+		+		+
" sp.E	+		+	+	+	+	+		
Meranoplus inermis					+				
Myrmicaria eumenoides	+	+	+	+	+	+	+	+	+
Messor regalis		- seed harvester -							
" sp.B		- seed harvester -							
Pheidole sp.A	+		+	+	+	+	+		
Pheidole sp.B		+	+		+				
Pheidole sp.C		- seed harvester -							
Monomorium bicolor					+	+			
" sp.C	+								
" sp.D	+		+	+		+	+		
Carebara sp.A ^a	+								
Decamorium uelense				+	+		+		
Tetramorium sericeiventre		- predator of Pheidole -							
" guineense	+	+	+	+	+	+	+		
" angulinode				+	+				
" sp.F ^a	+								
" termitobium ^a	+								+

^a Found in mounds of termites marked +