CHEMICALS INVOLVED IN THE COMMUNICATION SYSTEM OF SOCIAL INSECTS: THEIR SOURCE AND METHODS OF ISOLATION AND IDENTIFICATION, WITH SPECIAL EMPHASIS ON ANTS

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The exchange of information among social insects is mainly regulated by compounds of diversified chemical nature, which are produced and stored in their exocrine glands in fairly small quantities. Special methods are used for the isolation and identification of these compounds. This review aims to provide information on the role that the compounds produced by social insects play in their mechanism of communication as well as to discuss the methods employed in the isolation and identification of these compounds.

Keywords: social insects; pheromones; exocrine secretions.

1. INTRODUCTION

Communication is a process which involves the transmission of signals between organisms, which confers, in some instances, advantages only to the emitter organism and its intraspecific group, only to the receiver organism or to both emitter and interspecific receiver organism.

Communication occurs throughout the plant and animal kingdom. In insects, it can be achieved by means of four different modes: visual, auditory, tactile and chemical.

A combination of two, three or even four modes of communication contributes occasionally to the communication system of a single species, however the supremacy of one mode seems to change the importance of the others. The dominance of a mode depends on the habitat and mode of life of a particular species.

Insects, particularly, use chemical communication to transmit information among themselves. When a chemical message is exchanged between members of the same or different species, the chemicals involved in these interactions are called semiochemicals (Gk. *semeon,* meaning a mark or signal)[1]. Semiochemicals are divided according to their intraspecific (pheromones) or interspecific (allelochemicals) effects.

Allelochemicals (Gk. *allelon,* meaning of one another)[2] are divided into three groups: allomones, kairomones and synomones[3,4]. Allomones (Gk. *allos,* other) are chemical messengers which give advantage to the organism which transmits the message. These chemicals are often used against predators. For example, formic acid, which is used by formicine ants when molested.

Kairomones (Gk. *kairos,* opportunistic) are messengers which give advantage to the organism which receives the message. Many kairomones are cues which help predators to find their prey, e.g. the chemical trail of the army ant *Neivamyrmex nigrescens* which feed on the brood of the ants. Synomones (Gk. *syn,* with or jointly) are chemicals which benefit both transmitter and receiver species, e.g. the scent released by the bee-polinated flowers which attracts pollinators.

Pheromones (Gk. *pherein,* to carry and hormon, to excite) is a chemical or a blend of chemicals which evoke physiological changes (primer pheromones) or behavioural responses (releaser pheromones)[5] in the organism recipient of the chemical message.

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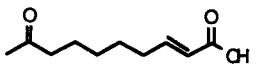
2. PRIMER PHEROMONES OF SOCIAL INSECTS

A classical example of a social insect primer pheromone is the 'queen substance' produced by the queen honeybee *Apis mellifera,* which supress the development of ovaries in workers honeybees and also the construction of queen cells. The so-called queen substance is 9-oxo-*(E)*-2-decenoic acid (9ODA) [1] which is produced in the mandibular glands; but further work demonstrated that a blend of five components is necessary to reproduce effectively the queen effects[6,7].

Primer pheromones were also found in wasps and ants. δ-Hexadecalactone [2] was isolated from the heads of the Oriental hornet queens *Vespa orientalis*[8]. This lactone showed to induce workers to construct queen cells in the absence of the queen at the end of the season. The acidic fractions collected from heads of fertile queens of the ant *Myrmica rubra* has been reported to diminish larval growth[9].



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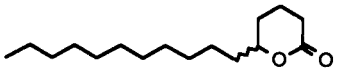

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3. RELEASER PHEROMONES OF SOCIAL INSECTS

Releaser pheromones are classified according to the behavioural response that the chemicals involved in these interactions elicit into: trail pheromones, alarm pheromones, territorial-marking pheromones, funeral pheromones, sex pheromones and recognition pheromones.

3.1. Trail pheromones

Trail pheromones are used almost exclusively by social insects such as ants, termites and bees to recruit nestmates to a newly discovered food source or new nest site. These insects use aerial (bees) or terrestrial trails (ants and termites) to guide their counterparts. Ants are known to use two types of recruitment: tandem running and trail-following. Tandem-running is a primitive form of recruitment in which only one nestmate is recruited at a time by the worker (scout) which has discovered the source of food or nest site and guidance is achieved by direct antennal contact with the leader until the pair reach the target area.

Trail-following recruitment, mediated by chemicals is the

most elaborated form of chemical communication[10]. In ants, it is believed to have evolved from tandem running recruitment. Trail-following behaviour, first observed by Bonnett[11] can be seen when an ant encounters a source of food or new site, and on its return to the nest, it starts to lay a path composed of a chemical or a mixture of chemicals, which attracts and induces nestmates to follow the pathway to the target site. On their way back to the nest, the recruited workers reinforce the trail, discharging the trail pheromone from their glandular source if food still available, otherwise they do not reinforce the trail, so the chemicals evaporate and the signal disappears.

Trail pheromones in ants arise from a number of glandular sources such as the poison gland, Dufour's gland, pygidial gland and hindgut. The trail is deposited on the ground by either the sting, anus, abdominal sternum or tarsi of the hind legs, as the ant moves along. The location of the general exocrine glands of a typical ant is shown in figure 1.

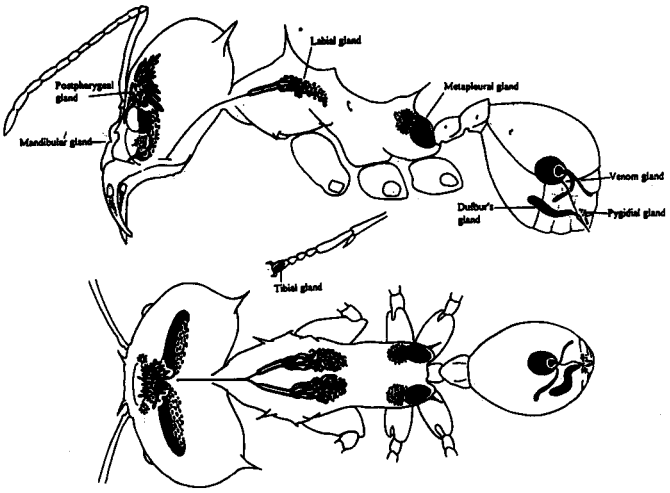
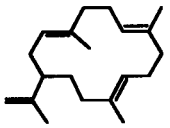


Figure 1. A sectional view of a typical ant showing the location of some exocrine glands.

The chemical nature of the compounds involved in trail-following recruitment is very diversified including terpenes hydrocarbons, oxygenated and nitrogenous heterocyclic compounds as shown in table 1.

The sternal glands are the only known source of the trail pheromones in termites. This gland, situated on the 4th or 5th sternite[40,41,42], lacks a reservoir and a duct, so that the pheromone has to be transported directly from the gland cells out through the cuticle. In primitive termites such as *Zootermopsis nevadensis,* the trail pheromones are used to recruit nestmates to repair breaches on the wall of the nest. However, in higher termites, the pheromones assumes a second function being used to direct termites to a food source. The trail phero-mone of the workers of nasute termite *Nasutitermes exitiosus* and *Trinevitermes bettonianus* was identified as cembrene-A, *(E,E,E)*-isopropenyl-4,8,12-trimethylcyclodeca-4,8,12-triene[41,42] [26]. The trail pheromone of *Reticulitermes virginicus* has been reported to be an unsaturated alcohol [27] [43] which also occurs in rotting wood and is believed to guide workers searching for food.



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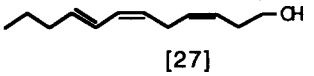
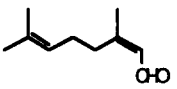


[27]

Many species of stingless bees use aerial odour trails which are placed between the food source and the nest. These trail pheromones are originated in the mandibular glands, and are used to recruit large number of nestmates over a short period of time [44]. Neral [28] and geranial [29] were found to be the trail pheromones used by *Trigona subterranea* to recruit counterparts to a food source[45].



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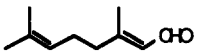


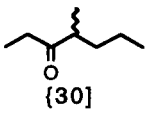
[29]

3.2. Alarm pheromones

Alarm pheromones are largely used by social insects to signal danger and recruit nestmates to a source of disturbance. These chemicals have been demonstrated to be present in several species of ants, two species of wasps, *Vespula germanica* and *V. vulgaris*[46], four species of honeybee, namely *Apis mellifera, A. cerana, A. florea* and *A. dorsata*[47,48,49,50], two species of stingless bees which belong to the genus *Trigona, T. pectoralis* and *T. cupira* and two species of the genus *Melipona, M. fasciata* and *M. interrupta triplaridis*[51] and also some termites[52,53].

Some exocrine glands are involved in the production of alarm pheromones in social insects, for example, the mandibular glands of the ants *Pogonomyrmex badius*[54] and *Lasius fuliginosus* [55], and of the meliponine bees *M. fasciata* and *M. interrupta triplaridis;* the anal glands of the ants *Iridomyrmex pruinosus, Monacis bispinosa* and *Tapinoma sessile*[56]; the sting gland of the honeybee[57,47] and the frontal glands of nasute soldiers of some termites[52].

Alarm pheromones are compounds designated to be effective over short distances and short periods of time. To fulfil these requests, these compounds must be highly volatile. The alarm pheromone substances identified so far are compounds with 5-12 carbon atoms and molecular weights varying between 100 and 200[58]. An updated list of substances involved in alarm communication in ants is given in the literature[59]. Responses of insects to alarm pheromones vary according to the concentration of the pheromone. Lower concentrations attract the insect towards the source of emission. However, responses to higher concentrations depends very much on the social organization of the colony. For ants which live in small, less well organized colonies[60], alarm pheromones are warning for evacuation, since they are not well equipped for defence. On the other hand, highly organized insect colonies are stimulated to attack when the concentration of the pheromone is high. Workers of *Pogonomyrmex barbatus,* for example will attack a wad of cotton wool treated with 4-methyl-3-heptanone [30], a component of its alarm pheromone. Longer exposure to this compound results in another sort of reaction with workers digging and carrying away pebbles.



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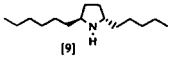
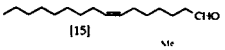
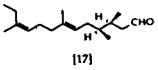
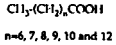
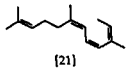
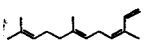
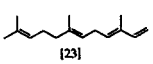
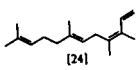
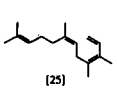
3.3. Territorial-marking pheromones

Territorial marking pheromones are chemicals used by workers of some social insects, particularly ants and bees either to mark a determined area where a particular colony has decided to settle (territorial marking) or to indicate the nest entrance of a colony and, thus helping foragers on their way back home (nest entrance marking). *Myrmica rubra* workers were found to mark its territory with odoriferous material which comes from the Dufour's gland[61]. *Oecophylla longinoda* workers[62,63] also mark a new terrain with drops of brown fluid from the

Table 1. Substances identified as trail pheromone components in ants.

Compound	Glandular source[1]	Subfamily	Species (References)	Chemical Structure
Nitrogenated compounds				
Methyl 4-methylpyrrole-2-carboxylate	PG	Myrmicinae	*Atta texana* [12], *Acromyrmex octospinosus* [13], *Acromyrmex subterraneus subterraneus* [14], *A. cephalotes* [15], *A. sexdens sexdens* [16]	 [3]
3-Ethyl-2,5-dimethylpyrazine (EDMP)	PG	Myrmicinae	*A. rubropilosa* [17], *A. s. sexdens* [16,18], *Manica rubida* [19], *Myrmica sp.* [20], *Tetramorium caespitum* [21], *T. meridionale* [22]	 [4]
2,5-Dimethylpyrazine (DMP)	PG	Myrmicinae	*T. caespitum* [21], *T. meridionale* [22]	 [5]
2,3,5-Trimethylpyrazine (TMP)	PG	Myrmicinae	*T. meridionale* [22]	 [6]
Indole	PG	Myrmicinae	*T. meridionale* [22]	 [7]
Monomorine I	PG	Myrmicinae	*Monomorium pharaonis* [23,24]	 [8]
Monomorine III	PG	Myrmicinae	*M. pharaonis* [23, 24]	 [9]
Methyl anthranilate	PPYG	Dorylinae	*Aenictus sp.*[25]	 [10]
Methyl nicotinate	PPYG	Dorylinae	*Aenictus sp.*[25]	 [11]
Oxygenated Compounds				
(3R, 4S)-4-Methyl-3-heptanol	PG	Ponerinae	*Leptogenys diminuta* [26]	 [12]
(=)-4-Methyl-3-heptanone	PG	Myrmicinae	*Aphaenogaster albisetosus* [27]	 [13]
(R)-1-Phenylethanol	PG	Myrmicinae	*Aphaenogaster cockerelli* [27]	 [14]
(Z) -9-Hexadecenal	PvG	Dolichoderinae	*Iridomyrmex humilis* [28,29,30,31]	 [15]
Methyl 6-methylsalicilate	PG	Myrmicinae	*T. impurum* [32]	 [16]
Faranal	DG	Myrmicinae	*M. pharaonis* [33]	 [17]
C_6-C_{10} and C_{12} acids	HG	Formicinae	*Lasius fuliginosus* [34]	 [18]
3, 4-Dihydro-8-hydroxy-3,5,7-trimethylisocoumarin	HG	Formicinae	*L. niger* [35]	 [19]
Cis- Isogeraniol	PYG	Ponerinae	*Leptogenys diminuta*[36]	 [20]
Terpenes hydrocarbons				
				 [21]
Z,Z,Z-Allofarnesene	DG	Myrmicinae	*Solenopsis invicta* [37]	 [22]
Z, E-a- Farnesene E, E- a-Farnesene	DG	Myrmicinae	*S. invicta* [37, 38, 39]	 [23]
				 [24]
				 [25]
Z, E-a-Homofarnesene	DG	Myrmicinae	*S. invicta, S. richteri* [38, 39]	

[1]PG, Poison gland; DG, Dufour's gland; PYG, Pygidial gland; PPYG, Post-pygidial gland; HG, Hindgut; PvG, Pavan's gland

anus by touching the substrate with the tip of the abdomen. Other species of ants mark their nest entrance to orient themselves on their way back home or to distinguish their own nest from alien nests. *Atta laevigata,* for example, marks its territory and nest entrance with a pheromone produced in the tip of the gaster[64]. The extracts of Dufour's glands of *A. laevigata* were shown to elicit strong territorial-marking behaviour. Heptadecane, 8,11-nonadecadiene, *(Z)*-9-nonadecene, and *(Z)*-9-tricosene were identified as components of the territorial odour[65].

The honeybee *Apis mellifera* and the wasp *Vespa vulgaris* attract nestmates with 'footprint' substance that they deposit in the entrance of the nest[66]. Some male solitary bees of the genus *Centris (Centris nitida* and *C. trigonoides subtarsata)* use a tibial gland secretion to mark the boundaries of defended territories[67]. However, other *Centris* species mark their territories with the mandibular gland secretion[68]. The ground-dwelling bee, *Eucera palestinae* uses the Dufour's gland secretion to mark the nest entrance among densely aggregated nest[69]. Also, *Andrena vetula,* a species which construct its nest in caves, orientate themselves towards their nest entrance by its scent[70].

3.4. Funeral pheromones

Removal of dead nestmates (necrophoresis) and any other decomposing material from the nest is a behaviour shown by some species of ants. Leafcutters of the genus *Atta,* for example, place their dead nestmates in special refuse chambers[71,72]. Despite that no considerable evidence of 'ant cemeteries' has been shown in the literature, the existence of necrophoresis in ants has been confirmed by different authors[45,54,73,74]. Long chain fatty acids and their corresponding esters were identified as components which triggers necrophoresis behaviour in *Pogonomyrmex badius* and *Solenopsis invicta* workers[45,54]. Oleic acid [31] was found to be a very effective component to elicit necrophoric behaviour. When a live worker ant is spread with oleic acid, it is treated as a dead ant.

[31]

3.5. Sex pheromones

Sex pheromones are substances released by one sex of a particular species to attract conspecific mates with the purpose of mating. These pheromones have been extensively studied in lepidopteran, dipteran and coleopteran insects due to the perspective of use of these chemicals in the control of these insect pests. However, sex pheromones have also been demonstrated to regulate courtship behaviour of hymenopteran insect. The queen substance, *(E)*-9-oxo-2-decenoic acid [1], for example, beside its function as a primer pheromone is also a powerful attractant for airborne drones of *Apis mellifera*[75]. Male bumble bees use the mandibular gland secretions to mark their territories and, because it brings males and females to a common place where mating can occur, these secretions also act as sex pheromones. Neral [28] and geranial [29] were found to elicit sexual activity in males of the wasp *Itoplectis conquistor*[76].

The sexual activity of ants is restricted to a few days of the year, a factor which contributes to make the study of ant sex pheromones a difficult task. The patterns of the sexual behaviour of ants are divided into two broad categories: the female calling and the male aggregation syndromes. The female-calling is a characteristic behaviour which is often exhibited by wingless females and sometimes by fertile workers which do not travel far from the nest. These females stand on the ground or low vegetation near the nest, and release the sex pheromones to 'call' the winged males to mate. In the male-aggregation syndrome, males from many colonies gather at specific mating sites, such as forest borders and crowns of trees, and the females fly into the swarms in order to mate. After mating, males and females disperse before the females lose their wings and start to excavate a nest. A reverse of the swarming procedure, exhibited by males, was discovered in some *Pheidole* species, the winged females gather in swarms while attracting males with pheromones. The males fly into the females swarms and mate with individual females[77].

Several exocrine glands are responsible for the production of sex pheromones in ants. The reproductive workers of *Rhytidoponera metallica* release the sex pheromone from the pygidial gland[78]. Females of the myrmicine ant *Harpagoxenus sublaevis* use a sex pheromone from the poison gland[79]. The Dufour's gland of *Monomorium pharaonis* queens is responsible for the production of sex pheromones[80]. Males of *Pogonomyrmex* discharge collectively the secretion from the mandibular gland when they arrive at the mating sites, and thus attract virgin females.

Recently, the first sex pheromone components were identified in ants. These components consist of a mixture of undecane, tridecane and *(Z)*-4-tridecene, produced in the Dufour's gland of the females formicine ants *Formica lugubris* and released in a proportion of 100: 5.23: 4.25 when the females were calling males[81].

4. EXOCRINE GLANDS OF ANTS

The exocrine glands (i.e. those secreting to the outside) of ants are responsible for the production of an array of substances, with most of them being involved in communication. The location of the major exocrine glands in a typical ant is shown in figure 1. The mandibular, postpharyngeal, and propharyngeal glands are located in the head. The metapleural glands are located in the metathorax and the Dufour's, poison and pygidial glands are located in the abdomen. The tibial glands, the source of the trail pheromones in some *Crematogaster* species, are located in the hind legs.

4.1. Mandibular glands

Mandibular glands are a pair of a thin-walled sacs, which consists of an agglomerate of cells with a duct, from where the secretion is transported to an open slit near the anterior edge of the preoral cavity. These glands may be filled with an enormous diversity of chemicals such as straight and branched chain alcohols, aldehydes, ketones, terpenoids and pyrazines. The production of a specific class of compounds in the mandibular gland is often regarded as a subfamilial character. For example, the secretions from the mandibular glands of myrmicine ants are dominated by ethyl ketones and their corresponding alcohols[82] and those of formicine ant contain chiefly terpenoids. Defence and alarm communication are the functions attributed to the secretion from the mandibular glands.

4.2. Postpharyngeal glands

The postpharyngeal glands are a pair of glands situated above the brain and opened into the pharynx. In some species of ants, e. g. *Acromyrmex octospinosus,* the gland has several finger-like protrusions[83]. However, in most ponerine and some formicine ants, e.g. *Neoponera villosa, Diacamma vagans, Odontoponera transversa,* and *Formiça sanguinea,* the glands are winged shaped[84]. In some species of ants, the secretion from the gland, has a yellow oily colour while in other species it is colourless. Straight chain, branched and unsaturated hydrocarbons ranging from C_{21} to C_{33} are the dominant chemical components found in the secretions of many species of ants[85, 86, 87] however oxygenated compounds such as fatty acids and sterols were also found[83].

The function of the postpharyngeal gland is unknown, however it has been demonstrated for three species of formicine (*Formica selysi, Camponotus lateralis,* and *Camponotus vagus)* and two species of myrmicine, *Myrmica rubra* and *Manica rubida,* that the chemical composition of the gland is similar to that of cuticular hydrocarbons, which are regarded to be species-specific[87].

4.3. Metapleural glands

The metapleural glands, also called metathoracic glands, consist of a cluster of glandular cells located at the posteriolateral edge of the metathorax. Each cell is equipped with a duct, which transports the secretion to a common membranous collecting sac. The collecting sac leads directly to a reservoir, which is a sclerotized cavity, from where the secretion may be transported to the outside by capillarity[88].

The secretion from the metapleural glands consist mainly of carboxylic acids, however phenols have also been found as constituents of the glandular secretion in *Crematogaster deformis*[89].

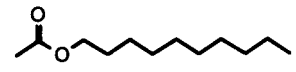
Some functions, such as production of nestmate recognition substances, territorial-marking pheromones[90,91], protection against microorganisms[92], antiseptical and repellent activities[89], and pollen germination inhibition[93,94], have been attributed to the secretion from the metapleural glands.

4.4. Dufour's gland

The Dufour's gland is a sac-like structure attached to the sting, which together with the poison gland, forms the venom apparatus of ants. This gland, first described by Dufour[95], is found in all aculeate hymenopterans (bees, wasps and ants). Its primary function is unknown, however, as the contents from the gland are oily, it may originally have provided a lubricant for eggs in the ovipositor.

Some pheromonal functions are attributed to chemical compounds encountered in the secretion from the gland, *Monomorium pharaonis* workers produce faranal, the active trail pheromone component of this species[96]. The Dufour's gland of virgin alate females of this species contains a sex pheromone which they use to attract and stimulate males[80], while mated queens produce cembrene-A [26] which is not present in workers or alate females and it is used as the queen recognition pheromones[91].

Four components were found to act as the territorial-marking pheromone of *Atta laevigata*[65]. The slaver-maker species, *Formica pergandei* and *F. subintegra* use three esters, decyl acetate [32], dodecyl acetate [33] and tetradecyl acetate [34] as defensive substances against intruders when the nest is disturbed. Also, in *F. pergandei* and *F. subsericea,* these esters operate as effective alarm substances[60], however, when crushed Dufour's glands or single gland equivalents of decyl, dodecyl and tetradecyl acetates were presented separately to workers of *F. subsericea,* the ants tended to separate suddenly and move in different directions within a few seconds after exposure.



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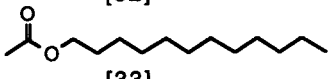

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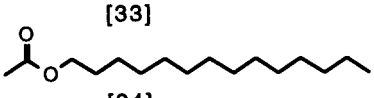


[34]

The hallmark of the Dufour's gland is the production of saturated and unsaturated linear hydrocarbons with an odd number of carbon atoms as its major components. However, hydrocarbons with an even number of carbon atoms, branched chain hydrocarbons and also many oxygenated compounds can be found in the gland. Aliphatic hydrocarbons ranging from C_9 to C_{27} are present in the Dufour's glands of dolichoderine, ponerine, myrmicine, doryline, pseudomyrmicine and formicine ants.

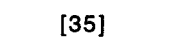
Oxygenated compounds (alcohols, aldehydes, ketones and esters) have been found in the secretion of formicine, myrmicine and ecitonine ants. In two species of *Labidus* and one of *Eciton,* a monoterpene, (*E*)-β-ocimene [35] was found as the major component of the Dufour's gland[98].

4.5. Venom gland

The venom gland is the source of the toxic defensive secretions in social insects[99]. This gland is basically divided into three parts: two secretory filaments, where the main biosynthetic activity takes place; a convoluted gland, which receives the preliminary secretion from the secretory filaments and converts it into the final products; and a reservoir sac, where the products are stored until the moment of discharge through the sting. In the Formicidae, the secretory filaments are extended either from the reservoir as a single filament that branches some distance from it as in ponerine, ecitonine, pseudomyrmicine, myrmeciinae, and nothomyrmeciine ants or are paired over their entire length as in the more evolved myrmicine, formicine and dolichoderine ants[100,101,102].

The chemical components found in the venoms of stinging ants are proteinaceous and alkaloidal. Proteinaceous venoms are widely spread in the subfamilies Myrmeciinae, Ponerinae, Dorylinae, Pseudomyrmicinae and Myrmicinae. However, some species of myrmicine ants possess the ability to biosynthesize a variety of alkaloids in their venom gland. *Solenopsis (Solenopsis)* species have a venom which is characterized by a predominance of 2-alkyl-6-methylpiperidines. The alkyl group usually contains an odd number of carbon atoms ranging from C_7 to C_{15} and sometimes contains a double bond, which when present in the side chain appears to have a *Z* configuration.

The *cis-* and *trans*-isomers of 2,6-disubstituted piperidines are usually present, however either the *cis*-isomer (in *Solenopsis xyloni* and *S. geminata)* or the *trans*-isomer (in *S. invicta*) tends to predominate. In addition to the usual piperidines, the venom of *S. xyloni* also contains 2-undecyl-6-methyl-2,3,4,5-tetrahydropyridine [36].



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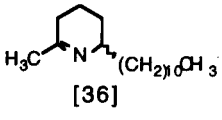
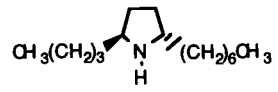


[36]

Another species of *Solenopsis, Solenopsis (Diplorhoptrum) fugax* utilizes 2-butyl-5-heptylpyrrolidine [37] to repel the hosts ants when they invade the nest of other species of ants to steal brood[103].



[37]

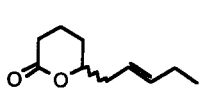
The venom of many species of *Monomorium* are also alkaloidal. Species of this genus all produce a species-specific mixtures of 2,5-alkyl and alkenyl-substituted pyrrolidines and pyrrolines[104]. The Pharaoh's ant, *Monomorium pharaonis,*

produces an indolizine (monomorine I) [8] and a pyrrolidine (monomorine III) [9] which were found to have some trail-following activity[105, 106], however the true trail pheromone was later identified as a component from the Dufour's gland[107].

The venom of formicine ants constitutes a rich source of formic acid, which the ants use as a defensive weapon. Formic acid was first identified by Wray[108] from workers of the wood ant, *Formica rufa*. This acid constitutes a diagnostic subfamilial character of formicine ants since it is now recognized that all formicine ants have a venom which consists of a strong aqueous solution of formic acid. Formic acid was reported as the only volatile compound present in formicine venom[109], however peptides and aminoacids have also been reported in the venoms of *Formica polyctena*[110] and *Camponotus pennsylvanicus*[111]. Acetic acid has also been identified as a minor component of two formicine species, *Camponotus floridanus* and *Anoplolepsis custodiens* [112,113]. Most recently, the convoluted gland of five formicine ants were found to possess microgram quantities of hexadecanol and its corresponding esters, hexadecyl formate and hexadecyl acetate, and it was suggested that hexadecanol forms a monolayer film on the secretory ducts to protect cells from the corrosive venom[114].

The poison gland is the source of the trail pheromones of some species of ants. In species of the tribe *Attini* , two substances were identified as trail pheromones, methyl 4-methylpyrrole-2-carboxylate [3] and 3-ethyl-2,5-dimethyl-pyrazine [4]. Indole [7], 2,5-dimethylpyrazine [5], 2,3,5-trimethylpyrazine [6] and methyl 6-methylsalicilate [16] either as a single component or a mixture of components were identified as the trail pheromones of *Tetramorini* ants (table 1).

The poison sac of queens of *Solenopsis invicta*[115] is the source of the queen recognition pheromone.This pheromone guides and attracts the worker ants towards the queen. Three components were chemically identified as the queen recognition pheromone, *(E)*-6-(1-pentenyl)-2H-pyran-2-one [38], tetrahydro-3,5-dimethyl-6-(1-methylbutyl)-2-H-pyran-2-one [39] and dihydroactinidiolide [40][116].



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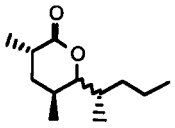

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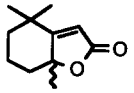
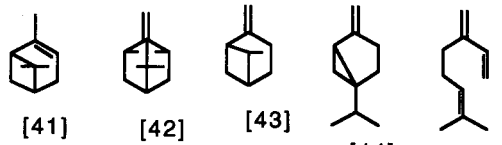
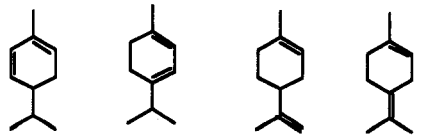


[40]

An unusual chemical composition is found in the secretion from the venom gland of the myrmicine ant *Myrmicaria natalensis* which has monoterpene hydrocarbons such as α-pinene [41], camphene [42], β-pinene [43], sabinene [44], β-myrcene [45], α-phellandrene [46], α-terpinene [47], limonene [48], and terpinolene [49][117].



[41] [42] [43] [44] [45



[46] [47] [48] [49]

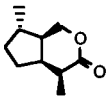
4.6. Pygidial gland

The pygidial gland, except for the Formicinae, occurs widely in ants[118].

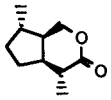
The gland consists of a pair of clusters of glandular cells located under the 6th abdominal tergite. Each cell sends a duct through the intersegmental membrane between the 6th and 7th abdominal tergites, and in many species this portion of the intersegmental membrane can be invaginated, thus forming two reservoir sacs[119].

The pygidial gland secretions play different roles in the chemical communication of some species of ants. It is involved in the production of pheromones to recruit nestmates for group predation and colony emmigration in *Leptogenys chinensis*[120] and *L. diminuta*[121]. In *Pachycondyla obscuricornis* it contains a tandem running recruitment pheromone[122] and reproductive females of *Rhytidoponera metallica* use the secretion as a sex pheromone[123]. In *Nothormyrmecia macrops,* the secretion has a function of alarm-defence. It is also used for defence in dolichoderine ants.

The chemistry of the pygidial gland secretion has been studied extensively in dolichoderine species by Pavan and Cavill and co-workers. Dolichoderine ants are unique in having iridoids as chemical components of their pygidial gland secretion. Three iridolactones, namely iridomyrmecin [50], isoiridomyrmecin [51] and isodihydroneptalactone [52] and two iridodials, iridodial [53] and dolichodial [54], cyclopentanoids and terpenoids and non-terpenoids ketones have been identified as chemical substances from the secretion[124,125].



[50]



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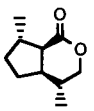

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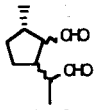

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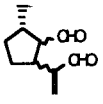
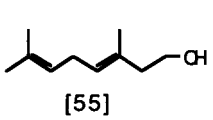


[54]

Beside the dolichoderine ants, the pygidial gland secretions of three other ant species have been analysed chemically. *Trans-* isogeraniol [55] together with *m-* hydroxybenzaldehyde [56], heptadecene and heptadecadiene was found in the secretion of *Rhytidoponera metallica* workers[119]. The pygidial gland secretion of *Leptogenys diminuta* was similarly found to contain *cis*-isogeraniol [20]. *Cis*-isogeraniol was found to be the active trail pheromone component, however a mixture of both *cis* and *trans*-isogeraniol isomers was also found to be active, which indicates that the *trans*-isomer does not inhibit the activity of the *cis*-isomer[121].

The secretion of *Nothomyrmecia macrops* workers was found to contain nine substances. Five of these substances were recognised as being part of the secretion. 3,7-Dimethyloct-6-en-2-one [57] was identified as the chief component, but 2,6-dimethyl-hept-5-enal, 2-nonanone, indole, and -dodecalactone were present in smaller amounts[126]. The other four components were hydrocarbons, which were found to be part of the Dufour's gland secretion.



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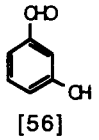

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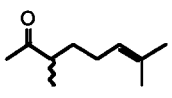

[57]

5. TECHNIQUES OF PHEROMONE RESEARCH

Insect pheromones are produced or stored and released in extremelly small amounts (micrograms to nanograms or less). They cannot be studied chemically by conventional techniques

of isolation and identification, unless an enormous amount of insect material is employed.

The first step for the isolation of an unknown pheromone is the separation of different parts of the insect's body to prepare extract. The extracts are then tested in order to identify the source of pheromone production or storage. Bioassays are normally designed according to the specific response that they require from the insect. General methods of bioassays have been reviewed[127]. Besides the use of live insects to test the activity of an extract, an insect antennae can also be employed to measure the response of the insect to components present in a pheromone source. This technique called electroantenno-graphy together with the single cell recording technique[128] has provided useful information related to the activity of compounds. However, despite its usefulness in indicating which substances have receptors on the insect's antennae, electroan-tennography does not indicate the type of behavioural response of the insect to an active component. The technique is usually employed to monitor the activity of compounds as they elute from the chromatographic column.

Once the source of a pheromone is located and its activity established, its chemical components are then isolated and chemically characterized. In the earlier years of pheromone research three techniques were widely used in the isolation of insect pheromones. They were: a) crushing and solvent extraction of the glands or parts of the insect's body where the pheromone is produced or stored; b) rinsing of the gland region using a suitable solvent; and c) steam distillation of suspensions or homogenates of insect parts, followed by subsequent solvent extraction.

These techniques have proved their usefulness in many cases, however they require large volumes of solvents and a massive amount of insect material, and then many steps of purification of the resulting extract with possible loss of more volatile components. They have, therefore been largely replaced by techniques which demand the use of only a few insects and far less solvent.

Airborne pheromones are often collected using adsorption materials such as silica gel, Porapak Q, and Tenax. Once the pheromone is trapped on the adsorbent, thermal desorption or solvent extraction takes place in order to release the volatile components from the surface of the adsorbent.

However, pheromones are usually collected in suitable solvents and then injected into the gas chromatograph, but solvents offer many disadvantages for pheromone analyses, such as dilution of the sample; it may react with certain components present in a pheromone blend, it may also brings impurities which can interfere with identification of components present at nanogram level, and in addition the very volatile components may be lost in the solvent peak. To avoid the inconveniences that the use of solvents may bring to pheromone analysis, several solventless techniques have been designed. An early design was introduced by Bowman and Karmen[129]. Subsequent devises were designed in order to place the insect material in the injection port using an aluminium capsule[130,131], a wire basket[132], a cooled wire plunger with a groove[133] or a capillary pushed through a hole in the injection septum[134]. The trapping system devised by Stälberg-Stenhagen[135] allows the volatiles from insect or plant to be first adsorbed in a Tenax precolumn, followed by subsequentt rapid thermal desorption of the volatiles onto the GC column.

A solid sampling technique, which allows a direct injection of samples without solvents was devised by Morgan and Wadhams[136]. In this technique, the insect tissue containing pheromones (e.g. exocrine glands, wings, antennae or cuticle) or fine micro capillaries with the glandular liquid, is sealed in soft glass capillaries which are then placed into the solid sampler (see Fig. 2) which is already connected to the GC injection port. The glass capillary is then crushed and the

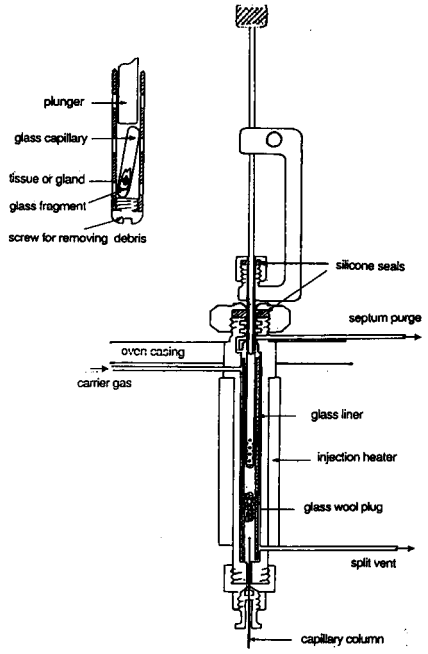

Figure 2. *Solid sample for crushing the glass capillaries in the injection port of a GC. Inset: cross section of tip of the sampler holding a glass cappilary before crushing. (From Morgan, 1990)*

volatiles, together with the carrier gas, are carried onto the column, where separation takes place.

The GC retention times and retention index provide useful information about the characteristics of a particular compound, such as its molecular size and polarity, however additional information is always required for the identification of a pheromone component. Gas chromatography coupled to mass spectrometry (GC-MS) is a powerful tool used in the identification of pheromones.

Electron impact (EI) is largely employed as the ionization technique. In this technique, the vaporized substances are bombarded with electrons and thus ionized. The resulting molecular ion subsequently collapses and give neutral radicals and positive ions, which are characteristic of the substance analysed. When the molecular ion is unstable or short lived because of thermal or energetic reasons, chemical ionization is the technique used. A reagent gas, present in excess in the ion source, is ionized by electron bombardment and the ionized gas can add or subtract a proton from the investigated substance. The mass spectrum then shows a stable *quasi*-molecular ion and only a small portion of the decay products. The mass spectrum provides information about the molecular mass of the compounds and the fragmentation patterns give information related to their structures. Identification is also aided by existing compilations of mass spectral data[137] and computer mass spectral libraries.

Mass spectrometers usually operate in order to give a complete mass spectum of a compound. However, the instrument can also be set to scan only one or a few ions of the substance, this technique is called mass fragmentography or selected ion detection. It is more sensitive than the conventional scan mode in which mass spectrometers normally operate, but it has the disadvantage of giving an incomplete mass spectrum.

Besides mass spectrometry, infrared spectroscopy is also useful to aid in the determination of a molecule structure, since it provides information about the functional groups present in a molecule. Using computer accumulation and Fourier transform equipment, spectra can be obtained at the nanogram level. An improved method of obtaining infrared spectra on small samples is the coupling of a gas chromatograph with a Fourier

transform infrared spectrometer (GC-FTIR). The compounds, eluting from the GC, flow into a heated light pipe which is installed in a Fourier transform infrared spectrometer, and are rapidly scanned.

The infrared spectra exhibit intense and narrower bands since the compounds are in the vapour phase. In addition, the samples emerging from the light pipe can be recovered.

Proton nuclear magnetic resonance spectroscopy (^{1}HNMR) is also useful in structure elucidation, but it has had a limited application in the determination of pheromone structures due to the large amount of sample that this technique requires. Fourier transform ^{1}H and ^{13}C NMR spectroscopy require 100 μg and 10 mg of pure material respectively. Since many insect pheromones are produced in nanogram quantities, thousands or millions of insects are required in order to get enough material for analysis by NMR. This technique is more suitable for the investigation of insect allelochemicals, especially defensive secretions, because they are produced by insects in large quantities.

In modern semiochemical research, NMR spectroscopy is used only when insuficient structural information is provided by more sensitive techniques.

There are cases when a mass and infrared spectra do not provide enough information to allow the full identification of a compound, in these instances, microchemical methods can be employed to aid in the elucidation of a compound structure. These microchemical methods have been reviewed by Attygalle and Morgan[138]. Microreactions are performed on glandular extracts or fractions collected after gas liquid chromatography.

Micro-reactions are particularly useful to determine the position of the double bonds, the degree of unsaturation, the carbon skeleton, and the presence of certain functional groups in a molecule.

Once the structure of a pheromone has been proposed, the next step is its synthesis to confirm the identification and also for behavioural bioassays. Since many pheromones contain double bonds and chiral centres, a high degree of stereochemical control is usually required for the synthetic methods normally employed.

Many reviews which deal with synthesis of insect pheromones are available[139,140,141].

The chirality of a pheromone molecule can be determined by gas chromatography using a capillary column coated with a chiral phase. Using this technique, a racemic mixture is injected into the GC column and its enantiomers are resolved into two distinct peaks with different retention times. A solution of one of the enantiomers is then analysed under the same conditions and its retention time compared with those of the racemic mixture. From this information, the identity of an unknown enantiomer can be determined.

Positional and geometrical isomers of mono- and diunsaturated compounds can be resolved with high-polarity phases containing large amounts of cyano groups and liquid crystal phases. The *E* -isomers elute before the *Z* -isomers on a cyano phase. On the liquid crystal phase column, *Z* -isomers elute first if the olefinic double bond is near the middle of the chain. However, as the double bond becomes nearer the end of the chain, the elution order reverses for *Z* - and *E* -isomers, and at some point they elute together.

There are two other methods used to find if double bonds are *cis* or *trans*. The first method requires examining the infrared spectrum of a substance for a weak absorption band at 970 cm^{-1} which is characteristic of *trans* olefinic bonds. The second method employed is to obtain a proton resonance magnetic nuclear spectrum of a molecule and examine the coupling constant values of the olefinic protons, which show to be 7-11 Hz for *cis* and 12-18 Hz for *trans* olefinic bonds.

Thin layer chromatography (TLC) using a stationary phase impregnated with silver nitrate is employed to separate *cis*- and *trans*-isomers. The ion Ag^+ readily forms a complex with the *cis*-isomers retaining these isomers longer whereas the *trans*-isomers elute with the solvent.

ACKNOWLEDGEMENT

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