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# CHEMICAL STUDIES ON THE CONTENTS OF THE DUFOUR GLAND OF *Manica rubida* (MYRMICINAE)

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**Summary**: The Dufour gland of *M. rubida* contains small amounts of very volatile compounds, dominated by acetone but including acetaldehyde, and a less volatile part consisting chiefly of (Z,E)-α-farnesene and (Z,E)-α-homofarnesene. These together make up 98% of the total, with several other farnesene isomers and homologues making up the remaining 2%. These farnesenes are all unstable in air and oxidize and polymerize rapidly. The Dufour glands of *Myrmica* species are similar in containing a very volatile fraction and a less volatile fraction also containing the same two farnesenes which are major components in *Manica*. In *Myrmica*, however, they are diluted with saturated and monounsaturated hydrocarbons which increase their stability in air. These chemical comparisons aid our understanding of the differences in behaviour, particularly in foraging and recruitment, between *Myrmica* and *Manica*.

**Key Words**: Ant, *Manica rubida*, Dufour gland, farnesenes, acetaldehyde, pheromones, foraging.

## Etude Chimique du Contenu de la Glande de Dufour de *Manica Rubida* (Myrmicinae)

**Résumé**: La glande de Dufour de *M. rubida* contient de petites quantités de substances très volatiles, telles que l'acétone (dominante) et l'acétaldehyde, et une fraction moins volatile comprenant notamment du (Z,E)-α-farnésène et du (Z,E)-α-homofarnésène. Ces deux dernières substances représentent, ensemble, les 98% de la sécrétion, plusieurs autres isomères et homologues du farnésène en formant les 2% restants. Les farnésènes sont tous instables dans l'air; ils s'oxydent et polymérisent rapidement. Les glandes de Dufour de *Myrmica* spp. contiennent aussi des substances très volatiles, et d'autres moins volatiles, parmi lesquelles les 2 farnésènes qui sont les constituants majeurs des glandes de Dufour de *M. rubida*. Chez *Myrmica* spp., cependant, ces farnésènes sont mêlés à des hydrocarbures saturés et insaturés ce qui en accroît la stabilité dans l'air. Ces comparaisons de nature chimique aident à comprendre les différences que présentent *Myrmica* spp. et *Manica rubida* au niveau de leur comportement, notamment lors de l'exploration de territoires et du recrutement de congénères.

**Mots-clés**: Fourmis, *Manica rubida*, glande de Dufour, farnésènes, acétaldéhyde, phéromone, exploration.

## INTRODUCTION

*Manica rubida* Latr. is a large myrmicine ant, the only European member of its genus. It was at one time included among the *Myrmica*, but is considered to be more primitive in its behaviour than *Myrmica* species. Since we have studied in detail the pheromonal chemistry and behaviour of *Myrmica* species in a number of publications, it was interesting to make a comparative study of *Manica rubida*. In particular, it would be illuminating to understand how *M. rubida* forms large and vigorous colonies, although CAMMAERTS and CAMMAERTS (1985) have shown that *M. rubida* workers do not recruit congeners to food gathering.

We have already described the chemical nature of the trail pheromone of this species (ATTYGALLE et al., 1985), and the substances of the mandibular gland (CAMMAERTS and ATTYGALLE, 1988). The other important gland of the exocrine system of myrmicine ants is the Dufour gland. We describe here the chemical contents of this gland in connection with the behavioural study of its contents (CAMMAERTS et al., this volume).

## MATERIALS AND METHODS

The collection and maintenance of the colonies of *M. rubida* are described in the previous paper. The poison apparatus (sting lance, venon reservoir, venom glands and Dufour gland) was dissected from freshly killed workers and sealed individually in glass capillary tubes until used for chromatography. These glands contained in sealed capillaries were introduced directly onto the gas chromatograph by the method of MORGAN and WADHAMS (1972), without the intervention of solvent.

For separation of the very volatile components, a fused silica capillary column (10m x 0.32mm) coated with a 10$\mu$m film of PoraPLOT Q (Chrompack UK, London) was used in a Carlo Erba Fractovap 4160 gas chromatograph (Fisons, Crawley, England), fitted with a flame ionization detector and a Shimadzu Chromatopac C-R3A data processor. The oven temperature was initially 50°C, then increased to 150°C at 20°C $min^{-1}$. Comparison of peak areas and retention times was made with standard solutions of the pure substances in water, of such concentration as to give peak areas similar to those obtained from the glands.

For examination of the less volatile portion, the individual glands were similarly treated, using a fused silica column (25m x 0.32mm) coated with OV-1 silicone of 0.4$\mu$m film thickness in a Hewlett Packard 5890 gas chromatograph directly linked to a Hewlett Packard 5970B Mass Selective Detector (mass spectrometer). The chromatographic carrier gas was helium at 4 psi (flow rate 1ml $min^{-1}$) with oven temperature at 30°C for 2 minutes then increased to 270°C at a rate of 4°C $min^{-1}$. The mass spectrometer was set to monitor m/z 35 to 350 using 70 eV ionization.

A mixture of farnesene isomers was prepared by heating a mixture of (Z)- and (E)-nerolidol (5g) (Aldrich, Gillingham, Dorset) with p-toluene sulphonic acid (0.1g) in toluene (100ml) in a Dean-Stark apparatus until no more water was produced (~ 2hr). The solution was cooled, neutralized with solid $NaHCO_3$ and chromatographed on a column of silica gel, eluting with light petroleum.

To follow its decomposition in air, a layer of the farnesene isomers, 5mm deep, was left in an open sample tube (1.5cm diameter). A small sample was removed each day and its refractive index measured in an Abbé refractometer until the mixture became too viscous to handle. In another experiment, air was blown through a solution of farnesene isomers in hexane (10mg/ℓ) and the ultraviolet spectrum recorded daily for 20 days.

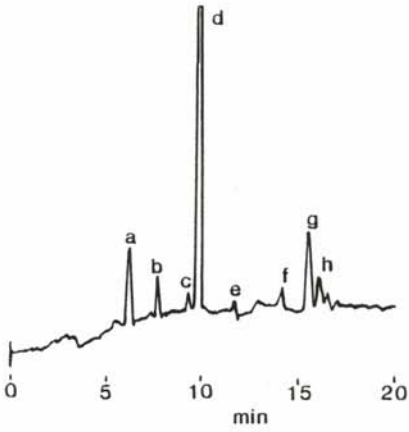


*Fig. 1 Gas chromarogram showing the very volatile substances in a single worker's Dufour gland of Manica rubida. The compounds are a, acetaldehyde, b, ethanol, c, propionaldehyde, d, acetone, e, propanol, f, isobutyraldehyde, g, butenone, h, butanone.*

*Fig. 1 Chromatographie en phase gazeuse montrant les substances très volatiles obtenues à partir d'une seule glande de Dufour d'ouvrière de Manica rubida. Les composés sont les suivants: a, acétaldéhyde, b, éthanol, c, propionaldéhyde, d, acétone, e, propanol, f, isobutyraldéhyde, g, buténone, h, butanone.*

## RESULTS

The most volatile part of the contents of the Dufour gland of *M. rubida* workers was studied by gas chromatography with the aid of a capillary column coated with a film of polymer made from styrene and divinylbenzene. This enabled us to obtain good separation of the very volatile $C_2$ to $C_4$ oxygenated compounds (Fig. 1). The chief compound present was acetone (average of 20ng) with smaller amounts of acetaldehyde (ethanal), ethanol, isobutyraldehyde (methylpropanal), and butenone, with propanal, propanol, butanone and 2-butanol visible in some samples. All were present in low nanogram quantities per gland.

The less volatile part of the secretion consisted of a simple mixture of (Z,E)-α-farnesene and (Z,E)-α-homofarnesene in varying proportions but with the latter always present in larger quantities and with an average ratio of 2:1. A small amount of other farnesene isomers and homologues (representing 2% of the total) were also present (Fig. 2).

When a mixture of farnesene isomers was exposed to air, it polymerized to an insoluble viscous gum in the course of one week. The course of this polymerization, as followed by the change in refractive index is shown in figure 3. MURRAY (1969) showed that a very thin film of farnesene was 50% decomposed in 4hr and completely in 24hr. However, a solution in hydrocarbon solvent is much more stable. When air was bubbled through a dilute solution of farnesene in hexane, the oxidation, as followed by the change in the ultraviolet spectrum of the farnesene changed slowly over 20 days, and decomposition was still not complete when the experiment was concluded (Fig.4).

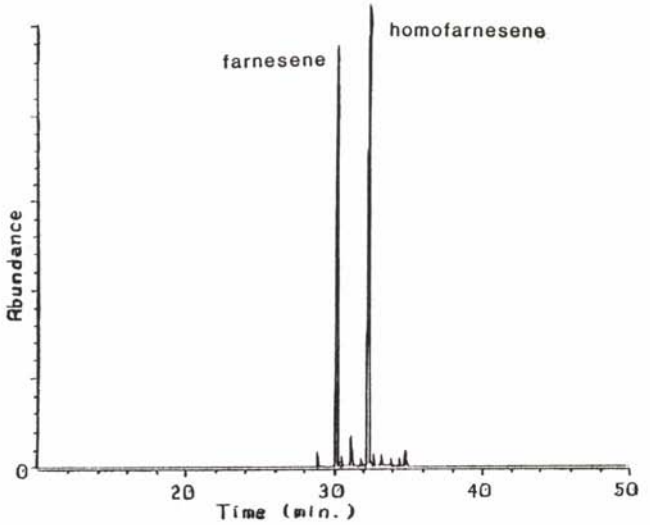


*Fig. 2 Gas chromatogram of a single Dufour gland from a worker of Manica rubida, showing the two major sesquiterpenes and the other compounds present in lower amounts.*

*Fig. 2 Chromatographie d'une seule glande de Dufour d'une ouvrière de Manica rubida montrant les deux sesquiterpènes majeurs et d'autres composés présents en faibles quantités*

## DISCUSSION

In our earlier work on a number of *Myrmica* species (ATTYGALLE et al., 1983; and earlier papers cited therein) we found that there was a very short-acting component in the Dufour gland secretion which induced activity of workers. This was shown to be due to the mixture of acetaldehyde, ethanol, acetone and other $C_2$ to $C_4$ alcohols, aldehydes and ketones present in the gland in low nanogram amounts. The acetaldehyde in the mixture was shown to induce attraction (CAMMAERTS-TRICOT et al., 1976). In the present work it was found that *M. rubida* workers did not respond to these volatile compounds in their own or *M. rubra* glands. However, *M. rubra* workers showed a short duration attraction to *Manica* glands which suggested that they also contain these highly volatile substances. This we were able to demonstrate, though there is less acetaldehyde in *M. rubida* glands than in *M. rubra* glands.

*Myrmica* Dufour glands contain mixtures of linear alkanes and alkenes always mixed with farnesene homologues in proportions varying with the species (ATTYGALLE and MORGAN, 1984). In *Manica rubida* we find only the farnesenes. The two genera therefore have similar but not identical compositions in this gland. If we postulate the odorous (to humans) farnesenes are also detected by the worker ants, then we can understand why there is cross activity of this secretion between *M. rubra* and *M. rubida*.

Farnesenes are, however, unstable in air, chiefly due to the $-CH=CH-CH_2-CH=CH-$ group they contain. The central $CH_2$ group is very susceptible to attack by oxygen. This decomposition and polymerization can be very rapid in thin films (MURRAY, 1969), less rapid in thicker films, and still slower when dissolved in a hydrocarbon solvent. Whether this hydrocarbon is hexane or heptadecane alters the conditions little. We may therefore expect the undiluted farnesene of *M. rubida* to give initially a stronger odour when deposited on a surface, but it would disappear more rapidly from a marked area than the fainter, diluted, but longer lasting odour from an area marked by *M. rubra* secretion.

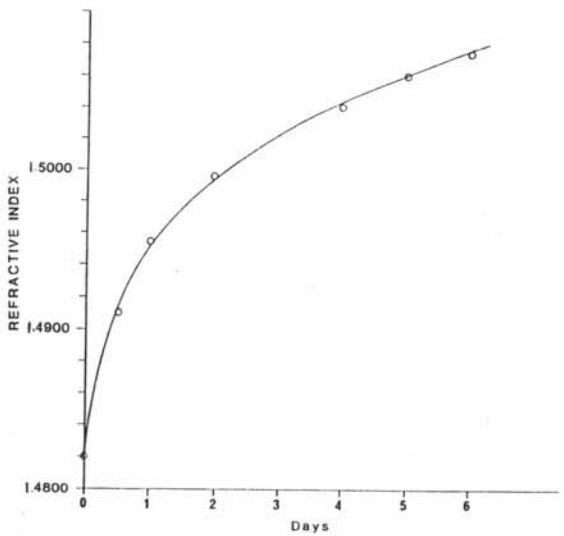


*Fig. 3 Change of refractive index with time for a mixture of farnesene isomers exposed to air.*

*Fig. 3 Changement de l'indice de réfraction en fonction du temps dans un mélange d'isomères du farnésène exposé à l'aire.*

We may conclude that the chemical evidence is entirely consistent with the behavioural observations on *M. rubida* and *M. rubra*. The pheromonal response of *M. rubida* is more primitive than that of *M. rubra*. We conclude that *M. rubida* can form such large and vigorous colonies, even though workers do not recruit nest mates, because they are much more vigorous foragers (c.f. VIENNE and ERRARD, this volume), and are continually marking the area near their nest as a foraging range.

We have now shown that eight species of *Myrmica* (CAMMAERTS et al., 1982; ATTYGALLE et al., 1983) and *M. rubida* all use their Dufour secretion to mark foraging areas. We tentatively suggest that this may be the primary function of the Dufour gland secretion in a large number of ant species, where it does not have some other, more specific function, such as providing the trail pheromone.

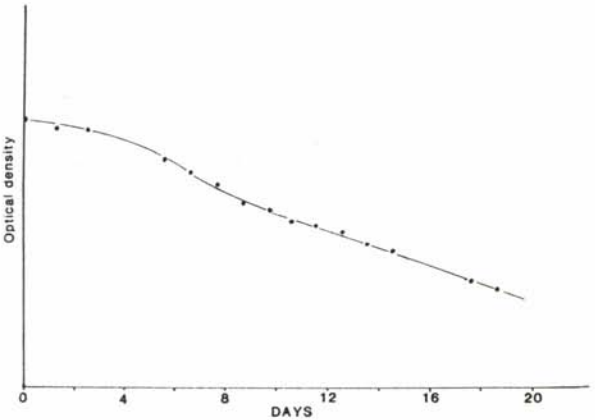


*Fig. 4 Change of optical density of a dilute solution of farnesene isomers in hexane with air continuously bubbled through it. The absorption was measured at 227nm, the maximum absorption of the mixture.*

*Fig. 4 Changement de densité optique d'une solution diluée d'isomères du farnésène dans l'hexane quand un courant d'air traverse la solution en permanence. L'absorption a été mesurée à 227nm, qui est le maximum d'absorption du mélange.*

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