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VOLATILE SECRETION OF DUFOUR GLAND OF WORKERS OF AN ARMY ANT, *Dorylus (Anomma) molestus*[1]

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Abstract—The Dufour glands of workers of *Dorylus (Anomma) molestus* contain chiefly linear alkenes and alkanes, with (*Z*)-9-tricosene and tricosane representing over 70%. The glands are relatively small with some indication of very small (nanogram or less) amounts of dihydrofarnesol. Minima, medium, and major workers of *Dorylus (Anomma) nigricans* contain a similar spectrum of compounds.

Key Words—Exocrine secretion, Dufour gland, *Dorylus (Anomma) molestus*, *Dorylus (Anomma) nigricans*, Dorylinae, Formicidae, Hymenoptera.

INTRODUCTION

The variation in the forms of organization of social insects is overwhelming. This can be illustrated by the variety of systems evolved within the Formicidae, which includes vegetarian seed-harvesting species and ferocious predators, strictly arboreal species and others that are entirely hypogaeic, while colony size ranges from as few as nine individuals in *Pachycondyla sublaevis* (Peeters,

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1990) up to several millions. The Old World army ants, which constitute the subfamily Dorylinae, undoubtedly represent one of the most fascinating groups among the Formicinae. They comprise the Aenictini, the majority of which are found in tropical Asia and Northeastern Australia, and the Dorylini, which are mainly distributed in Africa. The New World army ants form the separate subfamily Ecitoninae.

The African Dorylini hold the record, by a wide margin, for size of colony in a monogynous ant, with approximately 22 million individuals in a colony of *Dorylus (Anomma) wilverthi* (Raignier and van Boven, 1955). Doryline ants do not have a permanent nest, and in their nomadic progress only occupy temporary bivouacs in between massive raiding bouts. In spite of their enormous colony size, their considerable impact upon the environment, and their unusual habits, Dorylinae have not received as much attention as other more amenable subfamilies of ants. The available papers deal mainly with their general biology and taxonomic aspects (reviewed by Raignier and van Boven, 1955, and Gotwald, 1982). A few reports have been published on the morphology of the exocrine glands of dorylines (Hölldobler and Engel, 1978; Billen, 1985). Nothing, however is known as yet on the ethological function or the chemical composition of the glandular secretions. This lack of information is more understandable when considering, on the one hand, the impossibility of keeping these nestless ants alive in the laboratory, and on the other, the practical problems of having sophisticated research equipment close to their African habitat.

Using the solid sampling technique of Morgan and Wadhams (1972), however, the site and time of collection of the material and the laboratory where it is chemically examined become independant of each other (cf., Billen et al., 1987). Using this technique we have been able to carry out the first chemical examination of the Dufour glands of the workers of *Dorylus (Anomma) molestus* (Gerstaecker) as part of a comparative taxonomic survey of this gland in various subfamilies of the Formicidae (Morgan, 1990a; Attygalle and Morgan, 1984). We report here this first chemical examination of a member of the subfamily Dorylinae, together with a preliminary examination of the Dufour glands of *Dorylus (Anomma) nigricans* Illiger carried out several years earlier and not yet fully reported.

METHODS AND MATERIALS

Live ants were collected near Nairobi, Kenya, and immediately flown to Leuven, Belgium, where they were dissected and the glands sealed in glass capillaries and sent by mail to Keele. Worker ants were immobilized by cooling over liquid nitrogen. Dissection was carried out in water under a binocular

microscope as described by Morgan (1990b). The dissected glands were dried and sealed in glass capillaries and kept in a refrigerator until ready for chemical analysis by combined gas chromatography–mass spectrometry.

Gas chromatography was carried out on a Hewlett Packard model 5890 gas chromatograph using helium (1 ml/min) as carrier gas on a fused silica capillary (12 m × 0.35 mm) coated with a 0.33-μm film of immobilized dimethylsilicone fluid (equivalent to OV-1) (Cambridge Capillaries, Cambridge, U.K.). The injector was set at 150°C, and the detector at 300°C. The column oven was programmed from 30°C to 250°C at 8°C/min and then held isothermally. Injection was splitless. The analytical column was linked to the Hewlett Packard 5970B mass selective detector through a deactivated, uncoated fused silica capillary (10 × 0.35 mm). The mass spectrometer was set to scan m/z 40–350 using 70 eV ionization with a scan time of about 1 scan/1.5 sec. Data were collected and processed by a Hewlett Packard ChemStation, model HP 59970C.

Formation of dimethylthioethers from alkenes for the location of double bonds was carried out on a nanogram scale as described by Billen et al. (1986) and Attygalle and Morgan (1988). A capillary containing 3, 5, or 10 Dufour glands was crushed under hexane, and to this was added dimethyl disulfide (50 μl) and a solution of iodine in ether (2 μl, 60 mg/ml). The mixture was placed in a Keele Microreactor (Attygalle and Morgan, 1986) sealed with a screw cap and heated overnight at 100°C. The reaction was halted by adding aqueous sodium thiosulfate (50 μl). For the linked gas chromatography–mass spectrometry of the reaction products, the injector was set at 280°C and the column programmed from 200°C to 320°C at 3°C/min.

The analyses of the Dufour glands of *Dorylus (Anomma) nigricans* were carried out by the same solid sampling technique of single dissected glands, but were performed some years earlier on the technology available then, i.e., packed gas chromatography column of polyethylene glycol (PEG 20M) operated at 210°C isothermally and without the benefit of mass spectrometry. The identifications of the alkanes and tricosene were based upon identity of retention times. The technique was as described in papers of that time (e.g., Billen et al. 1986).

RESULTS

The Dufour glands of *Dorylus (Anomma) molestus* are filled with linear alkanes and alkenes with (*Z*)-9-tricosene and tricosane together providing over 70% of the total (Table 1). No terpenes or oxygenated compounds were observed among the major components. A typical gas chromatogram is shown in Figure 1. The position of the double bonds in the alkenes was determined by conversion to the α,β-dimethylthioethers with dimethyl disulfide. The position of the

TABLE 1. AVERAGE PERCENTAGE COMPOSITION OF THE DUFOUR GLANDS OF WORKERS OF *D. molestus* BASED ON ANALYSES OF SEVEN INDIVIDUALS[a]

Number in Figure 1	Compound	%	SD
1	Pentadecene	0.72	0.7
2	Pentadecane		
3	Heptadecadiene	0.58	1.5
4	Heptadecene	10.24	7.9
5	(*Z*)-Dihydrofarnesol	0.3	0.9
6	Nonadecadiene	0.55	1.5
7	Nonadecene	0.28	0.7
8	Heneicosene	0.18	0.5
9	(*Z*)-9-Tricosene	58.4	22.1
10	Tricosane	13.7	3.1
11	Pentacosadiene	6.82	9.0
12	9-Pentacosene	5.55	7.5

[a] Mean amount per gland, 200 ng.

double bonds were easily located in tricosene and pentacosene from the mass spectral fragmentation of the dimethylthioethers. The geometry of tricosene was shown by comparison of the retention time of the derivative with that of an authentic specimen of (*Z*)-9-tricosene to be *Z*. That of the *E* isomer has a different retention time. Samples containing 3, 5, and 10 glands were each used for the formation of the dimethylthioethers, but there was insufficient material in each of them to locate the position of the double bond in heptadecene or nonadecene.

The only evidence of nonlinear compounds was in one sample that showed a peak eluting immediately after heptadecene, which had the mass spectrum and retention time of an authentic sample of (*Z*)-dihydrofarnesol made by the method of Dawson et al. (1988). In the remaining samples, this was too weak to be clearly seen.

The preliminary examination of *Dorylus (Anomma) nigricans* also showed that tricosane and probably tricosene were the major compounds present and that the general pattern of compounds was similar, but lack of identification of some of the other substances makes full comparison impossible. Five individual minima workers, one medium, and two major workers were examined. The major component in all of them was that tentatively identified as tricosene (mean value of 30 ng), followed by tricosane (27 ng); the third component eluting near docosane was unidentified.

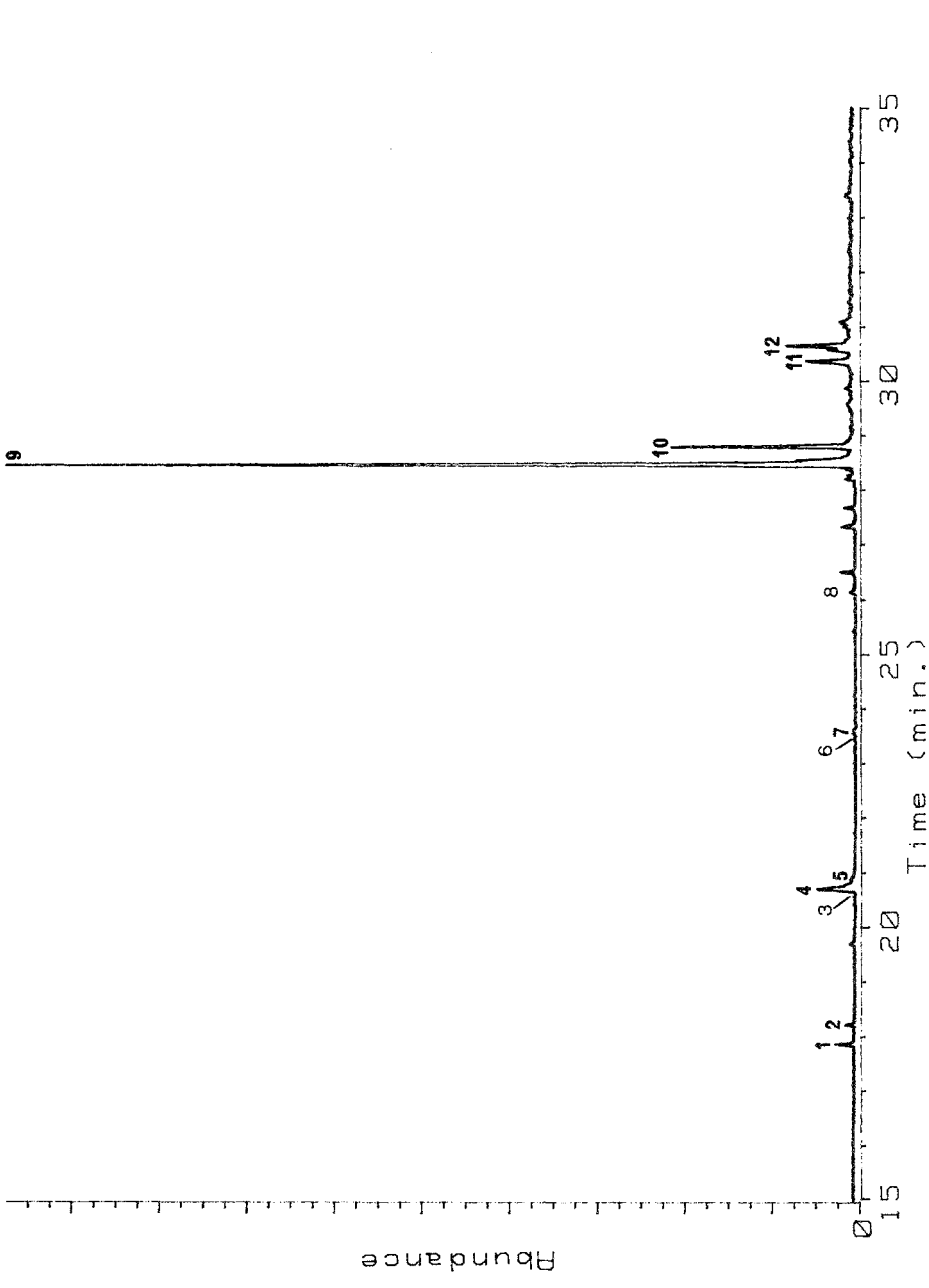

FIG. 1. Gas chromatogram of a single Dufour gland of a worker of *Dorylus (Anomma) molestus* on a capillary column. Labeled peaks are listed in Table 1.

DISCUSSION

Examination of Dufour glands of two species of *Dorylus* show that tricosene and tricosane are by far the major components in the glands of these members of the Dorylinae. Only seven single specimens of *D. molestus* were available, one of which showed a relatively high proportion of minor components, which included one terpenoid substance identified as dihydrofarnesol (Table 1) and one specimen in which the proportions of pentacosadiene and pentacosene were comparable with those of tricosene and tricosane. The result is relatively large standard deviations in the percentages in Table 1 for this small sample. The glands are comparatively small for ants, with this sample giving an average of 200 ng of secretion per gland.

The main points of interest are, first, the relatively high molecular mass (C_{23} and C_{25}) of the principal components. This we have noted previously (Attygalle et al., 1990) is characteristic of species living in a tropical climate, compared with species from a temperate climate, where C_{11}-C_{17} compounds usually predominate. Secondly, the tricosene is the same one encountered in *Atta* (Evershed and Morgan, 1981) and frequently elsewhere in insect cuticular hydrocarbons. Thirdly, although tricosane (mp 47.6°C) is a solid, tricosene is a liquid at room temperature, and the mixture of many alkenes will keep the tricosane in solution and keep the mixture liquid. Fourthly, the meager evidence for a sesquiterpene compound, dihydrofarnesol, already identified in a ecitonine ant (Morgan et al., in preparation) and now in its Old World counterpart, is interesting, although otherwise the chemistry of ecitonines and dorylines is very different.

The data on *D. nigricans* indicate that this species is not greatly different from *D. molestus*. The amount in the gland varied from 50 to 150 ng, rising with increasing size of the workers, but overall these glands are relatively small among ant Dufour glands. We may expect other species of *Dorylus* will give similar but not identical patterns of hydrocarbons in their Dufour gland.

At our present level of knowledge, there is nothing distinctive about this glandular secretion that correlates with the unusual nomadic existence of doryline ants.

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