

VOLATILE SECRETIONS OF OLD WORLD ARMY ANT
Aenictus rotundatus AND CHEMOTAXONOMIC
IMPLICATIONS OF ARMY ANT DUFOUR
GLAND CHEMISTRY

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Abstract—The Dufour glands of *Aenictus rotundatus* contain a complex mixture of terpenoids with geranylgeraniol comprising over 50% of the secretion. Some novel compounds have been tentatively identified as higher homologs of 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane based on GC-MS data. The Dufour gland secretion of *A. rotundatus* is more similar in composition to the secretions of members of the subfamily Ecitoninae than to its closer relatives from the tribe Dorylini, a result that further complicates studies on the phylogeny of army ants. The mandibular glands of *A. rotundatus* contain a mixture of 4-methyl-3-heptanone and limonene in trace amounts, and the well-developed postpygidial glands contain methyl anthranilate only.

Key Words—Hymenoptera, Formicidae, army ants, Dorylinae, *Aenictus*, *Dorylus*, *Eciton*, Ecitoninae, Dufour gland, mandibular gland, postpygidial gland, ant secretions, chemotaxonomy.

INTRODUCTION

Army ants are characterized by their nomadic existence and group predation behavior (Wilson, 1958). They are divided into two subfamilies, each with a distinct zoogeographical distribution: the Ecitoninae in the New World and the Dorylinae in the Old World tropics (Hölldobler and Wilson, 1990). The Dory-

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linae are further divided into two tribes: the Dorylini (single genus *Dorylus*) and the Aenictini [single genus *Aenictus* (Gotwald, 1982)]. Due to the considerable differences in external morphology and based on a geological interpretation of their geographical distribution, it was suggested that the two tribes developed separately (Gotwald, 1979). Furthermore, the common presence, in *Aenictus* and *Eciton*, of a conspicuous epithelium associated with the inner part of the seventh abdominal sternite has led to the suggestion that there is a possible relationship between the Aenictini and the Ecitoninae, in spite of their very different geographical distribution (Jessen, 1987). However, it has since been demonstrated that the Dufour glands of members of the Aenictini and Dorylini possess a unique crenellate lining, which distinguishes them from other ants and suggests both tribes should be classified within a single subfamily, the Dorylinae (Billen and Gotwald, 1988).

Previous work has been carried out in our laboratories on the volatile secretions of *Dorylus molestus* (Dorylini) (Bagnères et al., 1991), *Eciton burckhelli*, *Labidus praedator*, and *L. coecus* (Ecitoninae) (Keegans et al., 1993) as part of a chemotaxonomic survey of army ant secretions. Here we report a study of the Dufour gland, mandibular gland, and postpygidial gland secretions of *Aenictus rotundatus* (Aenictini) and draw attention to the phylogenetic implications of the results.

METHODS AND MATERIALS

Live worker ants of *Aenictus rotundatus* were collected, from their raiding column, in Nairobi, Kenya, and immediately flown to Leuven. The ants were immobilized by cooling over liquid nitrogen and dissection was carried out under a binocular microscope (as described by Morgan, 1990). The dissected glands were dried, sealed in glass capillaries, and stored in a refrigerator until ready for analysis by gas chromatography-mass spectrometry (GC-MS).

GC-MS was performed (as described by Bagnères et al., 1991) on a 5% phenyl-95% dimethylsiloxane phase of 0.25 μm film thickness in a fused silica capillary column (12 m \times 0.2 mm). Helium was used as the carrier gas at 1 ml/min. The oven temperature was programmed from 30°C to 270°C at 7°/min.

Commercially available samples of 6-methyl-5-hepten-2-one (Koch Light), geranylacetone (Aldrich), limonene (Aldrich), and methyl anthranilate (Aldrich) were used to confirm identifications based on mass spectra. Samples of β -springene (P. Baekström) and geranylinalool and geranylgeraniol (R. Lucas, Quest International) were received as gifts.

1,3,3-Trimethyl-2,7-dioxabicyclo[2,2,1]heptane was synthesized from 6-methyl-5-hepten-2-one via the γ,δ -epoxide (by a modification of the method

of Gaoni, 1968). To a solution of the ketone (0.5 g, 3.95 mmol) in THF-H₂O (3:4, 50 ml) a solution of magnesium monoperoxyphthalate (1.2 g, 2.43 mmol) in THF-H₂O (3:4, 30 ml) was added with stirring. The reaction mixture was left at room temperature for 2 hr before the addition of saturated sodium hydrogen carbonate solution (50 ml). The neutral solution was extracted with ether (50 ml) and the extract washed with water (20 ml), dried (magnesium sulfate), and concentrated by rotary evaporator to yield a colorless oil (0.51 g). GC-MS analysis of the product showed essentially a single peak corresponding to the γ,δ -epoxide of 6-methyl-5-hepten-2-one [M^+ 142(2), 127(3), 84(30), 83(11), 82(8), 72(16), 59(11), 43(100)]. NMR data (δ 1.23(s, 2 \times CH₃), δ 1.69(m, CH₂CH₂CO), δ 2.13(s, CH₃CO) and δ 2.50(m, epoxidic H and CH₂CO)] and IR data [ν 1718(carbonyl), 1377, 1358 and 1162 cm⁻¹ (epoxide)] were found to be identical to those previously reported (Gaoni, 1968).

Cyclization was achieved by heating the epoxyketone over a few grains of silica gel for 20 min to yield 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane (95%). GC-MS analysis revealed that the bicyclic compound had a similar mass spectrum to the epoxyketone but it eluted 4 min earlier [M^+ 142(3), 127(3), 84(20), 83(10), 82(16), 72(31), 59(5), 42(100)]. NMR data [δ 1.13(s, CH₃), δ 1.19(s, CH₃), δ 1.35-2.30(m, 2 \times CH₃), δ 1.51(s, CH₃), δ 4.16(d, CH)] was consistent with that previously reported (Gaoni, 1968).

A mixture of farnesene isomers was prepared by the dehydration of nerolidol (Parry, 1978). GC-MS analysis of the products showed a 70% conversion to the six farnesene isomers.

A mixture of geranylneral and geranylgeranial was prepared by PCC oxidation (Corey and Suggs, 1975) of geranylgeraniol. GC-MS analysis showed a 90% conversion to an equilibrium mixture of geranylneral [M^+ 288(2), 245(2), 136(8), 107(15), 84(20), 81(50), 69(97), 41(100)] and geranylgeranial [M^+ 288(2), 245(2), 136(9), 107(12), 84(31), 81(42), 69(99), 41(100)].

4-Methyl-3-heptanone was prepared in quantitative yield by hypochlorite oxidation of 4-methyl-3-heptanol.

RESULTS

The Dufour gland secretion of *A. rotundatus* was found to be dominated by terpenoids (Figures 1 and 2, Table 1). Geranylgeraniol (17) was the major component, comprising 50.8% of the secretion. The other principal components included an equilibrium mixture of geranylneral (16), which is masked by 17 in Figure 1, and geranylgeranial (18), and β -springene (12). The bicyclic acetal 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane (1), 6-methyl-5-hepten-2-one (2), geranylacetone (4), and geranyllinalool (15), present in the secretion, have been found in the Dufour glands of New World army ants (Keegans et al., 1993). We have also found two pairs of isomeric compounds in the gland of

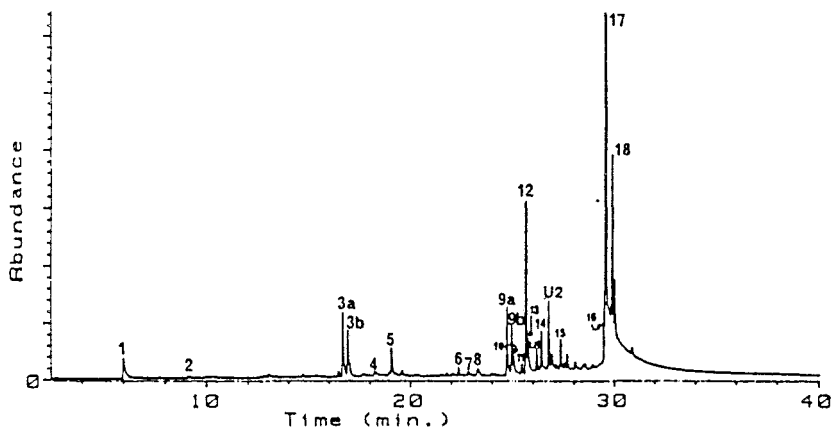


FIG. 1. Total ion chromatogram of the volatile compounds in the Dufour gland of *Aenictus rotundatus* (the numbering of the peaks corresponds to that in Figure 2 and Table 1).

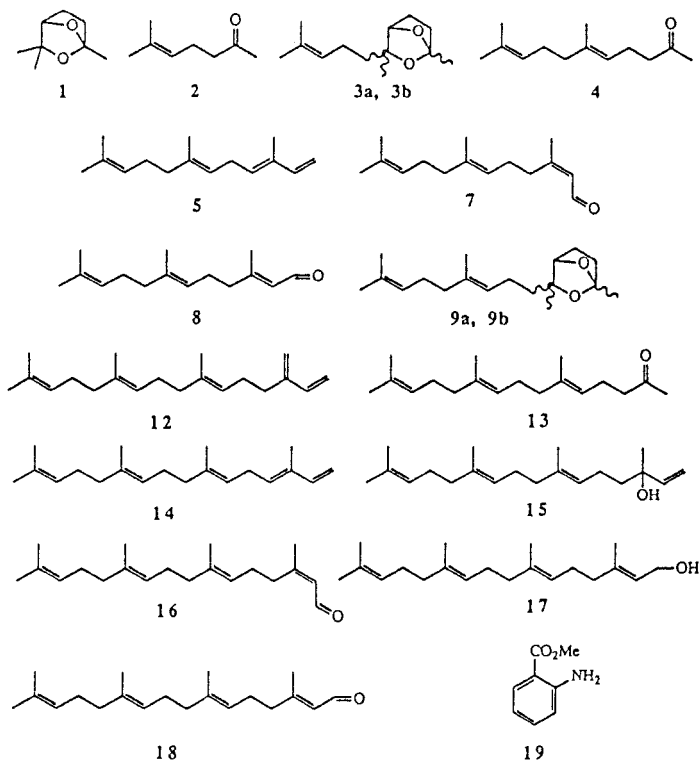


FIG. 2. The chemical structures of the volatile compounds from *Aenictus rotundatus*. The structures of 3a, 3b and 9a, 9b are tentative.

TABLE 1. PERCENTAGE COMPOSITION OF VOLATILES FROM DUFOUR GLAND SECRETION OF *A. rotundatus*

Compound	Mean % (N = 5)	SD
1. Bicyclic acetal	1.2	1.1
2. 6-Methyl-5-hepten-2-one	t ^a	
3a. Bicyclic acetal	2.9	1.9
3b. Bicyclic acetal	2.5	1.7
4. Geranylacetone	0.6	0.2
5. (<i>E,E</i>)- α -Farnesene	0.8	1.0
6. Heptadecane	0.6	0.1
7. (<i>Z,E</i>)-Farnesal	t	
8. (<i>E,E</i>)-Farnesal	0.6	0.3
9a. Bicyclic acetal	2.9	1.4
9b. Bicyclic acetal	2.1	0.9
10. Nonadecene	1.4	0.3
11. Nonadecane	0.6	0.1
12. β -Springene	7.6	1.9
13. Farnesylacetone	1.5	0.9
U1. Unknown 1	1.1	0.1
14. α -Springene	2.2	0.6
U2. Unknown 2	2.8	1.4
15. Geranylinalool	1.7	0.5
16. Geranylneral	4.6	1.0
17. Geranylgeraniol	50.8	12.6
18. Geranylgeranial	12.1	0.5
Total/ μ g	53.9	24.4

^at = trace component (<0.5%).

A. rotundatus, for which we propose the structures **3a**, **3b** and **9a**, **9b** (Figure 2), by analogy to **1**. These compounds were also found by Keegans et al. (1993) but remained unidentified at that time.

In addition to the terpenoids present in the Dufour gland, small amounts of heptadecane (**6**), nonadecene (**10**), and nonadecane (**11**) were also detected. The Dufour gland of *A. rotundatus* was the major source of volatiles of workers with each gland containing a mean of 54 (\pm 24) μ g.

Due to the small amount of volatile material in the heads of *A. rotundatus*, 10 heads were used for each injection (mean amount = 19 \pm 9 ng, N = 3 \times 10). The major component was identified as 4-methyl-3-heptanone (mean 78 \pm 13%) and the minor component as the monoterpene limonene (mean 22 \pm 13%). The amount reported may not be truly representative of normal conditions, for many species of ant tend to discharge their mandibular gland secretion when seriously disturbed.

The postpygidial glands of *A. rotundatus* are well developed and were found to contain methyl anthranilate (19) (mean 104 ± 100 ng, $N = 10$) as the only detectable component.

No volatiles were detected in the poison gland or sternal gland of *A. rotundatus*, although both elicited a trail-following response in the ants.

DISCUSSION

The Dufour gland secretion of *A. rotundatus* contains a complex mixture of structurally related terpenoids. Geranylgeraniol, the major component, has been previously identified as a component of the Dufour gland secretion of *Formica fusca*, *F. nigricans*, and *F. polyctena* (Bergström and Löfqvist, 1973). We have tentatively identified higher homologues of the bicyclic acetal (1). The mass spectra of 3a and 3b, shown in Figure 3, have the same general appearance as that of 1. The molecular ions at m/z 210 would correspond to the molecular formulae of 3a and 3b ($C_{13}H_{22}O_2$) and the spectra show fragment ions at m/z 127, 84, and 43, which are important ions in the mass spectrum of compound 1. The relatively low retention times of 3a and 3b provided further evidence for the bicyclic structure, since they eluted before geranylacetone 4 just as 1 eluted before 6-methyl-5-hepten-2-one (2). The two forms of 3 are probably due to *cis-trans* isomerism of the side chains about the rings.

Compounds 9a and 9b gave mass spectra of the same general appearance as 1, 3a and 3b and had molecular ions at m/z 278. We suggest therefore that they have the structure shown in Figure 2.

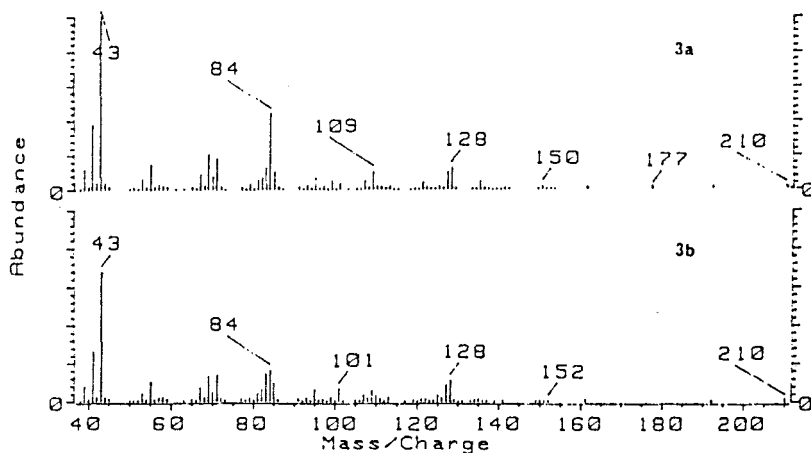


FIG. 3. The mass spectra of compounds 3a and 3b, tentatively identified as bicyclic acetals.

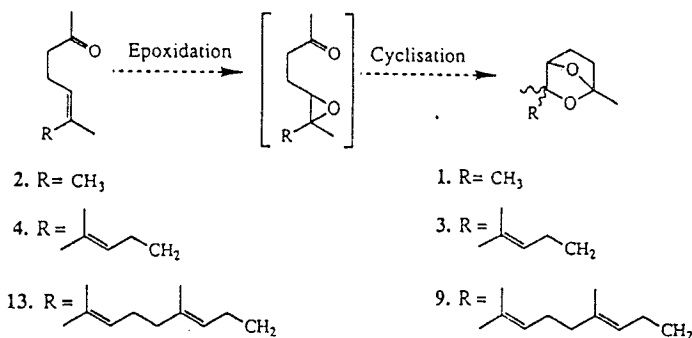


FIG. 4. A possible biosynthetic relationship between the isoprenylacetones and the bicyclic acetals.

The presence of 6-methyl-5-hepten-2-one (2), geranylacetone (4), and farnesylacetone (13) was yet further evidence for the bicyclic structure of 3 and 9, since the isoprenylacetones can be thought of as their biosynthetic precursors, via the γ,δ -epoxides (Figure 4). It is known that γ,δ -epoxides are relatively unstable and readily decompose to the bicyclic acetals on distillation and exposure to acid (Gaoni, 1968). We therefore coinjected a synthetic sample of the epoxide of 6-methyl-5-hepten-2-one into the GC-MS with an *A. rotundatus* Dufour gland. However, the epoxide did not cyclize, demonstrating that the bicyclic acetals were real components in the secretion and not decomposition products brought about by heating the samples in the injection port.

Several attempts to synthesize the γ,δ -epoxide of geranylacetone, and thus the bicyclic acetal, were made. Treatment of geranylacetone with one equivalent of epoxidizing agent gave the undesired 9,10-epoxide, while attempted selective reduction of the corresponding bis-epoxide with sodium cyclohexadienyldicarbonyliron gave no product. We were thus unable to confirm the structures of compounds 3 and 9.

Comparison of the Dufour gland secretion of *A. rotundatus* with those of the Dorylini [*Dorylus molestus* and *D. nigricans*, (Bagnères et al., 1991)] and Ectoninae [*Ecton burchelli*, *Labidus praedator*, and *L. coecus* (Keegans et al., 1993)] is very interesting, as, on the limited information available, the chemistry of *A. rotundatus* appears to be closer to the Ectoninae than the Dorylini. The Dufour glands of members of the Dorylini examined to date contain essentially linear hydrocarbons with (*Z*)-9-tricosene as the major component, while the Ectoninae contain a mixture of terpenoids and linear hydrocarbons. Compounds 1, 2, 3, 4, 6, 10, 11, and 15 from the Dufour gland of *A. rotundatus* are also present in the Dufour gland of *E. burchelli*. Thus, the chemical results appear to contradict the morphological study, which was used as evidence against the

triphyletic origin of army ants (Billen and Gotwald, 1988) and support that which linked the Aenictini and the Ecitoninae (Jessen, 1987). We hope to obtain more army ant species in the future to examine this curious result further.

The major volatile from the heads of *A. rotundatus*, like *E. burchelli* and *L. coecus*, is 4-methyl-3-heptanone, which is a common component of the mandibular gland secretions of myrmicine ants, where it often functions as an alarm pheromone (Attygalle and Morgan, 1984). However, its role in army ant communication is unknown, and the use of such a common compound as a guide in ant chemotaxonomy is limited.

Postpygidial glands are well developed in the Aenictini. The only component detected in the postpygidial glands of *A. rotundatus* was methyl anthranilate, which has been found previously in the mandibular glands of males of *Camponotus* species (Brand et al., 1973) where it is suspected to act as a sex pheromone. The function of methyl anthranilate in *A. rotundatus* is unknown, but in an unidentified *Aenictus* species (close to *A. laeviceps*) we have demonstrated that, together with methyl nicotinate, it acts as the trail pheromone (Oldham et al., 1994).

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