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VOLATILE GLANDULAR SECRETIONS OF THREE SPECIES OF NEW WORLD ARMY ANTS, *Eciton burchelli*, *Labidus coecus*, AND *Labidus praedator*

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Abstract—The Dufour glands of workers of *Eciton burchelli* contain a mixture of small quantities of oxygenated compounds, some of which are derived from terpenes, and C_{17}–C_{25} hydrocarbons. The secretion of the Dufour glands of soldiers was either similar to that of workers, with geranylacetone a significant component, or they contained geranyllinalool in large amounts. The glands of workers and soldiers of *Labidus praedator* and *Labidus coecus* contained (*E*)-β-ocimene, a new substance for the Dufour glands of ants. 4-Methyl-3-heptanone was the dominant compound in the mandibular glands of *E. burchelli* and *L. coecus*. Skatole and indole were found in the gasters of *L. praedator*, and skatole was present in the venom glands of some soldiers of *E. burchelli*.

Key Words—Hymenoptera, Formicidae, Ecitoninae, army ants, *Eciton*, *Labidus*, (*E*)-β-ocimene, Dufour gland, mandibular gland.

INTRODUCTION

The behavior of foraging along well-established trails, one of the characteristics of ant societies, has developed to exceptional proportions in the army ants, among which a migratory existence and group predation are the two essential

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and diagnostic characteristics (Wilson, 1958). The most spectacular and extreme form of this group-foraging and raiding is displayed by the Dorylinae and Ecitoninae, the army ants of the Old and New World, respectively. Most of the available knowledge on army ants is on the Ecitoninae, and in particular the genus *Eciton*, including reports on its taxonomy (Watkins, 1976), anatomy and glandular ultrastructure (Whelden, 1963; Billen, 1985), social behavior and foraging strategy (Topoff, 1972; Rettenmeyer et al., 1983; Franks, 1985), thermoregulation (Franks, 1989), and review papers on their general biology (Schneirla, 1971; Gotwald, 1982). The few reports available on their use of pheromones deal with the existence of trail substances (Blum and Portocarrero, 1964; Watkins et al., 1967; Torgerson and Akre, 1970) and the existence of sternal and tergal glands (Hölldobler and Engel, 1978; Hölldobler and Wilson, 1990, p. 235), and there is a contribution by Franks and Hölldobler (1987) on sexual competition and possible queen pheromones. Until now, only behavioral studies have been carried out; this report is the first chemical identification of any of their exocrine secretions.

Three species of the Ecitoninae have been studied here. *Labidus coecus* and *Labidus praedator* are "column raiders," where the small and medium workers run along the trails while the soldiers remain stationary on either side. *Eciton burchelli* is known as a "swarm raider," where workers spread out into a broad fan-shaped raiding front.

We have examined the chemical contents of the mandibular gland, venom gland, Dufour gland, postpharyngeal gland, cuticle, and sixth and seventh abdominal sternites of workers and soldiers of *Eciton burchelli*.

We report the composition of mandibular gland and Dufour gland secretions of workers of *Labidus praedator* as well as the volatile products from their whole abdomens, and the contents of the Dufour glands of soldiers. We also report on the mandibular and Dufour gland contents of minor and medium workers and soldiers of *Labidus coecus*. These data form part of a comparative survey of representative species of the major ant subfamilies. We have recently reported the first such chemical study on the secretions of an Old World army ant (subfamily Dorylinae) *Dorylus* (*Anomma*) *molestus* (Bagnères et al., 1991).

METHODS AND MATERIALS

Collection

Eciton burchelli (Westwood 1842) was collected at Reserva Ducke in Manaus, AM Brazil in March 1991. *Labidus coecus* (Latreille 1802) was collected at Bowmer Ranch near Waco, Texas, U.S.A. in June 1985 and *L. praedator* (Fr. Smith 1858) near Rio Claro, SP Brazil in November 1989. Raiding workers and soldiers were taken live to the laboratory and dissected. Whole

abdomens, heads, venom glands, Dufour glands with sting attached, and isolated Dufour glands (free of other tissues) were sealed singly or in groups in soft glass capillaries (Morgan, 1990), which were then posted to Keele, where they were kept in a refrigerator until ready for analysis.

Analysis

The sealed capillaries were placed in the injector area of the gas chromatograph, and after 2 min heating, were crushed in the device described by Morgan and Wadhams (1972) to introduce the volatile material onto the column without the intervention of solvents. Gas chromatography linked to mass spectrometry was performed as described by Bagnères et al. (1991). A fused silica capillary column (12 m × 0.2 mm) coated with OV-1 of 0.33-μm film thickness was used with helium carrier gas at 1 ml/min. The oven temperature was programmed from 30°C at 8°C/min to 250°C.

Some samples were analyzed by gas chromatography on a Carlo Erba Fractovap Series 4160 using a column of the same type and dimensions as for GC-MS. The carrier gas was helium at 1 ml/min, and the oven was programmed from 100°C at 6°C/min to 270°C. The detector was connected to a Shimadzu CR-3A recording integrator for quantification.

The double bond position in tricosene of the cuticular hydrocarbons was determined by GC-MS of the dimethylthioether derivative, prepared with dimethyl disulfide as described by Billen et al. (1986).

Preparation of Reference Compounds

2,3-Dihydrofarnesol. 2,3-Dihydrofarnesol was prepared essentially as described by Dawson et al. (1988). Farnesol (5 g, 25 mmol, Aldrich) was stirred at room temperature in an atmosphere of hydrogen in the presence of platinum oxide (0.1 g) until 500 ml (25 mmol) of hydrogen had been absorbed. The catalyst was filtered off and the solvent removed to give a mixture of dihydrofarnesol isomers, IR, liquid film, 3500–3300 cm^{-1} (OH), 1720 cm^{-1} (C=C), NMR (60 MHz) δ 0.89 (d, CH_3) δ 1.2–1.3 (m, 5H, CH_2 and CH) δ 1.65 (m, 9H CH_3—C=), δ 2.0 (m, 6H, CH_2), δ 3.2 (t, CH_2—OH), δ 5.04 (broadened t, 2H, CH=). GC—MS resolved the crude product into a number of peaks with R_t between 20 and 22 min identified as isomeric dihydrofarnesols and tetrahydrofarnesol. The main peak at R_t 20.79 min, representing 60% of the total, was (*E*)-2,3-dihydrofarnesol, identified by its mass spectrum (M^+ 224 (0.1%), *m/z* 209 (0.1), 181 (3.3), 164 (0.3), 162 (2.1), 149 (0.6), 139 (0.8), 123 (10.2), 121 (2.5), 119 (0.2), 109 (6.4), 107 (26), 95 (16), 91 (24), 82 (10.4), 81 (33), 71 (3.4), 69 (100).

2,3-Dihydrofarnesyl Acetate. Crude dihydrofarnesol (2.24 g) in dry pyridine (3 ml) and acetyl chloride (0.86 g), held at −10°C for 10 min and then

at room temperature for 2 hr, gave, after the usual work-up, crude dihydrofarnesyl acetate, IR, 1740 cm^{-1} (C=O), 1230 cm^{-1} (C—O), NMR 60 MHz, δ 0.7 (d, CH_3), δ 1.1–1.3 (m, 5H, CH_2 and CH), δ 1.4 (br, 9H, CH_3), δ 1.9 (s CH_3CO), δ 2.0 (m, 6H, CH_2) δ 4.1–4.3 (t, CH_2—O), δ 5.0 (m, 2H, CH=). GC—MS gave only two peaks, R_t 21.46 and 21.85 min, identified as the *E* and *Z* isomers, respectively, of 2,3-dihydrofarnesyl acetate. Mass spectrum of (*E*)-2,3-dihydrofarnesyl acetate gave M^+ 224 (0.1%), *m/z* 223 (2), 191 (0.2), 189 (0.5), 163 (2.7), 149 (0.8), 135 (1.9), 123 (11), 109 (7), 95 (16), 93 (14), 81 (48), 79 (7), 69 (100), 67 (73), 57 (3), 55 (19), 53 (14), 43 (91), 41 (87).

7-Methyl-2-undecanone. The Grignard reagent prepared from 2-bromohexane (8.2 g, 50 mmol) in tetrahydrofuran, was added to a cooled (0°C), stirred solution of 1,4-dibromobutane (10.7 g, 50 mmol) and dilithium tetrachlorocuprate (10 mmol) in tetrahydrofuran (Friedman and Shani, 1974). After 1 hr, isolation and distillation gave 1-bromo-5-methylnonane. The Grignard reagent prepared in turn from 1-bromo-5-methylnonane was treated with acetyl chloride at −70°C. Work-up in the usual way gave 7-methyl-2-undecanone (bp 70°/0.05 mm in bulb-tube distillation), NMR (270 MHz) δ 0.9 (m, 6H, CH_3), δ 1.1 (m, 12H, CH_2), δ 1.4 (m, 1H, CH), δ 2.2 (s, CH_3CO), δ 2.4 (t, CH_2CO); mass spectrum M^+ 184 (0.1%), *m/z* 166 (1), 151 (1), 124 (10), 109 (8), 95 (12), 71 (25), 58 (75), 43 (100), 41 (30).

7-Methyl-2-undecanol. Reduction of 7-methyl-2-undecanone (200 μl) with sodium borohydride (40 mg) in methanol (2 ml) gave 7-methyl-2-undecanol, NMR (270 MHz) δ 0.9 (m, 6H, CH_3) δ 1.1 (m, 12H, CH_2), δ 1.4 (m, CH and CH_3—C—OH, 4H), δ 1.7 (m, 2H, CH_2—C—OH), δ 3.2 (s, OH), δ 3.8 (m, CH—OH); mass spectrum, M^+ 186 (not seen), *m/z* 153 (2%), 126 (3), 111 (15), 84 (20), 69 (40), 55 (38), 45 (100), 43 (80), 41 (48).

Other compounds were identified by comparison of their retention times and mass spectra with authentic specimens, except for the cyclic acetal **4**, dimethyldisulfide and trisulfide, and the alkenes, which were identified by their mass spectral fragmentation patterns alone.

RESULTS

The presentation of the analyses of Dufour glands of soldiers and workers of *Eciton burchelli* provided difficulties because the composition of the secretion varied considerably among the 23 individuals examined (13 workers and 10 soldiers). There was a pattern of linear hydrocarbons present that was more or less constant in proportions throughout the sample, but when these were taken together with the very variable amounts of oxygenated compounds also present, the percentage composition was rather variable, as indicated by the standard deviations found in Table 1. Of these hydrocarbons, tricosene was the major

TABLE 1. MAJOR COMPOUNDS IN DUFOUR GLAND SECRETION OF WORKER AND SOLDIER CASTES OF *Eciton burchelli*, PRESENTED, WHERE POSSIBLE, AS MEAN PERCENTAGE OF TOTAL SECRETION IN GLAND WITH STANDARD DEVIATION[a]

Compound	Workers ($N = 13$)[b] ($\bar{X} \pm$ SD)	Soldiers I ($N = 5$)[c] ($\bar{X} \pm$ SD)	Soldiers II ($N = 5$)[c] ($\bar{X} \pm$ SD)
2-Methylcyclopentanone	0.2 ± 0.2	t[d]	t
2-Methylcyclopentanol	0.3 ± 0.2	t	t
Cyclic acetal **4**	5.7 ± 1.9	—	c
6-Methyl-5-hepten-2-one	0.4 ± 0.3	t	c
6-Methyl-3-octanone	t		
(*Z*)-β-Ocimene	—	t	t
(*E*)-β-Ocimene	1.1 ± 2.0	[e]	t
7-Methyl-2-undecanone	1.1 ± 0.8		
7-Methyl-2-undecanol	0.8 ± 0.8		
Geranylacetone	4.9 ± 3.0	0.4 ± 0.6	4.4 ± 5.3
Pentadecane	0.6 ± 1.1		
Farnesol	(12.7)[f]		
Heptadecane	1.7 ± 0.4		
Nonadecene	2.1 ± 1.4		
Nonadecane	1.0 ± 0.4		
Geranyllinalool	—	44.2 ± 14.4	
Eicosene	2.0 ± 0.2		
Eicosane	0.2 ± 0.2		
Heneicosadiene	—	—	2.3 ± 1.5
Heneicosene	20.7 ± 5.2	8.6 ± 5.1	15.6 ± 9.6
Heneicosane	4.5 ± 1.4	— —	5.1 ± 1.0
Docosene	2.4 ± 1.3	2.6 ± 1.3	3.1 ± 0.2
Tricosene	42.2 ± 3.0	19.9 ± 8.9	37.0 ± 3.0
Tricosane	3.0 ± 1.1	1.6 ± 0.8	2.3 ± 1.0
Mean total amount (μg)	1.4 ± 0.6	4.7 ± 2.4	2.7 ± 1.7

[a] Soldiers have been divided into two groups, those containing (I) or not containing (II) geranyllinalool.
[b] Not all minor components listed, so total does not equal 100%, 36 substances were quantified, 20 of them hydrocarbons.
[c] Gaps in the columns means either the substance was not present or that amounts were small and quantitation too difficult.
[d] t = trace constituent, <0.5%, — = not detected.
[e] Present but very variable.
[f] Identified in only one individual.

one, followed by heneicosane, and thereafter a large number of C_{17}–C_{25} alkanes and alkenes in smaller proportion. The analysis was further complicated by finding geranyllinalool **(1)** (Figure 1) in large quantities in five of the soldiers' Dufour glands, together with variable amounts of (*E*)-β-ocimene **(2a)**, while

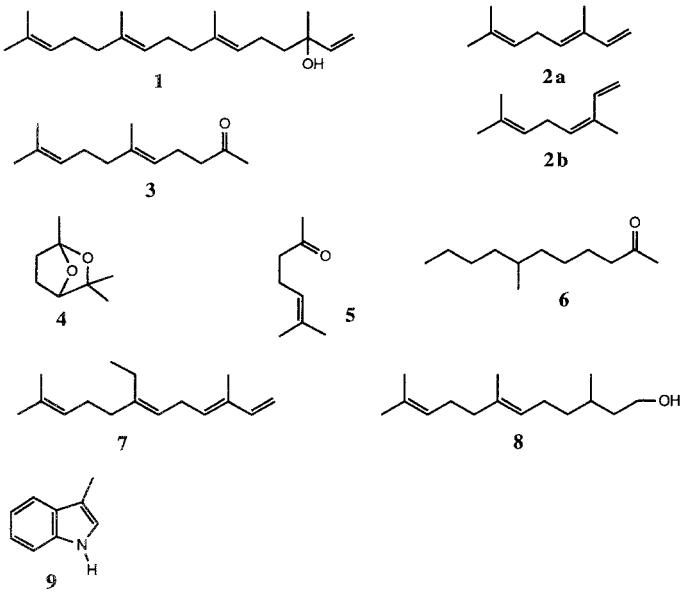

FIG. 1. Chemical structures: **1,** geranyllinalool; **2a,** (*E*)-β-ocimene; **2b,** (*Z*)-β-ocimine; **3,** geranylacetone; **4,** 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane; **5,** 6-methyl-5-hepten-2-one; **6,** 7-methyl-2-undecanone; **7,** (*E,E*)-α-homofarnesene; **8,** 2,3-dihydrofarnesol; **9,** skatole.

the other five soldiers did not contain geranyllinalool but had more geranylacetone **(3)** (Figure 2). Chemically, the latter group of soldiers more closely resembled the workers, which all contained geranylacetone but had no geranyllinalool. The workers' Dufour glands also contained the cyclic acetal 1,3,3-trimethyl-2,7-dioxabicyclo(2,2,1)heptane **(4)** and 6-methyl-5-hepten-2-one **(5),** which can be regarded as a biosynthetic precursor of **4.** Three groups of results are therefore presented in Table 1, and many minor components have had to be omitted for lack of useful quantitation. The species was notable for containing chemicals of very wide range of volatility from the very volatile cyclic acetal **4** to pentacosane. This is unusual in Dufour glands. It was also notable for having present very small quantities (individually less than 1% of total) of many other compounds, most of them identified by their mass spectra, e.g., methyl-branched hydrocarbons related to the major hydrocarbons, but also some other unidentified compounds. The quantities of secretion in glands of both workers and soldiers were large for ants of their size, with more than 1 μg secretion in workers and up to 8 μg in soldiers that contained geranyllinalool.

Geranyllinalool was identified by comparison of mass spectrum and retention time with an authentic sample contained in jasmine oil (Demole and Led-

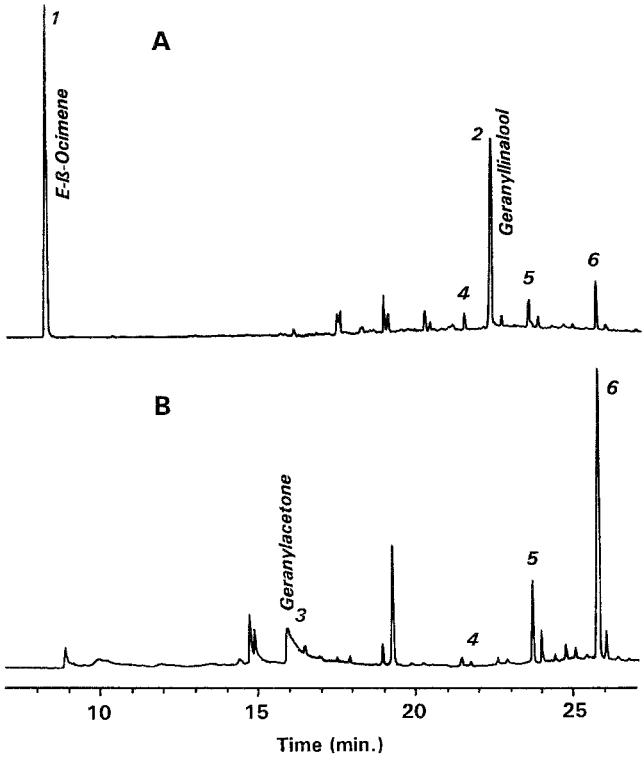

FIG. 2. Total ion chromatograms of the two different patterns seen in the secretions of Dufour glands of soldiers of *Eciton burchelli*: (A) one dominated by (*E*)-β-ocimene (peak 1) and geranyllinalool (peak 2), and (B) one where these are replaced by geranylacetone (peak 3); other peaks are nonadecane (4), heneicosene (5), and tricosene (6).

erer, 1958). 7-Methyl-2-undecanone **(6)** and 7-methyl-2-undecanol are not previously recorded in the chemical literature. The ketone was recognized as a methyl-branched 2-ketone by its mass spectrum and retention time. Possible structures were deduced using biosynthetic considerations and the correct structure was proved by synthesis. When the ketone was reduced to the corresponding alcohol, that was found to be identical with another minor product in the gland.

The Dufour glands of workers of *L. praedator* contained principally (*E*)-β-ocimene (**2a,** 83% of the total secretion, Table 2). The linear hydrocarbon nonadecene was the second most important compound. Minor amounts of (*Z*)-β-ocimene **(2b),** (*E,E*)-β-homofarnesene **(7),** (*E*)-2,3-dihydrofarnesol **(8)** and its acetate and hexadecyl acetate were also present. (*Z*)-β-Ocimene was identified by comparison with authentic material in a mixture of (*E*)- and (*Z*)-β-ocimene. (*E,E*)-β-homofarnesene was identical with that described by Attygalle and Mor-

TABLE 2. PERCENTAGE COMPOSITION OF DUFOUR GLAND SECRETIONS OF WORKERS AND SOLDIERS OF *Labidus praedator*[a]

Compound	Clean worker glands (% ± SD)	All worker glands (% ± SD)	Worker abdomens (%)	Soldier abdomens (%)
(*Z*)-β-Ocimene	1.2 ± 0.3	0.5 ± 0.1	1.4	0.5
(*E*)-β-Ocimene	83.1 ± 0.8	74.7 ± 11.2	42.1	74.7
Monoterpene isomer **1**	—	1.0 ± 0.9		1.0
Homoocimene	—	1.8 ± 0.7	0.3	0.3
Monoterpene isomer **2**	—	1.9 ± 0.6		0.4
2-Decanone	—	—	0.2	
Geraniol	—	—	0.4	
Indole	—	—	1.2	—
2-Undecanone	—	—	—	0.2
Methyl 6-methylsalicylate	—	0.4 ± 0.1		
Skatole	—	—	36.5	
(*E*,*E*)-β-Homofarnesene	—	0.1 ± 0.1	0.3	0.4
(*E*)-2,3-Dihydrofarnesol	1.7 ± 0.6	0.6 ± 0.3	0.8	1.6
Heptadecene	2.0 ± 0.3	1.2 ± 0.5	0.8	2.5
Heptadecane	1.2 ± 0.5	0.1 ± 0.1		0.8
(*E*,*E*)-Farnesol	—	0.3 ± 0.1	0.8	t
Farnesal	—			t
Dihydrofarnesyl acetate	0.6 ± 0.2	0.4 ± 0.2		0.5
Nonadecadiene	0.5 ± 0.1	1.6 ± 2.0	0.2	0.8
Nonadecene	9.6 ± 0.3	12.0 ± 4.6	5.7	7.7
Nonadecane	0.4 ± 0.2	0.6 ± 0.3	0.3	0.6
Hexadecyl acetate	0.6 ± 0.2	1.4 ± 1.8		
Geranyl-linalool	—	—	0.5	
Heneicosene	—	—	0.3	
Heneicosane	—	—	2.2	
Tricosadiene	—	—	0.6	
Tricosene	—	—	4.0	
Tricosane	—	—	1.2	
Mean total amount (μg)	86	90	34	490

[a]Worker samples were either carefully dissected free of other tissues ($N = 2$), or had some tissue attached ($N = 8$). Mean values for the two and ten samples are given. The mean values for whole abdomens of worker ($N = 20$) and soldiers ($N = 2$) are also given. — indicates substance not detected.

gan (1982). 2,3-Dihydrofarnesol and its acetate, prepared as described above, were identical in mass spectra and retention times with the ant compounds. Small differences in composition were found between cleanly dissected workers' Dufour glands and those to which the sting (but not the venom gland) and some tissue were still attached (Table 2). Some minor amounts of farnesol and some

unidentified monoterpenes were present in samples of the sting apparatus but were not found in the cleanly dissected Dufour glands, and it is presumed they come from some other structure. Whole abdomens were found to contain the same mixture of substances as the Dufour glands with the addition of skatole (3-methylindole, **9**), in quantity similar to that of the (*E*)-β-ocimene (Figure 3), and some higher hydrocarbons (C_{21} and C_{23}) probably from the cuticle (Table 2). The glands of soldiers were very similar to those of workers. The glands of *L. praedator* workers contained much less secretion than those of *E. burchelli* but soldiers' glands of *L. praedator* were correspondingly larger than those of workers of the same species.

The Dufour glands of *Labidus coecus* contained a much simpler mixture of substances, essentially (*E*)-β-ocimene **(2a)** in minor and medium workers and soldiers, with over 90% of this compound in all but one sample of a medium worker gland (Table 3). The amount of glandular secretion was intermediate in amount between that of *E. burchelli* and *L. praedator*.

The heads of both workers and soldiers of *E. burchelli* contained essentially 4-methyl-3-heptanone with much smaller amounts of 4-methyl-3-heptanol, two substances already identified in the mandibular glands of a number of ant species (Table 4).

The heads of *L. praedator* contained very small amounts of 2-nonanone, 2-decanone, and 2-undecanone, evidently from the mandibular gland since such volatile ketones are often found in mandibular glands (Attygalle and Morgan, 1984). There were rather large amounts of tricosane, tricosene, tricosadiene, heneicosane, and heneicosene, characteristic substances of the postpharyngeal

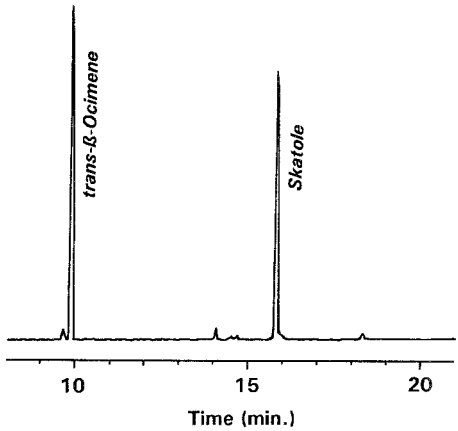

FIG. 3. Total ion chromatogram of the volatile compounds in a whole gaster of a worker of *L. praedator*. A Dufour gland chromatographed under the same conditions showed only one peak for β-ocimene.

TABLE 3. PERCENTAGE COMPOSITION OF DUFOUR GLAND SECRETIONS OF MINOR WORKERS, MEDIUM WORKERS, AND SOLDIERS OF *Labidus coecus* AND MEAN TOTAL AMOUNT OF SECRETION PER GLAND

Compound	Minor workers ($N = 6$)	Medium workers ($N = 3$)	Soldiers ($N = 4$)
(*Z*)-β-Ocimene	1.6	—	1.4
(*E*)-β-Ocimene	97.8	80.0	98
2-Nonanone	—	2.4	—
Nonanal	—	8.0	—
Decanal	—	10.4	—
Nonadecene	0.1	—	—
Tricosene	0.4	—	—
Mean total amount (μg)	0.62	0.25	0.94

TABLE 4. MEAN VALUES OF PERCENTAGE COMPOSITION OF VOLATILE COMPOUNDS IN HEADS OF WORKERS ($N = 5$) AND SOLDIERS ($N = 2$) OF *E. Burchelli*, WORKERS OF *L. praedator* (10 Heads in One Sample), AND MEDIUM WORKERS OF *L. coecus* ($N = 4$).

Compound	*E. burchelli*		*L. praedator* workers	*L. coecus* workers
	Workers	Soldiers		
4-Methyl-3-heptanone	92.6	91.2	—	89.6
4-Methyl-3-heptanol	7.4	8.9	—	8.3
2-Carene	—	—	9.8	—
1-Hexanol	—	—	11.2	—
Hexanal	—	—	4.5	—
2-Nonanone	—	—	14.2	—
Nonanal	—	—	17.1	—
2-Decanone	—	—	19.4	—
1-Decanol	—	—	—	2.1
2-Undecanone	—	—	17.0	—
Tridecane	—	—	6.7	—
Mean total amount (ng)	2,200	3,900	4.8	760

gland (Bagnères and Morgan, 1991), also present in this head sample, which are not included in Table 4.

The heads of *L. coecus* workers contained chiefly 4-methyl-3-heptanone. In this, and in the amount of secretion, they resembled *E. burchelli* much more than *L. praedator* (Table 4).

Venom glands were also dissected from three workers and two soldiers of

E. burchelli. The worker glands contained a very variable mixture of alkylpyrazines among other things (Table 5). The soldier glands were without volatile substances. Samples of sixth and seventh abdominal sternites from *E. burchelli* were also available. No volatile substances were detected in the samples of worker sternites. Of six soldiers' sternites prepared, two contained relatively large amounts of (~30 ng) of skatole, two contained small amounts (1–2 ng) of skatole, and two contained no detectable amount of skatole (<0.1 ng).

Samples of antennae, legs, abdominal cuticle, and the postpharyngeal glands of soldiers and workers of *E. burchelli* were also examined. The composition of hydrocarbons on the legs and abdominal cuticle and in the postpharyngeal glands was very similar; 9-tricosene, tricosane, heneicosane, pentacosane, and heptacosane (in decreasing order of importance) were all present. The antennae also contained these hydrocarbons but also up to 40% of cholesterol, not previously identified in cuticular wax.

DISCUSSION

Although the New World army ants of the subfamily Ecitoninae have been extensively studied in the field, the difficulty of confining them in a laboratory and keeping them alive with normal behavior has until now prevented laboratory-based studies of their exocrine secretions or pheromones. By the use of our microchemical studies on single isolated glands sealed in glass capillaries (Morgan, 1990), it has been possible to prepare glands from freshly collected ants to transport the samples and to carry out the analyses later. The great interest in this subfamily and the absence of chemical data on them made it interesting

TABLE 5. PERCENTAGE COMPOSITION OF VENOM GLANDS OF THREE SAMPLES OF WORKERS OF *Eciton burchelli*[a]

	Worker		
Compound	1	2	3
Dimethyldisulfide	24.8	—	—
Dimethyltrisulfide	20.7	—	—
2,5-Dimethylpyrazine	7.3	—	20.2
Trimethylpyrazine	—	35.5	—
3-Ethyl-2,5-dimethylpyrazine	4.4	64.5	27.5
Indole	32.8	—	52.2
2-Decanone	9.6	—	—
Total amount (ng)	642	4.5	2185

[a] Venom glands from two soldiers gave no volatile compounds.

to record these preliminary and fragmentary studies of three species of Ecitoninae. Of the five genera in the subfamily, we have examined two species of *Labidus* and one of *Eciton*. The Dufour glands of the two *Labidus* species were notable for having the relatively volatile monoterpene substance (*E*)-β-ocimene **(2a)** as the major substance while it was a minor substance in *E. burchelli*. Ocimene ($C_{10}H_{16}$) is a highly volatile substance with a pleasant odor for humans. It was first isolated from the leaves of the herb sweet basil (*Ocimum basilicum*). It is found in the leaves and flowers of many plants (cf. Sutton et al., 1992), along with other monoterpenes, and is a constituent of many perfumes. Although Dufour gland substances are usually less volatile than ocimene, monoterpenes have been found in other ants. *Myrmicaria natalensis* venom glands contain a mixture of pinene, sabinene, phellandrene, and camphene (Brand et al., 1974). β-Pinene was reported in the mandibular glands of *Atta sexdens* (Schildknecht, 1976) and perillene in the mandibular glands of *Lasius fuliginosus* (Bernardi et al., 1967). β-Ocimene is found frequently in the Isoptera, for example, in the frontal glands of soldiers of several species of termites (Baker et al., 1981). This is, however, the first time a monoterpene has been found as the major substance of the Dufour gland of an ant.

Ocimene was usually absent from the Dufour glands of *E. burchelli* workers or present only as minor substances, but it appeared as a major substance in some of the soldier glands. The workers of *E. burchelli* were more like that of many other ant species, having some oxygenated and terpenoid compounds in a mixture of higher alkanes, alkenes, and methyl-branched alkanes.

Another minor component of the Dufour glands was found to have a mass spectrum similar to that of ocimene **(2a, 2b)** but contains one more carbon atom (Figure 4). We have tentatively identified it as a homoocimene with the structure 3,7-dimethyl-1,3,6-nonatriene. Its mass spectrum does not correspond to the known 4,8-dimethyl-1,3,7-nonatriene. The presence of homofarnesene accompanying farnesene is a common occurrence in ants (Attygalle and Morgan, 1982). There are a number of other homologous sesquiterpenes, such as the juvenile hormones, but homomonoterpenes are very rare. Complete identification of the homoocimene will depend upon its unambiguous synthesis.

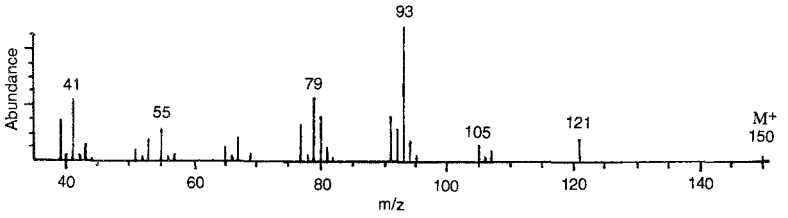

FIG. 4. Mass spectrum of the minor compound in the Dufour glands of *Labidus praedator*, identified as homoocimene.

E. burchelli is distinguishable from the two *Labidus* species by its very variable Dufour gland secretion. That of *E. burchelli* is much more like that of many formicine ants with a mixture of odorous terpene and oxygenated chemicals in a hydrocarbon mixture. The two cleanly dissected Dufour glands of *L. praedator* workers gave surprisingly consistent results (Table 2). The *Labidus* species, with their predominance of ocimene, are quite unusual. *L. praedator* and *L. coecus* can nevertheless be distinguished by the absence of linear hydrocarbons in *L. coecus*, but also they can be distinguished very easily by their mandibular gland secretions (Table 4).

Billen (1992) found that an extremely powerful and persistent trail pheromone was located in the seventh abdominal sternite of *E. burchelli*, and he was able to dissect and prepare samples of sixth and seventh sternite cuticle of several workers and soldiers of this species. We were unable to detect any volatile substances in the worker sternites but we identified skatole **(9)** in some soldier sternites. Since no skatole was detected in the Dufour glands of *E. burchelli*, it is possible the skatole is a product of the well-developed glandular epithelium on the seventh sternite described by Hölldobler and Engel (1978) for *E. hamatum* (see Hölldobler and Wilson, 1990, p. 235), and the possibility of skatole being the trail pheromone must be considered. The presence of relatively large quantities of skatole (and some of its homolog, indole) in the abdomens (but not the Dufour glands) of *L. praeditor* is also very interesting. Skatole has been found in the poison gland of major workers of *Pheidole fallax*, but no pheromone function has been assigned to it (Law et al., 1965). Indole has recently been shown to be one of the components of the trail pheromone from the poison gland of the ant *Tetramorium meridionale* (Jackson et al., 1990). We must await the opportunity to test the behavioral effect of skatole.

Eciton burchelli will require a more detailed examination to understand the variation in our results on the Dufour and venom glands. Is it possible that the two chemically distinct groups of soldiers reflect some morphologically or behaviorally distinct castes in this species or do they represent individuals at different stages of development in the nomadic–stationary cycle of colony life? The presence of cholesterol on the worker antennae also needs further study. We have discovered some interesting substances, unusual in ant secretions, some of them readily available, which we hope will stimulate interest in behavioral studies of the Ecitoninae.

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