Note

Volatile Constituents of the Green Ant *Oecophylla smaragdina*

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Oecophylla smaragdina (Fabricius) inhabits tropical woodland regions from Southern Asia to Northern Australia and many western pacific islands where they are commonly known as green tree ants.¹⁾ The ant contains 19.8 g/100 g of carbohydrates and 25 g/100 g of fat, and is considered as a food by the native aboriginal communities in the northern part of Australia.²⁾ A hot water extract of this ant is also used by the aborigines as an expectorant, antiseptic, and antimicrobial agent for scabies and other itchy conditions.³⁾ This study presents the first report of the volatile and hydrocarbon constituents of *Oecophylla smaragdina*. Brand *et al.*²⁾ have obtained a very high fatty component yield (25%) in green ants from the Northern Territory area of Australia by using chloroform-methanol extraction, while our yield after five replicated experiments, using dichloromethane extraction, did not exceed 8%. We also repeated the extraction of fatty components by using chloroform-methanol, but similar results were obtained. A seasonal variation in the amount of fatty components in green ants could be possible, but could not be expected to account for the high value reported by Brand *et al.* Ants of the sub-family Formicinae are characterized by their widespread use of formic acid as a venom and defensive substance. Formic acid has been identified as the sole or major constituent of the venom sacs of numerous species of the formicine genera *Oecophylla*, *Formica*, *Camponotus* and *Polyergus*.⁴⁾ The chemistry of the Dufour and mandibular glands of formicine genera *Formica*, *Lasius* and *Camponotus* has also been explored, and Dufour's gland was identified as a major source of hydrocarbons and their oxygenated derivatives.⁵⁻⁷⁾ The volatile hydrocarbon constituents of the whole ant are given in Table I. The compounds were identified from mass spectral data and/or by co-injection with reference compounds where available, or from reference data.⁸⁻¹¹⁾ Six hydrocarbons were identified from C₁₁ to C₁₇ carbon atoms, in the total and gaster extracts. Of these, undecane (8) and tridecane (17) were predominant (approx. 64%),

Table I. VOLATILE CONSTITUENTS IN A METHYLENE CHLORIDE EXTRACT OF *O. smaragdina*

No.	Neutral and basic compounds from total extraction	Head	Source gaster	Mass spectral reference
1	Ethylbenzene		+	9
2	Styrene		+	9
3	Bromobenzene		+	11
4	Unknown		+	
5	Unknown		+	
6	Decane		+	11
7	Octanol	+		9
8	Undecane		+	11
9	Unknown (suspected mol. wt. 152)		+	
10	Borneol	+		9
11	Napthalene		+	9
12	Decan-3-one	+		9
13	Dodecane	+		11
14	Nerol	+		9
15	Unknown (suspected mol. wt. 182)		+	
16	Unknown (suspected mol. wt. 182)		+	
17	Tridecane	+	+	11
18	Hexyl hexanoate	+		11
19	Tetradecane		+	9
20	Butylhydrogen phthalate		+	11
21	Pentadecane	+		11
22	Unknown		+	
23	Hexadecane		+	11
24	Heptadecane		+	11

while hexadecane (23) and heptadecane (24) were minor constituents. By gas chromatography, the constituents isolated from the heads of *Oecophylla smaragdina* were found to be predominantly (56%) tridecane (17) and pentadecane (21), while the rest were essentially higher molecular weight hydrocarbon atoms. Such higher molecular weight hydrocarbons are normally constituents of the cuticle was¹²⁾ and were not examined in detail. The alkanols, 1-octanol (7), nerol (14) and borneol (10), were predominant (40%) in the head extract, but were minor components in the total extract. A longer Soxhlet extraction (10 hr) with dichloromethane gave the formate esters of these alcohols. Authentic formate esters of the alcohols were produced and the peaks identified. When whole ants were ground with sodium hydrogen carbonate and then simply extracted with dichloromethane at room temperature (25°C), the original unesterified alcohols were found. Only one ketone, 3-decanone (12), was identified in the head and gaster extracts. Hexyl hexanoate (18), a new ester, was present in the head extract, this ester not having been previously reported from an insect secretion and being identified against an authentic sample.

The acidic fraction from the total extract, after methylation, showed the presence of methyl formate. The characterization of formic acid, the major constituent of the venom from the ant of sub-family Formicinae, a representative of the genus *Oecophylla*, needs no comment. Additionally, the following fatty acids were characterized as their methyl esters: caproic, enanthic, caprylic, pelargonic, capric, lauric, myristic, palmitoleic, heptadecanoic, and oleic. Two major components of fatty from sub-family Formicinae, palmitic and stearic acid,¹³⁾ were absent in *Oecophylla smaragdina*. Oleic, palmitoleic and linolenic acids were the major components, accounting for approx. 80% of the acids, while the others listed made up the remainder.

Workers of *Oecophylla smaragdina* are chemically characterized by the narrow range of alkanes, and by the presence of nerol, borneol, ketone and other related compounds. The likely functions of Dufour's gland secretions in alarm and defence roles are known,^{14,15)} but the behavioural functions of volatile hydrocarbons in *Oecophylla smaragdina*, if any, are yet to be studied.

Experimental

Isolation of the volatile constituents of Oecophylla smaragdina

(a) A small branch with a nest of green ants was cut and placed into a plastic bag (*Eucalyptus tetradonta*) from a tree. The bag was frozen at -10°C for 15 min and the dead ants collected. *Oecophylla smaragdina* workers (14.6 g) were ground in a glass mortar with anhydrous sodium sulphate (3.0 g), and the mixture was extracted (Soxhlet) with freshly distilled methylene chloride (100 ml) for 10 hr. The extract was washed with aqueous sodium hydrogen carbonate (2%, 25 ml $\times$ 3) and water (25 ml $\times$ 3), and then dried over anhydrous sodium sulphate. The solvent was finally removed under reduced pressure, and the non-acidic constituents (1.2 g, 8%) were subjected to a gas chromatographic-mass spectrometric analysis.

The combined aqueous layer were carefully acidified (dil. HCl) and extracted with diethyl ether for 24 hr. The ethereal solution, after drying with anhydrous magnesium sulfate, was evaporated to a small volume at atmospheric pressure under N_2 . Diazomethane in diethylether was added until in excess, and the methylated material, after concentrating, was used for analysis.

(b) Whole ants were divided into the head, thorax and gaster, and separate extractions were carried out on the heads (10.5 g) and gasters (21.1 g), as described in (a). The two extracts, after removing the acidic components, were used for comparative gas chromatographic-mass spectrometric studies.

(c) Whole ants (20.1 g) were ground in a glass mortar with sodium hydrogen carbonate (20.1 g) and anhydrous sodium sulphate (3.0 g). The mixture was extracted with dichloromethane at room temperature (25°C), washed with water, dried over anhydrous magnesium sulfate, and the

solvent evaporated as described in (a). The neutral components were used for comparative gas chromatographic-mass spectrometric studies.

Capillary GLC was carried out by using at 25.0 m OV101 glass capillary column. The column was programmed at 2°C from 90°C to 250°C in a Pye 104 series chromatograph. For combined gas chromatography-mass spectrometry, the 25.0 m OV101 glass capillary column was used, the mass spectrometer being operated at a 70 eV ionization voltage with the ion source at 190° . The spectra produced were acquired and processed by a Digital PDP 11/73 computer system.

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