2-Hydroxy-5-methyl-1,4-benzoquinone from the salivary gland of the soldier termites *Odontotermes magdalenae*

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Summary. By gas chromatography – mass spectrometry and comparison with authentic samples, 2-hydroxy-5-methyl-1,4-benzoquinone was identified for the first time in the salivary gland secretion of the termite soldiers of *Odontotermes magdalenae*, in addition to previously reported benzoquinones.

Key words. 2-Hydroxy-5-methyl-1,4-benzoquinone; *Odontotermes magdalenae*; scrub savannah; salivary glands; chemical defence; chemical ecology.

Termites form an important component of the soil fauna of the scrub savannah region of Nigeria. The common tree climber *Odontotermes magdalenae*, a garden and house pest in Nigeria, is often responsible for the destruction of timber used in housing construction. The success of termites over the years is due in large part to the development of elaborate behavioural and chemical defences[2–4]. Termite soldier chemical defences are very novel and variable. The bizarre morphological and glandular devices that produce and deliver these weapons are equally impressive[5]. As a contribution to the chemical ecology of the social insects of tropical grassland, we hereby report on the chemical composition of the salivary gland secretion from the soldier termite *Odontotermes magdalenae*, on which no chemical work has been previously recorded[6–8].

Methods. Odontotermes magdalenae adults were collected at night from several localities in the scrub savannah regions of Kano and Bauchi States, on the campuses of Bayero and Abubakar Tafawa Balewa Universities respectively. They were immediately transported to the laboratory where they were chilled prior to the isolation of scent materials. The salivary gland was excised under a 200 mM NaCl solution and the scent volatiles extracted in chromatoquality and freshly distilled ether. Extracts (sample, vol. ca 5 µl) were stored in sealed glass ampoules at −15 °C.

Preliminary analysis of the scent volatiles was carried out on a Packard model 427 gas chromatograph equipped with a flame ionization detector and a glass capillary column (2 m × 2 mm) packed with 5% OV-225 on a 100–120 mesh gas chrom G. The carrier gas (nitrogen) flow rate was 30 ml/min and oven temperature was 70 °C isothermal for 15 min, then programmed to 270 °C at 6 °C/min.

Gas chromatography-mass spectrometry (GC-MS) in the EI mode was performed at 70 eV and 100 µA ionizing current using a KRATOS MS25-mass spectrometer interfaced to a CARLO-ELBO-FRACTOVAP 4200 gas chromatograph. Separations were accomplished on a 2 m × 2 mm 5% OV-225 silicone on a 100–120 mesh gas chrom Q at 30 °C/min. The oven temperature was 70 °C isothermal for 15 min and then programmed to 200 °C at 6 °C/min. Authentic samples of 1,4-benzoquinone (**2**), 2-ethyl-1,4-benzoquinone (**3**), 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) and 2-methyl-1,4-benzoquinone (**6**) were available for comparison. The 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) was synthesized by the method of Woodward et al.[9] and the GC identity of (**4**) was corroborated with the synthetic sample.

Results. The secretion from the salivary gland of *Odontotermes magdalenae* is a highly odoriferous oil. The EI mass spectra of six peaks revealed by GC and GC-MS (OV225) column are given in the table.

The identities of the peaks were indicated by computer library search on their mass spectra and confirmed by comparisons of retention times and mass spectra with authentic samples. The reconstructed ion current trace of the salivary gland secretion of *Odontotermes magdalenae*, and the mass spectrum of 2-hydroxy-5-methyl,1,4-benzoquinone (**4**), are shown in figures 1 and 2 respectively.

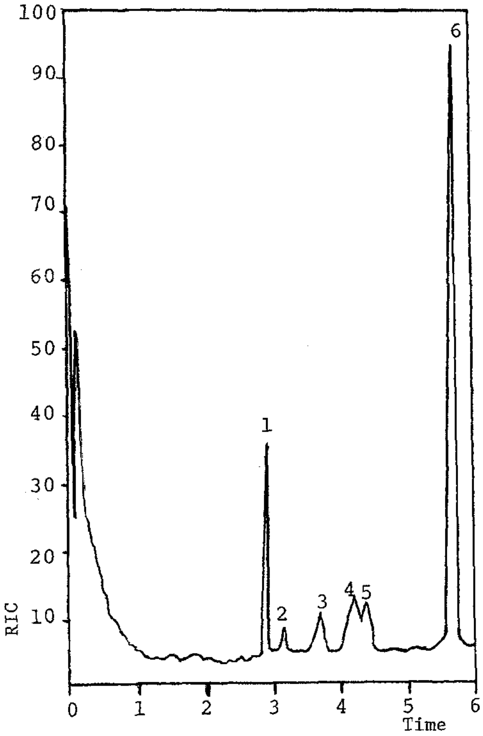

Figure 1. Reconstructed ion current trace of salivary gland secretions from *Odontotermes magdalenae*, obtained by EI-GC-MS. GC conditions: 2 m × 2 mm glass column packed with 5% OV 225 on 100–120 mesh Gas Chrom. G. Injection temperature 180 °C; oven temperature 70 °C for 15 min and then temperature programmed to 270 °C at 6 °C per min.

Discussion. Of the 1,4-benzoquinones (**2**), (**3**) and (**6**) identified in the salivary gland secretion from termite soldier *Odontotermes magdalenae*, the 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) has not been previously reported from any *Odontotermes* species. 2-Hydroxy-5-methyl-1,4-benzoquinone (**4**) was identified by co-chromatography with a synthetic sample which gave a retention time and mass spectral fragmentation pattern identical with that of the natural product. The chemistry of arthropod natural products used in defence and alarm has been an area of primary interest to chemists and entomologists, as recent investigations into the substances used as chemical warfare by termite soldiers have revealed a wide range of structural variations[10]. The basic weapons available to soldier termites are their mandibles and

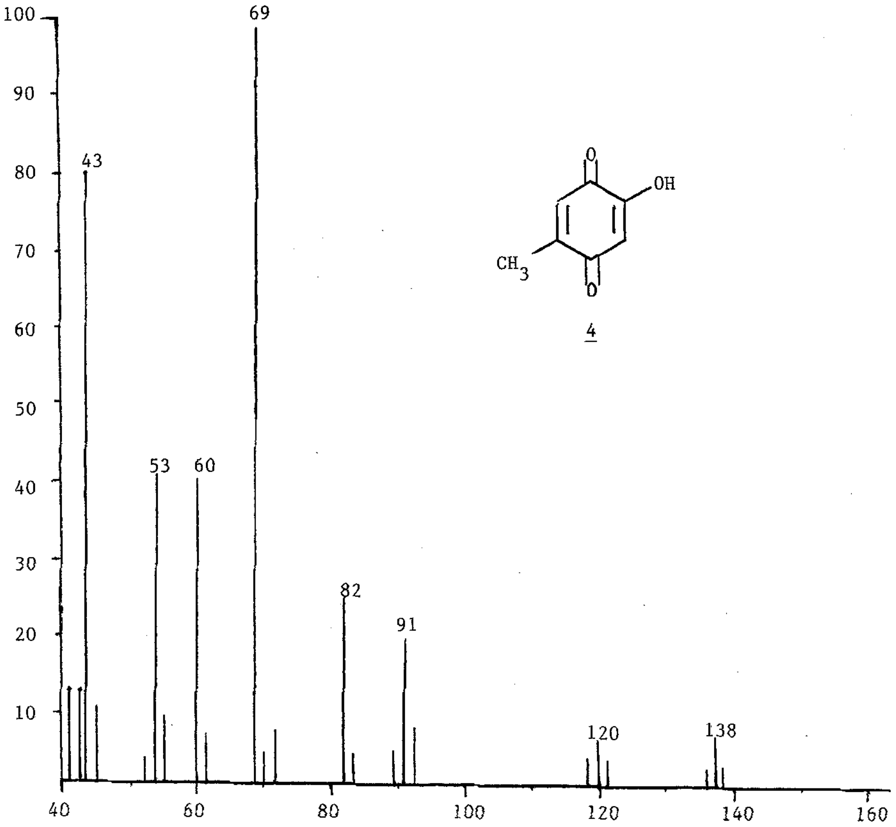

Figure 2. Mass spectrum of 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) from *Odontotermes magdalenea*.

Component of salivary gland secretion of *Odontotermes magdalenae*

Peak No.		Composition	Mol. wt	EI mass spectrum m/z (% abundance)
1	Toluene	2	92	92(M$^+$,15), 66(75), 54(100)
2	1,4-benzoquinone	2	122	122(M$^+$,26), 94(60), 82(35), 67(30), 66(79), 54(199), 43(90)
3	2-ethyl-1,4-benzoquinone	3	136	136(M$^+$,32), 108(44), 79(5), 71(33), 54(60), 43(100)
4	2-hydroxy-5-methyl-1,4-benzoquinone	37	138	138(M$^+$,8), 120(8), 91(20), 82(25), 69(100), 60(41), 53(41), 43(80)
5	Unidentified	2	166	166(M$^+$,23), 50(29), 91(77), 53(29), 45(54), 43(100)
6	2-methyl-1,4-benzoquinone	54	124	124(M$^+$,100), 123(39), 95(35), 77(30), 67(40), 55(35), 41(30)

defensive chemicals[11]. It is known that termite soldiers may repel, kill or immobilise an attacking ant by biting, with the addition of a toxic or irritant chemical secretion from the frontal gland reservoir; by daubing, in which a brush-like elongated labrum is employed in the topical application of a toxic mixture from the frontal gland, or by squirting, in which a sticky glue-like secretion is fired by the soldiers from a distance of up to 10 cm[11]. Soldiers of the subfamily Microtermitinae, represented by *Odontotermes badius*, a subterranean fungus-growing termite, and *O. stercorivorus*, were found to bite with the concomitant expulsion of an aqueous mixture of proteins and 1,4-benzoquinones from the salivary glands opening into the mouth[12], and 2-methyl-1,4-benzoquinone was also found in the same gland of *Odontotermes radamanni*[11]. The quinones found in *O. badius* and *O. radamanni* are obviously used for defence against predators. We suggest here that the previously unreported 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) along with 1,4-benzoquinone (**2**), 2-ethyl-1,4-benzoquinone (**3**) and 2-methyl-1,4-benzoquinone (**6**) are used as defence chemicals by *O. magdalenae*, possibly as a nonspecific irritant to reduce ant predation. Earlier studies on defensive compounds of millipedes, identified quinones (**2, 6**); 2-methoxy-3-methyl-1,4-benzoquinone; 2,3-dimethoxy-1,4-benzoquinone, and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (ubiquinone), which were found to be oozed or sprayed by four species of African millipede[13,14].

It is interesting to note that the biosynthetic products of the salivary gland of *Odontotermes magdalenae* are of the same biochemical origin; that is, they are mainly acetate-derived, and that 2-hydroxy-5-methyl-1,4-benzoquinone (**4**) is related to ubiquinone, which has been isolated from an African millipede[15].

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Air ventilation in nests of two African stingless bees *Trigona denoiti* and *Trigona gribodoi*

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Summary. The ground nesting *Trigona denoiti* as well as the arboreal nesting *T. gribodoi* have nest cavities with only a single narrow entrance tube. For air exchange in the nest to occur, the bees have to ventilate the nest actively. As a consequence the air is exchanged in a similar way as in vertebrates with lungs. Phases of inspiration follow phases of expiration ('breathing' frequency in the range of 2–3 breaths/min) and tidal volumes range from 0.63 ± 0.24 ml in *T. denoiti* to 1.13 ± 0.32 in *T. gribodoi*. Ventilation during night time was strongly reduced.
Key words. Respiration; social behavior; *Trigona denoiti; Trigona gribodoi.*

The regulation of the nest climate is a crucial problem for all eusocial insects. The brood is in many species highly sensitive to temperature extremes and quickly dies if the nest humidity is not regulated. Various strategies for nest climate control have been developed within the social insects, ranging from the transport of brood within the nest to warmer or cool areas (e.g. in ant nests[1,2]) to the highly complex regulation of nest climate in honeybees (*Apis mellifera*).

Though the control of nest climate in honey bees is well understood[3–7], in stingless bees data are very scarce[8]. In particular African species have been neglected, although they have interesting nest architectures which might impose specific behavioral requirements for nest climate control. A nest cavity with a single narrow entrance tube is typical for all *Trigona* species in southern Africa[9]. The stingless bees either nest in hollow trees or subterranean cavities. The cavities are coated with batumen, the typical nest material of the stingless bees. It consists of water-proof resin and is a good insulator.

T. denoiti nests 60–100 cm deep in the ground. The nest as well as the entrance tube is lined with batumen. The brood nest has a spiral structure and uses the small nest cavity most efficiently. The brood nest temperature is regulated by the bees, though a mechanism for active cooling seems to be absent[10]. A long, narrow entrance tube (1 cm diameter, more than 60 cm long) connects the nest with the outside, which may make cooling of the nest by fanning extremely difficult. The bees apparently nest at a great enough depth to achieve a stable nest climate even when the outside temperatures are high.

T. gribodoi nests in cavities of hollow trees[11]. In Transvaal they are mainly found in the Red Bush Willow (*Combretum apiculatum*). They also have only a single narrow nest entrance tube (0.7 cm diameter, 10–20 cm long). The nest does not overheat because of the shade and insulation provided by the tree. Also in this species no active cooling has been observed, though they actively produce heat if the temperature drops below 31 °C (B. Weissenbacher, unpublished data).

Although a small nest entrance with a long connective tube to the nest is effective in reducing water loss and buffering against ambient temperature variation, it may create substantial problems for the regulation of the concentration of the respiratory gases within the nest. The narrow entrance tube allows no flow-through system for fresh air as found in termite mounds[12,13]. Unless the bees can withstand extremely low oxygen levels in the nest, they have need for a ventilatory system which exchanges old stale air with fresh air.

Southwick and Moritz[14] showed that colonies of honeybees are able to control the concentration of the respiratory gases even if there is only a single narrow entrance tube to the hive. The honeybee workers actively fanned stale air out of the hive, producing a pattern similar to that of breathing of mammals, with an inspiration and expiration phase. Nests of honeybees, however, very often have a large entrance or even multiple entrances to the nest cavity and it is believed that in most cases the honeybee workers ventilate their nest in such a way that there is a continuous unidirectional airflow through the nest. The observed phenomenon therefore might be of small relevance in natural colonies of honeybees.

In the African stingless bees, on the contrary, the single entrance tube is definitely a physiological phenomenon. Since there is only one connection to the outside world, and the nest cavity is lined with airtight batumen, the bees should generate a type of 'colony breathing', similar to that found in the experimental setup with honeybees. In this paper we study how air exchange and the ventilatory system in ground nesting and arboreal stingless bees operates.

Materials and methods. Two nests of each species were localized in the Lapalala Game Reserve, Transvaal, South Africa. A copper/constantane thermocouple connected to a recorder, was placed at different levels into the entrance hole, to document the temperature gradient from the outside air to the nest. Thereafter the thermocouples were positioned 10 cm inside the entrance tube and the changes in temperature were recorded for at least 20 min (fig. 1). During the day, when the ground surface temperature exceeded 60 °C, an increase in