



Male specific tyramides from three additional myrmicine genera

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ARTICLE INFO

Article history:

Received 16 February 2010

Accepted 14 March 2010

Keywords:

Tyramide

Megalomyrmex

Trachymyrmex

Cyphomyrmex

Male ants

Semiochemical

1. Subject and source

It has been estimated that there are over 20 thousand ant species (Hölldobler and Wilson, 1990; Smith et al., 2009), over 12,500 currently described (antbase.org, 15 Jan. 2010) some of which play a large role in destroying (Lessard et al., 2009; Porter and Savignano, 1990) or maintaining the structure of ecological communities (Hölldobler and Wilson, 1990). The Myrmicinae is the largest of the 21 extant subfamilies in the ants (Hymenoptera: Formicidae) (Bolton, 2003). Ants use a complex chemosensory system involving a mixture of semiochemicals for within and between species interactions (i.e., recruitment, nestmate recognition, alarms, territorial marking, competition, etc.) (Attygalle and Morgan, 1984; Hölldobler and Carlin, 1987). Many of the glandular substances are small volatile molecules and although many still need to be studied to determine their function, a large number have been identified (Attygalle and Morgan, 1984).

There are two ant tribes represented in this study, Solenopsidini (*Megalomyrmex modestus*, *Megalomyrmex wallacei*, *Megalomyrmex wettereri*, *Megalomyrmex symmetochus*, *Megalomyrmex staudingeri*) and Attini (*Cyphomyrmex faunulus* and *Trachymyrmex septentrionalis*). All species occur in wet Neotropical rainforests in Central and South America besides *T. septentrionalis* which occurs in the Midwestern, Southern and Eastern parts of the United States (Rabeling et al., 2007). *M. modestus* and *M. wallacei* were collected at two sites in Costa Rica (10.33333N,84.08333W and 10.4264N, 84.0258W, respectively), *M. staudingeri* in Peru (3.44S, 72.85W), *M. wettereri* and *M. symmetochus* in Panama (9.120858N, 79.715906W), *T. septentrionalis* in the United States (37.37057N, 78.683217W), and *C. faunulus* in Suriname and French Guiana (4.73019444N, 56.768W and 4.08835N, 52.67718W, respectively) (Table 1). *M. modestus*, *M. staudingeri* and *M. wallacei* are free-living species while *M. wettereri* and *M. symmetochus* associate with fungus-growing ant species (Adams et al., 2000; Brandão, 1990, 2003).

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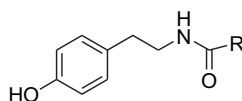
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Table 1

Male tyramides. Collection number includes the initials of the collector, the date (yr/month/day), and the series. The numbers reported are relative ratios of the compounds and X indicates that only one compound was detected.



Species	Collection number and country	CH ₃	R n-C ₃ H ₇	n-C ₅ H ₁₁
<i>Megalomyrmex modestus</i>	RMMA030812-02 Costa Rica		1.5	1
<i>Megalomyrmex staudingeri</i>	RMMA040613-05 Peru		1.5	1
<i>Megalomyrmex wallacei</i>	RMMA050710-01 Costa Rica		1	1
<i>Megalomyrmex wettereri</i>	RMMA010330-01 Panama		2	1
<i>Megalomyrmex symmetochus</i>	RMMA050729-12(B) Panama		9	1
<i>Trachymyrmex septentrionalis</i>	SAR040627-01 United States	X		
<i>Cyphomyrmex faunulus</i>	JSC060307-05 Suriname		X	
<i>Cyphomyrmex faunulus</i>	TRS050729-18 French Guiana		X	

T. septentrionalis and *C. faunulus* are fungus-growing ants that maintain a symbiotic relationship with their fungal cultivar and are in two distinct phylogenetic clades (Schultz and Brady, 2008). Voucher specimens have been deposited at the Smithsonian Institution National Museum of Natural History, Washington, DC.

2. Previous work

Surprisingly semiochemicals are largely unknown in male ants (but see Blum et al., 1987; Brand et al., 1973). Methyl 6-methyl salicylate, 2,4-dimethyl-2-hexenoic acid, and methyl anthranilate are sex specific compounds found in *Camponotus* males but these are from the mandibular glands (Brand et al., 1973). A number of N-acyltyramines, or tyramides, were recently discovered in three ant genera and nine ant species (Jones et al., 2010). The amides were reported to be specific to the gaster of male ants and were not found in females of the same species tested. Although their function is unknown, it has been suggested that they may play a role in male aggregations and/or male-female aggregations (Jones et al., 2010). The only other report of these compounds that are larger than N-acetyltyramine, has been a mixture detected in marine bacteria (Böröczky et al., 2006).

3. Present study

Two to 15 winged males of the above mentioned species were taken from live colonies from the field or laboratory and placed in 100% methanol. Gas chromatography-mass spectrometry was carried out in the EI mode using a Shimadzu QP 2010 GC/MS equipped with a RTX-5, 30 m × 0.25 mm i.d., column. The instrument was programmed from 60 °C to 250 °C at 10°/min. High-resolution mass spectrometry was performed on a Waters GCT high-resolution mass spectrometer (using an electron impact volume) coupled to an Agilent HP-6890 series GC system, using a Restek RTX-5MS (5% diphenyl-95% dimethyl polysiloxane, 30 m length, 0.25 mm ID, 0.25 μm film thickness) column operating with a temperature program of 100 °C (1 min) to 280 °C (10 min) at a rate of 10 °C/min, and with a constant flow of He of 1 ml/min as the gas carrier.

The previously described tyramides (R=CH₃, n-C₃H₇) were identified by direct GC/MS comparison with authentic samples (Böröczky et al., 2006; Jones et al., 2010). N-[2-(4-hydroxyphenyl)ethyl]hexanamide (R=n-C₅H₁₁) was suggested by its mass spectra: EIMS{M⁺} *m/z* 235 (2), 121 (13), 120 (100), 116(12), 107 (40), 99 (3), 91 (1), 77 (2), 71 (7), 43 (16); HRMS *m/z* 235.1573 calcd. for C₁₄H₂₁NO₂ 235.1572. It was identical in every respect to an authentic sample prepared by the condensation of tyramine with hexanoyl chloride (Janusz et al., 1993). The results of this investigation are summarized in Table 1.

4. Chemotaxonomic significance

All ants in this and a previous study (Jones et al., 2010) occupy two large phylogenetic clades in the subfamily Myrmicinae (Brady et al., 2006) and represent three different tribes of ants (Myrmicariini: *Myrmecaria*; Solenopsidini: *Megalomyrmex*, *Monomorium*, and *Solenopsis*; Attini: *Cyphomyrmex* and *Trachymyrmex*).

In the present study, the novel hexanamide (R=C₅H₁₁) and the propanamide (R=C₂H₅) are present in *Megalomyrmex*, in varying ratios that may be species specific, while, *Cyphomyrmex* has only the propanamide (R=C₂H₅) which had been previously reported in *Monomorium* and *Myrmecaria* males. Finally, *Trachymyrmex* has the acetamide (R=CH₃) which has not been previously reported in ants (Table 1). It appears that these tyramides are not ant tribe specific and have none or little phylogenetic signal. *Cyphomyrmex*, *Monomorium* and *Myrmecaria* are all distant relatives belonging to different ant tribes yet share the same propanamide where as *Cyphomyrmex* and *Trachymyrmex* are both fungus-growing ants and members of the

tribe Attini and do not have any amides in common. It is not yet known if tyramides are widespread among the ants or if it is only found in myrmicines. Numerous chemical studies of *Camponotus* species (Hymenoptera: Formicidae) have been conducted over the past thirty years and no tyramine amides have been detected (see Voegtle et al., 2008 and references therein).

Future behavioral assays with tyramides will elucidate the function of these unusual compounds. Males are thought to locate females by using aggregation pheromones that attract male and female reproductives (Hölldobler and Bartz, 1985). The exact pheromones used by males are uncertain (Smith et al., 2009) but the tyramides reported here and in Jones et al. (2010) are the most promising candidates to date and should be tested in behavioral studies in the future.

Acknowledgements

This work was supported by the Organization of Tropical Biology/Smithsonian Mellon Foundation, the Amazon Conservation Association, the Society of Systematic Biologists, a P.E.O. Scholar Award, the National Science Foundation DDIG program (DEB-0508698) and the University of Texas (Hartman Summer Merit Stipend) awarded to RMMA. For field research support, we are grateful to the Smithsonian Tropical Research Institute, Panama; Amazon Explorama Lodge, Peru; and La Selva Biological Station and El Ceibo Ranger Station, Costa Rica and the respective permitting agencies. We would also like to thank Steve A. Rehner, Ted R. Schultz, Jeffery Sossa-Calvo for ant specimens and Dr. H. M. Garraffo and Dr. T.F. Spande from the Laboratory of Bioorganic Chemistry NIDDK/NIH, Bethesda, MD for obtaining the high-resolution mass spectrum.

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