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Update on the defensive chemicals of the little black ant, *Monomorium minimum* (Hymenoptera: Formicidae)

Jian Chen [a,*], Charles L. Cantrell [b], David Oi [c], Michael J. Grodowitz [a]

[a] USDA-ARS, National Biological Control Laboratory, 59 Lee Road, Stoneville, MS 38776, USA
[b] USDA-ARS, Natural Products Utilization Research, University, MS 38677, USA
[c] USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

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ABSTRACT

Alkaloids, including 2,5-dialkylpyrrolidines and 2,5-dialkylpyrrolines, have been reported to be components in the venom of little black ants, *Monomorium minimum* (Buckley). Two fatty amines were recently reported as minor compounds. By analyzing the discharge collected from the stinger apparatus (milking), this study revealed the presence of an additional seven compounds in the defensive secretion of this ant species. Compounds identified were 9-decenyl-1-amine, *N*-methylenedecan-1-amine, *N*-methylenedodecan-1-amine, 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline, *N*-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine, β-springene ((*E*,*E*)-7,11,15-trimethyl-3-methylene-1,6,10,14-hexadecatetraene) and neocembrene ((*E*,*E*,*E*)-1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene). β-springene and neocembrene were found only in the defensive secretion of queens. Analyses of the contents of isolated poison and Dufour's glands of the queen indicated that all amines and alkaloids were from the poison gland and that β-springene and neocembrene were from the Dufour's gland. This demonstrated that the defensive secretion in *M. minimum* queens consists of components from both glands. This is also the first report on the natural occurrence of 9-decenyl-1-amine, N-methylenedecan-1-amine, and N-methylenedodecan-1-amine.

1. Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is an invasive ant species that was introduced into the United States from South America in the 1930's and has become ecologically dominant in areas it infests. Several native ant species have been reported to compete effectively with *S. invicta* (Baroni Urbani and Kannowski, 1974; Helms and Vinson, 2001; Rao and Vinson, 2004); among them is the little black ant, *Monomorium minimum* (Buckley) (Smith, 1965; Thompson, 1990). Little black ants can build their nests close to fire ant colonies and successfully compete with *S. invicta* for food and territory (Thompson, 1990; Rao and Vinson, 2004). Like *S. invicta*, both workers and queens of *M. minimum* possess a venom apparatus, also known as a stinger apparatus. The venom apparatus is composed of the sting and two glands, the venom gland and Dufour's gland. When discharged, the venom gland secretion may be accompanied by chemicals from Dufour's gland. The venom is used by *M. minimum* workers in competition with other ants for food and territory (Adams and Traniello, 1981). *M. minimum* venom has been shown to repel *S. invicta* workers (Baroni Urbani and Kannowski, 1974) and may be one of several important factor why *M. minimum* can compete effectively against *S. invicta*.

Alkaloids are the dominant components of *M. minimum* venom, including; saturated and unsaturated 2,5-dialkyl-pyrrolidines (Jones et al., 1982; Lange et al., 1989), unsaturated 2,5-dialkyl-pyrrolines (Lange et al., 1989), and unsaturated N-methyl-2,5-dialkyl-pyrrolidine (Jones et al., 1982). Two primary amines, decylamine and dodecylamine were recently reported (Wang and Chen, 2015) (see Table 1 for the details of compounds that have been published for *M. minimum*).

It is not unusual for venom compositions to differ between workers and queens, particularly in the proportion of some major components (Maile et al., 2000; Eliyahu et al., 2011; Chen et al., 2012). However, research on venom chemistry in *Monomorium* species have mainly focused on workers. As part of our effort in searching for insecticidal substances from natural products, we

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* Corresponding author. USDA-ARS, BCPRU, 59 Lee Road, Stoneville, MS 38776, USA.
E-mail address: jian.chen@ars.usda.gov (J. Chen).

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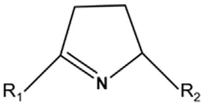
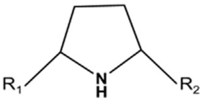
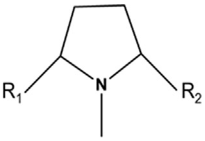
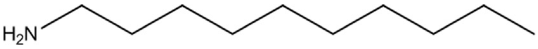
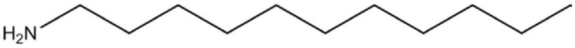
Table 1
Compounds that have been previously identified in the venom of little black fire ants, *Monomorium minimum*.

Compounds	R_1	R_2	References
	$(CH_2)_4CH{=}CH_2$ $(CH_2)_4CH{=}CH_2$	$(CH_2)_7CH{=}CH_2$ $(CH_2)_8CH_3$	Lange et al., 1989
	$(CH_2)_4CH{=}CH_2$ $(CH_2)_4CH{=}CH_2$ $(CH_2)_5CH_3$	$(CH_2)_7CH{=}CH_2$ $(CH_2)_8CH_3$ $(CH_2)_8CH_3$	Jones et al., 1982; Lange et al., 1989 Lange et al., 1989 Lange et al., 1989
	$(CH_2)_4CH{=}CH_2$	$(CH_2)_7CH{=}CH_2$	Jones et al., 1982
			Wang and Chen, 2015
			Wang and Chen, 2015

analyzed chemical components in the venom of *M. minimum* for both workers and queens. We report here the discovery of previously unknown compounds and differences in venom composition between workers and queens.

2. Materials and methods

2.1. Ants

Ten colonies of *Monomorium minimum* were collected from Washington County, Mississippi. Colonies were reared in plastic trays with distilled water, 10% w/w sugar water, and mealworm beetle pupae, *Tenebrio molitor*, under laboratory conditions at 28 °C and 45% RH.

2.2. Venom composition

Venom is defined here as the discharge released by ants through the stinger during manual stimulation. Venom was collected directly on a fiber used for solid phase microextraction (SPME) (fused silica fiber with 85 μm polyacrylate, Supelco®, Bellefonte, PA). A fine forceps was used to hold an ant until a drop of venom formed on the tip of its stinger (Fig. S1). Under a stereo microscope, the venom was collected by touching the drop with a SPME fiber. The ant was then stimulated to release more venom by gently touching the stinger using the fiber. At least three drops were collected on a fiber from each individual ant. The sample was then directly analyzed using gas chromatography–mass spectrometry (GC-MS). The SPME fiber was injected into the GC injection port within 30 s of venom collection. The absolute concentration of each compound was not determined; however, relative abundances were estimated based on the GC TIC (total ion chromatogram) peak areas without using correction factors. For SPME samples, some alkaloid peaks were not well separated due to their higher concentrations. In order to obtain relative peak area of each alkaloid in relation to the total alkaloid, diluted hexane ant extraction was used to ensure adequate separation of each alkaloid peak in the total ion chromatogram. For those samples, one ant was placed in a 200 μl glass conical limited volume insert (J.G Finneran Associates, Inc., Vineland, New Jersey, USA) with 100 μl hexane. The ant was then crushed using a micropipette and 2 μl liquid was injected into the GC-MS.

2.3. Gland content analysis

The content of poison and Dufour's glands were analyzed separately for three queens from each of two colonies. For gland removal, queens were pinned dorsal side up on a raised wax-based dissecting tray using a stereo microscope at 7 to 10× magnification. Individuals were secured through the pronotum using a minuten pin and the abdomen immobilized by laying pins cross-ways over the narrow pedicle. The poison and Dufour's gland were removed by grasping the terminal abdominal segments or the stinger itself with a fine forceps and pulling posteriorly. After removal, the entire venom apparatus was placed in a drop of phosphate buffered saline on a microscope slide. The poison and Dufour's glands were separated using Vannas Spring Scissors with a 2 mm cutting edge. After separation each gland was placed in 100 μl dichlormethane and a two microliter sample injected into the GC-MS system. While the poison/Dufour's gland apparatus was successfully removed from worker ants a clear delineation of the two was not achieved due to the extremely small size of the workers.

2.4. Gas chromatography – mass spectrometry

The GC-MS system consisted of an Agilent 7890A gas chromatograph with a HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5975 mass selective detector. The GC temperature was programmed at an initial temperature of 50 °C, held for 1 min, increased to 240 °C at a rate of 20 °C/min and held for 20 min, and then increased to 280 °C and held for 8 min. Splitless mode was used. Injection temperature was 250 °C, and transfer line temperature was 270 °C. The mass spectrometer was operated at 70 eV in the electron impact mode. Chemical identification was confirmed by comparing the retention times and mass spectra of samples with those of standards that were either purchased commercially or synthesized in our laboratory. 9-decenyl-1-amine was synthesized and β-springene (purity ≥ 95%) was purchased from Allichem LLC (Baltimore, Maryland, USA). If standards were not available, identification was made by comparison of their mass spectra with those in either NIST library (National Institute of Standards and Technology, USA) or the literature.

2.5. Synthesis of 9-decenyl-1-amine

The synthesis scheme for 9-decenyl-1-amine is illustrated in Fig. 1. For synthesis of 1-bromo-9-decene (**2**), 9-decen-1-ol (**1**, 0.506 g 3.24 mmol, Sigma-Aldrich, St. Louis, MO) was dissolved in 10 mL of CH_2Cl_2 in a 50 mL three-necked round bottom flask equipped with a condenser. After addition of tetrabromoethane (1.2806 g, 3.86 mmol), the reaction mixture was cooled to 0 °C and triphenylphosphine (1.0124 g, 3.86 mmol) was added over a period of 30 min. The mixture was warmed to room temperature and refluxed overnight. The reaction mixture was cooled to room temperature and 10 mL of pentane was added. Triphenylphosphine oxide precipitated as a colorless solid. The suspension was filtered over a 30 mL fritted funnel and the residue was washed with pentane (3 × 10 mL). The filtrate was evaporated under vacuum to yield a colorless oil. The residue was separated by silica gel column chromatography on a Biotage 100+M column (40–63 μm, 60 Å, 39 × 157 mm) running at 50 mL/min using a hexane: EtOAc gradient beginning with 100:0 to 80:20 over 1540 mL, followed by 80:20 to 0:100 over 1540 mL. 22 mL fractions were collected and recombined based on TLC similarities into compound **2** (0.650 g, 2.97 mmol, 92%). (^{1}H NMR (400 MHz in $CDCl_3$), δ 5.74–5.84 (m, 1H), 4.96 dd (2H, J = 2, 17), 4.92 dd (2H, J = 2, 10), 3.38 t (2H, J = 7), 1.99–2.05 (m, 2H), 1.79–1.87 (m), 1.28 (s) ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.32, 114.39, 77.55, 77.23, 76.91, 34.22, 33.98, 33.04, 29.49, 29.21, 29.08, 28.93, 28.37. EI-MS (70eV): 55 (17), 69 (11), 97 (11), 83 (6), 148 (4)).

For synthesis of 1-azidodec-9-ene (**3**), 1-bromo-9-decene (**2**, 0.625 g, 2.85 mmol) was dissolved in 5 mL dry DMSO in a 50 mL Schlenk flask under inert gas atmosphere and sodium azide (0.368 g, 5.67 mmol) was added. The reaction mixture was stirred at room temperature for 30 min and then heated to 60 °C for 2 h. The mixture was cooled to room temperature and 5 mL of CH_2Cl_2 and 5 mL of H_2O were added. The organic phase was separated, washed with water (3 × 5 mL) and dried with Na_2SO_4. The solvents were evaporated in a vacuum to yield compound **3** as a light yellow oil, which was used in the following reaction step without further purification (0.326 g, 1.79 mmol, 63%). (^{1}H NMR (400 MHz in $CDCl_3$), δ 5.72–5.82 (m, 1H), 4.95 dd (2H, J = 2, 17), 4.91 dd (2H, J = 2, 10), 3.21 t (2H, J = 7), 1.97–2.03 (m, 2H), 1.52–1.59 (m), 1.26 (s) ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.24, 114.32, 77.55, 77.23, 76.91, 51.61, 33.91, 29.45, 29.24, 29.14, 29.01, 28.97, 26.84. EI-MS (70eV): 55 (11), 122 (8), 70 (8), 69 (6), 56 (6)).

For synthesis of 9-decen-1-amine (**4**), in a 250 mL three necked round bottom flask equipped with a condenser and 50 mL of dry THF were cooled to 0 °C in an ice/water bath. $LiAlH_4$ (0.303 g, 7.98 mmol) was added slowly to produce a grey suspension, which was stirred for 5 min 1-azido-9-decene (**3**, 0.818 g, 4.51 mmol) was dissolved in 10 mL dry THF and slowly added to the $LiAlH_4$ suspension over a period of 20 min, stirred for another 30 min at 0 °C and then refluxed for an additional 1.5 h. The reaction mixture was cooled to 0 °C and water was subsequently added drop-wise until hydrogen gas evolution ceased. After further addition of 10 mL H_2O and 5 mL of 20% aq. NaOH solution, the suspension filtered over a Büchner funnel. The residue was rinsed with 25 mL THF. The filtrates were combined and the solvents were evaporated in a vacuum to a volume of about 30 mL. 50 mL of CH_2Cl_2 and 50 mL of H_2O were added, the organic phase separated and dried over $MgSO_4$. The solvents were evaporated in a vacuum to yield compound **4** (0.406 g, 2.61 mmol, 58%) as a light yellow oil, which solidified over 3 days (^{1}H NMR (500 MHz in $CDCl_3$), δ 5.75–5.83 (m, 1H), 4.97 d (1H, J = 17), 4.91 d (1H, J = 10), 2.77 t (1H, J = 7), 2.00–2.04 (m, 2H), 1.53–1.57 (m), 1.27 (s) ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.04, 114.16, 77.33, 77.08, 76.82, 41.11, 33.76, 31.06, 29.41, 29.29, 29.05, 28.88, 26.80. EI-MS (70eV): 55 (9), 56 (8), 114 (5), 67 (5), 86 (4)).

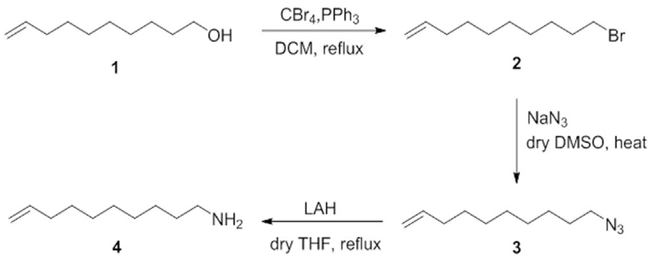


Fig. 1. Synthesis scheme for 9-decenyl-1-amine (4).

3. Results

3.1. Venom of workers

In addition to 2,5-dialkylpyrrolines, 2,5-dialkylpyrrolines and fatty amines, 9-decenyl-1-amine, *N*-methylenedecan-1-amine and *N*-methylenedodecan-1-amine were identified (Figs. 2 and 3). The identity of 9-decenyl-1-amine was confirmed using the synthetic standard. Both retention time and mass spectra were matched between the sample and standard (Fig. S2). The identity of N-methylenedecan-1-amine and N-methylenedodecan-1-amine were confirmed by direct comparison of the MS with the corresponding compound in the NIST library search (Figs. S3 and S4). Three 2,5-dialkylpyrrolines, three 2,5-dialkylpyrrolidines, and two

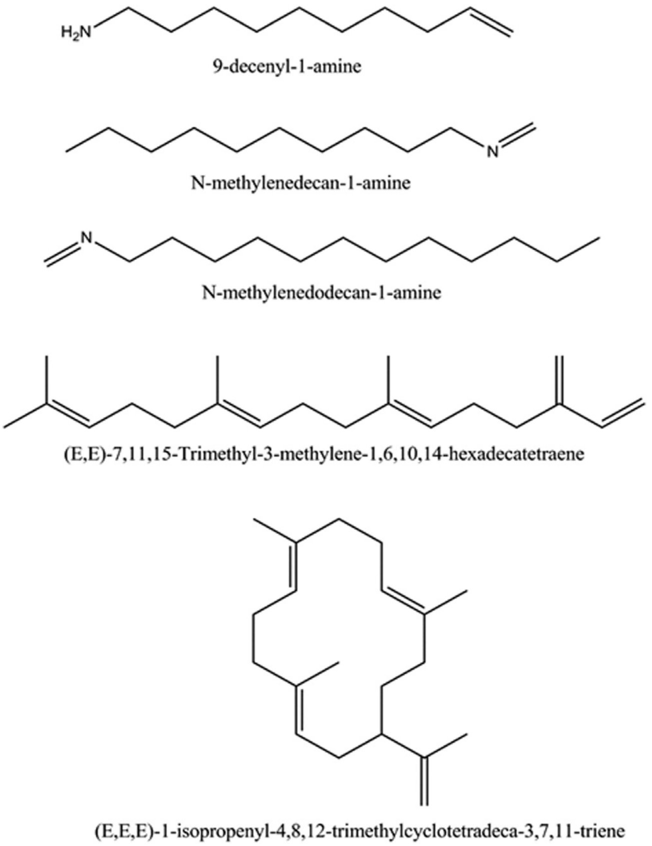


Fig. 2. New amines and terpenes identified in the venom of little black ants *Monomorium minimum*. (E,E)-7,11,15-trimethyl-3-methylene-1,6,10,14-hexadecatetraene (β-springene) and (E,E,E)-1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene (neocembrene) were found only in queens.

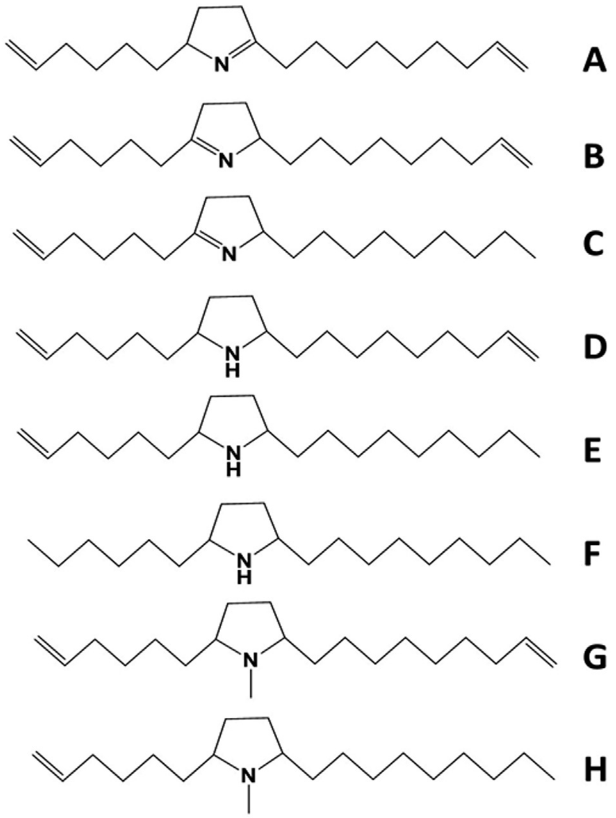


Fig. 3. Alkaloids found in the venom of the little black ants, *Monomorium minimum*. A, B, C, D, and E were detected in every sample of both workers and queens and F only in workers. G and H was observed in both workers and queens, but not in every sample. Relative peak area of each alkaloid in the peak area of total alkaloids is shown in Fig. S15.

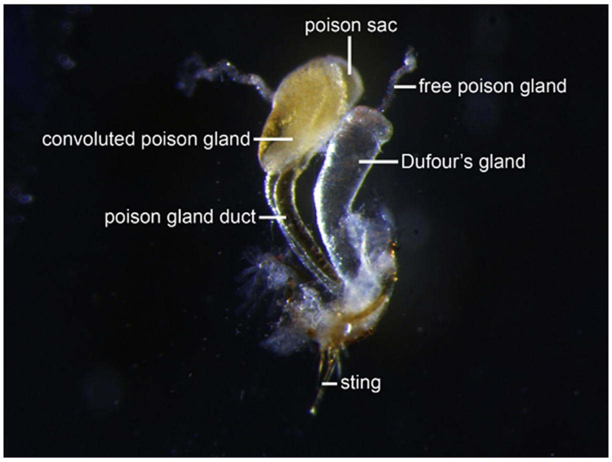


Fig. 4. Poison and Dufour's glands in *Monomorium minimum* queen. β-springene and neocembrene were only found in the Dufour's gland.

N- 2,5-dialkylpyrrolidines were identified, including 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline (Fig. S5), 2-(1-hex-5-enyl)-5-(1-non-8-enyl)-1-pyrroline (Fig. S6), 2-(1-hex-5-enyl)-5-nonanyl-1-pyrroline (Fig. S7), 2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine (Fig. S8), 2-(hex-5-enyl)-5-nonanylpyrrolidine (Fig. S9), 2-hexyl-5-nonanylpyrrolidine (Fig. S10), N-methyl-2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine (Fig. S11), and N-methyl-2-(hex-5-enyl)-5-nonanylpyrrolidine (Fig. S12). The relative peak areas of these identified compounds are shown in Table S1 and Fig. S15. The venom composition seemed to be dominated by 2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine (39.0%) and 2-(hex-5-enyl)-5-nonanylpyrrolidine (55.4%). Since the relative peak area of 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline and N-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine was very low (<0.004%) and not detected in every sample, they were not included in calculating the relative peak area of each alkaloid (Fig. 4). All amines and alkaloids found in the whole worker extract also exist in the collected venom.

3.2. *Venom of queens*

The queen venom did not contain 2-hexyl-5-nonanylpyrrolidine. In addition to 2,5-dialkylpyrrolines, 2,5-dialkylpyrrolines, decylamine, and dodecylamine; 9-decenyl-1-amine, N-methylenedecan-1-amine and N-methylenedodecan-1-amine, β-springene ((*E*,*E*)-7,11,15-trimethyl-3-methylene-1,6,10,14-hexadecatetraene) and neocembrene ((*E*,*E*,*E*)-1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene) were also identified. Identity of β-springene was confirmed by a synthetic standard (Fig. S13). The identity of neocembrene was confirmed by comparing the mass spectrum of the sample with that in the literature. This compound has been reported in the queen of the pharaoh ant, *Monomorium pharaonis* (Edwards and Chambers, 1984). Extracts of *M. pharaonis* were also analyzed using the same GC-MS conditions. The retention time and mass spectrum of neocembrene in *M. pharaonis* were matched to those of neocembrene in *M. minimum* queen (Fig. S14). The relative peak areas of identified compounds are shown in Table S1. The venom composition seemed to be dominated by 2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine (81.3%), followed by 2-(hex-5-enyl)-5-nonanylpyrrolidine (7.5%) (Fig. S15). Since 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline and N-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine was detected in only a minimal number of queen venom samples, they were not included in calculating the relative peak area of each alkaloid (Fig. S15). β-springene and neocembrene were only found in the Dufour's gland. Stinger apparatus with poison and Dufour's glands of *M. minimum* queen is shown in Fig. 4.

4. Discussion

To our knowledge, 9-decenyl-1-amine, N-methylenedecan-1-amine, and N-methyllenedodecan-1-amine have never been reported as naturally occurring compounds. Two fatty amines, decylamine and dodecylamine, from *M. minimum* were reported to be insecticidal against the red imported fire ants (Wang and Chen, 2015). Dodecylamine repelled the common house fly, *Musca domestica* and decylamine repelled the honey bee, *Apis mellifera* (Atkins et al., 1975). Dodecylamine deterred oviposition of European corn borer, *Ostrinia nubilalis* (Binder and Robbins, 1997). Decylamine and dodecylamine also showed high nematicidal activity against pine wood nematode, *Bursaphelenchus Lignicolus* (Nagase et al., 1982). Due to its similarity in structure to decylamine, 9-decenyl-1-amine may also have similar toxic and repellant effects. Both N-methylenedecan-1-amine and N-methyllenedodecan-1-amine have a carbon-nitrogen double bond in their chemical structures, an imine functional group. Toxicity data of these two amines are not available in the literature. What functions they may serve in the ant venom warrants further research.

This study has demonstrated a clear difference in venom composition between workers and queens. β-springene and neocembrene were found only in the venom of queens while 2-hexyl-5-nonanylpyrrolidine did not occur in the queen. β-springene, a diterpene, was originally isolated from a skin (dorsal) gland of the springbok antelope, *Antidorcas marsupialis*, from Africa (Burger et al., 1978, 1981). It has been found in other mammals such as the white-lipped peccary, *Tayassu pecari* (Waterhouse et al., 2001), and collared peccary, *Tayassu tajacu* (Waterhouse et al., 1996); two reptilians, Cuvier's dwarf caiman, *Paleosuchus palpebrosus*, and the smooth-fronted caiman, *Paleosuchus trigonatus* (Schulz et al., 2003); four species of oribatid mites, *Oribotritia banksi*, *O. berlesei*, *O. hermanni*, and *O. storkani* (Raspotnig et al., 2011); five species of bumblebees, *Bombus griseocollis* (Bertsch et al., 2004), *B. hypnorum* (Cahlikova et al., 2004), *B. morrisoni*, *B. rufocinctus* (Bertsch et al., 2008), *B.* semenoviellus (Hovorka et al., 2006); and two stingless bees, *Melipona beecheii* (Cruz-López et al., 2005) and *Nannotrigona testaceicornis* (Pianaro et al., 2009). β-springene was also found in the Dufour's gland of the ectoparasitoid *Habrobracon hebetor* (Say) where its abundance varied significantly with age (Howard et al., 2003). The only ant species that has been reported to produce β-springene is the Old World army ant, *Aenictus rotundatus* (Oldham et al., 1994). However, its isomer, *E*,*E*,*E*-α-springene, was found in the Dufour's gland of the Dinosaur ant, *Nothomyrmecia macrops* (Billen et al., 1988). The secretion of Dufour's gland of ants can have multiple functions (Morgan, 2008). The potential function of β-springene in the venom of *M. minimum* queens can only be elucidated by further experimentation.

There are several synonyms for neocembrene. The chemical structure of neocembrene was first assigned by Shmidt et al. (1970) while studying the diterpene hydrocarbons of the oleoresin in Siberian spruce, *Picea obvata* (Ledb.) and Korean pine, *Pinus koraensis* (Sieb Zucc.). Neocembrene was found by Moore (1966) as the trail pheromone of *Nasutitermes exitiosus* (Hill); however, the chemical structure of neocembrene in *N. exitiosus* was not elucidated until 1972 (Birch et al., 1972). Due to a slight difference in NMR spectrum compared to those of neocembrene from two conifers mentioned above, it was then named as neocembrene-A. Neocembrene was also isolated from the gum-resin of *Commiphora mukul* (Hook, ex Stocks) Engl and it was named as cembrene-A (Patil et al., 1973). Since its discovery, it has been found in numerous plants, corals, insects, and mammals. Vanderah et al. (1978) found it in soft coral, *Nephthea* species. In addition to *N. exitiosus*, neocembrene was found in 16 other termite species which are belong to 7 different genera, including *Constrictotermes cyphergaster* (Silvestri), *Nasutitermes corniger* (Motschulsky), *N. ephratae* (Holmgren), *N. exitiosus*, *N. guayanae*, *N. kemneri*, *N. lujae*, *N. voeltzkowi*, *Trinervitermes geminates* (Sillam-Dussès et al., 2010), and *T. trinervoides*. (Sillam-Dussès et al., 2010), *Cubitermes umbratus* (Wiemer et al., 1979), *Crenetermes mixtus* (Prestwich, 1979), *N. costalis* (Hall and Traniello, 1985), *Noditermes wasambaricus* (Meinwald et al., 1978), *Prorhinotermes canalifrons* (Sillam-Dussès et al., 2005), *P. simplex* (Sillam-Dussès et al., 2009), *T. bettonianus* (McDowell and Oloo, 1984). Neocembrene was found in only two insect species outside isoptera: a sandfly, *Lutzomyia longipalpis* (Hamilton et al., 2004) and a myrmicine ant, *Monomorium pharaonis* (Edwards and Chambers, 1984). Neocembrene has exhibited cytotoxicity against A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (mouse lymphocytic leukemia) cell lines (Duh et al., 1999). In *M. pharaonis*, neocembrene was found only in the Dufour's gland of the fertile queens. Since alates and young queens do not contain neocembrene, its potential role in suppressing the development of new sexual forms (males and queens) in an ant colony was suggested (Edwards and Chambers, 1984). The function of neocembrene in *M. minimum* is another interesting area and more research is warranted.

2,5-dialkylpyrrolidines are dominant components of *M. minimum* venom for both workers and queens. 2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine and N-methyl-2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine were reported in *M. minimum* by Jones et al. (1982). Lange et al. (1989) reported the following 5 alkaloids in this species, including 2-(1-hex-5-enyl)-5-(1-non-8-enyl)-1-pyrroline, 2-(1-hex-5-enyl)-5-nonanyl-1-pyrroline, 2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine, 2-(hex-5-enyl)-5-nonanylpyrrolidine, and 2-hexyl-5-nonanylpyrrolidine. The presence of all these alkaloids were confirmed in this study. In addition, it is the first time that 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline and N-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine were identified in the venom of *M. minimum.* However, both alkaloids have been found in other ant species in the genus of *Monomorium*. 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline was found in *M. near metoecus*, *M. virdum*, and *M. ebeninum* and N-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine in *M. near metoecus*, *M. virdum*, *M. near emersoni*, and *M. cyaneum* (Jones et al., 1982). Both 2,5-dialkyl-pyrrolidines and 2,5-dialkyl-pyrrolines are insecticidal (Bacos et al., 1988).

This study demonstrates that the venom in *M. minimum* queen consists of products from both the poison glands and the Dufour's glands. Due to the extremely small size of the workers, separation of the poison gland from the Dufour's gland in workers was not successful. Except 2-hexyl-5-nonanylpyrrolidine, all compounds found in worker's venom were also found in queen's poison gland. Although the possibility of Dufour's gland origin couldn't be completely ruled out, all the identified compounds for workers are most likely from the poison gland.

Defense is very important to the survival of an ant colony. Many ant species depend on chemicals for their defense. The results of this study extend our knowledge about chemicals produced in the venom of *M. minimum*, which will help advance our understanding of its biology.

Ethical Statement

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2016.09.009.

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