Toxicon 53 (2009) 479–486

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

# Re-investigation of venom chemistry of *Solenopsis* fire ants. II. Identification of novel alkaloids in *S. invicta*

Li Chen, Henry Y. Fadamiro*

*Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, USA*

ARTICLE INFO

*Article history:*
Received 18 October 2008
Received in revised form 16 January 2009
Accepted 20 January 2009
Available online 29 January 2009

*Keywords:*
Fire ant
*Solenopsis invicta*
Venom
Solenopsins
Alkaloid

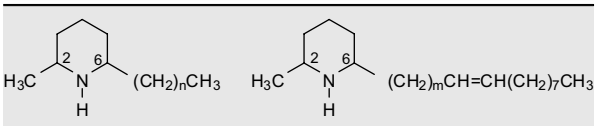
ABSTRACT

The venom of the red imported fire ant, *Solenopsis invicta*, is dominated by *trans* stereoisomers of 2,6-dialkylpiperidines. *cis* Stereoisomers of alkaloids in the venom of *S. invicta* were separated from *trans* stereoisomers by using silica gel short column chromatography and identified by coupled gas chromatography mass spectrometry (GC–MS). Seven pairs of *cis* and *trans* stereoisomers were identified based on relative retention times and mass spectral data. The GC trace of the *cis* stereoisomers of *S. invicta* alkaloids was presented for the first time. In addition to the previously described 2,6-dialkylpiperideines, eleven novel 2,6-dialkyl-$\Delta^{1,2}$-piperideines and 2,6-dialkyl-$\Delta^{1,6}$-piperideines were identified from *S. invicta* venom. The results are discussed in relation to the evolutionary significance of these piperideines and their possible biosynthetic pathways in *Solenopsis* fire ants.

Published by Elsevier Ltd.

## 1. Introduction

Since its introduction in 1930s, the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), has spread throughout the southern United States affecting agriculture, wildlife, and human well-being. Fire ants produce alkaloid-rich venoms, which have antibacterial, fungicidal, insecticidal and hemolytic properties (Blum et al., 1958; Jouvenaz et al., 1972; Javors et al., 1993). The venoms are stored in the poison sac and delivered through the stinger. The venom chemistry of *Solenopsis* fire ants has been characterized by various authors (MacConnell et al., 1970, 1971; Jones et al., 1982; Blum et al., 1992; Leclercq et al., 1994). The venom of *S. invicta* is composed mainly of 2-methyl-6-alkyl or -alkenylpiperidines known as solenopsins. Both *cis* and *trans* stereoisomers of the solenopsins are present in *S. invicta* with *trans* stereoisomers predominating. The absolute configurations of *cis* and *trans* stereoisomers are (2*R*,6*S*) and (2*R*,6*R*), respectively (Leclercq et al., 1994), and the side chain double bonds in the 6-alkenylpiperidines are always *cis* form (MacConnell et al., 1971). Seven *trans*-2,6-disubstituted piperidine alkaloids including *trans* $C_{11}$, *trans* $C_{13:1}$, *trans* $C_{13}$, *trans* $C_{15:1}$, *trans* $C_{15}$, *trans* $C_{17:1}$, *trans* $C_{17}$ have been identified from *S. invicta* venom (Brand et al., 1972; MacConnell et al., 1976; Blum et al., 1992). The venom of *S. invicta* also contains, as minor components, various *cis* 2,6-disubstituted piperidine alkaloids including *cis* $C_{11}$, *cis* $C_{13:1}$, *cis* $C_{13}$, *cis* $C_{15:1}$, *cis* $C_{15}$ (Brand et al., 1972).




$C_{11}$: n = 10
$C_{13}$: n = 12
$C_{15}$: n = 14
$C_{17}$: n = 16

$C_{11:1}$: m = 1
$C_{13:1}$: m = 3
$C_{15:1}$: m = 5
$C_{17:1}$: m = 7

* Corresponding author. Tel.: +1 334 844 5098; fax: +1 334 844 5005.
*E-mail address:* fadamhy@auburn.edu (H.Y. Fadamiro).

0041-0101/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.toxicon.2009.01.016

In a recent study published in this issue, we developed a practical fractionation procedure to separate *cis* and *trans* alkaloids from body extracts of workers of the black imported fire ant, *Solenopsis richteri* Forel (Chen and Fadamiro, 2009). In addition to the previously described components of *S. richteri* venom, seven novel 2,6-dialkyl-$\Delta^{1,2}$-piperideines and 2,6-dialkyl-$\Delta^{1,6}$-piperideines were detected. Further analyses by coupled gas chromatography electroantennogram detection (GC–EAD) demonstrated that some of these novel piperideines elicited GC–EAD responses (unpublished data) in *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae), a parasitoid of *Solenopsis* fire ants, which has been released in southeastern United States for their biological control. With the ultimate goal of identifying components of the venom alkaloids which may play a role in mediating interactions between imported fire ants and *Pseudacteon* phorid flies, we conducted two parallel studies on re-investigation of the venom chemistry of *S. richteri* and *S. invicta*. This paper reports on the separation of the *cis* and *trans* stereoisomers of venom alkaloids and the identification of novel alkaloids from venom of *S. invicta* workers. A similar paper in this issue reported on the novel alkaloids of its congener, *S. richteri* (Chen and Fadamiro, 2009).

## 2. Materials and methods

### 2.1. Source of ant colony

Workers of red imported fire ant, *S. invicta*, were collected from Auburn University campus, Alabama, USA. Colony was maintained in 1-gallon plastic jar coated with Fluon® (ICI, Wilmington, DE) to prevent escape, and was fed sugar water and crickets.

### 2.2. Extraction, isolation and identification of venom alkaloids

The procedures of extraction, isolation and identification of venom alkaloids from *S. invicta* were as described by Chen and Fadamiro (2009). Briefly, ant workers (~5 g) were killed by freezing and extracted with hexane for 24 h. The extract (0.4 ml) was loaded onto a silica gel column and eluted with hexane–acetone to obtain alkaloids. The chemistry of each collection (*ca* 1 mL) was analyzed by gas chromatography (GC) using a Shimadzu GC-17A equipped with a flame ionization detector (FID). The collections were pooled based on changes observed in the GC chromatograms of each collection. Three major GC fractions were obtained: cuticular hydrocarbons, *cis* alkaloid, and *trans* alkaloid fractions.

The two alkaloid fractions (*cis* and *trans* alkaloids) were subsequently analyzed by GC–MS using an Agilent 7890A GC coupled to a 5975C Mass Selective Detector, with an HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Mass spectra were obtained using electron impact (EI, 70 eV). The chemical identities of alkaloids were determined by analysis of their mass spectra, as well as by comparison of diagnostic ion fragments with published results on *Solenopsis* fire ants.

## 3. Results

Silica gel short column chromatography of hexane whole body extract of *S. invicta* produced three major fractions. The chemistry characteristics of these three fractions were verified by GC–MS analyses as cuticular hydrocarbons, *cis* alkaloid, and *trans* alkaloid fractions.

The third fraction, *trans* form alkaloids, contains seven apparent peaks which were identified by comparison with published GC profiles of alkaloids from *S. invicta* workers (Brand et al., 1972; MacConnell et al., 1976; Blum et al., 1992; Deslippe and Guo, 2000) and by mass spectrum of each peak obtained by GC–MS analysis (Figs. 1 and 2). These seven major peaks (Fig. 1), **2**, **5**, **9**, **13**, **17**, **21**, **24**, were identified as *trans* $C_{11}$, *trans* $C_{13:1}$, *trans* $C_{13}$, *trans* $C_{15:1}$, *trans* $C_{15}$, *trans* $C_{17:1}$, *trans* $C_{17,}$ respectively. The mass spectra of *trans* $C_{11}$, *trans* $C_{13:1}$, *trans* $C_{13}$, *trans* $C_{15:1}$, and *trans* $C_{15}$ have been reported in our parallel study of *S. richteri* venom alkaloids (Chen and Fadamiro, 2009). Fig. 2 depicts the mass spectra of *trans* $C_{17:1}$, *trans* $C_{17}$.

The mass spectra of peaks **6**, **14** are almost identical to those of major components **5**, **13**, respectively. Considering that these two peaks eluted right after corresponding major peaks **5** and **13**, we tentatively identified **6** and **14** as stereoisomers of **5** and **13** with a *trans* double bond on the alkyl side chain, respectively. We propose that the absolute configurations of **6**, **14** are the same as **5** and **13** (2*R*,6*R*) (Table 1). The spectra of peaks **10** and **18** have *m*/*z* 98 as the base peak, 111, 124, P-17, P-3, and P-2 (Fig. 3), presumably indicating the presence of a terminal double bond on alkyl side chain. As **10** and **18** eluted together with **9**, **17**, the configuration of both **10** and **18** might be same as **9**, **17** (2*R*,6*R*) (Table 1).

The mass spectra of minor peaks **1** and **3** (Fig. 4) were absolutely identical to that reported by Brand et al. (1972) for 2,6-dialkylpiperideines. The important mass peaks at *m*/*z* 96, 111 indicate an N–$C_6$ double bond, whereas *m*/*z* 96, 97, 110 indicate an N–$C_2$ double bond. Therefore, **1** and **3** were identified as 2-methyl-6-*n*-undecyl-$\Delta^{1,6}$-piperideine and 2-methyl-6-*n*-undecyl-$\Delta^{1,2}$-piperideine, respectively. The GC retention times and mass spectra of **7**, **11**, **15** were exactly same as that presented for *S. richteri* (Chen and Fadamiro, 2009). Peaks **19** and **22** had same characteristic mass ions as peaks **1**, **3**, **7**, **11**, and **15**. The identities of peaks **7**, **11**, **15**, **19**, and **22** were established as shown in Fig. 4 and Table 1. Peaks **4**, **8**, **12**, **16** have been identified as piperideines with a double bond on alkyl side chain in our study on *S. richteri* (Chen and Fadamiro, 2009). The structures of **20** and **23** were established in the same manner (Fig. 4).

The *cis* stereoisomers always eluted before the corresponding *trans* stereoisomers. Fig. 5 shows GC trace of *cis* alkaloid fraction (the second fraction). Since mass spectra of **2′**, **5′**, **9′**, **13′**, **17′**, **21′** and **24′** are identical to that of corresponding *trans* stereoisomers, peaks **2′**, **5′**, **9′**, **13′**, **17′**, **21′** and **24′** are identified as *cis* $C_{11}$, *cis* $C_{13:1}$, *cis* $C_{13}$, *cis* $C_{15:1}$, *cis* $C_{15}$, *cis* $C_{17:1}$, *cis* $C_{17,}$ respectively. Following the results of MacConnell et al. (1971) and Leclercq et al. (1994), we propose that the absolute configurations of these *cis* stereoisomers are (2*R*,6*S*) and that the double bonds in 6-alkyl side chains are *cis*. In addition to the above *cis*

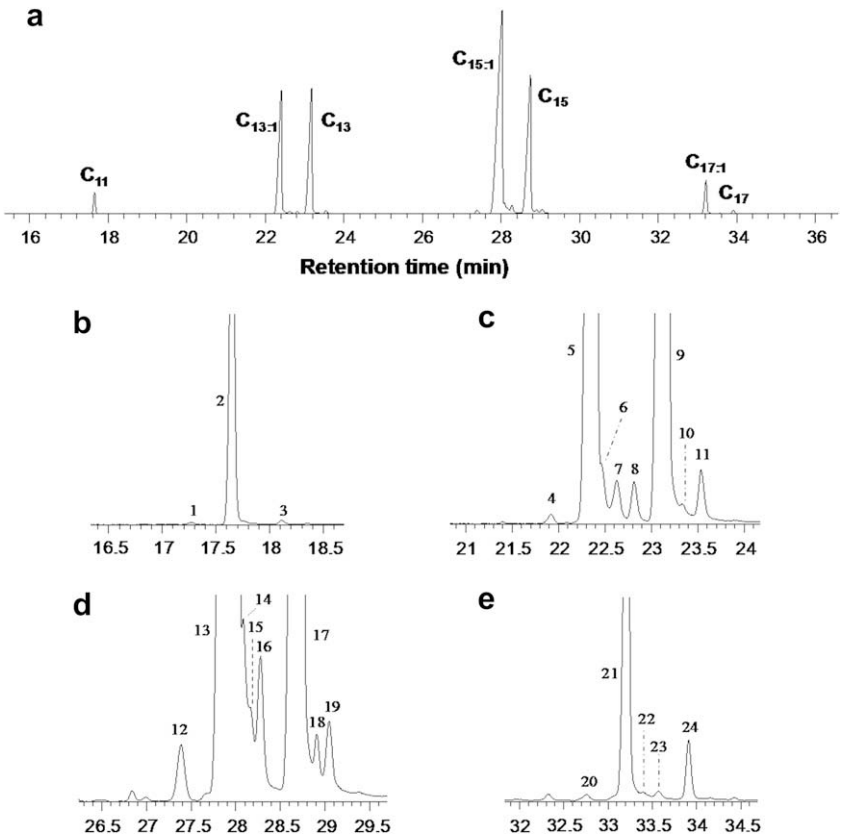



**Fig. 1.** Typical GC trace of *trans* alkaloids from *S. invicta*. (a) Visible peak area of GC chromatogram; (b)–(e) amplified GC peaks in (a).

stereoisomers, the second fraction contains 2,6-dialkylpiperideines whose mass spectra are identical to those found in the third fraction. The GC retention times of 2,6-dialkylpiperideines with same number in the second and third fractions (for instance, **4** and **4′**) are exactly the same (Figs. 1 and 5). As a pair of enantioisomers (for example, 2*R* and 2*S* $\Delta^{1,6}$-piperideines or 6*R* and 6*S* $\Delta^{1,2}$-piperideines) cannot be separated by silica gel column chromatography, **4** and **4′** must be the same compound. $\Delta^{1,6}$-piperideines of **4′**, **7′**, **12′**, **15′**, **20′**, **22′** are major peaks of the second fraction (Fig. 5).

## 4. Discussion

In this study, both the *cis* and *trans* stereoisomers of venom alkaloids of *S. invicta* were easily separated by silica gel column chromatography with the *cis* stereoisomers always eluting before the corresponding *trans* stereoisomers. Separation of hexane body extract of *S. invicta* workers yielded three fractions: cuticular hydrocarbons (first fraction), *cis* alkaloids (second fraction) and *trans* alkaloids (third fraction). As previously reported by other authors (MacConnell et al., 1971; Blum et al., 1992),

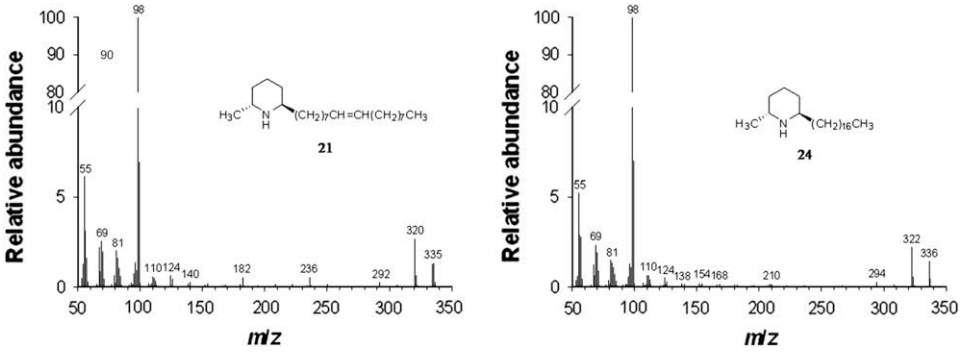



**Fig. 2.** Mass spectra of *trans* $C_{17:1}$ and *trans* $C_{17}$ from *S. invicta*.

**Table 1**
Chemical identity of alkaloids from *S. invicta*.

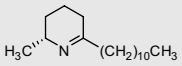
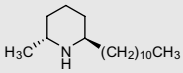
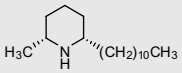
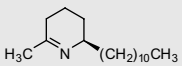
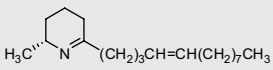
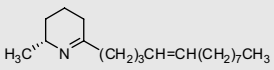
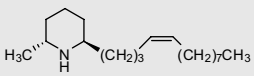
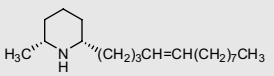
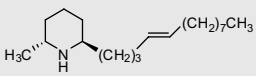
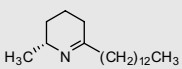
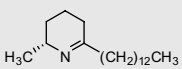
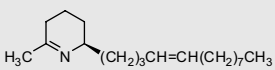
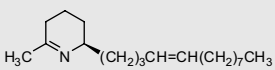
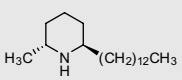
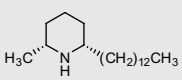
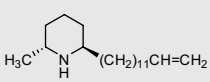
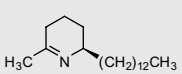
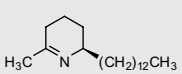
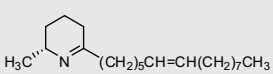
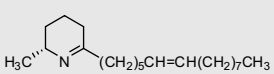
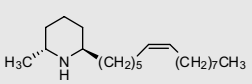
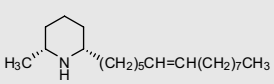
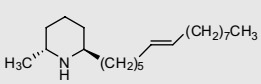
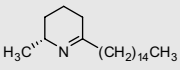
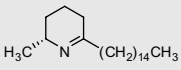
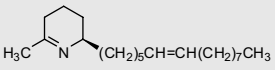
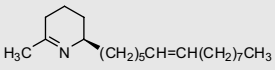
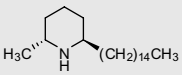
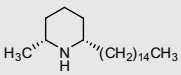
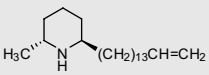
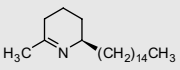
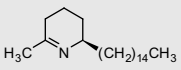
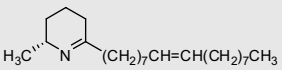
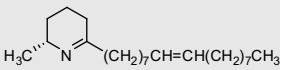
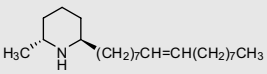
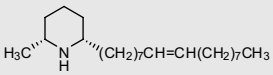
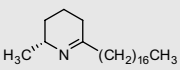
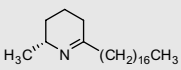
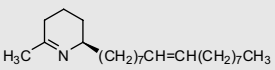
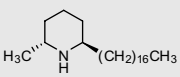
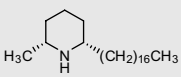
| *trans* Alkaloids | | | *cis* Alkaloids | | |
|---|---|---|---|---|---|
| Peak | Configuration | Structure | Peak | Configuration | Structure |
| **1** | 2*R* |  | | | |
| **2** | 2*R*,6*R* |  | **2′** | 2*R*,6*S* |  |
| **3** | 6*R* |  | | | |
| **4** | 2*R* |  | **4′** | 2*R* |  |
| **5** | 2*R*,6*R* |  | **5′** | 2*R*,6*S* |  |
| **6** | 2*R*,6*R* |  | | | |
| **7** | 2*R* |  | **7′** | 2*R* |  |
| **8** | 6*R* |  | **8′** | 6*R* |  |
| **9** | 2*R*,6*R* |  | **9′** | 2*R*,6*S* |  |
| **10** | 2*R*,6*R* |  | | | |
| **11** | 6*R* |  | **11′** | 6*R* |  |
| **12** | 2*R* |  | **12′** | 2*R* |  |
| **13** | 2*R*,6*R* |  | **13′** | 2*R*,6*S* |  |
| **14** | 2*R*,6*R* |  | | | |

**Table 1** (*continued*)

| *trans* Alkaloids | | | *cis* Alkaloids | | |
|---|---|---|---|---|---|
| Peak | Configuration | Structure | Peak | Configuration | Structure |
| **15** | 2*R* |  | **15′** | 2*R* |  |
| **16** | 6*R* |  | **16′** | 6*R* |  |
| **17** | 2*R*,6*R* |  | **17′** | 2*R*,6*S* |  |
| **18** | 2*R*,6*R* |  | | | |
| **19** | 6*R* |  | **19′** | 6*R* |  |
| **20** | 2*R* |  | **20′** | 2*R* |  |
| **21** | 2*R*,6*R* |  | **21′** | 2*R*,6*S* |  |
| **22** | 2*R* |  | **22′** | 2*R* |  |
| **23** | 6*R* |  | | | |
| **24** | 2*R*,6*R* |  | **24′** | 2*R*,6*S* |  |

the *trans* alkaloid fraction contained seven major components all of which are *trans*-2-methyl-6-alkyl (or alkenyl) piperidines. These *trans* form alkaloids were identified as *trans* $C_{11}$, *trans* $C_{13:1}$, *trans* $C_{13}$, *trans* $C_{15:1}$, *trans* $C_{15}$, *trans* $C_{17:1}$, *trans* $C_{17}$. All *cis* stereoisomers of these *trans* form alkaloids were present in the *cis* alkaloid fraction. Silica gel chromatography verified that the *trans* form alkaloids predominate while the corresponding *cis* form alkaloids are present only in trace amounts, as previously reported (Brand et al., 1972; Blum et al., 1992). The shape of GC trace of *cis* alkaloids is relatively similar to that of *trans* alkaloids.

In addition to these seven pairs of diasteroisomers, eleven novel alkaloids were identified in both alkaloid fractions of *S. invicta* venom. These included two $\Delta^{1,6}$-piperideines and two $\Delta^{1,2}$-piperideines for $C_{13}$ and $C_{15}$, and one $\Delta^{1,6}$-piperideine and two $\Delta^{1,2}$-piperideines for $C_{17}$. Seven (two $\Delta^{1,6}$-piperideines and two $\Delta^{1,2}$-piperideines for $C_{13,}$ and two $\Delta^{1,6}$-piperideines and one $\Delta^{1,2}$-piperideines for $C_{15}$) of these eleven novel alkaloids were also recently identified from the venom of *S. richteri*, a congener of *S. invicta* (Chen and Fadamiro, 2009). Although $\Delta^{1,6}$-piperideines and $\Delta^{1,2}$-piperideines for $C_{11}$, and $C_{17}$ are not complete in *S. invicta* and *S. richteri* (Chen and Fadamiro, 2009), we can predict that other members of *Solenopsis* genus might possess two $\Delta^{1,6}$-piperideines and two $\Delta^{1,2}$-piperideines for $C_{11}$ or $C_{17}$ as those found in *S. invicta* for $C_{13}$ and $C_{15}$. GC trace of the second fraction contains distinctive $\Delta^{1,6}$-piperideine peaks, **4′**, **7′**, **12′**, **15′**, **20′** and **22′**, but fairly visible $\Delta^{1,2}$-piperideine peaks (e.g., **8′**, **11′**, **16′**, **19′**).

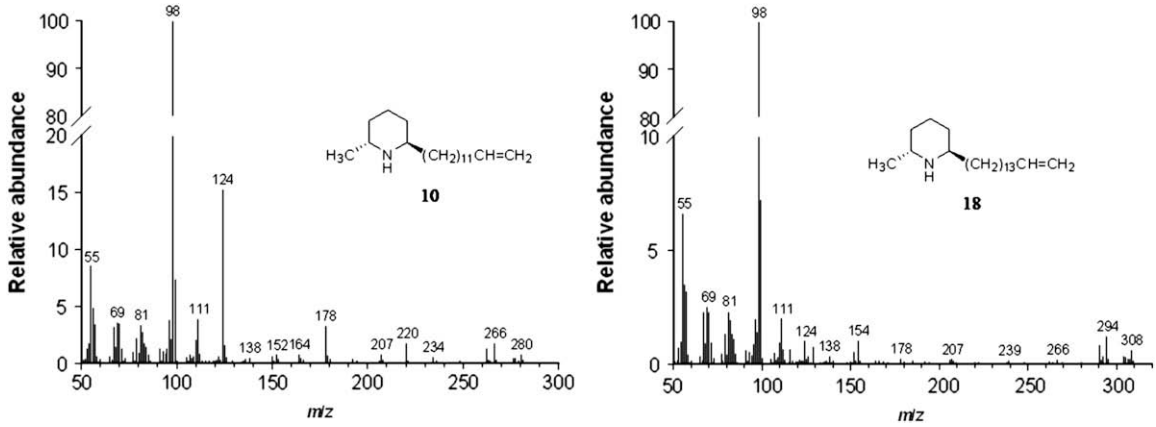



**Fig. 3.** Mass spectra of alkaloids with a terminal double bond in alkyl side chain (**10** and **18**) from *S. invicta*.

The co-occurrence in *S. invicta*, as well as in *S. richteri* (Chen and Fadamiro, 2009), of piperideines and piperidines with certain number of carbons in alkyl side chain may support our hypothesis that $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines function as precursors for biosynthesis of imported fire ant alkaloids (Chen and Fadamiro, 2009). As *S. invicta* has the biosynthetic capacity to synthesize mainly the *trans* form alkaloids, it is likely that enantioselective enzymes are present in this species which can reduce $\Delta^{1,2}$-piperideines exclusively into (2*R*,6*R*)

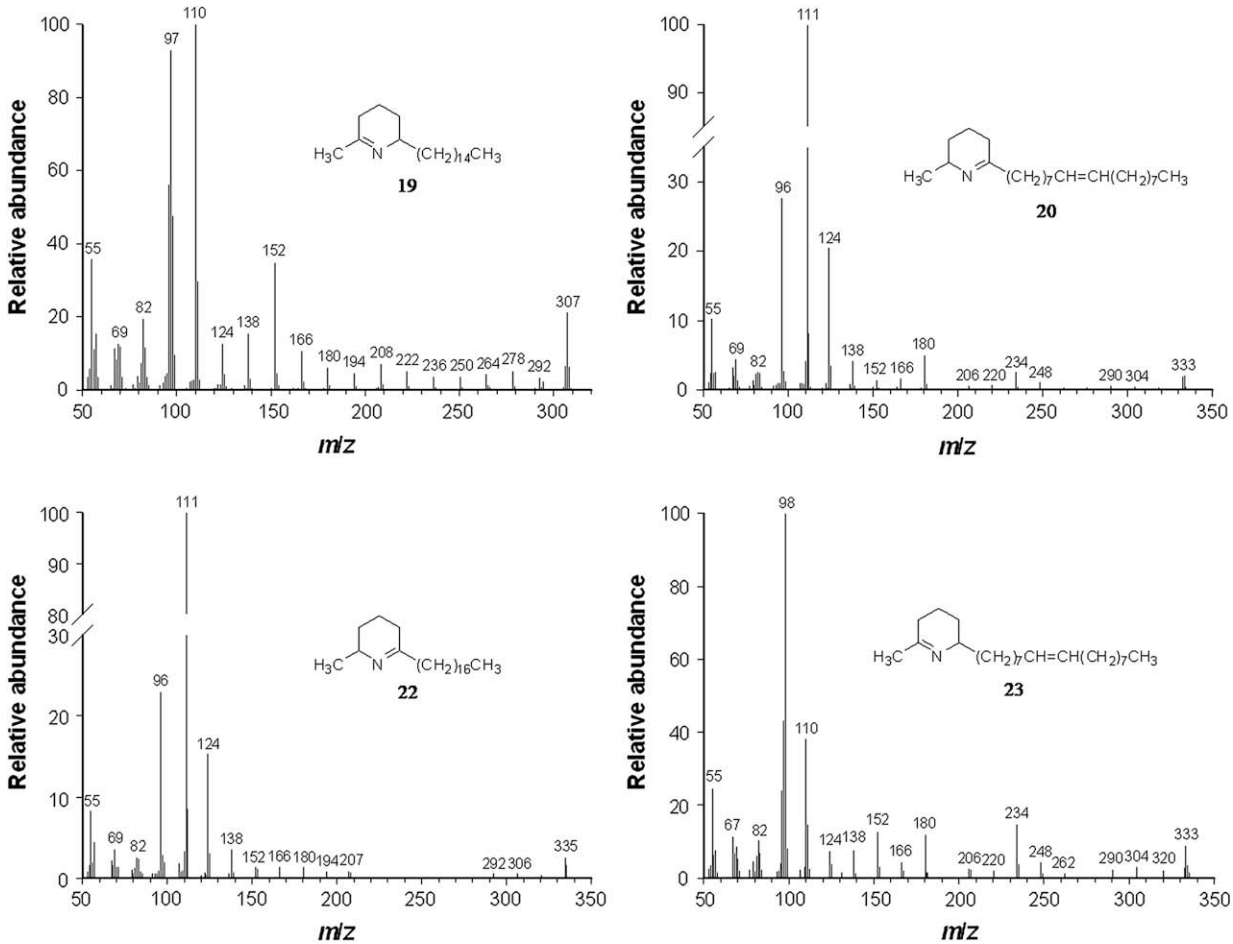



**Fig. 4.** Mass spectra of piperideines from *S. invicta*.

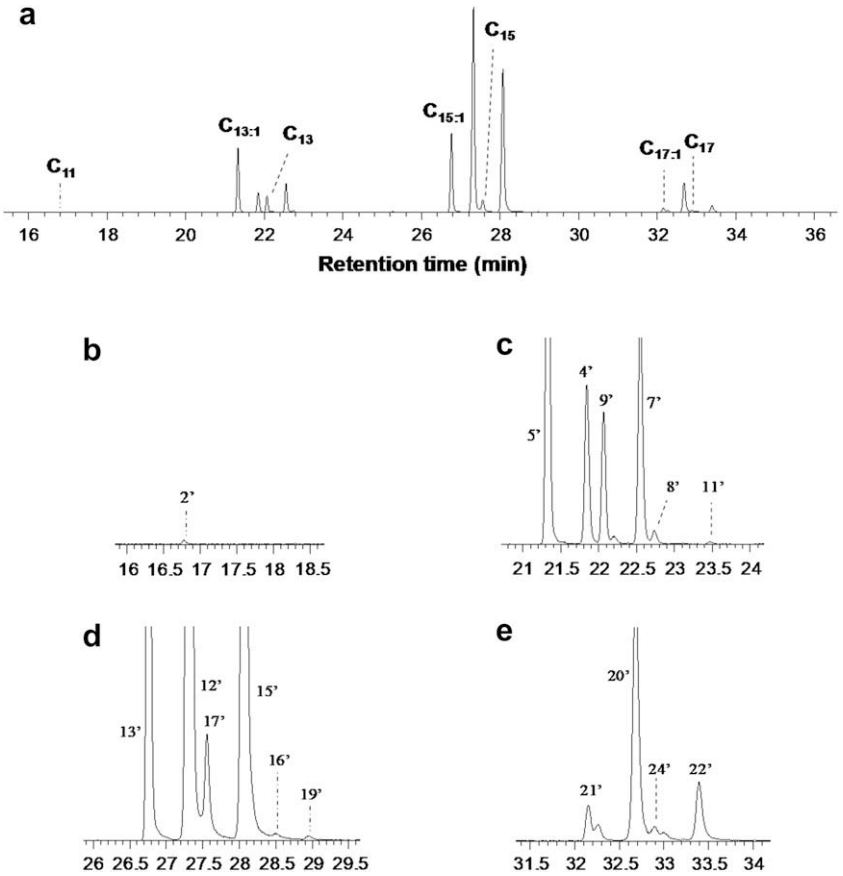



**Fig. 5.** Typical GC trace of *cis* alkaloids from *S. invicta*. (a) Visible peak area of GC chromatogram; (b)–(e), amplified GC peaks in (a).

dialkylpiperidines and $\Delta^{1,6}$-piperideines mainly into (2*R*,6*R*) and partially into (2*R*,6*S*) dialkylpiperidines. Thus, the configurations of $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines in *S. invicta* must be (6*R*) and (2*R*), respectively. It seems likely that $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines are equally important in the biosynthesis of alkaloids in *S. invicta* venom since they both exist almost across $C_{11}$, $C_{13}$, $C_{15}$, $C_{17}$.

Studies on the chemistry of alkaloidal components in the venom of various fire ant species have led to a hypothetical construct for the biochemical evolution of these compounds (Brand, 1978). The venom of *S. invicta*, which is dominated by piperidines with $C_{13}$ and $C_{15}$ side chains, is easily distinguished from that of *S. richteri*, which is dominated by piperidines with $C_{11}$ and $C_{13}$ side chains (Brand et al., 1972; Vander Meer and Lofgren, 1988; Chen and Fadamiro, 2009). Comparative analyses of the venom components of five species of *Solenopsis* fire ants (*Solenopsis aurea*, *Solenopsis xyloni*, *Solenopsis geminata*, *S. richteri* and *S. invicta*) demonstrated that their alkaloidal compositions are species-specific (Brand et al., 1972, 1973b; Blum et al., 1973; MacConnell et al., 1976). The first three species (*S. aurea*, *S. xyloni*, *S. geminata*) contain primarily *cis* $C_{11}$ and *trans* $C_{11}$ with traces of *cis* $C_{13}$ and/or *cis* $C_{13:1}$. The ratios of *cis* $C_{11}$ and *trans* $C_{11}$ in *S. aurea* and *S. xyloni* are about 4:1 (Blum et al., 1973) whereas the ratio in *S. geminata* is about 1.5:1 (Brand et al., 1973a). The composition of *cis* and *trans* isomers in *S. aurea* and *S. xyloni* matched that of the equilibrium mixture formed during their chemical synthesis in the laboratory. For instance, the chemical synthesis of $C_{11}$ (solenopsin A) through $\Delta^{1,6}$-piperideine or $\Delta^{2,3}$-piperideine gave a ratio of the *cis* isomer to the *trans* isomer of 4:1 (Hill and Yuri, 1977; Jefford and Wang, 1993). This match-up together with the existence of $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines in the venom of *Solenopsis* fire ants may provide further indirect evidence that $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines function as precursors for biosynthesis of fire ant alkaloids. Brand et al. (1973b) hypothesized that the venom of ancestral *Solenopsis* species was simple (i.e., contained few components) and showed little biosynthetic "sophistication". The evolution of the venoms would have begun with a condition in which the venom contained abundant $C_{11}$ and minute traces of $C_{13}$, with the *cis* isomers dominating and the *trans* isomers at a much lower level. Two evolutionary steps can be addressed here: (a) a switch from a probable normal production ratio of *cis* and *trans* $C_{11}$ to a thermodynamically highly unfavorable decrease in *cis* and an increase in *trans* isomers; and (b) further evolution of abundant *trans* $C_{13}$, and then the addition of *trans* $C_{15}$ and *trans* $C_{17}$ accompanied by a reduction in the production of *trans* $C_{11}$. On

this basis, *S. aurea*, *S. xyloni*, and *S. geminata* would appear to be the more primitive species, whereas *S. richteri* and *S. invicta* are relatively newer species. The venom of *Solenopsis eduardi* (a close relative of *S. geminata*) collected from Colombia consisted of more than 98% *trans* $C_{11}$ and about 1% of the corresponding *cis* isomer (MacConnell et al., 1976), which represented a major shift from *cis* $C_{11}$ to *trans* $C_{11}$. The newer *Solenopsis* species seemed to possess enzymes that could synthesize piperidines with longer and unsaturated side chains and with stereochemical compositions diverging rather markedly from the thermodynamic equilibrium mixture. Therefore, $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines may play an important role in the evolutionary progression. The elucidation of the biosynthetic pathways leading to these piperidine alkaloids would likely provide a direct evidence for the proposed fire ant evolution model.

In summary, eleven novel $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines were identified in this study from *S. invicta* venom, seven of which were also identified from *S. richteri* venom (Chen and Fadamiro, 2009). These piperideines are likely as species-specific as major *trans* form of 2,6-dialkylpiperidines, and thus may be used as an additional character in defining populations of the different species of *Solenopsis* fire ants. In addition, our GC profiles indicated *cis* and *trans* alkaloids in the venom of *S. invicta* separately with the presence of *cis* $C_{17:1}$ and *cis* $C_{17}$. Preliminary electrophysiological studies with *Pseudacteon* phorid fly antennae showed that both the second and third fractions were capable of eliciting significant EAG responses in *P. tricuspis*. Further analyses by GC–EAD demonstrated that *P. tricuspis* antenna responded to the *cis*, *trans* alkaloids and $\Delta^{1,6}$-piperideines (unpublished data). The role of these piperidines and piperideines in mediating fire ant-phorid fly interactions merits further investigation.

## Ethical statement

This is not relevant to this manuscript, since the study was performed on insects (ants), not on animals.

## Acknowledgements

We thank Dr. Aijun Zhang (Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA-ARS, Beltsville, Maryland, USA) for reviewing an earlier version of this manuscript. This research was funded in part by the Alabama Fire Ant Management Program and the Auburn University Biogrants Program.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## References

Blum, M.S., Walder, J.R., Callahan, P.S., 1958. Chemical, insecticidal, and antibiotic properties of fire ant venom. Science 128 (3319), 307–308.
Blum, M.S., Brand, J.M., Duffield, R.M., Snelling, R.R., 1973. Chemistry of the venom of *Solenopsis aurea* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 66 (3), 702.
Blum, M.S., Fales, H.M., Leadbetter, G., Leonhardt, B.A., Duffield, R.M., 1992. A new dialkylpiperidine in the venom of the fire ant *Solenopsis invicta*. Journal of Natural Toxins 1 (2), 57–63.
Brand, J.M., 1978. Fire ant venom alkaloids: their contribution to chemosystematics and biochemical evolution. Biochemical Systematics and Ecology 6 (4), 337–340.
Brand, J.M., Blum, M.S., Fales, H.M., MacConnell, J.G., 1972. Fire ant venoms: comparative analyses of alkaloidal components. Toxicon 10 (3), 259–271.
Brand, J.M., Blum, M.S., Barlin, M.R., 1973a. Fire ant venoms: intraspecific and interspecific variation among castes and individuals. Toxicon 11 (4), 325–331.
Brand, J.M., Blum, M.S., Ross, H.H., 1973b. Biochemical evolution in fire ant venoms. Insect Biochemistry 3 (1), 45–51.
Chen, L., Fadamiro, H.Y., 2009. Re-investigation of venom chemistry in *Solenopsis* fire ants. I. Identification of novel alkaloids in *S. richteri*. Toxicon 53 (5), 469–478.
Deslippe, R.J., Guo, Y., 2000. Venom alkaloids of fire ants in relation to worker size and age. Toxicon 38 (2), 223–232.
Hill, R.K., Yuri, T., 1977. An approach to natural 2-alkyl-6-methylpiperidines via *N*-acyllactam rearrangement. Tetrahedron 33 (13), 1569–1571.
Javors, M.A., Zhou, W., Maas, J.W., Han, S., Keenan, R.W., 1993. Effects of fire ant venom alkaloids on platelet and neutrophil function. Life Sciences 53 (14), 1105–1112.
Jefford, C.W., Wang, J.B., 1993. An enantiospecific synthesis of solenopsin A. Tetrahedron Letters 34 (18), 2911–2914.
Jones, T.H., Blum, M.S., Fales, H.M., 1982. Ant venom alkaloids from *Solenopsis* and *Monomorium* species: recent developments. Tetrahedron 38 (13), 1949–1958.
Jouvenaz, D.P., Blum, M.S., MacConnell, J.G., 1972. Antibacterial activity of venom alkaloids from the imported fire ant, *Solenopsis invicta* Buren. Antimicrobial Agents and Chemotherapy 2 (4), 291–293.
Leclercq, S., Thirionet, I., Broeders, F., Daloze, D., Vander Meer, R.K., Braekman, J.C., 1994. Absolute configuration of the solenopsins, venom alkaloids of the fire ants. Tetrahedron 50 (28), 8465–8478.
MacConnell, J.G., Blum, M.S., Fales, H.M., 1970. Alkaloid from fire ant venom: identification and synthesis. Science 168 (3933), 840–841.
MacConnell, J.G., Blum, M.S., Fales, H.M., 1971. Chemistry of fire ant venom. Tetrahedron 27 (6), 1129–1139.
MacConnell, J.G., Blum, M.S., Buren, W.F., Williams, R.N., Fales, H.M., 1976. Fire ant venoms: chemotaxonomic correlations with alkaloidal compositions. Toxicon 14 (1), 69–78.
Vander Meer, R.K., Lofgren, C.S., 1988. Use of chemical characters in defining populations of fire ants, *Solenopsis saevissima* complex, (Hymenoptera: Formicidae). Florida Entomologist 71 (3), 323–332.