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Re-investigation of venom chemistry of *Solenopsis* fire ants. I. Identification of novel alkaloids in *S. richteri*

Li Chen, Henry Y. Fadamiro*

Department of Entomology & Plant Pathology, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

Dialkylpiperidines are characteristic of fire ants in the genus *Solenopsis* (Hymenoptera: Formicidae). Workers of the black imported fire ant, *S. richteri* produce *cis* and *trans* stereoisomers of 2,6-dialkylpiperidines with the *trans* isomer predominating. We used silica gel short column chromatography to separate both stereoisomers (*cis* and *trans*) of *S. richteri* venom alkaloids and coupled gas chromatography mass spectrometry (GC-MS) to identify novel minor components. The identities of various peaks in GC-MS analyses of the venom fractions were based on relative retention times and mass spectral data. GC profiles verified the presence of both *cis* and *trans* stereoisomers of $C_{15:1}$ and C_{15} in *S. richteri*. The GC trace of the *cis* stereoisomers of *S. richteri* alkaloids was presented for the first time. 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. The chemical identities of these minor components were determined by comparing with fragmentations of known compounds. Possible biosynthetic pathways for the production of *cis* and *trans* solenopsins by *S. richteri* are discussed.

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1. Introduction

Fire ants *Solenopsis* spp. (Hymenoptera: Formicidae) produce a wide variety of alkaloids in their poison glands, which are stored in the poison sac (venom) (MacConnell et al., 1971). These alkaloids function primarily as defensive compounds but may also possess antibacterial, antifungal, phytotoxic, insecticidal, and hemolytic properties (Blum et al., 1958; Javors et al., 1993). The venoms of the imported fire ants, *S. richteri* Forel and *S. invicta* Buren, consist of a complex mixture of 2-methyl-6-alkylpiperidines accompanied by side chain saturated or unsaturated derivatives (MacConnell et al., 1970, 1971, 1974, 1976; Brand et al., 1972, 1973; Jones et al., 1982; Blum et al., 1992; Leclercq et al., 1994). These piperidine alkaloids (i.e. dialkylpiperidines) have been classified as solenopsins, isosolenopsins, dehydrosolenopsins, or dehydroisosolenopsins, depending on the relative configuration of their substituents, length, and unsaturation of the alkyl chain (Brand et al., 1972, 1973; MacConnell et al., 1976). Carbon numbers (e.g., in *trans* $C_{11:1}$ with 11 referring to the chain length and 1 referring to the number of double bonds) have been widely used to represent these trivial names (Brand et al., 1972). The relative proportions of piperidine alkaloids in the venom may differ between nestmates and non-nestmate conspecifics, as well as between different species of imported fire ants (Brand et al., 1973; Deslippe and Guo, 2000) (Table 1).

A practical and effective procedure for determining the absolute configuration of solenopsins based on the transformation of the natural secondary amines into diastereoisomeric amides by reaction with (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid was developed by Leclercq et al. (1994). The authors reported that the absolute configuration of the *trans* alkaloids is always (2*R*,6*R*) while that of the *cis* alkaloids is (2*R*,6*S*). The side chain double bonds in the piperidines (dehydrosolenopsins A, B, C, D) are always *Z* (*cis*) form (MacConnell et al., 1971).

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

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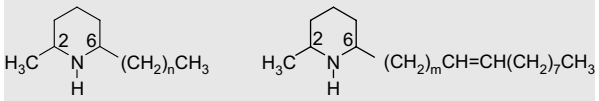
Table 1
Piperidine alkaloids in fire ants.



Solenopsin A: $n = 10$, *trans* C_{11}	Dehydrosolenopsin A: $m = 1$, *trans* $C_{11:1}$
Isosolenopsin A: $n = 10$, *cis* C_{11}	Dehydroisosolenopsin A: $m = 1$, *cis* $C_{11:1}$
Solenopsin B: $n = 12$, *trans* C_{13}	Dehydrosolenopsin B: $m = 3$, *trans* $C_{13:1}$
Isosolenopsin B: $n = 12$, *cis* C_{13}	Dehydroisosolenopsin B: $m = 3$, *cis* $C_{13:1}$
Solenopsin C: $n = 14$, *trans* C_{15}	Dehydrosolenopsin C: $m = 5$, *trans* $C_{15:1}$
Isosolenopsin C: $n = 14$, *cis* C_{15}	Dehydroisosolenopsin C: $m = 5$, *cis* $C_{15:1}$
Solenopsin D: $n = 16$, *trans* C_{17}	Dehydrosolenopsin D: $m = 7$, *trans* $C_{17:1}$
Isosolenopsin D: $n = 16$, *cis* C_{17}	Dehydroisosolenopsin D: $m = 7$, *cis* $C_{17:1}$

trans-2, 6-Disubstitution about the piperidine ring predominated in *Solenopsis* spp. 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} (Brand et al., 1972; MacConnell et al., 1976; Blum et al., 1992). The venoms of imported fire ants may also contain, as minor components, various *cis* 2,6-disubstituted piperidines, such as *cis* C_{11}, *cis* $C_{13:1}$, *cis* C_{13}, *cis* $C_{15:1}$, *cis* C_{15} (Brand et al., 1972). The GC profile of *trans* stereoisomers of venom alkaloids has been widely used to identify *Solenopsis* fire ant populations (Vander Meer and Lofgren, 1988). However, the GC trace of *cis* stereoisomers of fire ant venom alkaloids have not been well presented to date, although a previous study suggested that synthetic *cis* and *trans* stereoisomers of fire ant venom alkaloids could be separated easily via column chromatography over alumina (MacConnell et al., 1971).

In a recent study of chemical mediated interactions between imported fire ants and parasitic phorid flies (*Pseudacteon* spp.) (Diptera:Phoridae), we demonstrated the behavioral and electrophysiological responses of the phorid fly, *P. tricuspis* Borgmeier, to body extracts of *S. invicta* workers (Chen and Fadamiro, 2007). Results of a follow-up study in which we screened for glandular sources of active compounds from workers of *Solenopsis* spp., which elicited EAG response in *P. tricuspis* showed that the poison gland and sac elicited significantly greater EAG response than any other gland or body part (unpublished data). Further analyses by coupled gas chromatography electroantennogram detection (GC-EAD) suggested the existence of additional unidentified minor components in the venoms of imported fire ants. This finding prompted us to conduct a re-investigation of the chemistry of venoms of imported fire ants with the ultimate goal of identifying components of the venom alkaloids which may be involved in fire ant–phorid fly interactions. In this paper, we report on the separation of the *cis* and *trans* stereoisomers of alkaloids and the identification of novel alkaloids from venom of *S. richteri* workers. Identification of novel alkaloids from venom of *S. invicta* (a congener of *S. richteri*) is reported in another paper in this issue (Chen and Fadamiro, 2009).

2. Materials and methods

2.1. Source of ant colonies

Workers of the black imported fire ant, *S. richteri*, were collected from Bolivar, Tennessee, USA. Colonies were maintained in 1-gallon plastic jars coated with Fluon® (ICI, Wilmington, DE) to prevent escape, and were fed sugar water and crickets.

2.2. Extraction, isolation and identification of venom alkaloids

Ant workers (~5 g) were killed by freezing and extracted with hexane (enough hexane to cover ant bodies) for 24 h. The extract (0.4 ml) was loaded onto a silica gel (0.75 g in Pasteur glass pipette) column and eluted with hexane containing increasing amounts of acetone to obtain alkaloids. The chemistry of each collection (*ca* 1 mL) was analyzed by gas chromatography (GC). GC analysis was performed on a Shimadzu GC-17A equipped with a flame ionization detector (FID). The dimensions of the capillary column used were as follows: Rtx®-1MS, 30 m × 0.25 mm i.d., 0.25 μm (Restek, Bellefonte, PA). The injector was operated in splitless mode with the split opened after 2 min. Helium was used as carrier (1.0 ml/min) and make-up gas. The GC program used was as follows: injection at 90 °C, increase at 15 °C/min up to 270 °C and hold for 16 min more. The collections were pooled based on changes observed in the GC chromatograms of each collection.

Three major GC fractions were obtained and were subsequently analyzed by gas chromatography–mass spectrometry (GC-MS) using an Agilent 7890A GC coupled to a 5975C mass selective detector, with a 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 GC oven temperature was programmed from 90 °C (isothermal form 2 min) to 210 °C at 15 °C/min, then to 280 °C at 2 °C/min, and held for 10 min. The injection temperature was set at 270 °C, and the transfer line temperature was set at 280 °C. The first fraction was cuticular hydrocarbons (unpublished data), and the remaining two fractions were alkaloids based on comparison with published GC profiles of *S. richteri* alkaloids. Alkaloids were identified by analysis of their mass spectra, as well as by comparison of diagnostic ion fragments with published data on *Solenopsis* fire ants.

3. Results

Venom alkaloids were easily extracted with hexane from the body of *S. richteri* workers and the major components of the hexane whole body extract were cuticular hydrocarbons and alkaloids. Preliminary studies using silica gel short column chromatography of the hexane whole body extract indicated that alkaloids could be separated easily from cuticular hydrocarbons. Therefore we developed a practical separation procedure to purify alkaloids from the whole body extract (Fig. 1). Following this procedure, the hexane whole body extract was separated into three major fractions: cuticular hydrocarbons, *cis* alkaloids, and *trans* alkaloids. The chemistry characteristics of these three fractions were verified by GC and GC-MS analyses. The cuticular hydrocarbons fraction will be presented in a separate manuscript.

The third fraction, *trans* form alkaloids, contains five apparent peaks which were identified by comparison with

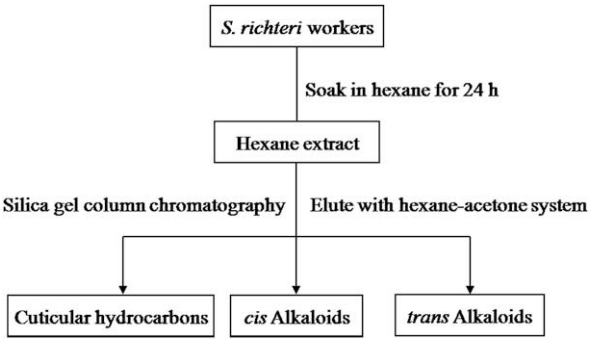


Fig. 1. A sketch of the procedure used for isolation of alkaloids from *S. richteri* workers.

published GC profiles of alkaloids from *S. richteri* workers (Brand et al., 1972; MacConnell et al., 1976) and by the mass spectrum of each peak obtained by GC-MS (Figs. 2 and 3). These five major peaks, **4**, **9**, **12**, **17**, **20**, are *trans* C_{11}, *trans* $C_{13:1}$, *trans* C_{13}, *trans* $C_{15:1}$, *trans* C_{15}, respectively (Fig. 2). Fig. 3 depicts the mass spectra of these five major peaks illustrating the characteristic base peak *m*/*z* 98, parent ion and P-15.

The mass spectra of minor peaks **3** and **5** (Fig. 4) were absolutely identical to that given 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, **3** and **5** were identified as 2-methyl-6-*n*-undecyl-$\Delta^{1,6}$-piperideine and 2-methyl-6-*n*-undecyl-$\Delta^{1,2}$-piperideine, respectively. In the same manner, the identities of peaks **10**, **13**, and **18** have been established as shown in Fig. 4 and Table 2.

The mass spectra of peaks **7**, **11**, **15**, **19** showed similar characteristic mass ions as the above 2,6-dialkylpiperideines containing unsaturated alkyl side-chains. Prominent parent ion, P-99 and P-153 (e.g., **15**, **19**) due to β-cleavage on either side of the double bond, and P-125 /126 (e.g., **7**, **11**, **15**) due to cleavage of the double bond indicate the existence and position of a double bond on the alkyl side-chain. Probable fragmentation pathways for all major fragment ions observed in the mass spectra of **7** and **11** are indicated in Fig. 5. Peaks **1**, **6**, **8**, **14**, **16** are unidentified alkaloids with base peak ion *m*/*z* 98.

Gas chromatographic retention times of both configurations (*cis*/*trans*) of a specific alkaloid are quite different. The *cis* stereoisomers always elute first because of steric hindrance of nitrogen by the 2,6-diequatorial substituents, which can be seen from GC-MS analysis of the second fraction (Fig. 6). The mass spectra of **4′**, **9′**, **12′**, **17′**, and **20′** (Fig. 6) are identical to their corresponding *trans* stereoisomers by electron impact. According to Leclercq et al. (1994), the absolute configuration of these *cis* stereoisomers is (2*R*,6*S*). The double bond in the *cis* form alkaloids is in the same position and has the same stereochemistry (*cis*) as the double bond previously identified in *trans* alkaloids (Brand et al., 1972). Aside of the above *cis* 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 the same number in the second and third fractions (for instance, **3** and **3′**) are exactly the same (Figs. 2 and 6). Therefore, the molecular structure of **3** in the third fraction must be the same as that of **3′** in the second fraction. It is interesting that $\Delta^{1,6}$-piperideines of **7′**, **10′**, **15′**, **18′** are apparently visible with **18′** as one of the major peaks in the second fraction (Fig. 6).

4. Discussion

The results of this study confirm that the venom of black imported fire ant, *S. richteri* contains five major components all of which are *trans*-2-methyl-6-alkyl (or alkenyl) piperidines, as previously reported (Brand et al., 1972; MacConnell et al., 1976). These *trans* form alkaloids predominate in imported fire ants, while the corresponding *cis* form alkaloids are present only in trace amounts (Brand et al., 1972; Vander Meer et al., 1985; Vander Meer and Lofgren, 1988). MacConnell et al. (1971) reported on the separation of the *cis* and *trans*

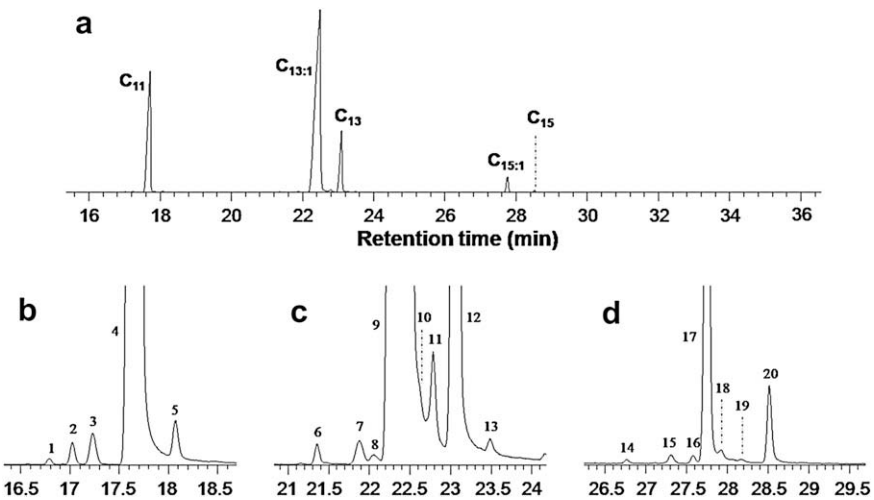


Fig. 2. Typical GC trace of *trans* alkaloids from *S. richteri*. (a), visible peak area of GC chromatogram; (b)–(d), amplified GC peaks in (a).

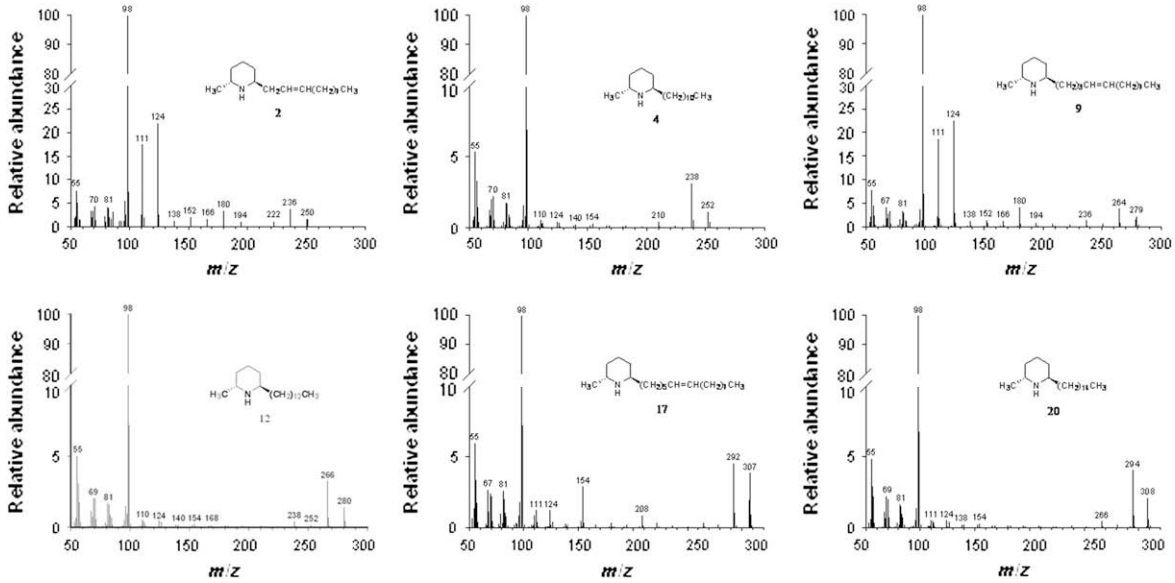


Fig. 3. Mass spectra of *trans* alkaloids from *S. richteri*.

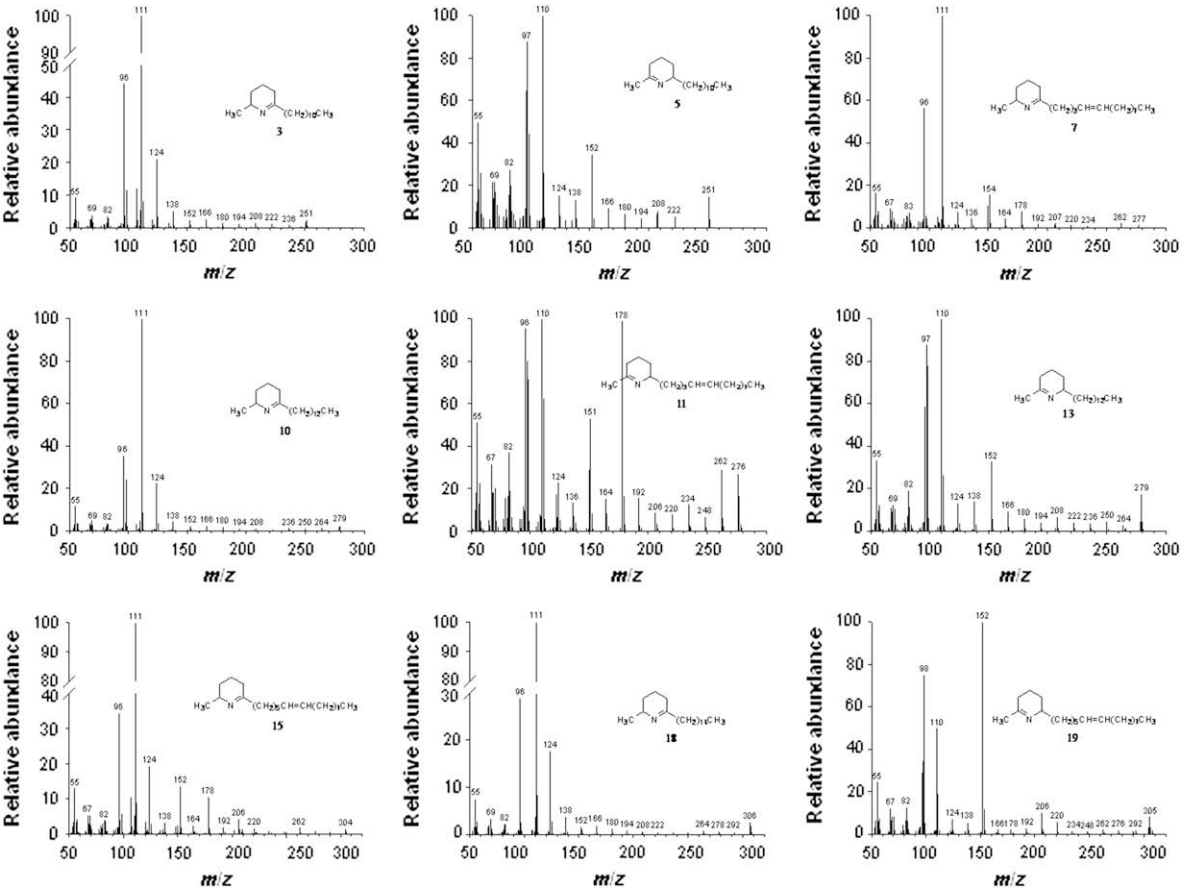
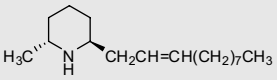
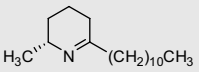
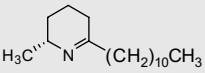
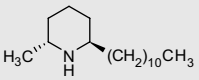
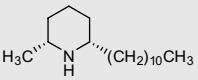
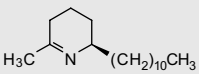
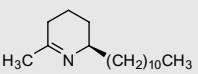
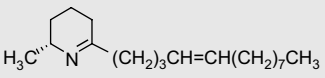
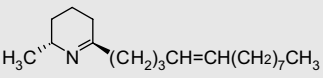
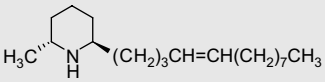
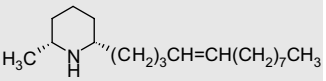
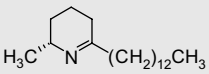
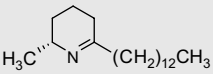
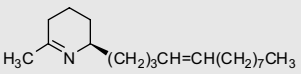
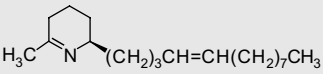
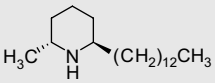
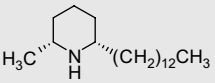
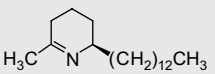
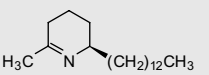
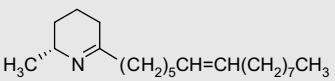
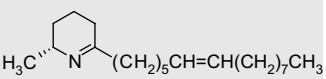
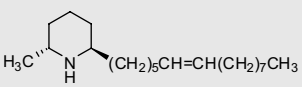
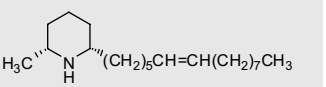


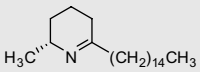
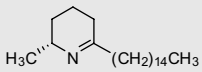
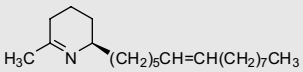
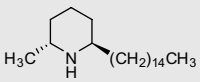
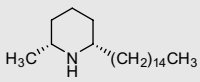
Fig. 4. Mass spectra of minor components of the third fraction from *S. richteri*.

Table 2
Chemical identity of alkaloids from *S. richteri*

trans Alkaloids			*cis* Alkaloids		
Peak	Configuration	Structure	Peak	Configuration	Structure
2	2*R*,6*R*				
3	2*R*		**3′**	2*R*	
4	2*R*,6*R*		**4′**	2*R*,6*S*	
5	6*R*		**5′**	6*R*	
7	2*R*		**7′**	2*R*	
9	2*R*,6*R*		**9′**	2*R*,6*S*	
10	2*R*		**10′**	2*R*	
11	6*R*		**11′**	6*R*	
12	2*R*,6*R*		**12′**	2*R*,6*S*	
13	6*R*		**13′**	6*R*	
15	2*R*		**15′**	2*R*	
17	2*R*,6*R*		**17′**	2*R*,6*S*	

(*continued on next page*)

Table 2 (*continued*)

trans Alkaloids			*cis* Alkaloids		
Peak	Configuration	Structure	Peak	Configuration	Structure
18	2*R*		**18′**	2*R*	
19	6*R*				
20	2*R*,6*R*		**20′**	2*R*,6*S*	

stereoisomers of synthetic solenopsins by alumina column chromatography. In the present study, the *cis* and *trans* stereoisomers of venom alkaloids of *S. richteri* were easily separated by silica gel column chromatography with the *cis* stereoisomers always eluting before the corresponding *trans* stereoisomers. This easy separation illustrated the difference in polarity of a *cis* and *trans* pair of diastereoisomers. The 2,6-disubstituted piperidine ring has the hydrogen atom and electron lone pair of the nitrogen atom considerably more exposed when the substituents are *trans*. Diequatorial substituents in these positions tend to shield both the hydrogen atom and the electron lone pair of the nitrogen to a considerable extent. If the biological activity of these alkaloids is dependent to any degree on the availability of the nitrogen atom, it is likely that the *trans* form isomer will be more active than the *cis* form isomer (Brand et al., 1972).

In the study, separation of the hexane body extract of *S. richteri* workers by silica gel column chromatography yielded three fractions: cuticular hydrocarbons (first fraction), *cis* alkaloids (second fraction) and *trans* alkaloids (third fraction). All *cis* stereoisomers of *trans* C_{11}, *trans* $C_{13:1}$, *trans* C_{13}, *trans* $C_{15:1}$, and *trans* C_{15} were present in the second fraction. The shape of the GC trace of the *cis* alkaloids is relatively similar to that of the *trans* alkaloids. The GC traces in previous reports (e.g., Brand et al., 1972; Vander Meer and Lofgren, 1988) only illustrated the existence of C_{11}, $C_{13:1}$ and C_{13} alkaloids in the venom of *S. richteri*. Our GC traces, however, clearly showed the presence of both *cis* and *trans* stereoisomers of $C_{15:1}$ and C_{15} in *S. richteri*. Brand et al. (1972) and MacConnell et al. (1976) mentioned the presence of only trace amounts of both *cis* and *trans* $C_{15:1}$ in the venom of the *S. richteri*. In our GC profile, a very small peak (retention time 12.86 min) with a base mass peak *m*/*z* 98 eluting before *trans* C_{11} was identified as *trans* C_9 (**21**, *trans*-2-methyl-6-*n*-nonylpiperidine) (Fig. 7). Both *cis* and *trans* C_9 have been detected from the venom of alate females of *S. richteri* (MacConnell et al., 1974) and from the venom of workers of *Solenopsis* (*Diplohoptrum*) species collected from Puerto Rico (Jones et al., 1982, 1996; Blum et al., 1985). We did not detect *cis* C_9 from the second fraction of the venom of *S. richteri* workers.

In addition to the above previously identified five pairs of diasteroisomers, seven novel alkaloids were identified in both alkaloid fractions of *S. richteri* venom in this study. These include two $\Delta^{1,6}$-piperideines and two $\Delta^{1,2}$-piperideines for C_{13}, and two $\Delta^{1,6}$-piperideines and one $\Delta^{1,2}$-piperideine for C_{15}. A parallel study on the venom chemistry of *S. invicta*, a congener of *S. richteri*, also revealed the presence of these seven novel alkaloids plus additional four newly identified alkaloids (Chen and Fadamiro, 2009). Although $\Delta^{1,6}$-piperideines and $\Delta^{1,2}$-piperideines for C_{11} in *S. richteri*, C_{11} and C_{17} in *S. invicta* are not complete, we can predict that other *Solenopsis* spp. might possess two $\Delta^{1,6}$-piperideines and two $\Delta^{1,2}$-piperideines for C_{11} or C_{17} as those found in *S. richteri* for C_{13} and in *S. invicta* for C_{13} and C_{15}. The GC trace of the second fraction from silica gel column chromatography contains distinctive $\Delta^{1,6}$-piperideine peaks **7′**, **10′**, **15′**, and **18′**, but fairly visible $\Delta^{1,2}$-piperideine peaks (e.g., **11′**). In the third fraction, GC peaks of $\Delta^{1,6}$-piperideines and $\Delta^{1,2}$-piperideines are in similar amounts. The reason why the second (*cis*) and third (*trans*) fractions contain same $\Delta^{1,6}$-piperideines and $\Delta^{1,2}$-piperideines of silica gel column chromatography is unclear and deserves further study.

Brand et al. (1972) first reported the identification of compound **3** from *S. xyloni* venom, but this compound has not been previously reported in *S. richteri* or *S. invicta*. A pair of structurally similar pyrrolines, 2-ethyl-5-alkyl-$\Delta^{1,2}$-pyrroline and 2-ethyl-5-alkyl-$\Delta^{1,5}$-pyrroline have been identified in the venom of *Monomorium* ants, a myrmicine genus related to *Solenopsis* (Jones et al., 1988). The mass spectra of two unidentified analogous compounds eluting before 2-ethyl-5-alkyl-pyrrolidine ($M^+ = 279$) were very similar to 2-methyl-6-alkenyl-$\Delta^{1,6}$-piperideine (Fig. 5a) and 2-methyl-6-alkenyl-$\Delta^{1,2}$-piperideine (Fig. 5b): MS *m*/*z* (rel. intensity) 277 (3, M^+), 124 (30), 111 (100), 82 (35); and MS *m*/*z* (rel. intensity) 277 (25, M^+), 152 (20), 138 (15), 124 (8), 111 (70), 110 (45), 97 (100), 96 (50), 82 (18). The existence of these pyrrolines in *Monomorium* spp. indirectly supports our identification of piperideines in the present study.

Based on the above results, we hypothesize that $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines may function as precursors for fire ant alkaloids. Brand et al. (1972) described the

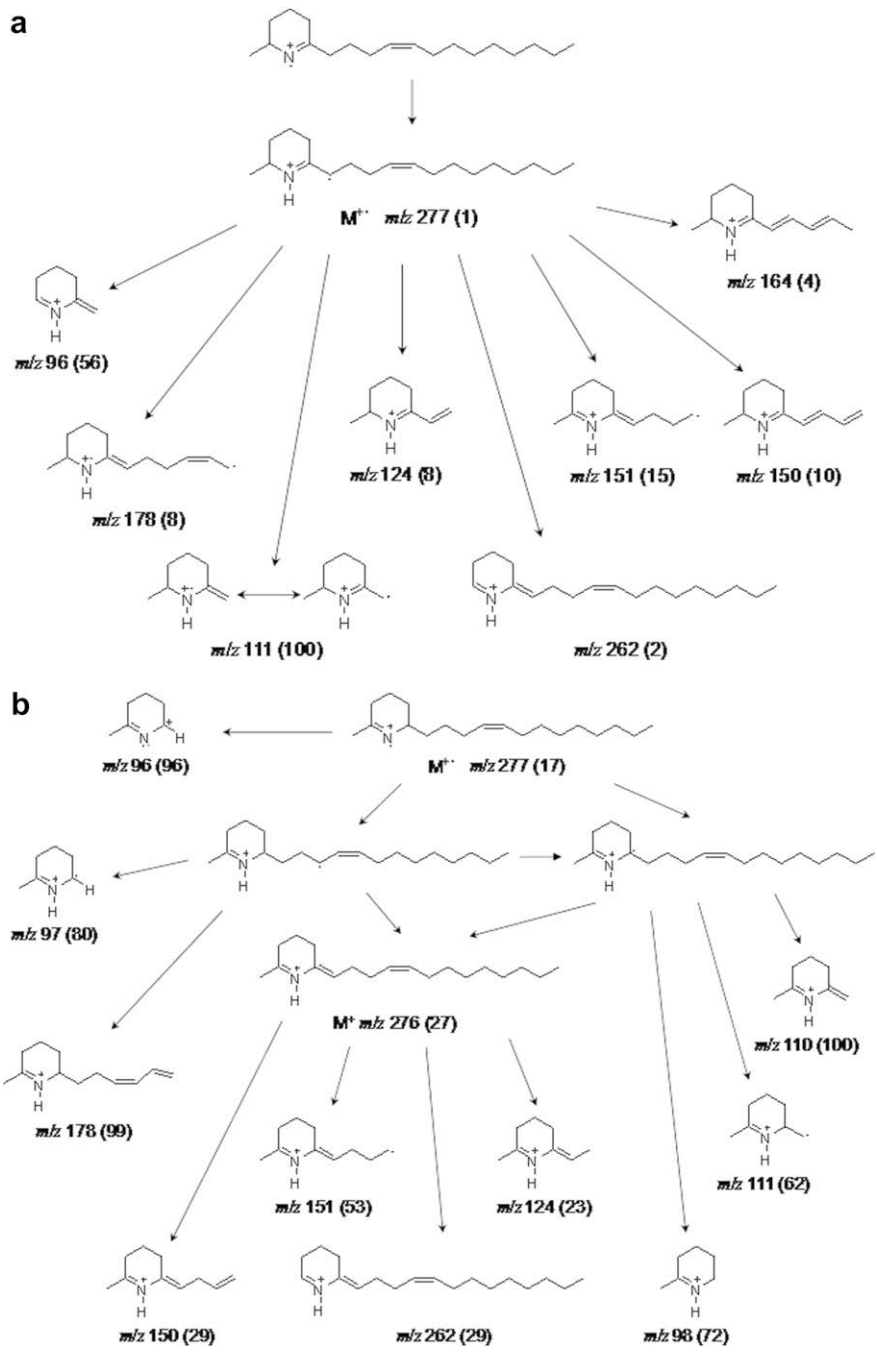


Fig. 5. Proposed mass spectral fragmentations for **7** (a) and **11** (b); percentages in parentheses are relative abundance of electron-impact mass peaks.

transformation of synthetic *cis* C_{11} as below (depicted in Fig. 8), which may support this hypothesis. The treatment of compound **2′** (*cis* C_{11}) with *t*-butylhypochlorite resulted in a mixture of compounds **3** and **5**. Reduction of this mixture with $NaBD_4$ caused the disappearance of both compounds and deuterium was found in the parent ion and *m*/*z* 98 of *cis* C_{11}. Only a few percentage of the *trans* C_{11} was formed in the reduction, which meant that reduction of $\Delta^{1,2}$-piperideines favors (2*R*)-CH_3 and reduction of $\Delta^{1,6}$-piperideines favors (6*S*)-$C_{11}H_{23}$. Therefore, the absolute configuration at C-2 and C-6 can be maintained when going from one series of derivatives to the other. Theoretically, 2,6-dialkylpiperideines can be reduced to either *cis*- (2*R*,6*S* and 2*S*,6*R*) or *trans*-2,6-dialkylpiperidines (2*R*,6*R* and 2*S*,6*S*). However, the absolute configurations of major components in the venom of *Solenopsis* fire ants are always (2*R*,6*R*) for *trans* alkaloids and (2*R*,6*S*) for *cis* alkaloids (Leclercq et al., 1994).

Based on previous reports (Leclercq et al., 1994, 1996) and our data, we proposed a biosynthetic pathway for *cis*- and *trans*-solenopsin A, which is shown in Fig. 9. Leclercq

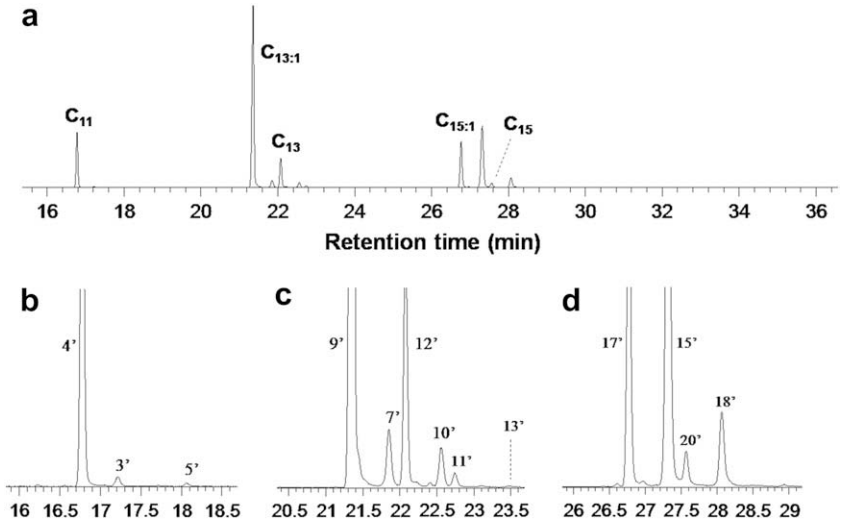


Fig. 6. Typical GC trace of *cis* alkaloids from *S. richteri*. (a), visible peak area of GC chromatogram; (b)–(d), amplified GC peaks in (a).

et al. (1996) demonstrated the polyacetate origin of the *cis*- and *trans*-solenopsin A and further presumed that both *cis*- and *trans*-solenopsins A are formed from an 18-carbon polyacetate chain produced by the condensation of acetyl-coenzyme A with subsequent units of malonyl-coenzyme A. As *S. richteri* 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*) dialkylpiperidines (Route A, Fig. 9) and $\Delta^{1,6}$-piperideines mainly into (2*R*,6*R*) and partially into (2*R*,6*S*) dialkylpiperidines (Route B, Fig. 9). Therefore, we propose that the configurations of $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines (Table 2) are (6*R*) and (2*R*), respectively. Reductive enzymes are essential to maintain such biosynthetic bias since *cis* stereoisomers, with the 2,6-substituents oriented diequatorially, are more stable than their *trans* stereoisomers. These (6*R*) $\Delta^{1,2}$-piperideines and (2*R*) $\Delta^{1,6}$-piperideines are likely to function as precursors for fire ant alkaloids but not as intermediates in the interconversion of the *cis* and *trans* ring isomers, as proposed by Brand et al. (1972). The combined peak areas of the same two (6*R*) $\Delta^{1,2}$-piperideines from both the second and third fractions are similar to those of the same two (2*R*) $\Delta^{1,6}$-piperideines, suggesting that Route A and Route B are equally important in the biosynthesis of alkaloids in *S. richteri* venom.

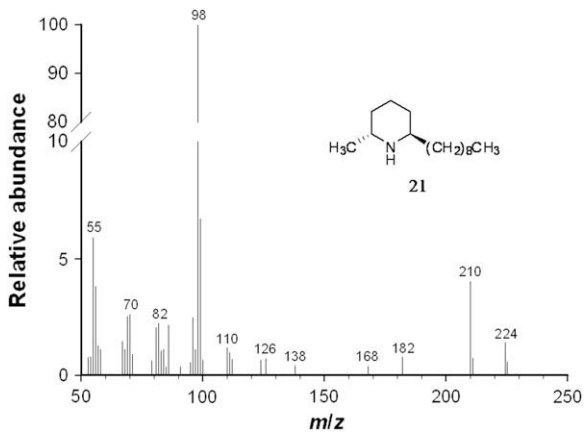


Fig. 7. Mass spectrum of *trans* C_9.

The following interesting phenomena may further support our hypothesis regarding the possible role of $\Delta^{1,2}$-piperideines and $\Delta^{1,6}$-piperideines as precursors for fire ant alkaloids. There were two pairs (saturated side chain versus unsaturated side chain) of piperideines at *trans* C_{13} area (**7**, **10**, **11**, **13**, Fig. 2c). As in *trans* C_{11} area, a pair of (6*R*) $\Delta^{1,2}$-piperideines were missing because **2,** *trans* $C_{11:1}$, only presented in trace amount (Fig. 2b). Even though (6*R*) $\Delta^{1,2}$-piperideine with unsaturated side chain, **19**, was present in trace amount, (6*R*) $\Delta^{1,2}$-piperideine with saturated side chain in the *trans* C_{15} area which should elute after **20** (*trans* C_{15}) was still missing (Fig. 2d). In the second fraction, the presence of these (6*R*) $\Delta^{1,2}$-piperideines and (2*R*) $\Delta^{1,6}$-piperideines is similar to that in the third fraction. Since *cis* alkaloids are very minor components, (2*R*) $\Delta^{1,6}$-piperideines may not be reduced only into *cis* alkaloids (2*R*,6*S*) but also to a large degree into *trans* alkaloids (2*R*,6*R*). Furthermore, when GC traces of the second and third fractions are overlaid together, the combined peak areas of piperideines with a certain carbon number appear to correlate with the combined peak areas of the corresponding piperidines. Feeding experiments of labeled compounds with live ants are needed to further refine the biosynthetic pathway outlined in Fig. 9.

In summary, seven novel alkaloids were identified in this study from *S. richteri* venom including three $\Delta^{1,2}$-piperideines and four $\Delta^{1,6}$-piperideines. For the first time, our GC profiles clearly demonstrated the presence of both *cis* and *trans* stereoisomers of $C_{15:1}$ and C_{15} in *S. richteri*. Alkaloids in the fire ant venom constitute key weapons in ant defensive behavior (Blum et al., 1958). The possible role of these novel

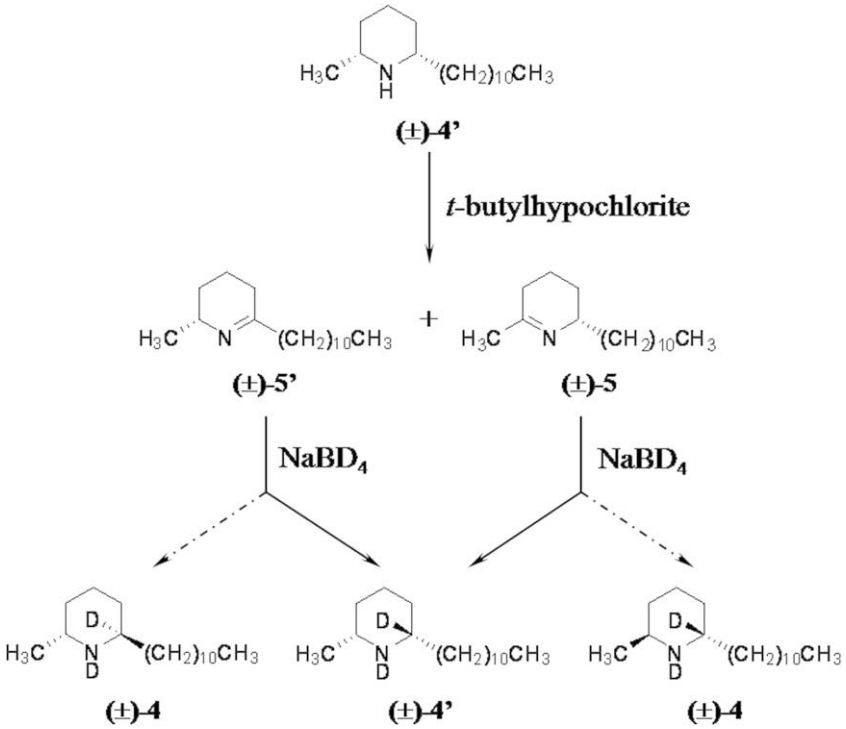


Fig. 8. Transformation of *cis* C_{11} (dashed lines mean secondary pathway, Brand et al., 1972).

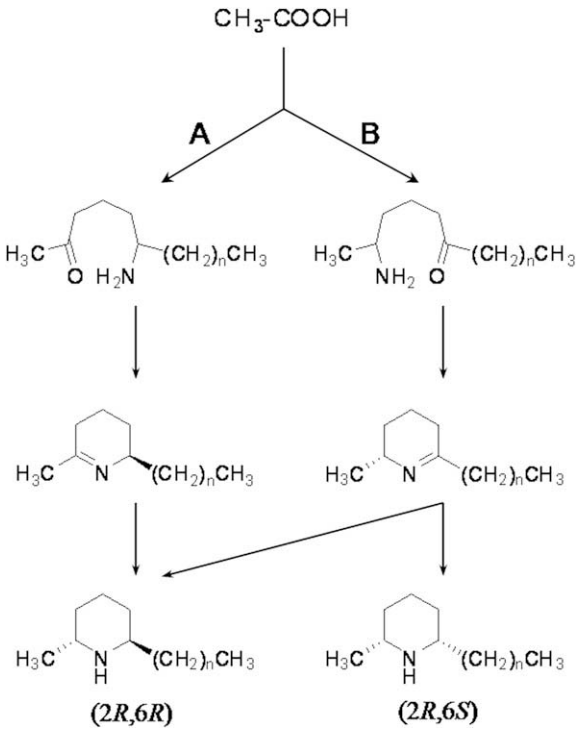


Fig. 9. Proposed biosynthetic pathways for *cis*- and *trans*-solenopsins. (A) $\Delta^{1,2}$-piperideine pathway; (B) $\Delta^{1,6}$-piperideine pathway.

alkaloids and other dialkylpiperidines in mediating ant–parasitoid interactions, such as between *S. richteri* and *Pseudacteon* phorid flies, remains to be determined.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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