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Rapid configuration analysis of the solenopsins

Adriana Pianaro [a], Eduardo G.P. Fox [b], Odair C. Bueno [b], Anita J. Marsaioli [a,*]

[a] *Chemistry Institute, University of Campinas - UNICAMP, R. Josué de Castro,13083-970 Campinas, SP, Brazil*
[b] *Research Center of Social Insect, Institute of Biosciences, São Paulo State University - UNESP, Av. 24A, 1515- Bela Vista, 13506-900 Rio Claro, SP, Brazil*

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ABSTRACT

A protocol for rapid access to the relative and absolute configurations of the solenopsins, the venom alkaloids of fire ants (*Solenopsis* spp.), was developed based on chiral capillary gas chromatography. The synthesis of racemic mixtures of 2-methyl-6-alkylpiperidines and the isolation of natural (2*R*,6*R*)- and (2*R*,6*S*)-2-methyl-6-undecylpiperidines allowed for the standardization of the chromatographic method. Application of this protocol revealed the previously unknown natural occurrence of four stereoisomers of 2-methyl-6-undecylpiperidine in venom samples from workers and gynes of *Solenopsis saevissima*.

1. Introduction

Solenopsis ants are known worldwide as fire ants because of the pain inflicted by their stings. These ants are trivially known in Brazil as 'formigas de fogo' or 'lava-pés'. These ants are aggressive and considered a serious pest affecting humans, agriculture, and livestock. Among the various *Solenopsis* species, the most studied ones worldwide are *Solenopsis invicta*, *Solenopsis richteri*, *Solenopsis geminata*, *Solenopsis saevissima*, *Solenopsis xyloni*, and *Solenopsis aurea*.[1,2a]

The venom produced by the *Solenopsis* ants consists of proteins (0.1%) and alkaloids: 2,6-dialkylpiperidines, 2,5-dialkylpyrrolidines, 2,5-dialkyl-1-pyrrolines, and 3,5-dialkylindolizidines.[2,3] As for the alkaloids themselves, they have insecticide, fungicide, bactericide, and hemolytic properties.[4]

Among the venom alkaloids of the *Solenopsis* fire ants, dialkylpiperidines are the most abundant and have been named as solenopsins or isosolenopsins according to the *trans* or *cis* relative configuration of the C-2 and C-6 piperidine ring substituents.[2b] The relative configurations of these alkaloids can be assessed by GC-FT-IR, the *cis*-isomers exhibit significant Bohlmann bands (at ~2810–2600 cm^{-1}) while the *trans*-isomers exhibit only very weak bands.[5] The absolute configurations of the solenopsins were first determined by derivatizing the natural secondary amines into diastereoisomeric amides with (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid chloride (MTPA-Cl), (Mosher's method). The ^1H NMR analyses of the chemical shift differences induced by derivatization, observing hydrogens located on right and left hand sides of the amide nitrogen taking the most preferred conformation (trifluoromethyl group and carbonyl amide group lies in the same plane), were correlated to the absolute configuration at C-2 and C-6. The absolute configuration of the major *trans*-alkaloids was determined as (2*R*,6*R*), while that of the *cis*-alkaloids was (2*R*,6*S*). It was further concluded that the predominant naturally occurring alkaloids of *Solenopsis* were always (2*R*,6*R*) and (2*R*,6*S*).[6]

Herein we report a chiral chromatographic method (GC–FID and GC–MS) to assess the relative and absolute configurations of solenopsins in a large range of *Solenopsis* fire ant venom samples. An appropriate chiral column for chiral gas chromatography analyses was selected with synthetic racemic standards of the piperidine alkaloids: (±)-*cis*- and (±)-*trans*-2-methyl-6-undecylpiperidines **6**, (±)-*cis*- and (±)-*trans*-2-methyl-6-tridecylpiperidines **7**, and (±)-*cis*- and (±)-*trans*-2-methyl-6-pentadecylpiperidines **8**.

2. Results and discussion

The synthetic route is depicted in Scheme 1, and used lutedine **1** as the starting material; we did not attempt to achieve any selectivity or yield optimization as the only objective was to obtain *cis*- and *trans*-solenopsin and isosolenopsin racemic standards for the chromatographic analyses, with diastereomeric ratios different from 1:1 in order to allow recognition by their relative abundances. The synthetic racemic mixtures were fully characterized by GC–MS, IR, ^1H and ^{13}C NMR, thus establishing the diastereomer ratios (Table 1, entries 4–6; Section 4).

Our second task was to select the appropriate chiral stationary phase from fused silica capillary column Chrompack CP-chirasil-Dex CB, Lipodex E octakis-(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin, and heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin for the GC and GC–MS analyses using racemic alkaloid mixtures. None gave satisfactory diastereomeric and enantiomeric discrimination of the synthetic alkaloids. Derivatization with acetyl chloride and trifluoroacetic anhydride produced the piperidine alkaloid acetamides and trifluoroacetamides, respectively (Scheme 2 and Table 2; see Section 4), which were submitted to

* Corresponding author. Tel.: +55 19 3521 3067; fax: +55 19 3521 3023.
E-mail address: anita@iqm.unicamp.br (A.J. Marsaioli).

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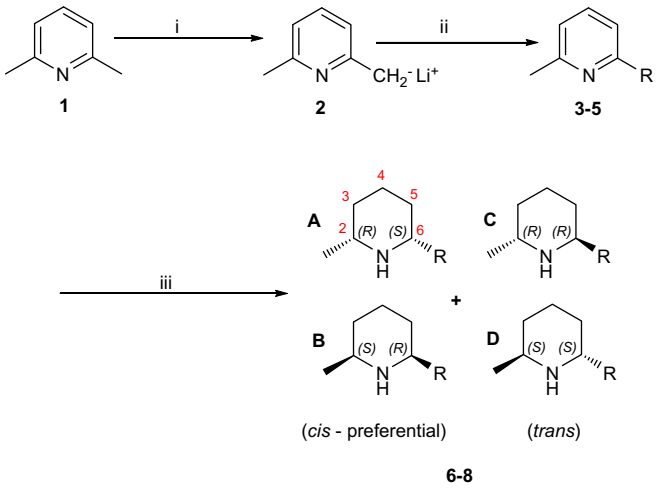
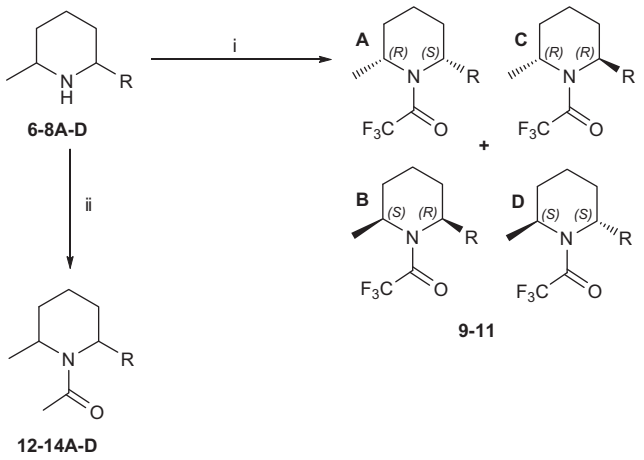


Scheme 1. Reagents and conditions: (i) Dry CH_2Cl_2, *n*-BuLi, Ar (g), 0 °C to rt; (ii) in situ reaction, tosylated alcohol; (iii) CH_3COOH/MeOH (1:5), Pt/C 10%, 70 psi H_2 (g).



Scheme 2. Reagents and conditions: (i) Trifluoroacetic anhydride/trifluoroacetic acid (8:1), pyridine, 30 °C; (ii) acetyl chloride, K_2CO_3 (2 mol/L).

Table 1
Synthesis of the piperidinic alkaloids

Entry	Compound	R	Isolated yields (%)	dr (*cis*:*trans*)
1	**3**	*n*-$C_{11}H_{23}$	19	–
2	**4**	*n*-$C_{13}H_{27}$	24	–
3	**5**	*n*-$C_{15}H_{31}$	15	–
4	(±)-**6**	*n*-$C_{11}H_{23}$	99	81:19
5	(±)-**7**	*n*-$C_{13}H_{27}$	89	97:3
6	(±)-**8**	*n*-$C_{15}H_{31}$	65	93:7

Table 2
Derivatization of the synthetic racemic mixture of piperidine alkaloids and diastereomeric and enantiomeric discrimination by chiral GC

Entry	Compound	R	dr *cis*:*trans*[b]	rt (min)-*trans*[b]	rt (min)-*cis*[b]
1	(±)-**9**	*n*-$C_{11}H_{23}$	77:23	49.78, 50.21	51.16, 51.51
2	(±)-**10**	*n*-$C_{13}H_{27}$	95:5	67.96, 68.29	69.45, 69.73
3	(±)-**11**[a]	*n*-$C_{15}H_{31}$	97:3	89.04	91.84
4	(±)-**12**	*n*-$C_{11}H_{23}$	95:5	24.52, 24.85	25.47, 25.79
5	(±)-**13**	*n*-$C_{13}H_{27}$	93:8	47.00, 48.00	48.52, 49.18
6	(±)-**14**	*n*-$C_{15}H_{31}$	94:6	94.76, 96.55	98.22, 99.53

[a] Trifluoroacetamides **11** did not separate on any available chiral capillary column .
[b] dr = diastereomer ratio; rt = retention time; using Chrompack CP-chirasil-Dex CB chiral column in GC.

GC and GC–MS analyses using the fused silica capillary columns with chiral stationary phases. Successful diastereomeric and enantiomeric discrimination of trifluoroacetamides **6** and **7** was achieved using Chrompack CP-chirasil-Dex CB as the chiral stationary phase, producing chromatographic signals from two pairs of enantiomers for each derivatized *cis* and *trans* piperidinic alkaloids (Table 2, entries 1 and 2; Scheme 2).

The stereoisomeric ratio was determined by comparing the relative abundances of the *cis*:*trans* isomers of (±)-**9A**–**D** by GC or GC–MS analyses and ^{13}C NMR of (±)-**6A**–**D** (Table 2, entry 1).[6a,7] The same rationale of combining the ^{13}C NMR of (±)-**7A**–**D** and the GC of the trifluoroacetamide derivatives was applied to the (±)-*cis*- and *trans*-trifluoroacetamides **10**, which were also discriminated by the Chrompack cyclodextrin capillary column (Table 2, entry 2). However, the trifluoroacetamide derivatives of **11** were not resolved using this methodology (Table 2, entry 3). The resolution of the stereoisomers of these alkaloids was achieved with the same methodology and chiral capillary column but using acetamide derivatives **14** (Scheme 2 and Table 2, entry 6; see Section 4).

This methodology was applied to monitor the relative and absolute configurations of the *Solenopsis* venom alkaloids, within the same nest and between different nests, taking care to analyze three ant venom sacs at a time (in triplicate) and sorting workers and queens of *S. saevissima*. This procedure was applied to 24 ant samples of *S. saevissima* workers (minor, medium, and major) and *S. saevissima* gynes (unmated queen) (Table 3; details in Section 4).

The retention time and absolute configuration of each isomer of (±)-**9** were determined using venom samples from gynes with high enantiomeric ratios of each *cis*- and *trans*-isomer (Table 3, entries 3–4—GY). The diastereomeric mixture of gyne venom, (nests 3 and 4 with diastereomeric ratios of 55:45 and 66:34 of the *cis*- and *trans*-2-methyl-6-undecylpiperidines **6**, respectively), was purified on a silica gel column providing two fractions of *cis*- and *trans*-**6** alkaloids in high diastereomeric excess (see Section 4). The specific rotations of the hydrochloride derivatives **6** {*trans*-**6**·HCl: $[\alpha]_D^{20} = -4$ (*c* 0.67, $CHCl_3$); *cis*-**6**·HCl: $[\alpha]_D^{20} = +7$ (*c* 0.6, $CHCl_3$)} were compared to the reported data {*trans*-(2*R*,6*R*)-**6**·HCl: lit.[7a] $[\alpha]_D^{20} = -7.7$ (*c* 0.51, $CHCl_3$); *cis*-(2*R*,6*S*)-**6**·HCl: lit.[7b] $[\alpha]_D^{24} = +10.0$ (*c* 1.1, $CHCl_3$)} to obtain the absolute configuration of each isomer (see Section 4). The isolated natural *cis*-(2*R*,6*S*)-**6** and *trans*-(2*R*,6*R*)-**6** isomers were derivatized with trifluoroacetic anhydride and co-injected with the synthetic racemic mixture of the four isomers of **9** (Fig. 1). Consequently, the co-elution of the *trans*-(2*R*,6*R*)-**9** standard with one of the chromatographic peaks of the synthetic racemic mixture provided the retention time of two *trans*-isomers [retention times: *trans*-(2*R*,6*R*)-**9** = 49.24 min, *trans*-(2*S*,6*S*)-**9** = 49.67 min, Fig. 1b and c]. Analogous procedures were applied to the *cis*-(2*R*,6*S*)-**9** standard (retention times: *cis*-(2*R*,6*S*)-**9** = 50.63 min, *cis*-(2*S*,6*R*)-**9** = 50.96 min, Fig. 1d–e), thus the retention times of all four isomers of **9** were characterized by chiral GC–FID or chiral GC–MS using the Chrompack fused silica capillary column.

The results of monitoring the four stereoisomers of **9** in 24 samples of *S. saevissima* venom are shown in Table 3. *Solenopsis saevissima* gynes display a *cis*:*trans* ratio with a predominance of

Table 3
Monitoring of diastereomeric and enantiomeric ratios of trifluoroacetamide derivatives **9** in *Solenopsis saevissima* venom samples

Entry[a]	I[b]	dr[c] (*cis*:*trans*)	er[c] (2*R*,6*S*:2*S*,6*R*) (*cis*)	er[c] (2*R*,6*R*:2*S*,6*S*) (*trans*)
1	W	—	—	—
	GY	39:61	90:10	7:93
2	MNW	1:99	100:0	100:0
	MDW	2:98	100:0	100:0
	MJW	5:95	100:0	100:0
	GY	87:13	100:0	100:0
3	MNW	6:94	100:0	100:0
	MDW	9:91	100:0	100:0
	MJW	16:84	100:0	100:0
	GY	55:45	100:0	100:0
4	MNW	9:91	100:0	100:0
	MDW	7:93	100:0	100:0
	MJW	11:89	100:0	100:0
	GY	66:34	100:0	100:0
5	MNW	5:95	100:0	100:0
	MDW	9:91	100:0	100:0
	MJW	17:83	100:0	100:0
	GY	53:47	100:0	100:0
6	MNW	37:63	59:41	43:57
	GY	20:80	99:1	15:85
7	MNW	0:100	—	100:0
	GY	68:32	100:0	100:0
8	MNW	17:83	100:0	100:0
	GY	62:37	100:0	100:0

[a] Entries 1–5 are nests that were collected in Pedro do Rio, RJ, Brazil and entries 6–8 are nests from Ubatuba, SP, Brazil.
[b] I = individuals analyzed. Workers were separated in different size classes: MNW, minor workers (1–2 mm), MDW, medium workers (3–4 mm), and MJW, major workers (5–6 mm). W = all sizes of workers and GY = gynes (unmated queens).
[c] Analyses by GC with Chrompack CP-chirasil-Dex CB chiral column, dr = diastereomer ratio, and er = enantiomer ratio. (—): These stereoisomers were not found in this sample.

the *cis*-isomer (Table 3, entries 2–5, 7, and 8—GY), except for two gyne samples displaying a predominance of the *trans*-isomer (Table 3, entries 1 and 6—GY). These analyses also revealed that the absolute configuration of the *trans* alkaloids of *S. saevissima* is not always (2*R*,6*R*) while *cis*-alkaloids are not always (2*R*,6*S*) (Fig. 2, Table 3—entries 1 and 6), as previously reported with *S. invicta* and *S. geminata*.[6] The absolute configuration of the *trans*-isomers of some gynes was predominantly (2*S*,6*S*)-**9D** (Table 3, entries 1 and 6—GY, Fig. 2), a fact not mentioned before. Moreover, some minor workers displayed a completely unusual *cis:trans* diastereomeric ratio (*dr* 37:63, Table 3, entry 6—MNW; Fig. 2).

3. Conclusion

These results raise deeper questions with regard to the innate chirality of the *S. saevissima* piperidine alkaloids and whether these glandular secretions and isomeric ratios play a role in the communication of the nestmates. Moreover, this methodology for the determination of the absolute configuration by chiral GC using trifluoroacetamide and acetamide derivates of *Solenopsis* piperidine alkaloids is useful to check the diastereomeric and enantiomeric ratios in samples of fire ant venoms.

4. Experimental

4.1. General

All reagents were commercially available. Pyridine, dichloromethane, and 2,6-lutidine were treated with calcium hydride and distilled.[8a] Ethyl ether was treated with calcium chloride and calcium hydride, and distilled.[8a] Tetrahydrofuran (THF) was treated with sodium and benzophenone, and distilled.[8a] Acetyl chloride was treated with phosphore pentoxide and quinoline, and distilled.[8a] Other reagents were used without further purification. The solvents (Synth, São Paulo, Brazil) were distilled and bidistilled.[8a] TLC analyses were performed on TLC aluminum sheets pre-coated with silica gel 60 F_{254} (Merck); the spots were visualized by short-wavelength UV light (254 nm) and spraying with anisaldehyde-sulfuric acid reagent or Dragendorff reagent.[8b] Hydrogenations were carried out in a Parr 3926 apparatus. Infrared spectra were recorded with Thermo Scientific Nicolet 380 FT-IR as a thin film in the Smart Performer ATR accessory and are expressed in cm^{-1}. Optical rotation data were measured on a Perkin Elmer 341 polarimeter, SROT mode, using a Na/HaI lamp at $\lambda = 589$ nm and temperature of 20 °C.

Mass spectra were carried out with an Agilent/HP 6890/5973 GC–MS system with Agilent 7683B series automatic liquid sampler equipped with a Supelco MDN-5S fused silica capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas with a flow rate of 1 mL/min and the split injection mode with a 10:1 ratio for *Solenopsis saevissima* venom alkaloid analyses and a 30:1 ratio for synthetic alkaloid analyses. Spectra were taken at 70 eV and the scanning speed was 2.89 scans/s from *m*/*z* 40 to 550. The mass detector interface and the injector temperatures were maintained at 280 and 250 °C, respectively. The oven temperature program was 50–290 °C at 25 °C/min with a final hold time of 10 min. Bidistilled ethyl acetate was added to the samples in order to have 1 μg/μL and 1 μL of each sample was analyzed by GC–MS.

GC–FID analyses were conducted with an Agilent 6850 chromatograph equipped with a capillary chiral column, either Chrompack CPChirasil—Dex CB (25 m × 0.25 mm × 0.25 μm), Lipodex E octakis-(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin [(28 m × 0.25 mm × 0.25 μm), prepared by Professor Ademir F. Morel from Santa Maria Federal University, RS, Brazil], or heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (25 m × 0.25 mm × 0.25 μm). Injector and detector temperatures were 180 and 250 °C, respectively. The carrier gas was highly purified hydrogen. The piperidine alkaloid acetamides and trifluoroacetamides were dissolved in bidistilled ethyl acetate (0.5 μg/μL) and a volume of 1 μL of each sample was injected in splitless mode in

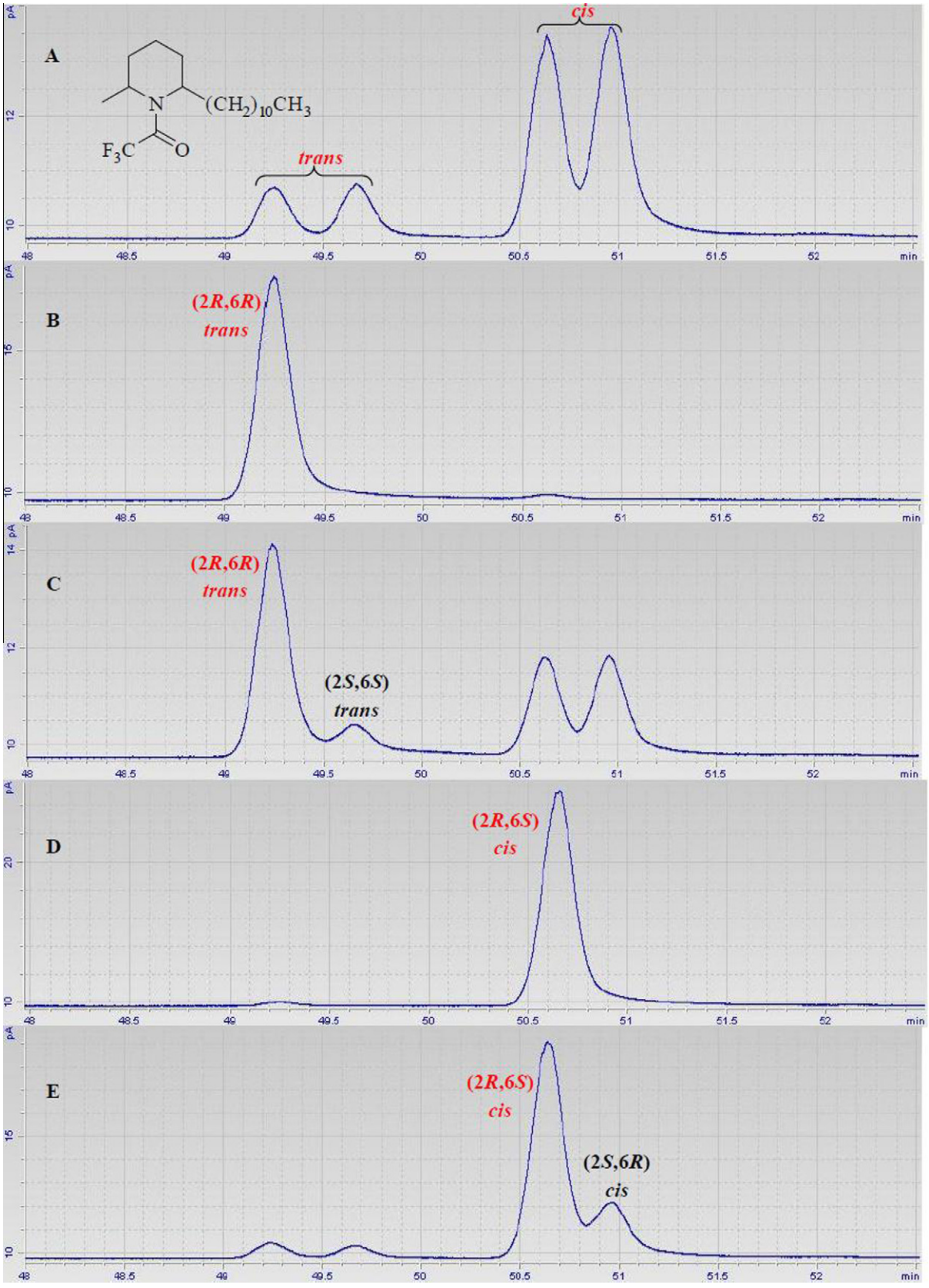


Figure 1. Chiral GC chromatograms (Chrompack CP-chirasil-Dex CB chiral column) for peak discrimination of two enantiomeric pairs of trifluoroacetamides **9**: (A) synthetic racemic mixture; (B) (2*R*,6*R*)-**9** standard; (C) co-injection of the racemic mixture with (2*R*,6*R*)-**9**; (D) (2*R*,6*S*)-**9** standard; (E) co-injection of the racemic mixture with (2*R*,6*S*)-**9**.

the Chrompack column. The carrier gas was maintained at a flow rate of 2.0 mL/min and the oven temperature program used to analyze trifluoroacetamides was 120–180 °C at 0.8 °C/min with a final hold time of 100 min (without this final hold time we could

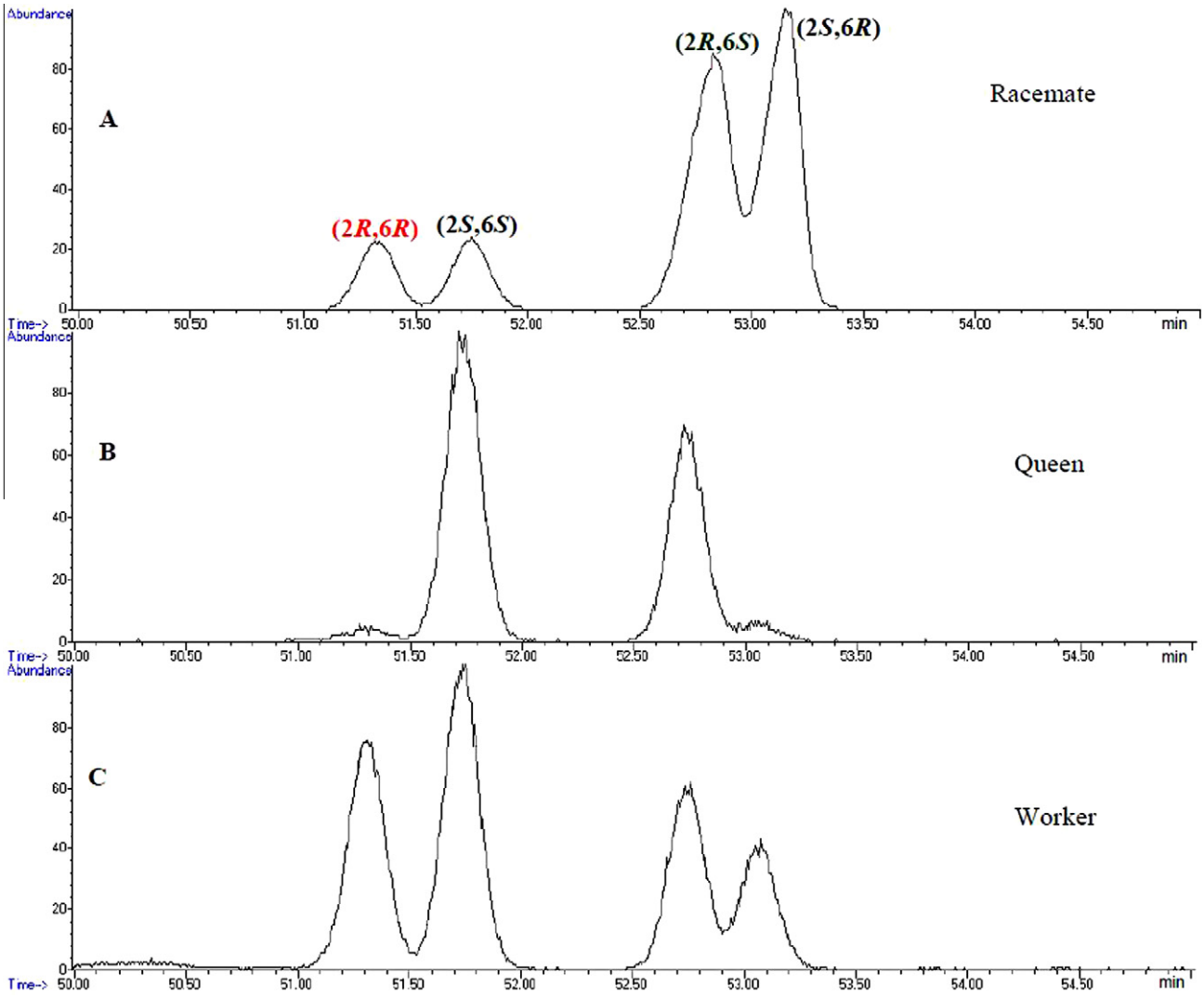


Figure 2. Ion total chromatograms of trifluoroacetamides **9** (Chrompack CP-chirasil-Dex CB chiral column, ion extraction mode: base peak *m*/*z* 194): (A) synthetic racemic mixture; (B) venom alkaloids of queen—Pedro do Rio/RJ; (C) venom alkaloids from minor worker—Ubatuba/SP.

only monitor 2-methyl-6-undecylpiperidine trifluoroacetamides) and for the acetamides it was 10 min at 170 °C and 170–180 °C at 1.0 °C/min with a final hold time of 100 min. These same methods were used for analysis by GC–MS with the Chrompack column. Various methods were tested by GC using the Lipodex E and heptakis cyclodextrin columns but satisfactory results were not obtained with these chiral capillary columns for the enantiomeric discrimination of the piperidine alkaloid acetamides and trifluoroacetamides.

^{1}H NMR spectra were recorded with Varian Inova 500 (499.88 MHz), Bruker Avance 500 (499.87 MHz), Bruker AvanceIII Oxford 400 (400.13 MHz), or Bruker Avance DPX 250 (250.13 MHz) spectrometers, equipped with 5 mm probes. Chemical shifts were reported in ppm using deuterated chloroform solvent ($CDCl_3$, δ 7.23 ppm) and trimethylsilane (TMS, δ 0.00 ppm) as internal standards. Multiplicity was reported as follows: s, singlet; d, doublet; t, triplet; quin, quintet; m, multiplet; dd, doublet of doublets. ^{13}C NMR spectra were recorded on Varian Inova 500 (125.71 MHz), Bruker Avance 500 (125.69 MHz), Bruker AvanceIII Oxford 400 (100.61 MHz), or Bruker Avance DPX 250 (62. 89 MHz) spectrometers, equipped with 5 mm probes or nanoprobes of 50 μL. Chemical shifts were reported in ppm using deuterated chloroform solvent ($CDCl_3$, δ 77.0 ppm) and trimethylsilane (TMS, δ 0.00 ppm) as internal standards. Methyl, methylene, methine, and carbon not bonded to hydrogen were discriminated using DEPT-135 and DEPT-90 spectra (Distortionless Enhancement by Polarization Transfer).

4.2. Solenopsis saevissima Fire ants

Individuals of each caste were obtained from five whole fire ant nests (entries 1–5, Table 3) collected in the municipality of Pedro do Rio, Rio de Janeiro state, Brazil (22°20′30″S 43°07′44″W) and three whole fire ant nests (entries 6–8, Table 3) in the city of Ubatuba, São Paulo state, Brazil (23°26′13″S 43°04′08″W) following the methods for handling and rearing these insects in the laboratory.[9a] Species identification was based on a series of characters and additional useful traits.[9b,c] Voucher specimens were deposited in the Adolph Hempel Entomological Collection of Insituto Biologico de São Paulo, São Paulo, Brazil. Workers from the whole nests were separated in the laboratory into different size classes (size interval, mean weight ± SD): minor workers (1–2 mm; 0.40 ± 0.08 mg),

medium workers (3–4 mm; 0.9 ± 0.18 mg), and major workers (5–6 mm; 2.0 ± 0.52 mg). Gynes (unmated queens; 3.1 ± 0.2 mg) were analyzed separately.[10]

4.3. Solenopsis saevissima venom alkaloids

Females of two different castes were cold-anesthetized and had their venom sacs dissected with a fine forceps. Each sample (three venom sacs, in triplicate) was macerated in bidistilled ethyl acetate and the venom extracts (1 μg/μL) were analyzed by GC–MS. Individuals from nests 2–5 have only one alkaloid: *cis*- and *trans*-2-methyl-6-undecylpiperidine **6**.[10]

4.4. Isolation of natural *cis*- and *trans*-2-methyl-undecylpiperidines 6

All minor workers ($N > 1000$) and gynes ($N \sim 50$) of nests 3 and 4 (entries 3–4, Table 3) were separated, cold-anesthetized, and macerated whole in distilled ethyl acetate (50 mL). The solvent was removed in vacuo and the extracts were analyzed by TLC and GC–MS. Hydrocarbons of the extracts were removed by a sintered glass Buchner funnel with silica gel (1 g), eluted with distilled ethyl acetate (300 mL) and distilled methanol/NH_4OH (9:1, 400 mL). The first fraction had only hydrocarbons, while the second fraction had only the alkaloids (minor workers—nest 3: 72.3 mg; minor workers—nest 4: 127.2 mg; gynes—nest 3: 10.2 mg; gynes—nest 4: 16.6 mg). The purified alkaloids of nest 3 (minor workers and gynes) were analyzed by ^{1}H and ^{13}C NMR. *Cis*- and *trans*-2-methyl-6-undecylpiperidines **6**—gynes from nest 3: ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.92 Hz, 6H), 1.25–1.80 (m, 58H), 1.89–1.95 (s, 2H), 2.85 (m, 1H), 3.03 (m, 1H), 3.27 (m, 1H), 3.51 (m, 1H). ^{13}C NMR (125.71 MHz, $CDCl_3$, TMS): δ 14.11 (CH_3), 14.12 (CH_3), 17.00 (CH_3), 17.46 (CH_2), 19.45 (CH_3), 22.67 (CH_2), 22.70 (CH_2), 23.10 (CH_2), 25.68 (CH_2), 25.94 (CH_2), 26.07 (CH_2), 27.67 (CH_2), 29.02 (CH_2), 29.36 (CH_2), 29.45 (CH_2), 29.48 (CH_2), 29.61 (CH_2), 29.63 (CH_2), 29.65 (CH_2), 29.67 (CH_2), 29.68 (CH_2), 30.60 (CH_2), 30.78 (CH_2), 31.93 (CH_2), 33.33 (CH_2), 47.71 (CH), 51.61 (CH), 53.98 (CH), 58.02 (CH). MS (70 eV), m/z (%)—same MS spectrum for both compounds: 253 (M^+, 1), 252 (2), 238 (3), 224 (1), 210 (1), 154 (1), 126 (1), 111 (1), 98 (base peak, 100), 81 (2), 70 (2), 55 (5), 41 (5).[7a] *Trans*-2-methyl-6-undecylpiperidine **6**—minor workers from nest 3: ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.95 Hz, 3H), 1.25–1.80 (m, 29H), 1.96 (s, 1H), 3.09 (m, 1H), 3.31 (m, 1H). ^{13}C NMR (125.71 MHz, $CDCl_3$, TMS): δ 14.12 (CH_3), 18.25 (CH_2), 18.52 (CH_3), 22.71 (CH_2), 26.13 (CH_2), 27.87 (CH_2), 29.37 (CH_2), 29.58 (CH_2), 29.60 (CH_2), 29.61 (CH_2), 29.65 (CH_2), 29.67 (CH_2), 29.75 (CH_2), 30.52 (CH_2), 31.93 (CH_2), 46.71 (CH), 51.07 (CH). MS (70 eV), m/z (%): 253 (M^+, 0.1), 252 (1), 238 (3), 224 (1), 210 (1), 154 (1), 126 (1), 111 (1), 98 (base peak, 100), 81 (2), 70 (2), 55 (5), 41 (5).[7a]

4.5. Hydrochlorides of natural alkaloids

Purified gyne venom alkaloids of the nests 3 and 4 (total amount of 26.8 mg; see Section 4.4 above), with only *cis*- and *trans*-2-methyl-6-undecylpiperidines **6** in high enantiomeric excess (Table 3, over 90% each), were separated by silica gel 60 column (0.035–0.070 mm, Merck, 9 g), eluted with distilled ethyl acetate, ethyl acetate/methanol (95:5), ethyl acetate/methanol (9:1), and ethyl acetate/methanol (4:1). Each chromatography column fraction was analyzed by GC–MS to monitor the elution of the *cis* and *trans* diastereomers. Pure fractions were assembled producing *cis*-**6** (6 mg, *de* 97%) and *trans*-**6** (4 mg, *de* 98%) which were solubilized with dry ethyl ether (1 mL) and a small amount of dry ethyl ether saturated with gaseous HCl (~3 mL). The solvent was evaporated at room temperature and the hydrochlorides were recrystallized from distilled dichloromethane/ethyl ether.[6a] *Cis*-**6**·HCl: $[\alpha]_D^{20} = +7$ (c 0.6, $CHCl_3$), same as (2*R*,6*S*)-**6A**·HCl: lit. [7b] $[\alpha]_D^{24} = +10.0$ (c 1.1, $CHCl_3$). ^{1}H NMR (499.87 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.95 Hz, 3H), 1.25–1.80 (m, 29H), 2.04 (s, 2H), 2.89 (m, 1H), 3.07 (m, 1H). ^{13}C NMR (125.69 MHz, $CDCl_3$, TMS): δ 14.12 (CH_3), 19.51 (CH_3), 22.70 (CH_2), 22.94 (CH_2), 25.74 (CH_2), 27.60 (CH_2), 29.36 (CH_2), 29.40 (CH_2), 29.58 (CH_2), 29.64 (CH_2), 29.71 (CH_2), 30.87 (CH_2), 31.93 (CH_2), 33.29 (CH_2), 54.64 (CH), 58.78 (CH).[7b] *Trans*-**6**·HCl: $[\alpha]_D^{20} = -4$ (c 0.67, $CHCl_3$), same as (2*R*,6*R*)-**6C**·HCl: lit.[6a] $[\alpha]_D^{20} = -7.7$ (c 0.51, $CHCl_3$). ^{1}H NMR (499.87 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.85 Hz, 3H), 1.25–1.80 (m, 29H), 1.98 (s, 2H), 3.28 (m, 1H), 3.54 (m, 1H). ^{13}C NMR (125.69 MHz, $CDCl_3$, TMS): δ 14.12 (CH_3), 16.92 (CH_3), 17.38 (CH_2), 22.70 (CH_2), 25.86 (CH_2), 26.22 (CH_2), 28.95 (CH_2), 29.36 (CH_2), 29.53 (CH_2), 29.58 (CH_2), 29.64 (CH_2), 29.72 (CH_2), 30.78 (CH_2), 31.92 (CH_2), 47.96 (CH), 51.79 (CH).[6a]

4.6. Tosylation of *n*-alcohols

Pyridine (4.57 mL), previously treated, was added to a solution of *n*-alcohol (3000 mg, 18.95 mmol) in 30 mL of dry dichloromethane in a round bottom flask, which was magnetically stirred at 0 °C. *p*-Toluenesulfonyl chloride (8670 mg, 45.48 mmol) was added slowly and the reaction mixture was magnetically stirred for 12 h at 25 °C, and monitored by TLC. After this time, an ice cold aqueous solution of HCl (at least 2 mol/L, 30 mL) and distilled dichloromethane (3 × 30 mL) were added. The organic phase was separated in a separating funnel, washed with a 5% aqueous solution of $NaHCO_3$ (30 mL), dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. The tosylate was purified by silica gel (40 g) column chromatography eluting with distilled hexane/ethyl acetate (7:3).[11] Decyl tosylate (4.22 g, 68.3% purified yield): ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 7.02 Hz, 3H), 1.23 (m, 14H), 1.63 (quin, J = 6.50, 7.50 Hz, 2H), 2.45 (s, 3H), 4.02 (t, J = 6.55 Hz, 2H), 7.34 (d, J = 8.05 Hz, 2H), 7.79 (d, 8.30 Hz, 2H). ^{13}C NMR (125.71 MHz, $CDCl_3$, TMS): δ 14.06 (CH_3), 21.59 (CH_3), 22.62 (CH_2), 25.29 (CH_2), 28.78 (CH_2), 28.88 (CH_2), 29.22 (CH_2), 29.35 (CH_2), 29.41 (CH_2), 31.82 (CH_2), 70.68 (OCH_2), 127.85 (CH), 129.76 (CH), 133.25 (C), 144.57 (C). IR (ν_{max}/cm^{-1}): 2956, 2924, 2857, 1598, 1465, 1362 and 1176 (two strong bands), 1098, 965–933, 830–814, 663. MS (70 eV), m/z (%): 312 (M^+, 0.1), 173 (base peak, 100), 172 (39), 155 (32), 140 (22), 112 (23), 97 (27), 91 (98), 83 (41), 70 (51), 55 (72), 43 (57), 41 (64). Dodecyl tosylate (3.52 g, 61.8% purified yield): ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.97 Hz, 3H), 1.25 (m, 18H), 1.63 (quin, J = 6.50, 7.50 Hz, 2H), 2.45 (s, 3H), 4.02 (t, J = 6.55 Hz, 2H), 7.34 (d, J = 8.00 Hz, 2H), 7.79 (d, J = 8.35 Hz, 2H). ^{13}C NMR (125.71 MHz, $CDCl_3$, TMS): δ 14.07 (CH_3), 21.59 (CH_3), 22.65 (CH_2), 25.29 (CH_2), 28.78 (CH_2), 28.89 (CH_2), 29.29 (CH_2), 29.35 (CH_2), 29.46 (CH_2), 29.57 (CH_2), 31.87 (CH_2), 70.68 (OCH_2), 127.85 (CH), 129.75 (CH), 133.25 (C), 144.57 (C). IR (ν_{max}/cm^{-1}): 2953, 2922, 2852, 1598, 1465, 1362 and 1176 (two strong bands), 1098, 952–920, 830–814, 664. MS (70 eV), m/z (%): 340 (M^+, 0.1), 173 (base peak, 100), 172 (38), 168 (24), 155 (24), 140 (13), 125 (7), 111 (16), 97 (24), 91 (48), 83 (24), 69 (23), 55 (23), 43 (19), 41 (17). Tetradecyl tosylate (3.14 g, 58.8% purified yield): ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.97 Hz, 3H), 1.25 (m, 22H), 1.63 (quin, J = 6.50, 7.50 Hz, 2H), 2.45 (s, 3H), 4.02 (t, J = 6.55 Hz, 2H), 7.34 (d, J = 8.00 Hz, 2H), 7.79 (d, J = 8.30 Hz, 2H). ^{13}C NMR (125.71 MHz, $CDCl_3$, TMS): δ 14.09 (CH_3), 21.59 (CH_3), 22.66 (CH_2), 25.30 (CH_2), 28.79 (CH_2), 28.90 (CH_2), 29.32 (CH_2), 29.36 (CH_2), 29.47 (CH_2), 29.57 (CH_2), 29.62 (CH_2), 29.63 (CH_2), 29.65 (CH_2), 31.89 (CH_2), 70.69 (OCH_2), 127.86 (CH), 129.76 (CH), 133.27 (C), 144.57 (C). IR (ν_{max}/cm^{-1}): 2957, 2915, 2848, 1596, 1473, 1356 and 1172 (two

strong bands), 1098, 956, 836–812, 667. MS (70 eV), *m*/*z* (%): 368 (M^+, 0.1), 196 (25), 173 (base peak, 100), 172 (37), 168 (10), 155 (23), 139 (5), 125 (10), 111 (20), 97 (33), 91 (48), 83 (32), 69 (28), 55 (28), 43 (24), 41 (20).[12]

4.7. Synthesis of 2-methyl-6-alkylpyridines

Treated 2,6-lutidine **1** (344 mg, 3.21 mmol) was solubilized in 10 mL of dry tetrahydrofuran (THF) under an argon atmosphere with magnetic stirring and ice bath (0 °C). A hexane solution of *n*-butyl-lithium (10 mol/L, 0.32 mL, 3.21 mmol) was added slowly and, after 15 min at room temperature with magnetic stirring, the reaction mixture was heated at reflux (35–40 °C) for 15 min for the formation of carbanion intermediate **2**. Subsequently, the corresponding tosylated alcohol (1000 mg, 3.21 mmol) was added slowly over 10 min. The reaction was monitored by TLC and stopped after 2.5 h with small ice chunks, maintaining the magnetic stirring and ice bath (0 °C). The reaction product was extracted with the addition of distilled water (10 mL) and distilled ethyl acetate (3 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo.[13] 2-Methyl-6-alkylpyridine was purified by silica gel (60 g) column chromatography with the following eluents: distilled dichloromethane and distilled dichloromethane/ethyl acetate (95:5). 2-Methyl-6-undecylpyridine **3** (153.7 mg, 19% purified yield): ^{1}H NMR (499.88 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.82 Hz, 3H), 1.29 (m, 16H), 1.68 (quin, J = 8.00, 7.60 Hz, 2H), 2.53 (s, 3H), 2.74 (t, J = 7.92 Hz, 2H), 6.94 (dd, J = 4.50, 4.40 Hz, 1H), 7.43 (d, J = 8.00 Hz, 1H), 7.46 (t, J = 7.65 Hz, 1H). ^{13}C NMR (62.89 MHz, $CDCl_3$, TMS): δ 14.08 (CH_3), 22.66 (CH_2), 24.51 (CH_3), 29.32 (CH_2), 29.47 (CH_2), 29.50 (CH_2), 29.55 (CH_2), 29.60 (CH_2), 29.62 (CH_2), 30.22 (CH_2), 31.89 (CH_2), 38.59 (CH_2), 119.40 (CH), 120.28 (CH), 136.40 (CH), 157.61(C), 161.94 (C). IR (ν_{max}/cm^{-1}): 2917–2851, 1590, 1575, 1454, 1302, 1140, 1083, 814–674. MS (70 eV), *m*/*z* (%): 247 (M^+, 2), 232 (0.1), 218 (1), 204 (2), 190 (2), 176 (2), 162 (3), 148 (3), 134 (11), 120 (19), 107 (base peak, 100), 93 (2), 79 (2), 65 (2), 53 (1), 41 (5). 2-Methyl-6-tridecylpyridine **4** (191.0 mg, 24% purified yield): ^{1}H NMR (400.13 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 7.00 Hz, 3H), 1.25 (m, 20H), 1.69 (quin, J = 8.00, 7.60 Hz, 2H), 2.53 (s, 3H), 2.74 (t, J = 7.90 Hz, 2H), 6.94 (dd, J = 3.44, 3.36 Hz, 1H), 7.43 (d, J = 8.80 Hz, 1H), 7.46 (t, J = 7.66 Hz, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$, TMS): δ 14.07 (CH_3), 22.65 (CH_2), 24.49 (CH_3), 29.31 (CH_2), 29.41 (CH_2), 29.46 (CH_2), 29.49 (CH_2), 29.54 (CH_2), 29.57 (CH_2), 29.61 (CH_2), 29.62 (CH_2), 29.65 (CH_2), 30.21 (CH_2), 31.88 (CH_2), 38.57 (CH_2), 119.38 (CH), 120.26 (CH), 136.38 (CH), 157.59 (C), 161.93 (C). IR (ν_{max}/cm^{-1}): 2917–2851, 1590, 1575, 1466, 1453, 1304, 1140, 1084, 814–674. MS (70 eV), *m*/*z* (%): 275 (M^+, 6), 260 (1), 246 (2), 232 (3), 218 (2), 204 (2), 190 (3), 176 (4), 162 (5), 148 (4), 134 (16), 120 (25), 107 (base peak, 100), 93 (2), 79 (1), 65 (1), 55 (1), 41 (3). 2-Methyl-6-pentadecylpyridine **5** (125.5 mg, 15% purified yield): ^{1}H NMR (400.13 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.82 Hz, 3H), 1.25 (m, 24H), 1.69 (quin, J = 8.00, 7.60 Hz, 2H), 2.53 (s, 3H), 2.74 (t, J = 7.90 Hz, 2H), 6.94 (dd, J = 3.60, 3.56 Hz, 1H), 7.43 (d, J = 8.40 Hz, 1H), 7.46 (t, J = 7.44 Hz, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$, TMS): δ 14.08 (CH_3), 22.66 (CH_2), 24.51 (CH_3), 29.33 (CH_2), 29.42 (CH_2), 29.50 (CH_2), 29.54 (CH_2), 29.62 (CH_2), 29.66 (CH_2), 30.21 (CH_2), 31.89 (CH_2), 38.59 (CH_2), 119.38 (CH), 120.26 (CH), 136.37 (CH), 157.60 (C), 161.94 (C). IR (ν_{max}/cm^{-1}): 2914–2849, 1589, 1573, 1467, 1454, 1302, 1140, 1083, 814–675. MS (70 eV), *m*/*z* (%): 303 (M^+, 4), 288 (0.1), 274 (1), 260 (2), 246 (1), 232 (1), 218 (1), 204 (1), 190 (2), 176 (3), 162 (4), 148 (3), 134 (12), 120 (20), 107 (base peak, 100), 93 (1), 79 (1), 65 (1), 55 (1), 41 (3).

4.8. Synthesis of 2-methyl-6-alkylpiperidines

2-Methyl-6-alkylpyridines **3–5** (59.8 mg undecyl, 91.1 mg tridecyl, and 152.6 mg pentadecyl) were solubilized in 8 mL of distilled methanol and 40 mL of glacial acetic acid. Next 110 mg of Pt/C 10% was added. The hydrogenation reaction was kept at a pressure of 70 psi of H_2 (g) with stirring for 72 h. The reaction mixture was filtered through a sintered glass Buchner funnel with Celite 545 (2 g) eluting with 50 mL of distilled methanol. The solvent was evaporated in vacuo and the product was analyzed by TLC and GC–MS.[14] 2-Methyl-6-alkylpyridines were purified by silica gel (10 g) column chromatography, eluting with distilled dichloromethane/ethyl acetate (9:1), dichloromethane/ethyl acetate (1:1), ethyl acetate, and methanol. (±)-*Cis* and (±)-*trans*-2-methyl-6-undecylpiperidines **6A–D** (97.3 mg, 99% purified yield): ^{1}H NMR (499.87 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.90 Hz, 3H), 1.25–1.80 (m, 29H), 1.95 (s, 1H), 2.69 (m, 1H), 2.86 (m, 1H). ^{13}C NMR (125.69 MHz, $CDCl_3$, TMS): δ 14.12 (CH_3), 21.00 (CH_3), 22.70 (CH_2), 23.91 (CH_2), 25.78 (CH_2), 29.36 (CH_2), 29.60 (CH_2), 29.65 (CH_2), 29.68 (CH_2), 31.93 (CH_2), 32.38 (CH_2), 35.13 (CH_2), 52.95 (CH), 57.27 (CH). IR (ν_{max}/cm^{-1}): 3310, 2917, 2851, 1648–1581, 1412–1339, 1088–1051, 650–621. MS (70 eV), *m*/*z* (%): 253 (M^+, 1), 252 (3), 238 (6), 224 (1), 210 (1), 154 (1), 126 (1), 111 (1), 98 (base peak, 100), 81 (1), 70 (2), 55 (3), 41 (3).[7a,c] (±)-*Cis* and (±)-*trans*-2-methyl-6-tridecylpiperidines **7A–D** (83.3 mg, 89% purified yield): ^{1}H NMR (250.13 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.49 Hz, 3H), 1.25–1.80 (m, 33H), 1.95 (s, 1H), 2.69 (m, 1H), 2.87 (m, 1H). ^{13}C NMR (62.89 MHz, $CDCl_3$, TMS): δ 14.10 (CH_3), 20.67 (CH_3), 22.68 (CH_2), 23.76 (CH_2), 25.73 (CH_2), 29.25 (CH_2), 29.35 (CH_2), 29.58 (CH_2), 29.65 (CH_2), 29.67 (CH_2), 31.91 (CH_2), 32.03 (CH_2), 34.75 (CH_2), 52.97 (CH), 57.23 (CH). IR (ν_{max}/cm^{-1}): 3307, 2912, 2846, 1561, 1403, 1084–1045, 641. MS (70 eV), *m*/*z* (%): 281 (M^+, 2), 280 (4), 266 (7), 252 (1), 238 (1), 224 (0.1), 154 (1), 126 (1), 111 (1), 98 (base peak, 100), 83 (1), 69 (2), 55 (3), 43 (3). (±)-*Cis* and (±)-*trans*-2-methyl-6-pentadecylpiperidines **8A–D** (102.7 mg, 65% purified yield): ^{1}H NMR (250.13 MHz, $CDCl_3$, TMS): δ 0.88 (t, J = 6.48 Hz, 3H), 1.25–1.80 (m, 37H), 1.96 (s, 1H), 2.68 (m, 1H), 2.84 (m, 1H). ^{13}C NMR (62.89 MHz, $CDCl_3$, TMS): δ 14.10 (CH_3), 20.91 (CH_3), 22.68 (CH_2), 23.87 (CH_2), 25.75 (CH_2), 29.35 (CH_2), 29.58 (CH_2), 29.69 (CH_2), 31.92 (CH_2), 32.28 (CH_2), 35.01 (CH_2), 52.93 (CH), 57.23 (CH). IR (ν_{max}/cm^{-1}): 3304, 2915, 2846, 1645–1566, 1409–1341, 1085–1046, 653–618. MS (70 eV), *m*/*z* (%): 309 (M^+, 2), 308 (4), 294 (6), 280 (0.1), 266 (1), 154 (1), 111 (3), 98 (base peak, 100), 84 (1), 70 (2), 55 (3), 41 (3).

4.9. Trifluoroacetylation of alkaloids

The alkaloids were solubilized with dry ethyl ether (1 mL) and pyridine (0.8 mL). Next, trifluoroacetic anhydride (0.8 mL) and trifluoroacetic acid (0.1 mL) were added slowly with magnetic stirring in a glycerin bath (30 °C) for 30 min.[15a] The reaction was stopped by the addition of distilled ethyl acetate (20 mL) and an aqueous solution of saturated copper sulfate (2 × 20 mL). The organic phase was separated in a separating funnel, dried with anhydrous Na_2SO_4, concentrated in vacuo, and analyzed by GC–MS and chiral GC. Trifluoroacetamides **9A–B**: MS (70 eV), *m*/*z* (%): 349 (M^+, 0.1), 334 (1), 280 (4), 194 (base peak, 100), 140 (5), 81 (7), 55 (11), 41 (4). Trifluoroacetamides **10A–B**: MS (70 eV), *m*/*z* (%): 377 (M^+, 0.1), 362 (1), 308 (4), 194 (base peak, 100), 140 (4), 81 (6), 55 (11), 41 (4). Trifluoroacetamides **11A–B**: MS (70 eV), *m*/*z* (%): 405 (M^+, 0.1), 390 (1), 336 (4),194 (base peak, 100), 140 (3), 81 (6), 55 (10), 41 (3).

4.10. Schotten-Baumann acylation

The alkaloids (1 equiv) were solubilized with distilled dichloromethane (10 mL) in a 50 mL flask after which an aqueous solution of K_2CO_3 (2 mol/L, 10 mL) was added. The reaction mixture was stirred vigorously and treated with acetyl chloride (3 equiv, solubilized in distilled DCM) which was slowly added. The mixture was left to stir at 25 °C overnight.[15b] The reaction was stopped by the addition of an aqueous solution of saturated Na_2CO_3 (being washed twice more) and the organic phase was separated in a separating funnel, dried over anhydrous Na_2SO_4, concentrated in vacuo, and analyzed by GC–MS and chiral GC. Acetamides **12A**–**B**: MS (70 eV), *m*/*z* (%): 295 (M^+, 2), 280 (3), 252 (3), 238 (4), 140 (base peak, 100), 98 (66), 55 (6), 43 (10). Acetamides **13A**–**B**: MS (70 eV), *m*/*z* (%): 323 (M^+, 2), 308 (3), 280 (2), 266 (3), 140 (base peak, 100), 98 (54), 55 (5), 43 (8). Acetamides **14A**–**B**: MS (70 eV), *m*/*z* (%): 351 (M^+, 2), 336 (3), 308 (3), 294 (3), 140 (base peak, 100), 98 (44), 55 (5), 43 (7).

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References

1. Attygalle, A. B.; Morgan, E. D. *Chem. Soc. Rev.* **1984**, *13*, 245–278.
2. (a) Chen, J.; Cantrell, C. L.; Shang, H. W.; Rojas, M. G. *J. Agric. Food Chem.* **2009**, *57*, 3128–3133; (b) Chen, L.; Fadamiro, H. Y. *Toxicon* **2009**, *53*, 469–478; (c) Chen, L.; Fadamiro, H. Y. *Toxicon* **2009**, *53*, 479–486; (d) Pedder, D. J.; Fales, H. M.; Jaouni, T.; Blum, M.; MacConnell, J.; Crewe, R. M. *Tetrahedron* **1976**, *32*, 2275–2279; (e) Jones, T. H.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1982**, *38*, 1949–1958; (f) Jones, T. H.; Torres, J. A.; Spande, T. F.; Garraffo, H. M.; Blum, M. S.; Snelling, R. R. *J. Chem. Ecol.* **1996**, *22*, 1221–1236; (g) Gorman, J. S. T.; Jones, T. H.; Spande, T. F.; Snelling, R. R.; Torres, J. A.; Garraffo, H. M. *J. Chem. Ecol.* **1998**, *24*, 933–943.
3. (a) Baer, H.; Liu, T.-Y.; Anderson, M. C.; Blum, M.; Schmid, W. H.; James, F. J. *Toxicon* **1979**, *17*, 397–405; (b) Borer, A. S.; Wassmann, P.; Schmidt, M.; Hoffman, D. R.; Zhou, J.-J.; Wright, C.; Schirmer, T.; Markovic-Housley, Z. J. *Mol. Biol.* **2012**, *415*, 635–648.
4. (a) Blum, M. S.; Walker, J. R.; Callahan, P. S.; Novak, A. F. *Science* **1958**, *128*, 306–307; (b) Jouvenaz, D. P.; Blum, M. S.; McConnell, J. G. *Antimicrob. Agents Chemother.* **1972**, *2*, 291–293; (c) Storey, G. K.; Vander Meer, R. K.; Boucias, D. G.; McCoy, C. W. *J. Invert. Pathol.* **1991**, *58*, 88–95; (d) Howell, G.; Butler, J.; DeShazo, R. D.; Farley, J. M.; Liu, H.-L.; Nanayakkara, N. P. D.; Yates, A.; Yi, G. B.; Rockhold, R. W. *Ann. Allergy Asthma Immunol.* **2005**, *94*, 380–386.
5. Garraffo, H. M.; Simon, L. D.; Daly, J. W.; Spande, T. F.; Jones, T. H. *Tetrahedron* **1994**, *50*, 11329–11338.
6. (a) Leclercq, S.; Thirionet, I.; Broeders, F.; Daloze, D.; Vander Meer, R.; Braekman, J. C. *Tetrahedron* **1994**, *50*, 8465–8478; (b) Leclercq, S.; Braekman, J. C.; Daloze, D.; Pasteels, J. M.; Van der Meer, R. K. *Naturwissenschaften* **1996**, *83*, 222–225.
7. (a) Jefford, C. W.; Wang, J. B. *Tetrahedron Lett.* **1993**, *34*, 2911–2914; (b) Poerwono, H.; Higashiyama, K.; Yamauchi, T.; Kubo, H.; Ohmiya, S.; Takahashi, H. *Tetrahedron* **1998**, *54*, 13955–13970; (c) Girard, N.; Gautier, C.; Malassene, R.; Hurvois, J.-P.; Moinet, C.; Toupet, L. *Synlett* **2004**, *2004*, 2005–2009; (d) Girard, N.; Hurvois, J.-P.; Toupet, L.; Moinet, C. *Synth. Commun.* **2005**, *35*, 711–723.
8. (a) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon Press: Oxford, 1980; (b) Touchstone, J. C.; Dobbins, M. F. *Practice of Thin Layer Chromatography*; John Wiley & Sons: New York, 1978.
9. (a) Banks, W. A.; Lofgren, C. S.; Jouvenaz, D. P.; Stringer, C. E.; Bishop, P. M.; Williams, D. F.; Wojcik, D. P.; Glancey, B. M. *Techniques for Collecting, Rearing, and Handling Imported Fire Ants*, New Orleans: USDA, SEA, 1981, AAT-S-21.; (b) Pitts, J. P.; Mchugh, J. V.; Ross, K. G. *Zool. Scr.* **2005**, *34*, 493–505; (c) Dall'Aglio-Holvorcem, C. G.; Benson, W. W.; Gilbert, L. E.; Trager, J. C.; Trigo, J. R. *Biochem. Syst. Ecol.* **2009**, *37*, 442–451.
10. Fox, E. G. P.; Pianaro, A.; Solis, D. R.; Delabie, J. H. C.; Vairo, B. C.; Machado, E. A.; Bueno, O. C. *Psyche* **2012**, 1–10.
11. Kabalka, G. W.; Varma, M.; Varma, R. S. J. *Org. Chem.* **1986**, *51*, 2386–2388.
12. Choudary, B. M.; Choudari, N. S.; Kantam, M. L. *Tetrahedron* **2000**, *56*, 7291–7298.
13. MacConnell, J. G.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1971**, *26*, 1129–1139.
14. Glorius, F.; Spielkamp, N.; Holle, S.; Goddard, R.; Lehmann, C. W. *Angew. Chem. Int. Ed.* **2004**, *43*, 2850–2852.
15. (a) Kusch, P.; Knupp, G.; Hergarten, M.; Kozupa, M.; Majchrzak, M. J. *Chromatogr. A* **2006**, *1113*, 198–205; (b) Bamane, R. V.; Rakholiya, V. K.; Chitre, T. S. *Heterocycl. Lett.* **2011**, *1*, 263–268.