CHEMISTRY OF VENOM ALKALOIDS IN SOME
Solenopsis (Diplorhoptrum) SPECIES FROM PUERTO RICO

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Abstract—A number of 15-carbon alkaloids have been identified in venom extracts of four Puerto Rican species of ants in the genus *Solenopsis (Diplorhoptrum)*. Workers of a species from El Verde produced the *cis* and *trans* isomers of 2-methyl-6-nonylpiperidine with the latter isomer predominating. The same compounds were identified in queens of a species from Río Grande, but in this species no alkaloids were detected in worker extracts. Workers of a *Diplorhoptrum* species collected on Mona Island produced primarily a *trans*-2-methyl-6-(*Z*-4-nonenyl)piperidine, **3,** with smaller amounts of the *cis* isomer, whereas the major compound found in the queens of the same species on Mona Island was (5*Z*,9*Z*)-3-hexyl-5-methylindolizidine, identical with the alkaloid produced by queens of a species collected on Cabo Rojo. Surprisingly, workers of the Cabo Rojo species produced (5*Z*,9*Z*)- and (5*E*,9*E*)-3-butyl-5-propylindolizidine (**4** and **5,** respectively) reported earlier as the 223AB indolizidines from skins of dendrobatid frogs. The possible significance of the qualitative and quantitative differences in the venom alkaloids synthesized by queens and workers is discussed as is the possibility that ants containing such

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alkaloids may serve as a dietary source for the skin alkaloids used by certain frogs in chemical defense.

Key Words—Venom, alkaloids, *Solenopsis*, Puerto Rico.

INTRODUCTION

A variety of saturated-ring nitrogen heterocycles have been identified in the venoms of ant species in the myrmicine genera *Monomorium* and *Solenopsis* (Jones et al., 1982a,b). The 3,5-dialkylindolizidines are one of the earliest reported structural classes of these compounds (Ritter et al., 1973). When they have been found with other structural types of ant venom alkaloids, their concomitants have been 2,5-dialkylpyrrolidines and 3,5-dialkylpyrrolizidines in *Monomorium* species (Ritter and Persoons, 1975; Jones et al., 1990a) and 2,6-dialkylpiperidines in *Solenopsis* species (Jones et al., 1984). The 3,5-dialkylindolizidines are often found among the skin alkaloids of dendrobatid frogs and occasionally occur in other amphibians while 2,5-disubstituted pyrrolidines, 3,5-disubstituted pyrrolizidines, and 2,6-disubstituted piperidines have also been reported as amphibian skin alkaloids (Daly et al., 1993).

The genus *Solenopsis* includes many minute species that are currently placed in a "subgenus" *Diplorhoptrum*, a large, worldwide group (Bolton, 1987) to which the species of the present study belong. In addition, in the past some "subgenera" (e.g., *Diphlorhoptrum*) have been elevated to the status of a genus, but they are now usually recognized as a synonym of *Solenopsis* (Ettershank, 1966). Here we use the name *Diplorhoptrum* as a matter of convenience without implying that it is a valid taxonomic entity.

In this report, the alkaloidal venom components of four collections of *Diplorhoptrum* spp. from Puerto Rico are described. Three of these collections, in which different castes were obtained, show conspicuous differences between alkaloids found in workers and in queens. In these collections 3,5-dialkylindolizidines and/or 2,6-dialkylpiperidines were detected, and of the former, the major alkaloid from the workers of one collection was found to be the indolizidine **223AB** (**5**) (Figure 1 below), first reported in dendrobatid frogs (Spande et al., 1981). While a number of alkaloids previously detected in ants have been found in amphibians, this is the first report of an alkaloid previously detected in amphibians, now being found in ants.

METHODS AND MATERIALS

Chemical Analyses

Gas chromatographic analyses and mass spectra were obtained using an LKB-2091 GC-MS, a Finnigan ITMS, or a Finnigan ion-trap model 800, all

equipped with 25-m × 0.32-mm-ID HP-5 fused-silica columns. The first two instruments used Hewlett-Packard 5890 gas chromatographs programmed from 60 to 250°C at 10°/min. The model 800 ion trap was interfaced with a Varian gas chromatograph model 3400 programmed from 100 to 280°C at 10°/min after a 1-min delay. FT-IR spectra were obtained using a Hewlett-Packard model 5965B detector (narrow band, 4000–750 cm^{-1}, resolution 8 cm^{-1}) interfaced with a Hewlett-Packard 5890 gas chromatograph fitted also with an HP-5 column programmed 100–280°C at 10°C/min after a 1-min delay. Data were analyzed with the 59970 IRD ChemStation. FT-IR spectra of neat liquids were obtained using a Perkin-Elmer series 1600 FT-IR instrument. High-resolution mass spectrometry (HRMS) used a JEOL SX102 instrument fitted with a 15-m × 0.20-mm HP-5 column.

Ants. Ants were collected in the areas (A–D) indicated in Table 1 below, and extracts were immediately prepared in glass vials containing methylene chloride. Voucher specimens of all samples were deposited in the collection of the Los Angeles County Museum of Natural History, Los Angeles, California. Each vial represented a different colony, and extracts of whole bodies were analyzed directly and individually. While the milking of stingers has demonstrated the venom glands to be the source of alkaloids detected in ants of the genera *Solenopsis* (cf. Brand et al., 1972; MacConnell et al., 1971) and *Monomorium* (unpublished work of Blum and Jones), the small size of the Puerto Rican *Diplorhoptrum* ants made this impractical. The alkaloid components of the extracts can be distinguished easily from the lipid components by their mass

TABLE 1. ALKALOIDS IDENTIFIED FROM PUERTO RICAN *Solenopsis (Diplorhoptrum)* SPECIES

| Collection site | Alkaloids | | | | | |
	cis-1	*trans*-1	2	3	4	5
A. El Verde						
workers	+	+ + + + +	−	−	−	−
B. Río Grande						
workers	−	−	−	−	−	−
queens	+ + + + +	+	−	−	−	−
C. Mona Island						
workers	−	−	+ + +	−	−	−
queens	−	−	t[a]	+ + +	−	−
D. Cabo Rojo						
workers	−	−	−	−	+ +	+ + +
queens	−	−	−	+ + +	t	t

[a] t = trace detected.

spectra, which generally have odd-mass molecular ions and even-mass fragments (for alkaloids with one nitrogen).

Based on the similarity of their size with ants of the genus *Monomorium* (cf. Jones et al., 1982b), we estimate very roughly that each *Diplorhoptrum* worker released, at most, 0.1–1.0 μg alkaloid.

Hydrogenation of Extract C. A slow stream of hydrogen was bubbled through a small portion of this extract in methanol in the presence of PtO$_2$ for 2–3 min.

Synthesis of Piperidines 2a and 2b

2-[3-(6-Methyl-2-pyridyl)propyl]-1,3-dioxolane (6). A solution of 2,6-luti-dine anion obtained from 6.4 g (60 mmol) of lutidine in 100 ml of diethyl ether and 38 ml of 1.6 M *n*-butyllithium in hexanes at 0°C was treated with a solution containing 7 ml of 2-(2-bromoethyl)-1,3-dioxolane in 30 ml of diethyl ether. After 0.5 hr, the mixture was poured into water and extracted with ether. The ether solution was dried over anhydrous K$_2$CO$_3$, and after filtration, the more volatile components were removed in vacuo to provide 9 g of an oil that was greater than 80% compound 6 by GC. IR data (neat) were 3054, 2948, 2874, 2769, 1590, 1574, 1457, 1410, 1373, 1135, 1097, 1034, 939, 817, 790 cm^{-1}; GC retention time was 9.1 min; MS *m/z* (relative intensity, percent, Finnigan model 800 ion trap mass detector): 208 (M + 1, 10), 162(13), 134(18), 107(100), 73(38). Compound 6 was accompanied by ca. 10% of a material of retention time 17.7 min; MS *m/z* 308 (M + 1, 100), 164(22), 162(17), 147(22), 134(15), 132(10), 107(22), 99(10), 73(32), which very likely was a bis-substituted luti-dine. HRMS, calculated for C$_{12}$H$_{17}$NO$_2$: 207.1255; observed, 207.1258.

2-Methyl-6-(Z,4-nonenyl)pyridine (7). A mixture containing 6 g of diox-olane 6, 50 ml of acetone, 50 ml of methanol, and 100 ml of water was carefully acidified with concentrated HCl to ca. pH 2 and stirred for 1.5 hr at room temperature. The mixture was neutralized with solid NaHCO$_3$, and most of the solvents were removed in vacuo. The mixture was extracted with diethyl ether, and the ether extracts were dried over anhydrous MgSO$_4$. Distillation provided 3.5 g of 4-(6-methyl-2-pyridyl)butanal, bp 64–67° (0.05 mm Hg); IR(neat) 3062, 2927, 2823, 2719, 1721, 1590, 1575, 1456, 791 cm^{-1}; GC retention time 5.6 min; MS *m/z* 164 (M + 1, 65), 146(3), 134(13), 120(5), 107(100), 65(8). HRMS, calculated for C$_{10}$H$_{13}$NO: 163.0094; observed, 163.0098. A mixture of 2.5 g (6 mmol) of *n*-pentyltriphenylphosphonium bromide in 25 ml of THF was cooled to 0°C and treated with 2.4 ml of 2.5 M *n*-butyllithium in hexanes. After 3 hr, a solution containing 1 g (6 mmol) of 4-(6-methyl-2-pyridyl)butanal in 5 ml of THF was added, and the mixture was stirred over-night. Following the addition of 50 ml of hexane and 25 ml of diethyl ether, the mixture was filtered through a Florisil pad and the solvent was removed in

vacuo. Redissolution and filtration was repeated to provide 1.25 g of crude **7** that was nearly 90% pure by GC analysis. IR(neat) 3067, 3005, 2943, 2923, 2861, 1656(w), 1591, 1578, 1456, 790 cm^{-1}; GC retention time 13.9 min; MS m/z 218 (M + 1, 12), 202(8), 132(8), 120(10), 107(100). HRMS, calculated for $C_{15}H_{23}N$: 217.1825; observed, 217.1819.

2-Methyl-6-(Z,4-nonenyl)piperidine (2). The procedure of Jones et al. (1990b) was applied to the preparation of **2.** A small portion (about 20 mg) of the Wittig product **7** in 1.0 ml of absolute ethanol in a 3-ml test tube was heated to 95°C in an oil bath and small chips of sodium metal added over a period of approximately 1 hr, adding fresh ethanol as necessary. On cooling to room temperature, the resulting solid was allowed to stand capped overnight at room temperature; then water was added and the solution stirred in a hot water bath under a stream of nitrogen to remove excess ethanol. The aqueous layer was extracted four times with small portions of ethyl acetate. These extracts were combined, dried (Na_2SO_4), and the solvent was evaporated. The residue was dissolved in 2.0 ml ethanol, the pH adjusted to 6 (checked with narrow range pH paper) with 10–15 drops of 0.5 N HCl, and a small portion (about 5 mg) of $NaCNBH_3$ (Aldrich) was added, followed by another, 1 hr later, as the originally turbid solution became clear. A third portion of $NaCNBH_3$ was added 2 hr later. The solution was stirred for 18 hr and then acidified with 0.5 N HCl until the evolution of gas ceased. The solution was evaporated under a nitrogen stream, made alkaline with 2 N KOH, and extracted four times with small portions of ethyl acetate. After drying, GC-MS showed two closely eluting peaks at 9.0 min and 9.4 min (3.2:1.0, respectively) with the same mass and infrared spectra as reported for the isomeric mixture **2a** and **2b,** respectively. HRMS calculated for $C_{15}H_{29}N$: 223.2293; observed, 223.2305.

RESULTS

Examination of the methylene chloride extracts of four collections (A–D) of *Diplorhoptrum* species from Puerto Rico revealed the presence of 2,6-dialkyl-piperidines and 3,5-dialkylindolizidines **1–5** (Table 1, Figure 1), whose structures and stereochemistry were assigned by comparison with authentic samples and/or by spectroscopic methods.

Collection A. An extract from workers collected at El Verde, PR, contained *cis* and *trans*-2-methyl-6-nonylpiperidine (**1**) in ca. a 1:8 ratio, characterized by nearly identical mass spectra but differing gas chromatographic retention times, which can be compared to those of authentic samples prepared in earlier studies (MacConnell et al., 1974; Jones et al., 1982a,b; Blum et al., 1985). The occurrence of piperidines and the preponderance of the *trans* stereoisomer is typical of other *Solenopsis* species (Brand et al., 1972).
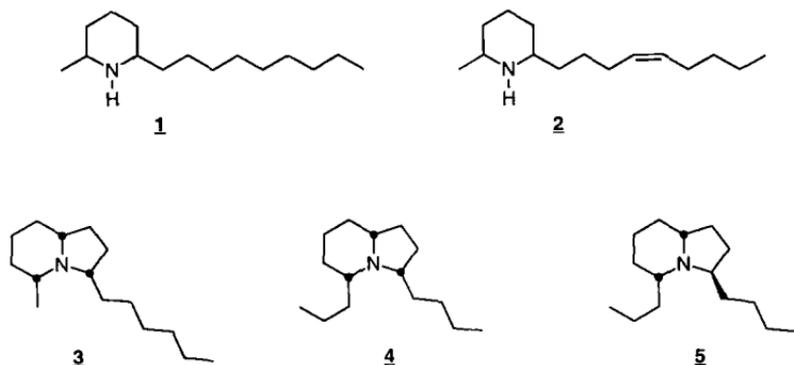
FIG. 1. Piperidines and indolizidines found in *Solenopsis* (*Diplorhoptrum*) species from Puerto Rico.

Collection B. An extract from workers collected at Río Grande, Puerto Rico, contained no alkaloids. The queens, however, contained the same alkaloids as above, *cis-* and *trans*-2-methyl-6-nonylpiperidine (1), but in ca. a 6:1 ratio. Additional confirmation that the major isomer was of the less commonly occurring ant alkaloid class of *cis* piperidines was provided by its FT-IR spectrum, which showed weak Bohlmann bands at 2803 and ca. 2730 cm^{-1} and was identical to that of a synthetic sample. These Bohlmann bands are absent in *trans* piperidines (Garraffo et al., 1994).

Collection C. An extract from workers collected at Mona Island, Puerto Rico, contained two components, **2a** and **2b,** in ca. a 1:4 ratio with virtually identical mass spectra: MS (*m/z*) (relative intensity), **2a** (minor): 223(M$^+$, 1), 222(1), 208(3), 180(5), 124(33), 111(15), 110(4), 99(7), 98(100), 96(8), 82(6), 81(8), 79(4), 70(9), 68(5), 69(27), 67(13), 56(10), 55(20); **2b** (major): 223(M$^+$, 2), 222(3), 208(5), 180(5), 124(15), 111(8), 99(8), 98(100), 81(4), 70(6), 69(4), 67(5), 57(8), 56(8), 55(13). The mass spectra indicated two unsaturated analogs of **1** that could be converted to a mixture of *cis-* and *trans*-**1** by catalytic hydrogenation (see Methods and Materials).

FT-IR spectra of **2a** and **2b** (Figure 2) indicated the first eluting component to be a *cis*-2,6-disubstituted piperidine and the second, its *trans* diastereomer. In addition, GC-FT-IR spectra showed a $\nu_{=CH}$ absorption at 3011 cm^{-1} and the absence of a terminal double bond as indicated by the lack of absorption at ca. 3080 ($\nu_{=CH}$ for a terminal olefin), 990 and 910 cm^{-1} ($\delta_{=CH}$ for a terminal olefin). An internal double bond of E stereochemistry was excluded by the absence of an absorption at ca. 964 cm^{-1} ($\delta_{=CH}$ for a *trans* olefin) and the presence of the 3011 cm^{-1} absorption, leaving only the possibility of an internal double bond of Z stereochemistry (cf. Attygalle, 1994). From the absence of
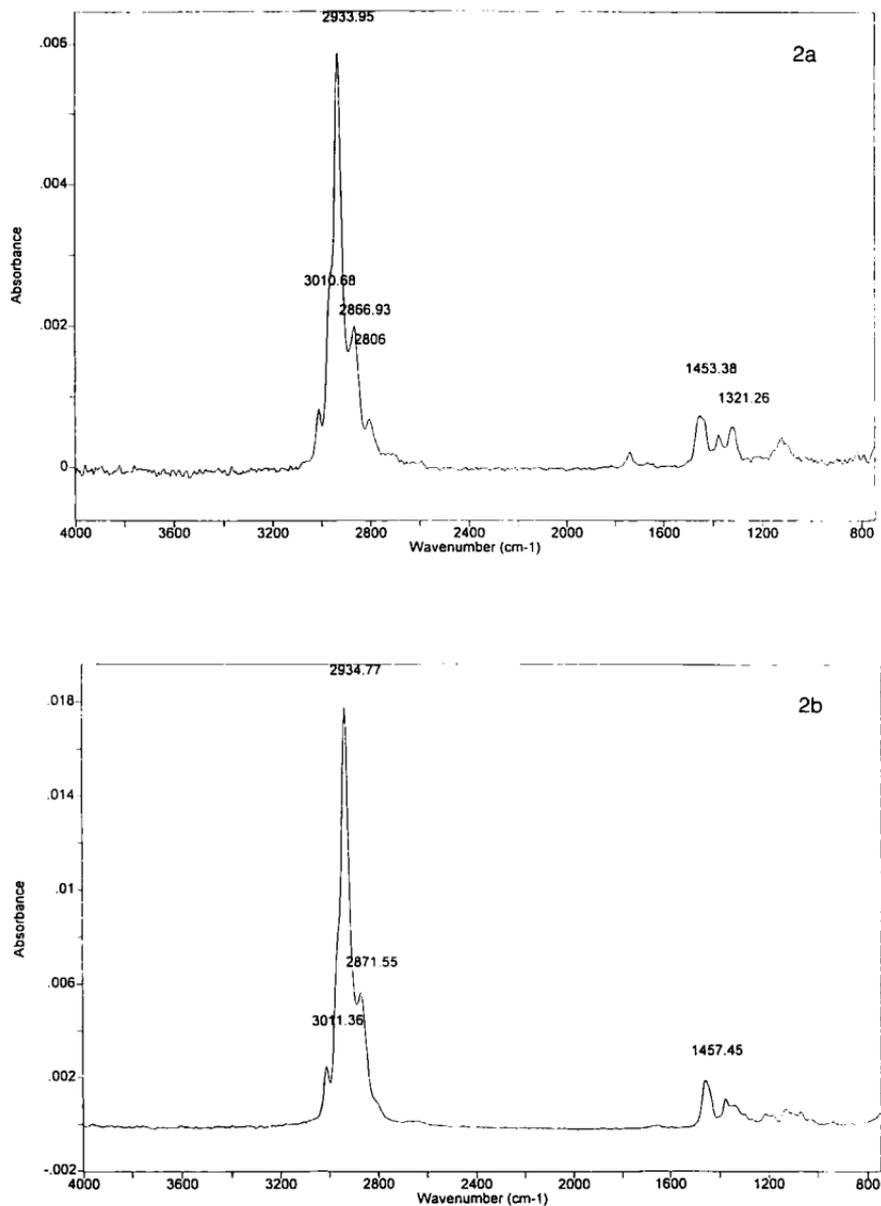
FIG. 2. GC-FT-IR spectra of the *cis*-ring piperidine **2a** and the *trans*-ring diastereomer **2b** from workers of *Diphlorhoptrum* sp. from Mona Island, Puerto Rico.

absorptions between 1600 and 1700 cm^{-1} in the infrared spectrum, the double bond in **2a/2b** was not imine or enamine unsaturation. Furthermore, vapor-phase FT-IR studies (Attygalle et al., 1994) on the position of the $\nu_{=CH}$ absorption on long-chain Z-monounsaturated primary acetates, excluded both Δ^{2-3} and Δ^{7-8} double bonds, since both show shifts to higher frequencies (ca. 3029 and 3022 cm^{-1}, respectively). A Δ^{3-4} position was also unlikely as their model indicated a 3018 cm^{-1} absorption for this structural class. Although the side-chain double bond in many ant venom alkaloids is in the terminal position, the loss of C$_7$H$_{15}$ from the molecular ion on mass spectrometry, yielding an ion at m/z 124 (20%), suggested the double bond in **2a/2b** was nearer the piperidine ring and supported the structural inferences from the FT-IR spectra. Incidentally, the mass spectrum also ruled out a Δ^{1-2} position, as an m/z 96 base peak (with only a weak 98 peak), has been reported for pinidine [2-methyl-6-(1-propenyl)piperidine] (NIST Data Base, 1992).

We considered a Δ^{4-5} position as the most probable location of the Z double bond since a weak loss of C$_3$H$_7$ was seen from the molecular ion (m/z 223) to give a peak (5%) at m/z 180. Such a cleavage most probably arises from an allylic fragmentation. An analogous cleavage (M$^+$-heptyl, m/z 180) has been noted previously in dehydrosolenopsin-B, i.e., *trans*-2-methyl-6-(Z-4-tridecenyl)piperidine, a C$_{19}$ homolog of **2b** from the venom of the red form of the fire ant, *Solenopsis invicta* (= *S. saevissma*) (MacConnell et al., 1971). Spectral data have been reported for the isomeric *cis*-2-methyl-6-(Z-4-tridecenyl)piperidine, where major fragments at m/z 124(20), 111(20) are also seen in addition to m/z 180(4) (Brand et al., 1972). In Scheme 1, we indicate probable fragmentation pathways to account for all major fragment ions observed in the mass spectra of **2a/2b**.

To prove this structure, the synthesis outlined in Scheme 2 was undertaken. The acetal intermediate **6** was synthesized from lutidine anion and 2-(2-bromoethyl)-1,3-dioxolane. This was deblocked to the aldehyde, which smoothly produced 2-methyl-6-(Z-4-nonenyl)pyridine (**7**) in a Wittig reaction (Johnson et al., 1982) with the phosphonium ylide (b) from 5-bromopentane. The olefin **7** was reduced, first with sodium in ethanol and then, after acidification to pH 6, by treatment with sodium cyanoborohydride to afford a mixture containing **2a** and **2b** (3.2 : 1, respectively). The GC retention times, and mass and GC-FT-IR spectra of the *cis* and *trans* disubstituted piperidines were identical in every respect to the natural alkaloids (see Methods and Materials). Coinjection on GC-MS of the synthetic mixture with the Mona Island workers' extract led to significant change in the ratio of **2a/2b** from the initial 1:4 to a 1:1.3 ratio, indicating each synthetic material to be identical in retention time to the corresponding natural material.

Along with traces of **2a/2b,** a 3-hexyl-5-methylindolizidine (**3**) was the predominant alkaloid in methylene chloride extracts from the queens of the
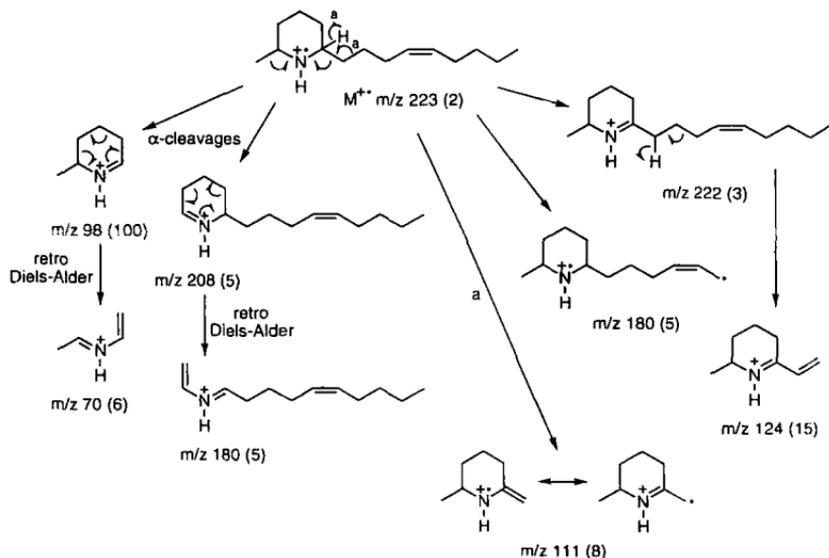
SCHEME 1. Proposed mass spectral fragmentations for **2b** (major isomer; *trans*-ring configuration); percentages in parentheses are electron-impact data obtained with an LKB-2091 instrument.

Mona Island collection. The mass spectrum [m/z 223 (M$^+$, 1), 222(1), 139(8), 138(100)] was the same as that reported for the compound found in the queens of an unidentified, but possibly identical, *Solenopsis species* collected in Marathon Key, Florida (Jones et al., 1984). The stereochemistry of **3** was established by examination of its FT-IR spectrum, which showed a broad range of Bohlmann bands decreasing in intensity from 2800 to 2500 cm^{-1}, which is characteristic
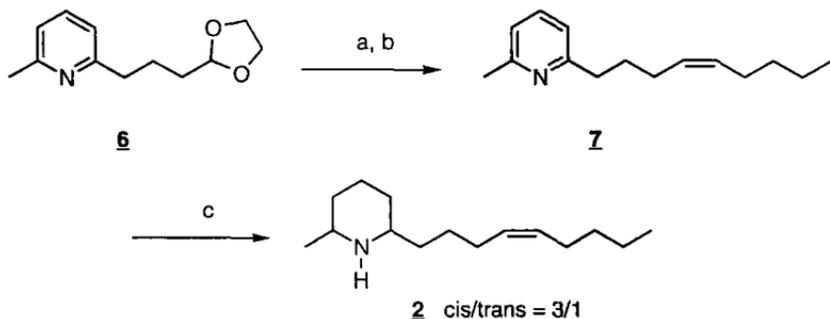
a) HCl/HClO$_4$, b) Ph$_3$P$^+$C$_5$H$_{11}$; BuLi, c) Na-EtOH; NaCNBH$_3$, pH 6

SCHEME 2. Chemical synthesis of piperidines **2a** and **2b**.
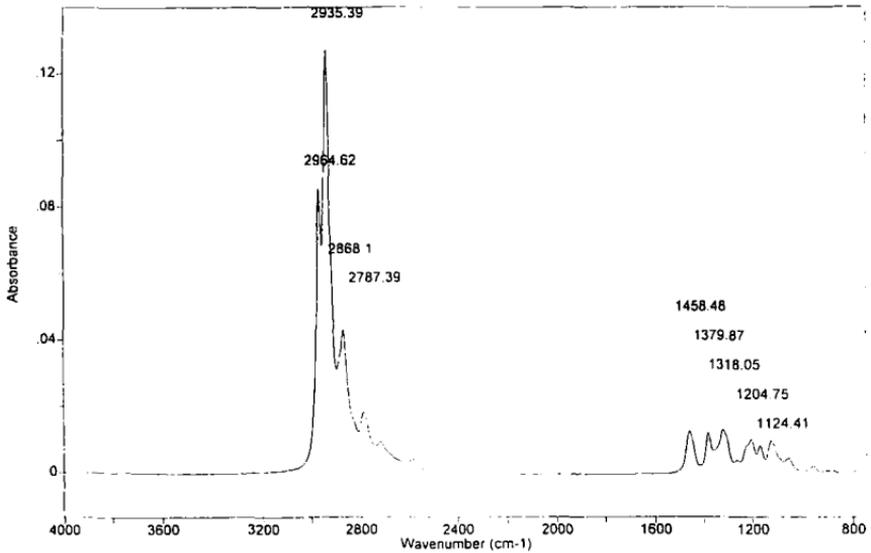
FIG. 3. GC-FT-IR spectrum of (5Z,9Z)-3-n-hexyl-5-methylindolizidine.

only of the 5Z,9Z isomer (Garraffo et al., 1993a). The E,Z nomenclature in the context of 3,5-disubstituted indolizidines refers to the hydrogen configurations at C-5 and C-9 with respect to that at C-3 and is either on the same molecular face (Z) or on the opposite face (E) (Sonnet et al., 1979). Compound **3** was identical with a synthetic sample of (5Z,9Z)-3-hexyl-5-methylindolizidine (Jones et al., 1984) (Figure 3). Indolizidines have been previously reported in various *Solenopsis* species (Jones et al., 1984). The Mona Island species is, at present, undescribed (Torres and Snelling, unpublished data).

Collection D. An extract from workers collected at Cabo Rojo, Puerto Rico, contained two components, **4** and **5,** in ca. 1:6 ratio, respectively, with the following mass spectra: (minor, **4**) MS m/z, 223(M$^+$, 2), 222(1), 180(30), 166(100), 124(5), 122(5); (major, **5**), MS m/z, 223(M$^+$, 4), 222(1), 180(100), 166(90), 124(7), 122(7). Extracts of two other populations of workers in the same area had ratios of these components of ca. 1:1.7 and 1:2.5. The mass and FT-IR spectra of **4** and **5** were identical to those reported for the amphibian alkaloids (5Z,9Z)-3-butyl-5-propylindolizidine and (5E,9E)-3-butyl-5-propylin-dolizidine, respectively (Garraffo et al., 1993a; Daly et al., 1993). Extracts from queens of the same colony contained trace amounts of **4** and **5** as well as one major component, which had mass and FT-IR spectra identical to those of **3,** the same indolizidine as found in queens from the Mona Island collection.

DISCUSSION

The alkaloids identified in this collection of *Diplorhoptrum* species from Puerto Rico may reflect the biological relationships in this group of ants. The unbranched 15-carbon skeleton of all these compounds is reminiscent of the unbranched 19-carbon alkaloid mixtures found in many New World and New Zealand *Monomorium* species (Jones et al., 1982b, 1988), where other carbon-chain lengths are not observed or are minor components. Additionally, while the presence of monocyclic alkaloids accompanying bicyclic alkaloids of the same carbon number in a particular ant venom has been reported, this is the first report of a monocyclic ant venom alkaloid (**2**) having side-chain functionality at a position that might permit the biosynthesis of a third C–N bond (in this case, by allylic activation), to yield the bicyclic alkaloid, **3,** found only in the queens. The *cis* ring diastereomer **2a,** detected as a minor component in Mona Island workers, would be the logical precursor.

Except for the queens of the Río Grande collection, where the unusual *cis*-**1** predominates, gas chromatographic retention times and FT-IR spectra show that the piperidine alkaloids **1** and **2** occur mainly as the usually encountered *trans* diastereomers with measurable amounts of *cis* diastereomers. It is noteworthy that the six-membered ring in the indolizidines **3, 4,** and **5** is *cis* disubstituted, of probable derivation from a *cis*-2,6-disubstituted piperidine, and suggesting biogenetic relationship with *Solenopsis conjurata*, whose venom contains *cis* and *trans* disubstituted piperidines along with a (5Z,9Z)-dialkylindolizidine (Jones et al., 1984).

In three of the four Puerto Rican *Diplorhoptrum* spp. for which queen and worker alkaloids have been compared (Table 1), significant differences are readily evident. Workers of the Río Grande species are extreme in not producing any detectable alkaloids in comparison with their queens. In addition, major quantitative and qualitative differences characterize the alkaloids synthesized by the Mona Island and Cabo Rojo species. Caste-specific differences in the mixtures of 2,6-dialkylpiperidines that dominate the venoms of fire ant (*Solenopsis geminata* group, sensu Trager spp.) have also been reported. For the most part, queens of these species produce a single diastereomeric pair of piperidines (*cis*- and *trans*-2-methyl-6-undecylpiperidine), whereas the workers of some species produce these and up to five pairs of *cis/trans* pyrrolidines (Brand et al., 1973). Since the queens are not actively involved in colony defense, their venoms may play another role. In the case of the queens of the Puerto Rican *Diplorhoptrum* spp., the presence of venom alkaloids not found in the workers strongly suggests a different function of these compounds in this caste, for example, as a pheromone or to protect the eggs from microbial infection (Vander Meer and Morel, 1994). A more systematic investigation of Puerto Rican *Diplorhoptrum* spp. is

being undertaken to determine the extent of chemical diversity in the venoms of these ants.

Most species of *Diplorhoptrum* are hypogeic, but some are known to be arboreal. An arboreal way of life may, in fact, be more common than generally realized, at least in tropical areas. Some of the species are facultative "thief ants," living in close proximity to nests of other, larger species. Often, their galleries may open into those of the larger ants. From these galleries, the *Diplorhoptrum* workers may raid the nest chambers of the other species for bits of food, but they are not above attacking the larvae and pupae of the unwilling host.

It is noteworthy that two of the four Puerto Rican *Diplorhoptrum* species studied here, the collections from Mona Island and Cabo Rojo, nest in trees, while the El Verde and Río Grande collections share the usual ground-nesting propensities of the group. An arboreal life may expose the workers to predators, such as lizards and frogs, which can readily forage on the tree and ingest large numbers of workers.

Rivero et al. (1963) found for the frog *Eleutherodactylus karlschmidti*, the most aquatic member of this genus in Puerto Rico, that most of its prey was terrestrial and 90% composed of insects. Forty-four percent of these, in turn, were comprised of dipterans, of which many were aquatic larvae. Trichopterans and hymenopterans each comprised about 15% of the insects consumed, and of the latter, most were ants. Lavigne and Drewry, who studied (1970) the prey of frogs and lizards at El Verde, found hymenopterans and dipterans to be dominant. Cintrón found (1970) a high consumption of coleopterans and hymenopterans (chiefly ants) by tree frogs of the genus *Eleutheradactylus* in palm (*Prestoea montana*) forests.

The present results are highly relevant to the proposal (Daly et al., 1994a,b) that dendrobatid frogs are sequestering, for defensive purposes, some of the venom components of dietary ants and other small arthropods. Thus, two further alkaloids (**4** and **5**), previously described only from dendrobatid frogs, have in the present paper been identified in an ant.

The indolizidine **5**, originally detected in dendrobatid frogs of the genus *Dendrobates* and named gephyrotoxin (GTX) **223AB,** has been the target of many syntheses (reviewed in Daly and Spande, 1986). It was originally thought that only the 5*E*,9*E* diastereomer occurred in dendrobatid frog skin based on the isolation of **5** from the Colombian species, *Dendrobates histrionicus* (Spande et al., 1981; Daly and Spande, 1986) while in the Panamanian species *Dendrobates speciosus* (Edwards et al., 1988) and one mantellid species from Madagascar (Garraffo et al., 1993b), only the 5*Z*,9*Z* diastereomer **4** was found. Additional work demonstrated that both **4** and **5** can also appear together in frog and toad skin extracts (Garraffo et al., 1993a; J. W. Daly, personal communication).

Dendrobatid frogs, raised in captivity on diets of fruit flies and crickets, have absolutely no skin alkaloids (Daly et al., 1992; 1994a,b) leading to the proposal that skin alkaloids are sequestered from dietary arthropods. Indeed, when fed fruit flies dusted with alkaloid-containing powder, such alkaloid-free frogs efficiently incorporated indolizidines, pyrrolizidines, and a number of other alkaloid classes (Daly et al., 1994a).

In addition, a captive-raised dendrobatid frog (*Dendrobates auratus*), when fed ants of the species *Monomorium pharaonis* (Daly et al., 1994a), incorporated the indolizidine, monomorine I, into skin. The frogs avidly ate these ants, so at least in this case the ant venom is not a deterrent to predation. These results indicate that the indolizidines **4** and **5** of the present paper should also be efficiently incorporated into dendrobatid frog skin.

At least half a dozen alkaloids of the indolizidine and pyrrolizidine classes have been found to be common to frogs and ants (Daly et al., 1993), strongly suggesting in the light of the above results that such alkaloids, when found in frog skin glands, have been sequestered from a diet containing ants. While this is not a large overlap—the total number of alkaloids detected in frog skins is over 400—it should be emphasized that only a few hundred of the many thousand species of myrmicine ants have so far been examined for alkaloids.

In Panama when captive-raised dendrobatid frogs were fed on leaf litter insects obtained through the use of Berlese funnels, some 20 alkaloids were found to have been sequestered into skin (Daly et al., 1994b). In this instance, no "ant" alkaloids were sequestered, perhaps suggesting that ants were poorly represented in the insects obtained from Berlese funnels. While the phenomenon of storing ingested alkaloids is not uncommon, having been encountered in a variety of vertebrate and invertebrate species, it may prove to be unusual in frogs, since only seven genera from four different families (Dendrobatidae, Bufonidae, Myobatrachidae, and Mantellidae) have lipophilic alkaloids with more than 80 genera examined to date. It is quite probable that this behavior reflects the presence of a transport and/or storage capacity for dietary alkaloids derived from ants or other small insects, but this has been demonstrated experimentally only for a few species of dendrobatid frogs, namely *Dendrobates auratus*, *Phyllobates bicolor*, and *Epipedobates tricolor* (Daly et al., 1994a,b).

Dendrobatid frogs are well known to include ants as a major food source (Donnelly, 1991; Toft, 1980); some dendrobatid frogs have even been referred to as "ant specialists" (Toft, 1995). *Solenopsis* species have been established by examination of stomach contents as among the food prey for frogs that are foreign to Puerto Rico (Silverstone, 1975; 1976). Ant species of the genus *Solenopsis* (*Diplorhoptrum*) related to the Puerto Rican species are sympatric with dendrobatids in Costa Rica (Jones et al., 1984) and are likely to be in other Central and South American areas as well. While the ant alkaloids have some taxonomic value, in that we have never observed collections of the same species

of ant with different alkaloids, in many cases, ants of different species do pro-
duce some of the same alkaloids, so that indolizidines **4** and **5** could very well
be components of venoms of species not yet collected.

While certain ants serve as food for dendrobatid frogs, there are many that
are rejected and shunned because of their bite or sting. It remains to be dem-
onstrated, whether other classes of frog-skin alkaloids besides the 3,5-disubsti-
tuted indolizidines, **4** and **5,** will be found in ants that constitute the food of
dendrobatid frogs. Dendrobatid frog skin also can contain precoccinelline, a
beetle alkaloid, and a pyrrolizidine oxime closely related in structure to a mil-
lipede alkaloid (Daly et al., 1993). Indeed, such alkaloids were found in skin
of dendrobatid frogs raised on leaf-litter insects in Panama (Daly et al., 1994b).
Small beetles and millipedes have been documented as prey items for dendro-
batid frogs and were present in the leaf litter collections.

It should be noted that almost all species of dendrobatid frogs are terrestrial.
Only one of two arboreal Panamanian species contained even trace amounts of
a putative ant alkaloid, in this case, alkaloid **195B,** 3-butyl-5-methylindolizidine
(Daly et al., 1987). The correlation, if any, between terrestrial and arboreal
ways of life of both insects and frogs in determining the skin alkaloids of the
latter should be more thoroughly explored.

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