Venom Chemistry of the Ant *Myrmicaria melanogaster* from Brunei

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Analysis of the extracts of the ant *Myrmicaria melanogaster* from Brunei in the Indonesian archipelago by GC-MS and GC-IR revealed the presence of five new alkaloids, identified as (9*Z*)-3-propylindolizidine (**1**), *cis*- and *trans*-2-butyl-5-propylpyrrolidine (**2** and **3**, respectively), (10*E*)-3-butyllehmizidine (**7**), and (5*Z*,8*Z*,9*Z*)-3-butyl-5-propyl-8-hydroxyindolizidine (**10a**), whose structures were established by comparison with synthetic samples. In addition the monoterpene hydrocarbons β-pinene, myrcene, and limonene were detected along with all four isomers of 3-butyl-5-methylindolizidine (**4a**−**d**), *cis*- and *trans*-2-butyl-5-(4-pentenyl)pyrrolidine (**5a** and **5b**), *trans*-2-butyl-5-pentylpyrrolidine (**6**), (5*Z*,9*Z*)-3-butyl-5-propylindolizidine (**8**), and (5*Z*,9*E*)-3-butyl-5-propylindolizidine (**9**), alkaloids well known from ants and frogs, whose structures were established on the basis of published spectra or comparison with authentic samples. This study utilized vapor-phase infrared analysis for the assignment of stereochemistry using Bohlmann bands for the bicyclic alkaloids and, in the case of **10a**, the detection of an intramolecular hydrogen bond. A biogenetic relationship between the mono- and bicyclic ring systems is proposed.

Ring-saturated nitrogen heterocycles are well-known components of the venoms of ants in the subfamily Myrmicinae, particularly in the genera *Megalomyrmex, Monomorium,* and *Solenopsis*.[1−3] These alkaloid structures are usually based on an unbranched carbon skeleton cyclized with nitrogen to form monocyclic pyrrolidines and piperidines, or bicyclic pyrrolizidines, indolizidines, quinolizidines, and decahydroquinolines.[4] In a number of species, the bicyclic compounds are present with their monocyclic homologues.[5−9] Monoterpene hydrocarbons have been reported in the poison glands of myrmicine ants in the genus *Myrmicaria* from Africa for over 30 years;[10] however, more recently indolizines reminiscent of the *Megalomyrmex*, *Monomorium*, and *Solenopsis* alkaloids, as well as complex polycyclic alkaloids, have been demonstrated from the venom glands of several African species in this genus.[11−14] In *Myrmicaria,* a close relative of *Megalomyrmex*,[15] the monoterpene hydrocarbons have a role as recruitment pheromones, while the alkaloids serve defensively.[13] In this report we describe the elucidation of the structures of three terpenes and nine bicyclic and five homologous monocyclic alkaloids detected in *Myrmicaria melanogaster* (Emery), a species reported only from Borneo and collected in the sultanate of Brunei Darussalam. These ants nest beneath leaves in the understory or subcanopy and travel to the canopy to tend homoptera (order Hemiptera). The venom gland contents are sprayed at enemies met head-on with the gaster doubled ventrally and its tip aimed forward. Eight of the 14 alkaloids from *M. melanogaster* have been reported from frog skin extracts.[16]

Results and Discussion

The initial gas chromatography−mass spectrometry (GC-MS) analysis of MeOH extracts of whole *M. melanogaster* collected in Brunei revealed the presence of three monoterpene hydrocarbons, β-pinene, myrcene, and limonene (peaks **A**, **B**, **C**) and 14 alkaloids (**1**−**10**) (Figure 1). The terpenes were identified from their mass spectra and by direct comparison with authentic samples. The alkaloids (Figure 2) were easily recognizable by the presence of abundant, even-mass fragment ions in their electron-impact mass spectra (EIMS). Authentic samples of a number of them were available from previous work. The four isomers of 3-butyl-5-methylindolizidine (**4a**−**d**) were identified by their retention times and EIMS and gas chromatography-Fourier-transform infrared spectra (GC-FTIR spectra), which were identical with those of authentic samples.[17] All four isomers have been detected in frog skin extracts.[18] The EIMS of the major components **5b** and **6** suggested 2-butylpyrrolidines with a five-carbon chain at C-5. Additionally, **5b** showed infrared absorption bands at 913, 993, 1641, and 3084 cm^{-1}, indicating the presence of a $-CH{=}CH_2$ group,[19] and was converted to **6** upon catalytic hydrogenation. Comparison with authentic samples showed **5a** and **5b** to be the *cis* and *trans* isomers of 2-butyl-5-(4-pentenyl)pyrrolidine, respectively,[20] and **6** to be *trans*-2-butyl-5-pentylpyrrolidine.[21] Pyrrolidine **6** is well known from ants[1] and has been detected in frog skin extracts and referred to as alkaloid **197B**.[18] The EIMS and GC-FTIR data of **8** and **9** (ca. 20:1) were identical to those published for (5*Z*,9*Z*)-3-butyl-5-propylindolizidine and (5*Z*,9*E*)-3-butyl-5-propylindolizidine, respectively.[18] Two diastereomers were found in *Solenopis* (*Diplorhoptrum*) ants of Puerto Rico, the 5*Z*,9*Z* and 5*E*,9*E* isomers in significantly different ratios in the three populations sampled.[9] The 5*E*,9*E* diastereomer was originally discovered in the dendrobatid frog *Oophaga silvatica* (formerly *Dendrobates histrionicus*), collected in Nariño, Colombia.[22] The 5*Z*,9*Z* diastereomer (**8**) was then found in *Oophaga speciosus* (formerly *Dendrobates speciosus*), from Chiriquí, Panama,[23] and all four diastereomers were found in extracts of the Argentinian bufonid toad *Melanophryniscus stelzneri*.[18] All isomers are referred to as alkaloid **223AB**. The remaining alkaloids, **1**−**3**, **7**, and **10**, were unchanged by hydrogenation, and their structures were suggested by their EIMS and GC-FTIR spectra, but required syntheses to confirm. None of these have been detected previously from either ant or frog skin extracts.

The EIMS of the 11-carbon alkaloids, **1**−**3**, all showed intense ions for the loss of propyl. In the case of **1**, the EIMS showed a molecular ion at *m*/*z* 167 and an intense ion at *m*/*z* 124 indicating a bicyclic structure and supporting the loss of a propyl group from the carbon next to nitrogen (α-cleavage). A (5*Z*,9*Z*)-5-propyl-

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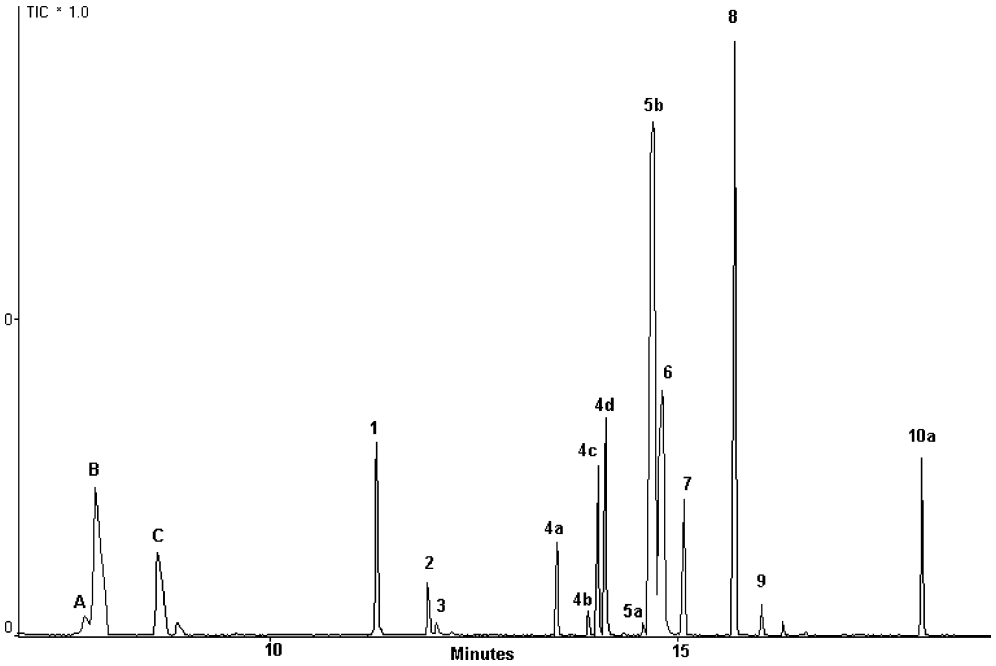


Figure 1. Total ion GC chromatogram of the venom gland extracts of the ant *Myrmicaria melanogaster* from Brunei. GC peaks **A**−**C** are terpenes, while GC peaks numbered **1**−**10** are alkaloids whose structure and synthesis are discussed in the text.

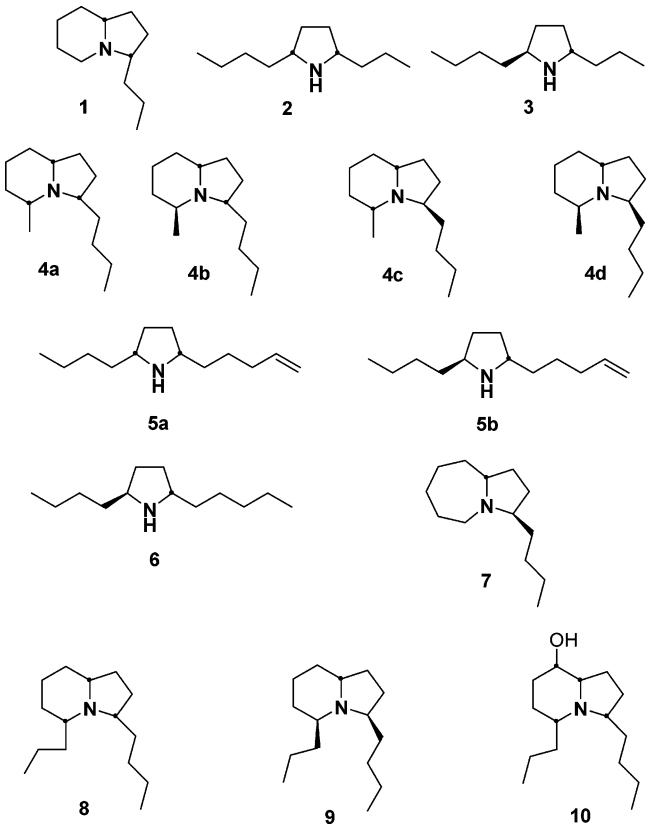
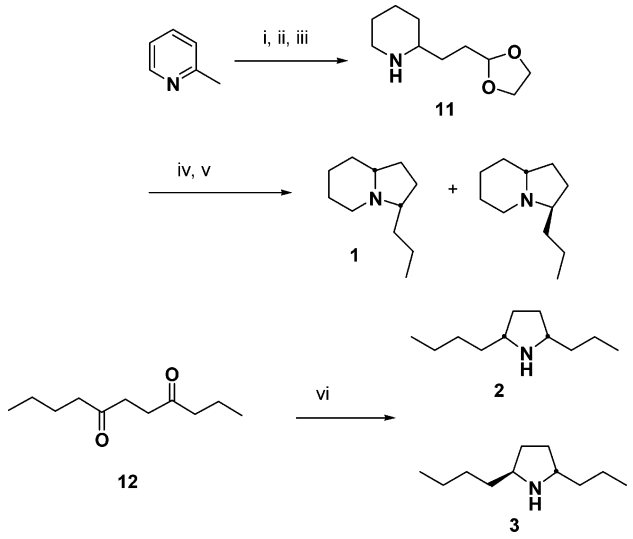


Figure 2. Alkaloids found in the ant venom extracts of *Myrmicaria melanogaster* workers from Brunei. See Figure 1 for the relative amounts. Only relative configurations are known. The occurrence of only one enantiomer is assumed.

indolizidine was originally reported as trace alkaloid **167B** in three dendrobatid frog extracts and has been synthesized by a number of groups (including our own, using the synthesis of ref 24). Shortly after original comparison work with synthetic material supplied by Holmes et al.,[25] alkaloid **167B** could no longer be detected in any of the frog skin extracts; it was presumably lost during handling

Scheme 1. Synthesis of Racemic (3,9*Z*)-(**1**) and (3,9*E*)-3-Propylindolizidine and Racemic *cis* and *trans* Isomers of 2-Propyl-5-butylpyrrolidines (**2** and **3**, respectively)[a]



[a] One enantiomer of the alkaloids **1**−**3** (relative configuration is shown) is presumed to be naturally occurring in the ant *Myrmicaria melanogaster* from Brunei (see text).

or storage due to its volatility. The structure for alkaloid **167B** proposed in 1982[26] was based only upon GC-EIMS and by analogy with other indolizidines in which odd-numbered side-chains were found invariably in the six-membered ring. Consequently, the natural occurrence of 5-propylindolizidine, referred to as alkaloid **167B**, should it be detected again, would require more rigorous proof, now available from GC-FTIR and CI-MS/MS spectra (see below). However, direct comparison showed **1** was not identical to an authentic sample of either of the two diastereomers of 5-propylindolizidine.[24] The desired two 3-propylindolizidine isomers were prepared from picoline in a straightforward manner (Scheme 1), and direct comparison of GC-EIMS, GC-retention times, and GC-FTIR spectra proved that **1** was identical to the first eluting propylindolizidine and had the broader, significant Bohlmann band pattern typical of the 3,9-*Z* configuration (see Figure 3) where the

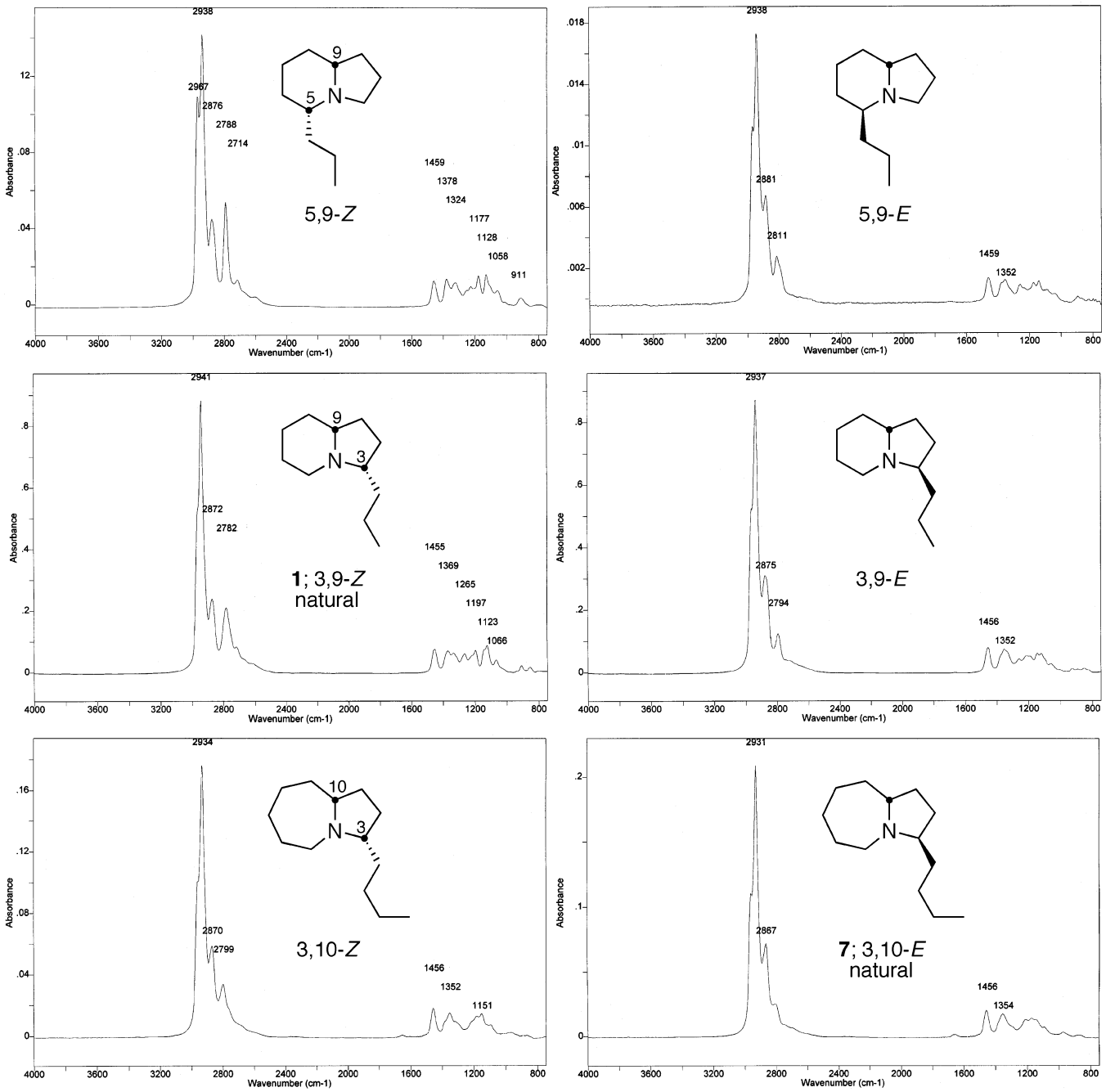


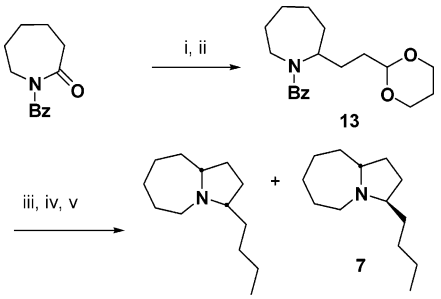
Figure 3. Vapor-phase FTIR spectra of the monosubstituted indolizidines and lehmizidines. The 3-substituted indolizidine **1** and lehmizidine **7** occur naturally in the venom of workers of the ant *Myrmicaria melanogaster* from Brunei (see text). Only relative configurations are depicted.

three C*H*N hydrogens are *trans* antiparallel to the nitrogen lone pair.[27] In Figure 3 are shown FTIR spectra of the two diastereomers of 3- and 5-propylindolizidine. It will be noted that the Bohlmann band pattern (ν_{CH} 2800−2600 cm^{-1}) of the 5,9-*Z* isomer of the 5-substituted indolizidine is enhanced relative to the 3,9-*Z* isomer of the 3-substituted indolizidine.

The application of a less commonly used mass spectrometric technique, that of chemical ionization with tandem mass spectrometry (CI-MS/MS), was extended here to monosubstituted "izidines". CI-MS/MS with ammonia reagent gas has proven extremely useful in determining the gross structures of "izidines" with an N−CH(R)− in each ring.[28] Here, however, rather than seeing the expected ions reflecting a ring and the particular ring substituent, only the ion corresponding to the *unsubstituted* ring was observed; the corresponding ion for the substituted ring was not apparent. This is not quite so useful as in the disubstituted "izidine" cases, but still indicates indirectly which ring is substituted. The 3- and 5-propyl indolizidines, for example, showed ions at *m*/*z* 84 and 70, respectively, demonstrating that a six-membered ring is unsubstituted in both of the 3-propylindolizidines and that the five-membered ring lacks a substituent in both the 5-propylindolizidines. By way of rationalization of this fragmentation, it is possible that the more substituted neutral fragment is preferentially extruded in analogy with the expected loss under EIMS conditions of the more substituted radical.

Alkaloids **2** and **3** appeared to be isomeric pyrrolidines of molecular weight 169, with the intense ions at *m*/*z* 126 and 112 indicating loss of propyl and butyl groups. Both isomers of 2-butyl-5-propyl pyrrolidine were easily prepared (Scheme 1) by reductive amination of 4,7-undecadione (**12**) and were identical to **2** and **3** by direct GC comparison, with the *cis* isomer eluting first, as is invariably the case with pyrrolidines.[21]

Scheme 2. Synthesis of Racemic (3,10*Z*)- and (3,10*E*)-3-Butyllehmizidine[a]



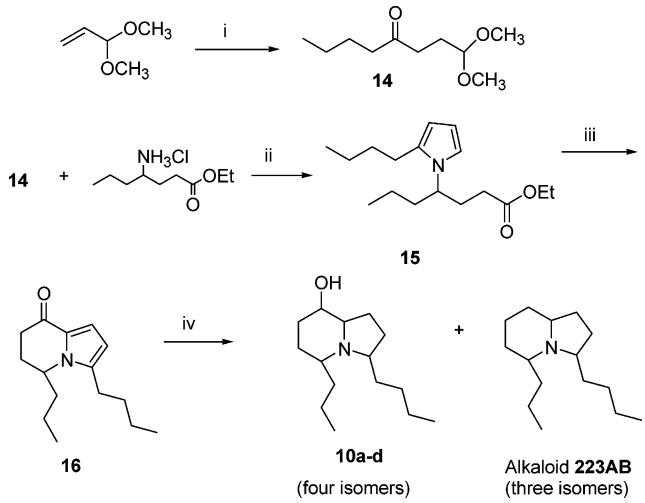
[a] Diastereomer **7** is naturally occurring in the ant *Myrmicaria melanogaster* from Brunei (see text).

Alkaloid **7**, isomeric with the 3-butyl-5-methylindolizidines **4a**−**d**, has not been described previously. The EIMS of **7** showed a molecular ion at *m*/*z* 195 and a base peak at *m*/*z* 138 (M − C_4H_9), reminiscent of the mass spectra of the 3-butyl-5-methylindolizidines, but without the weak ion at *m*/*z* 180 (M − CH_3). The presence of a pyrrolidine ring and a butyl group in alkaloids **4**−**6** suggested that **7** might be a 3-butyl-5*H*-octahydropyrrolo[1,2-*a*]azepine, whose trivial name would be 3-butyllehmizidine.[29] A number of lehmizidines with a methyl in the seven-membered ring and a nine-carbon moiety in the five-membered ring have been reported in frog skin extracts,[29] but none with only a single substituent in the five-membered ring. Both isomers of 3-butyllehmizidine were prepared (Scheme 2), and the GC retention time and EIMS and FTIR spectra of **7** (see Figure 3) were identical to those of the second eluting synthetic butyllehmizidine. On the basis of the weaker Bohlmann band in the FTIR spectrum, this isomer was assigned the 3,10*E* configuration. The CI-MS/MS technique described above yielded an ion at *m*/*z* 98 for both diastereomers, indicating that an unsubstituted seven-membered ring was present.

Alkaloid **10a**, of nominal molecular weight 239, possesses a molecular formula of $C_{15}H_{29}NO$ (high-resolution MS, HRMS), and the EIMS had intense ions at *m*/*z* 196 and 182, indicating the loss of propyl and butyl units, respectively. Additionally, the GC-FTIR spectrum suggested an all-*cis*-substituted (i.e., 5*Z*,9*Z*) indolizidine and showed an absorption at 3528 cm^{-1}, indicating an intramolecularly hydrogen-bonded hydroxyl group. As reference compounds, we hydrogenated the exo-alkylidene moiety of a small sample of pumiliotoxin **251D**, an indolizidine possessing an axial hydroxyl group at C-8 that shows hydrogen bonding to nitrogen. The reduction mixture showed on GC-FTIR a ν_{OH} of 3547 cm^{-1} for the major diastereomer (3 parts) and 3535 cm^{-1} for the minor diastereomer (1 part), supporting our suspicion that the axial hydroxyl of **10a** was at the 8-position, a conclusion further strengthened by the EI mass spectral fragmentation for **10a** and **10b** shown in Scheme 5.

The orientation of the hydroxyl group in alkaloid **10a** is likely axial at C-8 in a *trans*-fused indolizidine, which is the stereochemistry that is accessible by the elegant indolizidine synthesis of Jefford et al.[30] We used the Jefford methodology as an outline, but incorporated the butyl group from the start of the synthesis in our route to the 8-hydroxyindolizidines (Scheme 3). The intramolecular acylation of pyrrole ester **15** using BBr_3 formed the dihydro-8-indolizone (**16**) nearly quantitatively. Jefford's work described the stereospecific hydrogenation of a similar compound, which, however, was unsubstituted at C-3, using a rhodium-on-alumina catalyst in ethanol with a trace of acetic acid.[30] With the 3-butyl group present in **16**, we observed complete loss of the hydroxyl group by hydrogenolysis during the Jefford procedure, but when the reaction was carried out in ethyl acetate containing a trace of the base, triethylamine, four isomers, **10a**, **10b**, **10c**, and **10d**, were formed as the major products in a 3:1:6:4.7 ratio and hydrogenolysis was largely but not completely eliminated since **10a**−**d** were still

Scheme 3. Synthesis of the Four Racemic Diastereomers (**10a**−**d**) of 3-Butyl-5-propyl-8-hydroxyindolizidine[a]

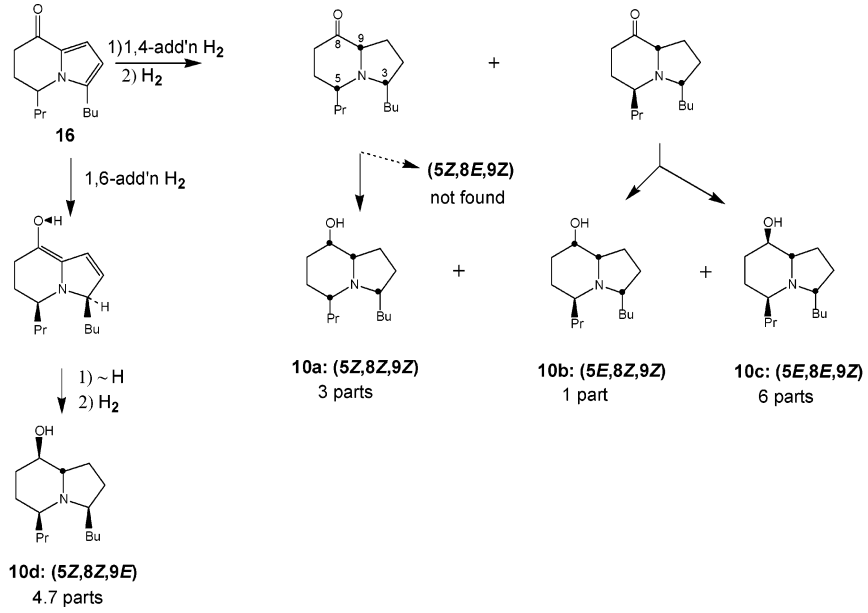


[a] Diastereomer **10a** (see Scheme 4) is naturally occurring in the ant *Myrmicaria melanogaster* from Brunei (see text).

accompanied by very small amounts of (5*Z*,9*Z*)- and (5*E*,9*Z*)-**223AB** and a totally unexpected third **223AB** diastereomer, with the 5*E*,9*E* stereochemistry. These minor side reactions evidently arise from hydrogenolysis ensuing when the keto group is reduced to an alcohol *before* the pyrrole moiety. Identifications were made by GC retention times, characteristic EIMS, and comparison to reference GC-FTIR spectra of the **223AB** diastereomers.[18] A small amount of starting material **16** and the 7,8-dehydro transformation product of **16** were also detected. The Bohlmann band patterns of the reference GC-FTIR spectra allowed the assignment of the relative stereochemistries of **10a**−**d**. Diastereomers **10a** and **10b** both possessed a hydrogen-bonded axial hydroxyl group with ν_{OH} of 3528 and 3539 cm^{-1}, respectively.[19] On the basis of their Bohlmann band patterns we have assigned them 5*Z*,8*Z*,9*Z* and 5*E*,8*Z*,9*Z* configurations, respectively. Diastereomers **10c** and **10d** possessed non-hydrogen-bonded equatorial hydroxyl groups with ν_{OH} of 3653 and 3649 cm^{-1} and ν_{C-O} of 1063 and 1055 cm^{-1}, respectively. The strong ν_{C-O} is typical of an equatorial C−O stretching frequency[19] and is not seen with the axial hydroxyl groups of **10a** and **10b**. The Bohlmann band patterns were closely matched with those of the **223AB** diastereomers of the 5*E*,9*Z* and 5*Z*,9*E* configurations; consequently we assigned the 5*E*,8*E*,9*Z* and 5*Z*,8*E*,9*E* configurations to **10c** and **10d**, respectively (Figure 4). Scheme 4 is a proposed mechanism for the formation of these four hydroxyindolizidines. Three have the 3- and 9-hydrogens on the same face, as expected for a concerted reduction of the pyrrole ring. These results are rationalized by assuming initial reduction of the pyrrole moiety of **16** followed by reduction of the 8-keto group with significant stereocontrol provided by the 5-propyl group. The diastereomer **10d**, with the 3- and 9-hydrogen on opposite faces, is proposed to result from a 1,6-addition of hydrogen and ketonization to give an axial-transfer of hydrogen, then a final reduction of the intermediate. This stereochemical result was totally unexpected, as was the absence of the 8*E* (equatorial) hydroxyl epimer of **10a**. Evidently the steric bulk of the two α-C-3 and C-5 substituents must block hydrogenation of the 8-ketone from the α-face.

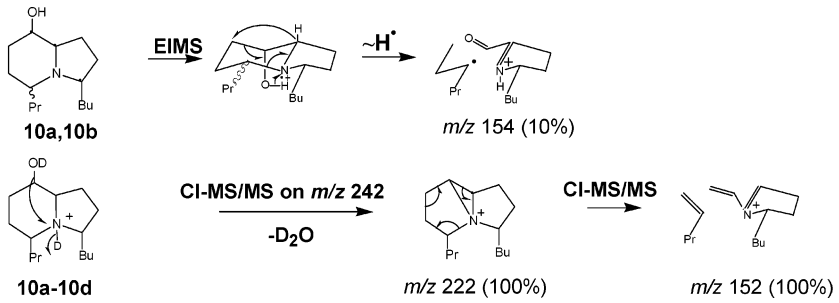
It was noted that the two H-bonded diastereomers, **10a** and **10b**, show a characteristic *m*/*z* 154 ion (10%) in the EIMS that is lacking in the non-hydrogen-bonded diastereomers, **10c** and **10d**. We propose the fragmentation pathway shown in Scheme 5, whereby a hexyl radical is extruded as a result of the transfer of a hydrogen radical from the 8α-hydroxyl group to the nitrogen radical ion. That concerted mechanism would be possible only with the axial 8-hydroxyl group and may provide the driving force for this

Scheme 4. Suggested Pathways for Formation of Racemic Diastereomers (**10a**−**d**) from Hydrogenation of Pyrrole Intermediate **16**[a]



[a] Only relative configurations are shown.

Scheme 5. Proposal for the Formation of the *m*/*z* 154 Ion in the EIMS of **10a** and **10b** and of the *m*/*z* 152 Ion in the CI-MS/MS of **10a**−**d**[a]



[a] The *m*/*z* 154 ion is diagnostic for the 8-axial hydroxyl group and is seen only with **10a** and **10b.** The *m*/*z* 152 ion is seen for all the 8-hydroxyindolizidines **10a**−**d** (see text).

fragmentation. All four diastereomers **10a**−**d** have similar fragmentation patterns in the CI-MS/MS. CI-MS/MS on the deuteronated **10a**−**d** led to a loss of D_2O via the proposed deuterium-free ion (*m*/*z* 222, 100%) that then gives a retro-Diels−Alder type cleavage to the *m*/*z* 152 ion as the base peak in a third stage of CI-MS/MS.

A ring-hydroxylated indolizidine, alkaloid **239X**, with the hydroxyl group not hydrogen-bonded (ν_{O-H} 3652 cm^{-1}) has been discovered in a dendrobatid frog, but is not identical to any of **10a**−**10d**. A gross structure for **239X** has been proposed,[16] but further analysis is required.

It should be noted that, while IR spectroscopy is being used considerably less frequently in characterization work on unknowns than a few decades ago, we have found that gas chromatography-vapor-phase IR spectroscopic analysis at the sub-microgram level is capable of quickly assigning essential structural details for ant and frog alkaloids where amounts and the need for purification remain significant barriers for even the present, very sensitive NMR techniques. The limited amounts and complex mixture of the alkaloids present in the Brunei ant extract precluded any attempt at isolation and ^1H NMR spectroscopy. In many cases, EI and CI mass spectrometric fragmentations, combined with study of FTIR Bohlmann band patterns, allow the preliminary assignment of ring systems and their stereochemistry. At this point, synthesis is undertaken to provide rigorous confirmation by spectroscopic comparisons and GC retention times with coinjections. For the synthetic work of this study, nonstereospecific syntheses were required, so as to obtain all possible diastereomers, for comparison purposes with the natural materials. In addition, racemic materials were desired as we plan chiral GC experiments in the future to determine whether the absolute stereochemistries of ant and frog alkaloids are the same, and in initial work we first need to show that enantiomers can be separated.

In terms of the known frog skin alkaloids, many of which derive from dietary ants, we observe here two new variations of known frog skin alkaloids: the 3-monosubstituted-indolizidine and -lehmizidine, which have substitution patterns yet undetected in frog skin. The known lehmizidines, all of which are disubsitituted, have so far been reported only in *Oophags lehmanni* (formerly *Dendrobates lehmanni*) from Colombia. Extensive studies on Madagascan frogs in the endemic genus *Mantella* have not yielded "izidines" with a substituent only in the five-membered ring.

All of the previous investigations of ants in the genus *Myrmicaria* have shown the presence of well-known monoterpene hydrocarbons in their venom glands, and while alkaloids were not detected in the South African species *M. natalensis*,[10] indolizidine ketones and more complex tri- and polycyclic alkaloids have been found in other African species collected in Kenya.[11−14] These alkaloids, reminiscent of the venom alkaloids found in *Monomorium* and *Solenopsis* species, are indolizines containing an unbranched carbon-chain cyclized to nitrogen, with the more complex compounds being based on a set of two or three condensed C_{15} indolizines. In contrast, *M. melanogaster* from Brunei contains a mixture of C_{11}, C_{13}, and

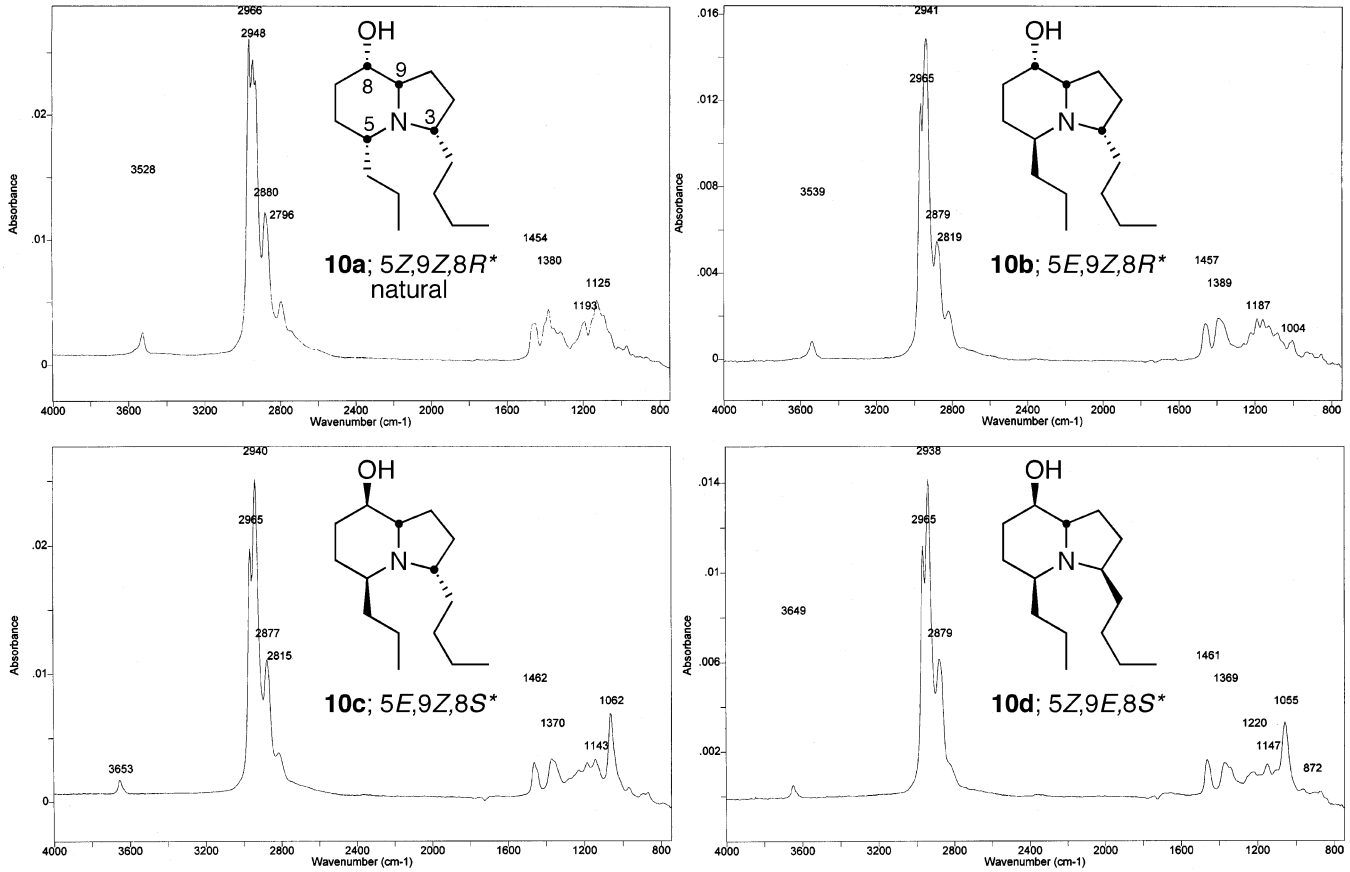


Figure 4. Vapor-phase GC-FTIR spectra of the four synthetic diastereomers **10a**−**d** of 3-butyl-5-propyl-8-hydroxyindolizidine. Diastereomer **10a** is found in the venom of the ant *Myrmicaria melanogaster* from Brunei (see text). Only relative configurations are depicted.

C_{15} alkaloids with homologous bi- and monocyclic compounds present, some of which have been previously reported from *Monomorium* and *Solenopsis* species. Recent work in phylogenetic systematics[15] places these two genera in the sister clade to the clade containing *Myrmicaria* and *Megalomyrmex*.

The presence of concomitant homologous bi- and monocyclic alkaloids, **4a** and **5b**, in an ant venom was first reported in *Monomorium pharaonis* in 1975.[5] This finding in other myrmicine species has suggested that the ring-type, regio- and diastereoisomer of the monocyclic compound might have been formed first, followed by formation of the bicyclic compound, or at least that these compounds had a common biosynthetic precursor.[6−9,31] In some cases, the stereochemistry of the monocyclic alkaloid is opposite that of the corresponding ring in the homologous bicyclic alkaloid.[5,7,8] In *M. melanogaster*, however, concomitance with identical stereochemistry is present in the C_{11} and C_{13} alkaloids, where 3-propylindolizidine **1** represents a cyclized form of the pyrrolidine **2**, and 3-butyllehmizidine **7** represents a cyclized form of **5b** or **6**. Surprisingly we did not detect the 2-(3-butenyl) analogue of **5b**, a putative precursor of **1** and **2**. Additionally, **1** and **7** are the first examples of monosubstituted bicyclic alkaloids from ants apparently resulting from cyclization at the terminal carbon of one of the pyrrolidine side-chains. On the other hand, **4a**−**d** can be seen as resulting from cyclization at the penultimate carbon of the C_5 chain of **5b** or **6**. Interestingly, all the bicyclic alkaloids reported here, **1**, **4a**−**d**, **7**−**9**, and **10a**−**d**, can be seen as derived from appropriately 5-substituted 2-*butyl*pyrrolidines. The ring stereochemistry of **1** and **7** also reflects that of the corresponding pyrrolidines. (3,9*Z*)-3-Propylindolizidine (**1**) has the *cis*-substituted pyrrolidine ring of **2**, and (3,10*E*)-3-butyllehmizidine (**7**) has the *trans*-substituted pyrrolidine ring of **5b** or **6**. In contrast, the four isomers of **4** would derive from both *cis*- and *trans*-pyrrolidines. These results contrast with *Monomorium pharaonis*, where the (5*Z*,9*Z*)-**4a** with a *cis*-substituted pyrrolidine ring occurs together with the *trans*-substituted **5b**.[5,6] While other isomers of 3-alkyl-5-methylindolizidines have been found along with their homologous piperidines in *Solenopsis (Diplorhoptrum*) species, *M. melanogaster* exhibits the first occurrence of a 5*E*,9*Z* isomer, **4b**, containing an axial 5-methyl group.[31]

Myrmicine venom alkaloids having an oxygen function are quite rare, and the few that have been reported have side-chain ketones and occur in *Myrmicaria* species.[11−13] Although isoprene-derived pyrrolidines containing side-chain alcohol groups have been reported in the ant *Harpagoxenus sublaevis*,[32] the presence of **10a** in *M. melanogaster* is the first reported ring-hydroxylated, acetate-derived ant venom alkaloid. Additionally, since the stereochemistry of **10a** is the same as that of **8**, the major **223AB** isomer present in *M. melanogaster*, its presence may be indicative of the biosynthetic pathway to indolizidines in this species. Among all the known ant venom alkaloids, the polyacetate pathway has been established by labeling studies only for the 2-alkyl-6-methylpiperidines from *Solenopsis* spp., in which case the oxygen functionality in the ring would be at the 4-position relative to the nitrogen.[33] Piperidines or pyrrolidines have been found in ants as concomitants of 3,5-dialkylindolizidines,[5−9,31] and in previous work, it has been suggested on the basis of stereochemical considerations that the first formed ring of ant-derived indolizidines is the same as in their monocyclic concomitants.[6] Along with a 2-butylpyrrolidine moiety in all of the alkaloids in *M. melanogaster*, the C-8-OH of **10a** strengthens that perception, since the hydroxyl would be at C-7 if the six-membered ring were formed first by the polyacetate pathway. Although it has been proposed that the six-membered ring is formed first in other *Myrmicaria* alkaloids, no monocyclic concomitants are reported in those cases.[13]

Experimental Section

General Experimental Procedures. GC-MS was carried out in the EI mode using a Shimadzu QP-5000 GC-MS equipped with an RTX-5, 30 m × 0.25 mm i.d. column. The instrument was programmed from 60 to 250 °C at 10 deg/min. Vapor-phase FT-IR spectra were obtained using a Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m × 0.25 mm RTX-5 amine column. NMR spectroscopy was carried out in $CDCl_3$ solutions using a Varian Mercury 400 NMR spectrometer. HRMS was performed on a JEOL SX102 instrument in the positive-ion fast-atom bombardment mode using a direct probe and a Waters LCT Premier time-of-flight instrument in the electrospray (ESI) mode. The monoterpenes were suggested from their mass spectra (NIST/EPA/NIH, 1999) and retention times and confirmed by comparison with commercial authentic samples.

Ants. Nine collections of *Myrmicaria melanogaster* (Emery) were made over a 4-year period (2001−2005) at various times of the year from two nests at the Kuala Belalong Field Studies Center, run by the Universiti Brunei Darussalem and located in the Batu Apoi Forest Reserve, Temburong District, Brunei, 4°32′ N, 115°10′ E. The workers were placed in vials containing a small amount of methanol for analysis. Voucher specimens have been deposited in the entomological collections of both the Brunei Museum and the Los Angeles County Natural History Museum.

The ion chromatograms of the methanol extracts were consistent for all of the extracts over the 5 years of collection. A GC-mass chromatogram of one such extract is shown in Figure 1. The mass spectra and retention times of peaks **A**, **B**, and **C** were identical to those of β-pinene, myrcene, and limonene, respectively. The mass spectra of peaks **1**−**10a** all showed fragmentations that suggested their structures. The FTIR spectra of peaks **5a** and **5b** showed an absorption band at ν_{max} 914 cm^{-1}. When a stream of hydrogen was passed through a few drops of the mixture in the presence of PtO_2, **5** was converted to **6**. The mass spectra and gas chromatographic retention times of peaks **4a**−**d**, **5a**, **5b**, and **6** were identical to those of authentic samples. The mass spectra and FTIR spectra of peaks **8** and **9** were identical to those previously published.[18] The structures of the remaining alkaloids were established by direct comparison with synthetic samples.

2-[2-(1,3-Dioxolan-2-yl)ethyl]piperidine (11). 2-Picoline (4.0 g, 43 mmol) was added dropwise to 25 mL of 1.6 M butyllithium in 50 mL of ether at 0 °C. After 0.5 h, 4.70 mL (40 mmol) of 2-(2-bromoethyl)-1,3-dioxolane was added dropwise. The resulting mixture was allowed to warm to ambient temperature and, after 0.5 h, carefully treated with 20 mL of H_2O. The organic layer was separated, dried over anhydrous K_2CO_3, and filtered and, after Kugelrohr distillation, provided 4.3 g (56% yield) of 2-[2-(1,3-dioxolan-2-yl)ethyl]pyridine, EIMS *m*/*z* 178 [M − 1^+] (1), 136 (5), 118 (10), 107 (15), 106 (25), 93 (47), 87 (17), 73 (100), 45 (62). A solution containing 1.0 g of this product in 50 mL of absolute ethanol containing 0.1 g of K_2CO_3 and 280 mg of 5% Rh on Al_2O_3 was shaken under 3 atm H_2 overnight. After filtration through Celite, Kugelrohr distillation provided 0.6 g (60% yield) of **11**, bp 87−90 °C/0.27 mmHg; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (1H, t, J = 6 Hz), 3.86 (2H, m), 3.75 (2H, m), 2.96 (1H, d, J = 12 Hz), 2.52 (1H, t, J = 12 Hz), 2.37 (1H, m), 1.68−0.96 (11H, complex m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.8, 65.06, 65.02, 56.88, 47.41, 33.08, 31.79, 30.54, 26.82, 25.06; EIMS *m*/*z* 184 [M − 1^+] (1), 113 (3), 85 (6), 84 (100), 73 (13), 56 (15); HRMS *m*/*z* 186.1509 ([M + H]$^+$), calcd for $C_{10}H_{20}NO_2$, 186.1494.

(3,9*E*) and (3,9*Z*)-3-Propylindolizidine (1). A solution containing 0.50 g (2.7 mmol) of **11** in 10 mL of 10% HCl (v/v) was stirred for 1.0 h. After the addition of 20 mL of CH_2Cl_2, the mixture was carefully brought to a methyl orange end point with KCN and stirred overnight. The mixture was then made basic with a slight excess of KCN, and the organic layer was separated and dried over K_2CO_3. The solvent was removed in vacuo, and the residue was taken up in 10 mL of THF and treated with an excess of *n*-propylmagnesium bromide to provide, after the usual workup,[4] 0.4 g (90% yield) of a 1:1 mixture of **3,9*Z*-** and **3,9*E*-1**. The GC-FTIR spectra for **3**,**9*Z*-** and **3,9*E*-1** are shown in Figure 3. GC-FTIR ν_{max} 2941, 2872, 2783, 1455, 1370, 1265, 1197, 1123, and 1066 cm^{-1} and 2938, 2877, 2795, 1456, 1352 cm^{-1}, respectively; EIMS *m*/*z* 167 [M$^+$] (1), 166 (2), 125 (10), 124 (100), 122 (4), 110 (2), 96 (2); HRMS for both (direct probe) *m*/*z* 168.1770 ([M + H]$^+$), calcd for $C_{11}H_{22}N$, 168.1752. The GC-MS and GC-FTIR data for the first eluting isomer were identical to those of the natural **1**.

4,7-Undecadione (12). A mixture of 0.45 g of butanal (6.25 mmol), 0.70 g of 1-heptene-3-one (6.25 mmol), and 0.2 g of 5-(2-hydroxyethyl)-4-methyl-3-benzylthiazolium chloride was treated with 2 mL of triethylamine and refluxed overnight under an argon atmosphere. The usual workup[21] provided 0.98 g (85% yield) of **12** that was 80% homogeneous by GC-MS. EIMS *m*/*z* 184 [M$^+$] (1), 142 (23), 127 (18), 113 (2), 99 (13), 85 (43), 71 (60), 57 (50), 55 (10), 43 (100), 41 (53); HRMS *m*/*z* 185.1554 ([M + H]$^+$), calcd for $C_{11}H_{21}O_2$, 185.1542.

***cis*- and *trans*-2-Butyl-5-propylpyrrolidine (2 and 3).** A solution containing 0.5 g (2.7 mmol) of **12**, 0.22 g of NH_4OAc, and 0.17 g (2.7 mmol) of $NaCNBH_3$ in 10 mL of methanol was stirred overnight and worked up in the usual manner[33] to provide 0.35 g (77% yield) of a 1:1 mixture of the pyrrolidines **2** and **3**, whose GC retention times were 11.88 and 11.98 min, respectively, and which exhibited identical mass spectra. EIMS *m*/*z* 169 [M$^+$] (1), 169 (2), 140 (2), 127 (4), 126 (63), 113 (5), 112 (100), 98 (10), 95 (11), 82 (12), 70 (12), 69 (20), 56 (20), 55 (24), 44 (30), 41 (60); HRMS for both (direct probe) *m*/*z* 170.1918 ([M + H]$^+$), calcd for $C_{11}H_{24}N$, 170.1909. The GC-MS data for **2** and **3** were identical to those of the natural compounds.

***N*-Benzyl-2-[2-(1,3-dioxan-2-yl)ethyl]azepane (13).** A solution containing 2.0 g (10 mmol) of *N*-benzylcaprolactam[35] in 20 mL of THF was cooled to 0 °C and treated with a 3-fold excess of the Grignard reagent prepared from 2-(2-bromoethyl)-1,3-dioxane[36] and stirred overnight at room temperature. The mixture was cooled to 0 °C, and 1.24 g of $NaCNBH_3$ was added followed by 8 mL of acetic acid. The literature-described workup,[37] followed by Kugelrohr distillation at 150−160 °C (0.15 mmHg), provided 1.5 g of **13** that was 81% pure by GC analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (5H, m), 4.48 (1H, t, J = 5.2 Hz), 4.09 (2H, dd, J = 11.6 and 5 Hz), 3.75 (4H, m), 2.78 (2H, m), 2.60 (1H, m), 2.07 (1H, m), 1.8−1.4 (13H, brm); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.43, 128.86 (2C), 128.31(2C), 126.72, 102.99, 67.16 (2C), 62.30, 55.30, 49.58, 33.54, 32.92, 28.67, 28.55, 27.34, 26.14, 25.98; EIMS *m*/*z* 303 [M$^+$] (1), 302 (1), 216 (2), 189 (10), 188 (100), 160 (2), 96(3), 91 (93); HRMS *m*/*z* 304.2275 ([M + 1]$^+$), calcd for $C_{19}H_{30}NO_2$, 304.2277.

(3,10*E*)- and (3,10*Z*)-3-Butyllehmizidine (7). A 0.50 g (1.65 mmol) sample of azepane **13** in 50 mL of methanol was refluxed overnight with 1.0 g of ammonium formate and 1.0 g of 10% Pd/C. The usual workup[29] provided 0.35 g (70% yield) of 2-[2-(1,3-dioxan-2-yl)ethyl]-azepane. EIMS *m*/*z* 213 [M$^+$] (1), 212 (1), 170 (1), 154 (1), 138 (3), 99 (5), 98 (100), 87 (6), 70 (16), 69 (14), 56 (10), 41 (25). The crude azepane acetal was stirred for 3 days in 10 mL of THF containing 5 mL of 10% HCl (v/v) and one drop of 70% $HClO_4$ (v/v), then as described for the cyclization of **11**, treated sequentially with KCN and an excess of *n*-butylmagnesium bromide. The usual workup provided a 1:1 mixture of the isomers of **7**. GC-FTIR ν_{max} 2934, 2871, 2799, 1457, 1353, 1151 cm^{-1} and 2932, 2867, 1457, 1353 cm^{-1} for the first and second eluting peaks, respectively. The GC-FTIR spectra for the isomers of **7** are shown in Figure 3. EIMS *m*/*z* 195 [M$^+$] (1), 166 (1), 152 (1), 139 (5). 138 (100), 136 (3), 110 (5), 96 (2), 82 (4), 68 (5), 55 (12), 41 (25); HRMS *m*/*z* 195.2005 ([M]$^+$), calcd for $C_{13}H_{25}N$, 195.1987, 138.1283 ([M − C_4H_9]$^+$), calcd for $C_9H_{16}N$, 138.1283. The GC-MS and GC-FTIR data for the second eluting isomer were identical to those of the natural **7**.

4-Oxooctanal dimethyl Acetal (14). A solution containing 10.2 g (100 mmol) of acrolein dimethyl acetal and 1.5 g of 2,2-azobisisobutyronitrile (AIBN) in 32 mL (300 mmol) of *n*-pentanal was heated at 80 °C for 36 h. Fractional distillation of the mixture provided 7.0 g (37% yield) of **14**, bp 69−74 °C (0.55 mmHg). GC-FTIR ν_{max} 2945, 2840, 1726 (s), 1448, 1364, 1192, 1125 (s), 1087 (s) cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.87, 104.05, 53.39 (2C), 42.85, 37.46, 29.74, 26.18, 22.55, 14.06; EIMS *m*/*z* 188 [M$^+$] (0.5), 187 (1), 157 (15), 114 (9), 99 (5), 88 (25), 75 (100), 71 (30), 57 (20), 41 (33); HRMS *m*/*z* 157.1268 ([M − OCH_3]$^+$), calcd for $C_9H_{17}O_2$, 157.1229; HR-CIMS (negative ion) 187.1310 (M − H^+)$^-$, calcd for $C_{10}H_{13}O_3$, 187.1334.

Ethyl-4-[*N*-2-butylpyrrolyl]heptanoate (15). A solution containing 3.2 g (18 mmol) of ethyl 4-oxoheptanoate,[38] 2.0 g (29 mmol) of hydroxylamine hydrochloride, and 2 mL of pyridine in 25 mL of EtOH was stirred overnight. After removal of the solvent in vacuo, the residue was partitioned between ether and water, and the organic layer provided 2.6 g of the corresponding oxime after the removal of solvent. EIMS *m*/*z* 187 [M$^+$] (1), 170 (18), 159 (2), 142 (58), 141 (15), 126 (50), 124 (25), 114 (32), 113 (38), 100 (5), 98 (7), 96 (16), 82 (16), 70 (9), 69 (9), 68 (10),55 (32), 54 (38), 43 (63), 41 (100). The crude oxime was taken up in 100 mL of EtOH containing 5 mL of $CHCl_3$ and

hydrogenated at 3 atm over 0.35 g of PtO_2 overnight.[39] After filtration, removal of the solvent in vacuo provided 2.8 g (75% yield) of the ethyl 4-aminoheptanoate hydrochloride. EIMS (unstable free base) *m*/*z* 173 [M^+] (0.1), 130 (29), 128 (7), 84 (89), 72 (100), 56 (36), 43 (17), 41 (38).

A solution containing 2.40 g (12.7 mmol) of acetal **14** in 20 mL of EtOH and 5 mL of 10% HCl (v/v) was stirred for 3 h at room temperature. After neutralization with solid $NaHCO_3$, the solvent was removed in vacuo and the residue was extracted with ether. The ether layer was dried over anhydrous $MgSO_4$, filtered, and concentrated, and the residue was taken up in 30 mL of CH_2Cl_2. This solution was added to 2.80 g (13.2 mmol) of ethyl 4-aminoheptanoate hydrochloride and 0.1 g of NaOAc in 50 mL of water, and the two-phase mixture was refluxed for 4 h. After checking an aliquot by GC-MS for completion, the cooled mixture was extracted with three 25 mL portions of ether, and the combined ether extracts were dried over anhydrous $MgSO_4$, filtered, and distilled, to provide 1.36 g of **15** (38% yield), bp (Kugelrohr) 125−130 °C (0.15 mmHg). GC-FTIR ν_{max} 3112, 2968, 2942, 2883, 1750 (s), 1543, 1476, 1375, 1277, 1170 (s), 1113, 1039, 877, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.56 (1H, s), 6.12 (1H, s), 5.83 (1H, s), 4.09 (2H, q, J = 7 Hz), 3.97 (1H, m), 2.47 (2H, t, J = 8 Hz), 2.09 (4H, br s), 1.95 (1H, m) 1.22 (3H, t, J = 7 Hz), 1.13 (2H, m), 0.94 (3H, t, J = 7 Hz), 0.87 (3H, t, J = 7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.48, 134.16, 115.41, 107.68, 104.59, 60.63, 54.46, 39.41, 31.79, 31.28, 30.83, 26.37, 22.88, 19.74, 14.42, 14.20, 14.17; EIMS *m*/*z* 279 [M^+] (18), 250 (5), 236 (17), 234 (12), 222 (10), 208 (5), 195 (10), 178 (8), 157 (10), 150 (25), 137 (9), 136 (8), 122 (28), 111 (18), 106 (15), 83 (25), 81 (15), 80 (100), 55 (43), 41 (52); HRMS *m*/*z* 279.2154, calcd for $C_{17}H_{29}NO_2$, 279.2198.

3-Butyl-5-propyl-8-oxo-5,6,7,8-tetrahydroindolizine (16). A solution containing 0.30 g (1.08 mmol) of pyrrole ester **15** in 10 mL of anhydrous CH_2Cl_2 under argon was treated with 1.7 mL of 2 M BBr_3 in CH_2Cl_2 at room temperature. After 40 min the solution was neutralized with an excess of saturated $NaHCO_3$ and the mixture was extracted with three 25 mL portions of ether. The combined ether extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to provide 0.23 g of **16** that was 81% pure by GC-MS. GC-FTIR ν_{max} 2963, 2947, 2882, 1685 (s), 1541, 1476, 1422, 1397, 1332, 1242, 1187, 1121, 775 cm^{-1}; EIMS *m*/*z* 233 [M^+] (43), 191 (18), 190 (100), 176 (10), 163 (10), 162 (17), 149 (30), 148 (88), 135 (17), 120 (18), 106 (23), 80 (25), 79 (22), 78 (23), 55 (16), 41 (75); HRMS *m*/*z* 234.1859 ([M + 1]$^+$), calcd for $C_{15}H_{24}NO$, 234.1858, 233.1823 ([M]$^+$), calcd for $C_{15}H_{23}NO$, 233.1780.

3-Butyl-5-propyl-8-hydroxyindolizidine (10a−d). In a typical hydrogenation, a solution containing 30 mg (0.13 mmol) of **16**, two drops of triethylamine, and 60 mg of 5% Rh/Al_2O_3 in 10 mL of EtOAc was shaken under 3 atm H_2 overnight. Gas chromatographic analysis revealed the presence of four isomers, **10a**, **10b**, **10c**, and **10d**, with an 88% combined yield in a 3:1:6:4.7 ratio having similar mass spectra. **10a**: EIMS *m*/*z* 239 [M^+] (1), 238 (1), 197 (3), 196 (29), 183 (11), 182 (100), 154 (13), 140 (9), 126 (4), 122 (3), 96 (5), 95 (4), 94 (7), 82 (10), 68 (12), 55 (30), 41 (50). **10b**: EIMS *m*/*z* 239 [M^+] (1), 238 (1), 197 (9), 196 (83), 183 (11), 182 (100), 154 (13), 140 (9), 126 (4), 122 (7), 96 (5), 95 (4), 94 (7), 82 (10), 68 (12), 55 (30), 41 (50). **10c**: EIMS *m*/*z* 239 [M^+] (1), 238 (1), 197 (3), 196 (100), 183 (11), 182 (90), 152 (3), 140 (9), 126 (2), 122 (5), 96 (5), 95 (4), 94 (7), 82 (10), 68 (12), 55 (30), 41 (50). **10d**: EIMS *m*/*z* 239 [M^+] (1), 238 (1), 197 (3), 196 (68), 183 (11), 182 (100), 152 (3), 140 (9), 126 (2), 122 (2), 96 (5), 95 (4), 94 (7), 82 (10), 68 (12), 55 (30), 41 (50). In a different hydrogenation experiment, run for 4 h, the ratio of **10a**, **10b**, **10c**, and **10d** was 3.5:1:3.6:1.8. The GC-FTIR spectra for **10a**, **10b**, **10c**, and **10d** are shown in Figure 4. HRMS for **10a**: *m*/*z* 239.2316 ([M]$^+$), calcd for $C_{15}H_{29}NO$, 239.2249; 196.1735 ([M − C_3H_7]$^+$), calcd for $C_{12}H_{22}NO$, 196.1701; 180.1567 ([M − C_4H_9]$^+$), calcd for $C_{11}H_{20}NO$, 180.1545. The GC-MS and GC-FTIR data for the first eluting isomer, **10a**, were identical to those of the natural **10a**. Minor amounts of unreacted starting material **16** were seen at a longer GC retention time than **10a**−**d**. At a shorter retention time, equivalent amounts of the 7,8-dehydro-transformation product of **16** were seen. EIMS *m*/*z* 217 (28), 174 (M − Pr, 100), 160 (18), 146 (8), 144 (13), 133 (15), 132 (38), 130 (33), 118 (15), 117 (15), 91 (8), 77 (13); GC-FTIR ν_{max} 3107, 3058, 2965, 2944, 2885, 1624, 1491, 1424, 1379, 1290, 1211, 1027, 762 cm^{-1}.

Additionally, small amounts of three isomers of alkaloid **223AB** were formed and identified by comparison with their literature GC-FTIR and mass spectra.

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