

Ammodendrine and *N*-Methylammodendrine Enantiomers: Isolation, Optical Rotation, and Toxicity

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Ammodendrine (**1**) was found to occur as a mixture of enantiomers in two different collections of plants identified as *Lupinus formosus*. The ammodendrine fraction was reacted in a peptide coupling reaction with 9-fluorenylmethoxycarbonyl-L-alanine (Fmoc-L-Ala-OH) to give diastereomers, which were separated by preparative HPLC. The pure D- and L-ammodendrine enantiomers were then obtained by Edman degradation. Optical rotation measurements revealed that the D- and L-enantiomers had optical rotations of $[\alpha]^{24}_D +5.4^\circ$ and -5.7° , respectively. D- and L-*N*-methylammodendrine enantiomers were synthesized from the corresponding ammodendrine enantiomers, and their optical rotations established as $[\alpha]^{23}_D +62.4^\circ$ and -59.0° , respectively. A mouse bioassay was used to determine the difference in toxicity between these two pairs of naturally occurring enantiomers. The LD₅₀ of (+)-D-ammodendrine in mice was determined to be 94.1 ± 7 mg/kg and that of (–)-L-ammodendrine as 115.0 ± 7 mg/kg. The LD₅₀ of (+)-D-*N*-methylammodendrine in mice was estimated to be 56.3 mg/kg, while that of (–)-L-*N*-methylammodendrine was determined to be 63.4 ± 5 mg/kg. These results establish the rotation values for pure ammodendrine and *N*-methylammodendrine and indicate that there is little difference in acute murine toxicity between the respective enantiomers.

Stereochemical integrity is a significant factor in determining the specificity of biological effects in both natural products and synthetic compounds.¹ Numerous examples of differential activity have been reported, with one of the most notorious being the drug thalidomide, since the *R*-enantiomer has sedative effects whereas the *S*-enantiomer is teratogenic. Thalidomide was synthesized as a racemic mixture of both enantiomers, but purification to provide only the *R*-enantiomer for therapeutic purposes was not a solution because a liver enzyme exists that converts the *R*- to the *S*-enantiomer.² Many naturally occurring alkaloids have chiral centers and correspondingly diverse modes of action. A recent example is that of the antimalarial alkaloids febrifugine and isofebrifugine, for which the natural compounds were found to have much higher activity than their synthetic antipodes.³ When describing plant-associated toxins, it is therefore also essential to describe the stereochemical structure of any chiral centers and accurately determine the optical rotation in order to validate their inclusion in toxicity studies.

Ingestion of *Lupinus* species by pregnant cows at specific gestational periods can result in calves with cleft palate and front limb contractures, commonly known as crooked calf disease.^{4–6} Ammodendrine (**1**) (Figure 1), a piperidine alkaloid found in *Lupinus formosus*, has a chiral center and is a reported teratogen.^{7,8} The variability of previously reported optical rotation values for ammodendrine (Table 1) led us to believe that lupine plants could be producing enantiomeric mixtures of **1**.

Results and Discussion

Lupine plant specimens collected near the Rio Vista Airport, Solano County, California, and at SMIP Ranch,

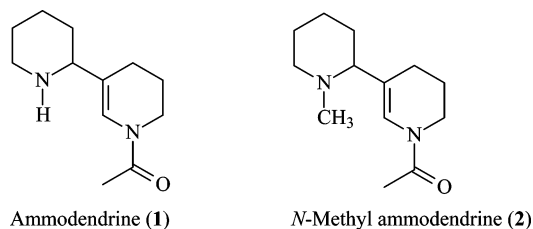


Figure 1. Chemical structures of ammodendrine (**1**) and *N*-methylammodendrine (**2**).

San Mateo County, California, were both characterized taxonomically as *Lupinus formosus* Greene (Leguminosae). GC-MS analysis of the alkaloids in the above-ground plant material from the Rio Vista and SMIP Ranch sites revealed significantly different alkaloid profiles (Figure 2). The chromatogram from the Rio Vista plant material shows that ammodendrine (**1**) and *N*-acetylhystrine (**3**) are the major piperidine alkaloids. Lower levels of quinolizidine alkaloids were also found in this plant material.⁷ *N*-Methylammodendrine (**2**) has also been found in this plant material in previous years.^{7–10} The chromatogram from the SMIP Ranch material shows that ammodendrine (**1**), *N*-acetylhystrine (**3**), and *N*-methylammodendrine (**2**) are the major piperidine alkaloids and that no quinolizidine alkaloids were detected in this plant material. Ammodendrine (**1**) was measured as 0.47% dry weight of the Rio Vista plant material and 0.63% dry weight of the SMIP Ranch plant material.

Ammodendrine (**1**) was isolated from *L. formosus* plant material collected in Solano County, California. The optical rotation of this ammodendrine isolate was $[\alpha]^{24}_D +2.9^\circ$, a value that did not compare well with previously reported measurements for this compound (Table 1). Ammodendrine was then isolated from *L. formosus* collected in San Mateo County, California, 10 days after the previous collection. The optical rotation of this isolate was $[\alpha]^{24}_D +1.8^\circ$, which

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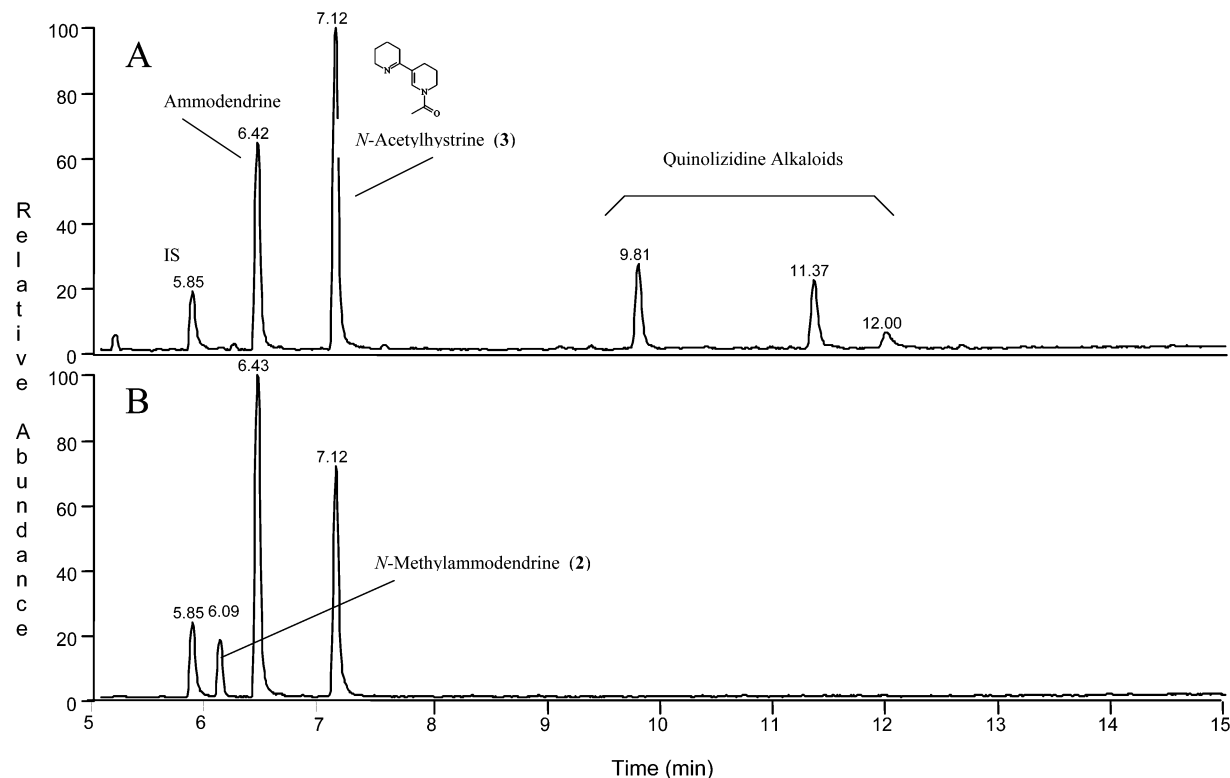
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Table 1. Optical Rotation and Toxicity Data for Ammodendrine (1) and *N*-Methylammodendrine (2)

compound	observed or reported optical rotations	LD ₅₀ ± CI ^a	plant source
D-ammodendrine ^b	[α] _D ²⁴ +5.4° (c 1.27, MeOH)	94.1 ± 6.8	<i>Lupinus formosus</i>
L-ammodendrine ^b	[α] _D ²⁴ −5.7° (c 1.66, MeOH)	115 ± 7.0	<i>L. formosus</i>
ammodendrine ¹¹	[α] _D ²⁴ +6.65° (c 3.9, EtOH)		<i>L. formosus</i>
ammodendrine ¹²	[α] _D ²⁴ +7.5° (c 0.22, EtOH)		<i>L. varius</i>
ammodendrine ¹³	[α] _D ²⁴ +7.1° (c 0.08, MeOH)		<i>L. varius</i>
ammodendrine ¹⁴	[α] _D ²³ −20° (c 0.004, CDCl ₃)		<i>Castilleja miniata</i>
ammodendrine ¹⁵	[α] _D ²⁴ +15° (EtOH)		
ammodendrine ¹⁶	[α] _D ²⁴ +11.8° (c 0.288, EtOH)		<i>L. hirsutus</i>
D- <i>N</i> -methylammodendrine ^b	[α] _D ²³ +62.4° (c 0.51, MeOH)	56.3	<i>L. formosus</i>
L- <i>N</i> -methylammodendrine ^b	[α] _D ²³ −59.0° (c 1.17, MeOH)	63.4 ± 4.7	<i>L. formosus</i>
<i>N</i> -methylammodendrine	[α] _D ²⁴ +40.5° (c 2.0, EtOH) ¹¹		<i>L. formosus</i>
<i>N</i> -methylammodendrine	[α] _D ²³ −44° (c 0.02, CDCl ₃) ¹³		<i>Castilleja miniata</i>

^a CI (95% confidence intervals). ^b This study.**Figure 2.** Gas chromatograms and alkaloid profiles of *Lupinus formosus* collected from (A) near the Rio Vista Airport and (B) SMIP Ranch.

was not in accord with the value reported for ammodendrine (+6.6°) from *L. formosus* collected at the same site 32 years previously.¹¹

Attempts to resolve the enantiomers by HPLC, using chiral columns, proved to be unsuccessful. We resorted to the conversion of the ammodendrine enantiomers into diastereomers by a peptide coupling reaction utilizing Fmoc-L-Ala-OH in the presence of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and hydroxybenzotriazole dissolved in *N,N*-dimethyl formamide (DMF) (Figure 3). The formation of Fmoc-L-Ala-ammodendrine (4) (MH⁺ 502) was monitored by atmospheric pressure chemical ionization–mass spectrometry (APCIMS). The conversion of 1 to 4 allowed reversed-phase HPLC analysis of the diastereomers (Figure S1) and confirmed that the ammodendrine enantiomers were present in both the Rio Vista Airport and SMIP Ranch *L. formosus* plants.

Treatment of the Fmoc-L-Ala-ammodendrine diastereomers (4) with piperidine in methylene chloride was monitored by electrospray ionization–mass spectrometry (ESIMS) for the formation of Ala-ammodendrine (5) (MH⁺ 280). The resultant L-Ala-ammodendrine diastereomers were separated from each other using reversed-phase

HPLC, and because of their earlier elution than their Fmoc analogues, this method was scaled up for the separation and isolation of milligram quantities of the respective diastereomers (Figure S2).

Conversion of the isolated diastereomers to their respective enantiomeric forms of ammodendrine (1) was accomplished by an Edman degradation procedure to remove the L-alanine. The progress of the reaction was monitored by ESIMS and indicated that the major portion of the reaction residue was ammodendrine (1) (MH⁺ 209). D- and L-ammodendrine were isolated from their respective reaction mixture using conventional silica gel column chromatography. Reversed-phase HPLC chromatography of the isolated ammodendrine enantiomers (1) after conversion to the Fmoc-L-Ala-ammodendrine (4) (Figure S3) established the D-ammodendrine enantiomer to be 91% and L-ammodendrine to be 98% enantiomerically pure.

The optical rotations of D-ammodendrine ([α]_D²⁴ +5.4°) and L-ammodendrine ([α]_D²⁴ −5.7°) are consistent with expected optical rotations of isolated paired enantiomers. However, these optical rotations differ from previously reported values (Table 1) where no measures were taken to ensure enantiomeric purity of the isolates. The greater

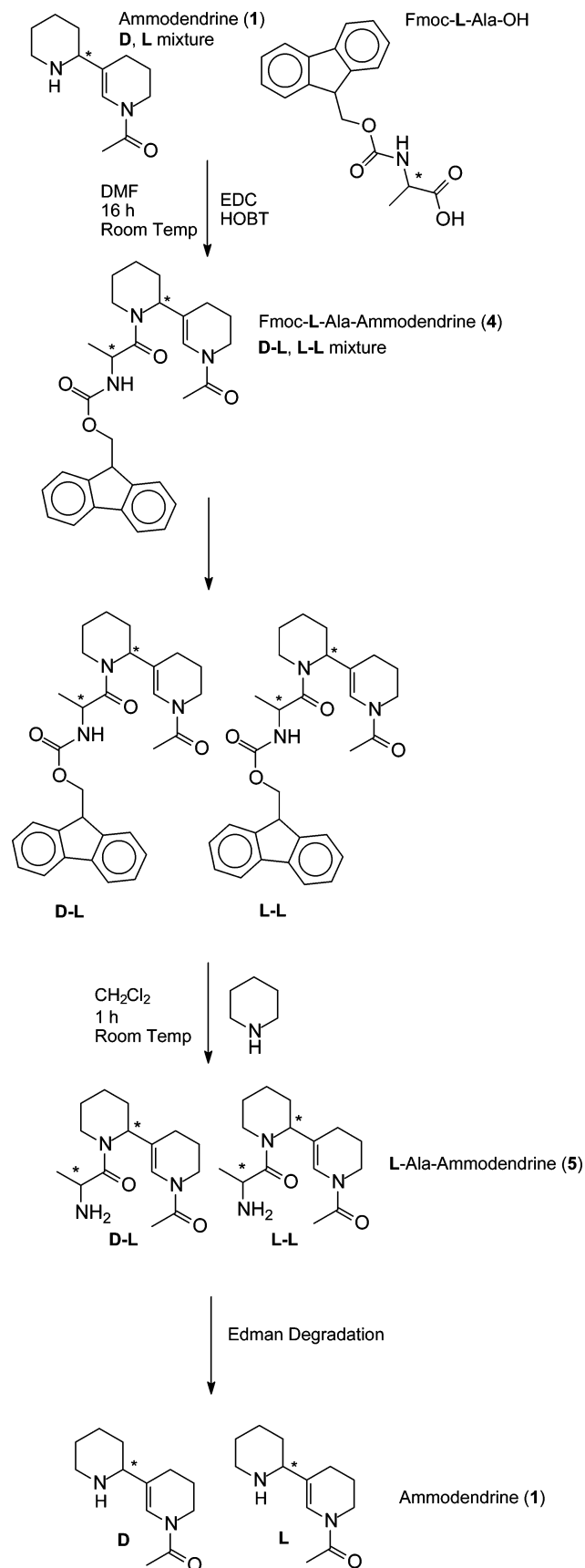


Figure 3. Conversion of ammodendrine (1) into diastereomers for separation and conversion of diastereomers back to enantiomerically pure ammodendrine isomers.

magnitude of the previously reported optical rotations than those reported in this study indicates that prior optical rotations for ammodendrine (1) are incorrect. The larger

rotations are possibly due to contamination of the samples by other alkaloids with higher rotation values, such as *N*-methyllummodendrine (2).

N-Methyllummodendrine (2) was detected in *L. formosus* collected from the SMIP Ranch and was present in previous collections of *L. formosus* from the Rio Vista site and has been found in other *Lupinus* species.^{7–10,17} We were therefore interested in determining the correct optical rotation values for the enantiomers of *N*-methyllummodendrine (2). The *N*-methyl derivatives were obtained by treating D- and L-ammodendrine (1) with iodomethane and were separated from their respective reaction mixtures using silica gel column chromatography.

The optical rotations of D-*N*-methyllummodendrine and L-*N*-methyllummodendrine were $[\alpha]_{\text{D}}^{23} +62.4^\circ$ and $[\alpha]_{\text{D}}^{23} -59.0^\circ$, respectively. As in the case of the ammodendrine enantiomers, these measurements are consistent for pairs of enantiomers and they are also substantially different from the measurements previously reported (Table 1).

Toxicity of ammodendrine (1) and *N*-methyllummodendrine (2) enantiomers was evaluated in a mouse bioassay. The LD_{50} of L-ammodendrine in mice was determined to be 115.0 ± 7.0 mg/kg, $n = 20$, and that of D-ammodendrine was 94.1 ± 7.8 mg/kg, $n = 23$. The LD_{50} of L-*N*-methyllummodendrine in mice was determined to be 63.4 ± 4.7 mg/kg, $n = 15$, and that of D-*N*-methyllummodendrine in mice was estimated to be 56.3 mg/kg (no CI), $n = 7$ (Table 1). The *N*-methyllummodendrine enantiomers were therefore appreciably more toxic than the ammodendrine isomers. Although there was a statistical difference ($P < 0.05$) in the measured toxicity between the D- and L-ammodendrine, the observed difference in physiological activity measured in the mouse bioassay was not as large as is often the case with enantiomers.^{1,3} It is possible that the receptor site responsible for toxicity is not highly chirally discriminatory. It is also possible that none of these compounds is the proximate toxin and that they are metabolized into compounds in which the asymmetric center is lost. This could occur either by isomerization of the existing double bond to a position between the two piperidine rings or by an oxidative process in the liver, resulting in the introduction of an additional double bond in the fully saturated piperidine ring. An analogy for the latter process exists with the pyrrolizidine alkaloids, which are not toxic per se but are converted to toxic dehydropyrrolizidines by P450 liver enzymes.¹⁸

It is recognized that the acute mouse toxicity model is not a surrogate for teratogenic crooked calf disease. Limitations in the amount of isolated enantiomeric material available only allowed us to investigate initially possible differences in toxicity based on a mouse model. However, a true measure of teratogenicity will need to be tested in a proper system such as a fetal goat model.¹⁹

In this study, the similarities in toxicity between the enantiomeric pairs of ammodendrine (1) and *N*-methyllummodendrine (2) indicate that, in the absence of evidence to the contrary, both enantiomers should be taken into account in predicting toxicity of plant samples to livestock, as has been done previously.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer model 241 polarimeter. Electrospray (ESI) and atmospheric pressure-chemical ionization (APCI) mass spectrometric data were acquired using an LCQ mass spectrometer (Finnigan, San Jose, CA). Samples were loop injected into the ESI or APCI source using a

methanol–20 mM ammonium acetate solution, 50:50 v/v, at a flow rate of 0.5 mL/min.

Chemicals and Reagents. Glacial acetic acid, ammonium hydroxide, *N,N*-dimethyl formamide (DMF), and sulfuric acid were purchased from Fisher Scientific (Pittsburgh, PA). Hydroxybenzotriazole, phenyl isothiocyanate (99%), piperidine (99.5%), and silica gel (70–230 mesh, 60 Å) for column chromatography were obtained from Aldrich Chemical (Milwaukee, WI). (L)-Fmoc-Ala-OH and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride were obtained from Fluka Chemical (Ronkonkoma, NY). Trifluoroacetic acid was from EM Science (Gibbstown, NJ), sodium sulfate from Baker (Phillipsburg, NJ), chloroform from Mallinckrodt Baker (Paris, KY), and ammonium acetate from VWR (Bristol, CT).

Plant Material. Lupine plant material was collected near Rio Vista Airport, Solano County, CA (38°10.50' N/121°44.38' W; elevation 7.9 m), on June 25, 2003, and at SMIP Ranch, San Mateo County, CA (37°21.66' N/122°17.87' W; elevation, 398 m), on July 2, 2003. The plant was in the flowering stage on these dates, and the samples consisted of the whole plant except for roots. The plant specimen collected near the Rio Vista Airport, accession #239432, and the plant specimen collected at the SMIP Ranch, accession #239414, were both taxonomically classified as *Lupinus formosus* by staff at the Intermountain Herbarium, Utah State University, where the voucher specimens are retained.

Extraction and Isolation of Lupine Alkaloids. Aerial plant material was air-dried and ground to pass through a 2 mm screen. The plant material (366.7 g) was extracted by steeping at room temperature for 16 h in methanol (4 × 4 L), the methanol extracts were combined, and the methanol was removed via rotary evaporation, leaving a dark green residue. The residue was first partitioned between 1% aqueous H₂SO₄ (2 L) and CHCl₃ (2 × 2 L). The CHCl₃ was discarded. The aqueous portion was made basic to pH 9, with the addition of NH₄OH, and then extracted with CHCl₃. The CHCl₃ extracted from the basic aqueous portion was combined, dried with anhydrous Na₂SO₄, filtered, and rotary evaporated to dryness. Ammodendrine (1) was isolated by chromatography on a 40 cm × 3.5 cm silica gel column using a mobile phase of CHCl₃–MeOH–NH₄OH (65:35:1, v/v/v).

Synthesis of Ammodendrine Diastereomers. Ammodendrine (1) (106.2 mg, 0.510 mmol), Fmoc-L-Ala-OH (182.0 mg, 0.553 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (128.5 mg, 0.670 mmol), and hydroxybenzotriazole (90.5 mg, 0.670 mmol) were weighed into a round-bottomed flask (10 mL) with a magnetic stirbar; *N,N*-dimethylformamide (DMF) (7 mL) was then added. The reaction was stirred under N₂ at RT for 16 h. DMF was evaporated with compressed air, the reaction mixture was dissolved in CHCl₃, and the organic layer was washed (2×) with distilled deionized water. The CHCl₃ phase was then dried over anhydrous Na₂SO₄, filtered, and rotary evaporated to an oily residue. The residue was analyzed using APCIMS, and the mass spectrum indicated that ammodendrine (1) had reacted to form Fmoc-L-Ala-ammodendrine (4) (MH⁺ 502). Based on reversed-phase HPLCMS analysis, approximately 50% of the ammodendrine (1) was consumed to form Fmoc-L-Ala-ammodendrine: HRESIMS *m/z* [M + Na]⁺ found, 524.2506, calcd for C₃₀H₃₃N₃O₄Na, 524.2525.

Plant samples and chiral isolates were derivatized by the procedure above and analyzed by HPLC using a 100 mm × 2 mm i.d., 5 μm, Betasil C₁₈ column (Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phase was 20 mM ammonium acetate–methanol (45:55, v/v) at a flow rate of 0.5 mL/min. The detector was a Finnigan LCQ mass spectrometer operating in the APCI mode.

The Fmoc portion of L-Fmoc-Ala-ammodendrine (4) was cleaved by treatment of 4 with a 25% solution of piperidine (1 mL) in methylene chloride (3 mL) for 1 h at RT. The solvent was evaporated from the reaction mixture with a gentle flow of N₂ and heat at 65 °C. The reaction mixture was dissolved in 1% H₂SO₄ and CHCl₃, and the aqueous layer was washed (3×) with CHCl₃ and then made basic to pH 9 with NH₄OH.

The basic aqueous layer was then extracted (3×) with CHCl₃. The CHCl₃ extracts from the basic aqueous portion were combined, dried with anhydrous Na₂SO₄, filtered, and rotary evaporated to dryness. The residue was sampled by flow injection ESIMS. The mass spectrum indicated that the residue was Ala-ammodendrine (5) (MH⁺ 280) and that this reaction had proceeded to completion, as Fmoc-Ala-ammodendrine (4) was not present in the mass spectrum: HRESIMS *m/z* [M + H]⁺ found, 280.2022; calcd for C₁₅H₂₆N₃O₂, 280.2025.

The Ala-ammodendrine diastereomers (5) were separated using a preparative scale, 250 mm × 21.2 mm i.d., 5 μm, Betasil C₁₈ HPLC column (Thermo Hypersil-Keystone). The mobile phase was 20 mM ammonium acetate–methanol (80:20, v/v) at a flow rate of 25 mL/min. Two major peaks eluted, and they were collected and combined with the corresponding peaks in subsequent runs. The mobile phase was evaporated to <500 mL and made basic to pH 9 with NH₄OH. The basic aqueous layer was then extracted with equal volumes of chloroform (3×). The CHCl₃ extracts were combined, dried with anhydrous Na₂SO₄, filtered, and rotary evaporated to dryness.

The Ala-ammodendrine (5) diastereomers were analyzed using HPLC using an analytical Betasil C₁₈ column with a 20 mM ammonium acetate–methanol mobile phase (80:20, v/v) at a flow rate of 0.5 mL/min; mass spectrometry in the ESIMS mode was used for detection.

Edman Degradation. The L-alanine portion of the Ala-ammodendrine (5) diastereomers was removed via Edman degradation. Each Ala-ammodendrine diastereomer (134.5 mg, 0.481 mmol) was treated with a methanol–water–triethylamine–phenylisothiocyanate (3.5:0.5:0.5:0.5, v/v/v/v) solution with stirring at 50 °C. After 1.5 h, trifluoroacetic acid (0.5 mL) was added and the reaction was stirred again at 50 °C for an additional 1.5 h. The solvents from the reaction were evaporated under a stream of N₂ at 65 °C. The reaction mixture was partitioned in 1% H₂SO₄ and CHCl₃. The CHCl₃ was discarded. The aqueous portion was made basic to pH 9, with the addition of NH₄OH, and then extracted with CHCl₃. The CHCl₃ extracted from the basic aqueous portion were combined, dried with anhydrous Na₂SO₄, filtered, and rotary evaporated to dryness. The residue was analyzed using ES-IMS, and the resultant mass spectrum indicated that the major portion of the residue was ammodendrine (1) (MH⁺ 209). The chiral ammodendrine (1) was isolated by chromatography on a 40 cm × 2.3 cm silica gel column using a mobile phase of CHCl₃–MeOH–NH₄OH (65:35:1, v/v/v). Finally, 1 was cleaned by acid/base extraction using 1% aqueous H₂SO₄ and extracting with CHCl₃, and then the aqueous portion made basic to pH 9 with the addition of NH₄OH and extracted with CHCl₃. These extracts were combined, dried with anhydrous Na₂SO₄, filtered, and rotary evaporated to dryness to give the pure compound (80.6 mg, 0.388 mmol, yield 80.6%). No isomerization of the chiral ammodendrine (1) occurred using these acid and base conditions.

Synthesis of D- and L-N-Methylammodendrine. D- or L-ammodendrine (1) (38.9 mg, 0.187 mmol) was weighed into a 1 mL Reactivial (Pierce, Rockford, IL) with a magnetic triangular stirbar. Acetone (200 μL) and iodomethane (27 μL) were added and the reaction was stirred at 57 °C for 1 h. The acetone was evaporated off under a stream of N₂ at 60 °C, the reaction mixture was dissolved in CHCl₃, and the organic layer was washed with 10% aqueous NaHCO₃. The CHCl₃ layer was then dried with anhydrous Na₂SO₄ and evaporated to dryness with N₂ at 60 °C. *N*-Methylammodendrine (2) was separated from the reaction contaminants using silica gel chromatography on a 35 cm × 1.25 cm column with a mobile phase of CHCl₃–MeOH–NH₄OH (65:35:1, v/v/v) to give 12.1 mg, 0.0544 mmol, yield 29%.

Mouse Bioassay. Known amounts of the individual alkaloids were dissolved in physiological buffered saline solution. The solutions were stored in sterile injection vials for toxicity testing.

Weanling White Swiss-Webster male mice, 15 to 20 g (Simonsen Labs, Gilroy, CA), were weighed after a 12 h fast and were dosed intravenously. Injections were performed via the tail vein in mice restrained in a plastic mouse block. The

mice were maintained under a heat lamp for 15 min to dilate the tail vein. The tail was cleaned with 70% ethanol, and i.v. injections were accomplished with a tuberculin syringe equipped with a 1.27 cm long 27-gauge needle. The volume injected varied depending on the dosage delivered. Time of injection, clinical effects, and time of death were noted and recorded.

The LD₅₀ for individual alkaloid toxicity was determined using a modified up-and-down method²⁰ and was calculated using the PROC PROBIT procedures of SAS (SAS Institute, Cary, NC) on a logistic distribution of the survival data. Confidence (fiducial) intervals (95%) were also calculated using the same program.

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Supporting Information Available: Figure S1 shows reconstructed HPLC ion chromatograms comparing ammodendrine-based diastereomers from *L. formosus* plant material collected at the Rio Vista and SMIP Ranch sites. Figures S2 and S3 show reconstructed ion chromatograms for L-Ala-ammodendrine (**5**) and Fmoc-L-Ala-ammodendrine diastereomers (**4**) isolated from *L. formosus* plant

material. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Brossi, A. *Med. Res. Rev.* **1994**, *14*, 665–691.
- (2) Eriksson, T.; Bjorkman, S.; Hoglund, P. *Eur. J. Clin. Pharmacol.* **2001**, *57*, 365–376.
- (3) Kobayashi, S.; Ueno, M.; Suzuki, R.; Ishitani, H.; Kim, H. S.; Wataya, Y. *J. Org. Chem.* **1999**, *64*, 6833–6841.
- (4) Shupe, J. L.; James, L. F.; Binns, W. J. *Am. Vet. Med. Assoc.* **1967**, *151*, 191–197.
- (5) Shupe, J. L.; Binns, W.; James, L. F.; Keeler, R. F. *J. Am. Vet. Med. Assoc.* **1967**, *151*, 198–203.
- (6) Shupe, J. L.; Binns, W.; James, L. F.; Keeler, R. F. *Aust. J. Agric. Res.* **1968**, *19*, 335–340.
- (7) Keeler, R. F.; Panter, K. E. *Teratology* **1989**, *40*, 423–432.
- (8) Panter, K. E.; Gardner, D. R.; Molyneux, R. J. *J. Nat. Toxins* **1998**, *7*, 131–140.
- (9) Wink, M.; Witte, L. Z. *Naturforsch.* **1987**, *42C*, 197–204.
- (10) Gardner, D. R.; Panter, K. E. *J. Nat. Toxins* **1994**, *3*, 107–116.
- (11) Fitch, W. L.; Dolinger, P. M.; Djerassi, C. *J. Org. Chem.* **1974**, *39*, 2974–2979.
- (12) Abel-Halim, O. B.; El-Gammal, A. A.; Abdel-Fattah, H.; Takeya, K. *Phytochemistry* **1999**, *51*, 5–9.
- (13) Mohamed, M. H.; Hassanean, H. A. *Phytochemistry* **1997**, *46*, 365–369.
- (14) McCoy, J. W.; Stermitz, F. R. *J. Nat. Prod.* **1983**, *46*, 902–907.
- (15) *Dictionary of Natural Products on CD-ROM*, version 7.2; Chapman and Hall: London, 1999.
- (16) Takamatsu, S.; Saito, K.; Sekine, T.; Ohmiya, S.; Kubo, H.; Otomasu, H.; Murakoshi, I. *Phytochemistry* **1990**, *12*, 3923–3926.
- (17) Panter, K. E.; Mayland, H. F.; Gardner, D. R.; Shewmaker, G. *Vet. Human Toxicol.* **2001**, *43*, 279–282.
- (18) Mattocks, A. R. *Chemistry and Toxicology of Pyrrolizidine Alkaloids*; Academic Press: Orlando, FL, 1986.
- (19) Panter, K. E.; Bunch, T. D.; Keeler, R. F.; Sisson, D. V.; Callan R. J. *J. Clin. Toxicol.* **1990**, *28*, 69–83.
- (20) Bruce, R. *Fundam. Appl. Toxicol.* **1987**, *8*, 97–101.

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