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Lupine Induced "Crooked Calf Disease" in Washington and Oregon: Identification of the Alkaloid Profiles in *Lupinus sulfureus, Lupinus leucophyllus,* and *Lupinus sericeus*

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Several lupines (*Lupinus spp.*) present on western U.S. rangelands contain alkaloids that are teratogenic to livestock and cause congenital birth defects in calves (crooked calf disease). Periodically, large losses of calves due to lupine-induced "crooked calf disease" occur in northern Oregon and eastern Washington state. Five lupine populations from this area representing three species (*L. leucophyllus, L. sulfureus, and L. sericeus*) were evaluated taxonomically and by gas chromatography/mass spectrometry, and the major alkaloids in each lupine species were identified. The teratogenic alkaloid anagyrine was present in both of the lupine species responsible for the high outbreaks in east-central Washington and northeastern Oregon. However, the alkaloid profiles of the two lupines identified as *L. leucophyllus* were dissimilar, as were the alkaloid profiles of the two lupines identified as *L. sulfureus*. Botanical classification is not sufficient to determine potential teratogenicity, and it must be followed by chemical characterization to determine risk to livestock.

KEYWORDS: Lupine; anagyrine; crooked calf disease; *Lupinus leucophyllus*; *Lupinus sulfureus*

INTRODUCTION

Lupines (*Lupinus* spp.) are a common plant species on western U.S. rangelands. Several species contain alkaloids that can be toxic and/or teratogenic to livestock and cause economic losses to cattle producers. Historically, acute intoxication from lupines was a major cause of sheep deaths (*1*). In the winter of 1898–1899, thousands of sheep died in the Judith Basin of Montana from feeding lupine hay that had been harvested with a large number seedpods. In one flock alone, 3600 of 7000 sheep died from eating lupine hay (*2*). Isolated cases where 80–100 sheep die from lupine poisoning still occur on rangelands heavily infested with lupine when the risk of lupine poisoning is high (personal communications).

Ingestion of lupine by cattle was first reported to cause congenital birth defects in calves (crooked calf disease) in the late 1950s (*3–5*). "Crooked calf disease" was described as a condition in which calves were born with a variety of deformities such as arthrogryposis, scoliosis, kyphosis, torticollis, and cleft palate (**Figure 1**) (*6–8*). The principle time of insult was identified as the 40–70th days of gestation and may extend to as late as day 100 (*6, 7*). The quinolizidine alkaloid anagyrine (*9*) and some piperidine alkaloids (*10, 11*) were shown to reduce fetal movement during this critical period of gestation (*12, 13*), causing the spine and limbs to develop in contracted or misaligned positions. Cattle losses due to lupine-induced crooked calf disease in several western states continue to the present.

Lupines are present in almost all rangelands in the Channel Scablands region of east-central Washington state. In this area, ranchers operate under the assumption that 1–5% of the calves born each year will be "crooked calves" (*14, 15*). However, periodically there are years with high incidences of "crooked calves". In 1997, approximately 4000 calves in Adams County alone were born with skeletal defects, most of which were so severe the affected calves had to be euthanized (*16*). In the same year, 13 specific ranches were surveyed and 628 of 2210 newborn calves (28%) were severely deformed and were euthanized. The incidence of calf losses ranged from 0 to 100% on these ranches (*14, 15*).

Lupinus leucophyllus Douglas ex. Lindl. (Velvet lupine), *Lupinus sulfureus* Douglas ex. Douglas Lindl. (Sulfur lupine), and *Lupinus sericeus* Pursh (Silky lupine) are all present in Adams County (**Figure 2A–C**). *L. leucophyllus* is widespread in Adams County and was determined to be the cause of the

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Figure 1. 18-Month old steer showing arthrogryposis typical of lupine-induced crooked calf disease born in 2006 to a cow grazing lupine-infested rangelands in Adams County, WA.

birth defects because it contained the teratogen anagyrine and was available to pregnant cows during the susceptible days of gestation (unpublished data). *L. sericeus* is also widespread in Adams County but does not contain anagyrine. *L. sulfureus* is present in isolated locations in Adams County and contains the reported teratogen ammodendrine. However, the pastures with *L. sulfureus* also contained *L. leucophyllus*, and while "crooked calves" also occurred on these pastures, it was not determined if *L. sulfureus* contributed to the incidence.

In the Fall of 1992, lupine-induced "crooked calf disease" occurred in a single herd of cows in Umatilla County in northeastern Oregon approximately 16 km east of Pendleton. Sixty seven calves from 131 cows (51%) were born with congenital skeletal malformations and/or cleft palates (*17*). *L. leucophyllus* and *L. sulfureus* were both present on the ranch (**Figure 2D** and **E**). However, *L. sulfureus* was determined to cause birth defects because it contained anagyrine while the *L. leucophyllus* did not contain anagyrine or any known teratogens (*17*). In addition, the breeding records revealed the cows were grazed on a pasture containing abundant *L. sulfureus* in the seedpod stage during the gestational period of 21–100 days. Futhermore, 70% of the calves born with "crooked calf disease" were born to cows that were grazed on the pasture during 60–80 days of gestation (*17*).

In this study, the lupine species present in Adams County, WA, and in Umatilla County, OR, were analyzed by gas chromatography/mass spectrometry (GC/MS) and the major alkaloids in each of the lupines were identified. Alkaloid content and composition were correlated with teratogenic potential and similarities and differences between lupine species.

MATERIALS AND METHODS

Chemicals and Reagents. Ammonium hydroxide was purchased from Fisher Scientific (Pittsburgh, PA). Sodium sulfate was from Baker (Phillipsburg, NJ), and chloroform was from Mallinckrodt Baker (Paris, KY). Caffeine and sparteine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, and Milwaukee, WI). Lupanine was obtained from Biomedical Research Co. (Los Angeles, CA). D-α-Isolupanine perchlorate was purchased from Koch-Light Laboratories Ltd. (Colnbrook, Buckinghamshire, England).

Plant Material. *L. leucophyllus*, accession no. 00237602, was collected near Ritzville, Adams County, WA (46° 50′68″ N/118° 3′83″ W), on July 17, 2006. A second *L. leucophyllus*, accession no. 00247372, was collected near Pendleton, Umatilla County, OR (45° 37′26″ N/118° 37′62″ W), on July 17, 2006. *L. sulfureus*, accession no. 00247073, was collected near Ritzville, Adams County, WA (47° 14′17″ N/118° 15′28″ W), on May 17, 2006. A second *L. sulfureus*, accession no. 00247072, was collected near Pendleton, Umatilla County, OR (45° 36′01″ N/118° 37′04″ W), on May 18, 2006. *L. sericeus*, accession no. 00247074, was collected near Ritzville, Adams County, WA (46° 55′04″ N/118° 5′05″ W), on May 16, 2006. The plants were in the flowering stage on these dates, and the samples consisted of above ground plant material (aerial parts). The *L. sulfureus*, *L. leucophyllus*, and *L. sericeus* plant specimens were taxonomically classified by staff at the Intermountain Herbarium, Utah State University, where the specimens are retained. A bulk collection of *L. leucophyllus* was collected near Ritzville, Adams County, WA (47° 56′45″ N/118° 13′82″ W), on June 6, 2002. *L. argenteus* was collected along the south side of State Highway 14, 3.1 km east of County Road 30, Jackson County, CO (40° 36′25″ N/106° 05′21″ W), on October 19, 2006.

Alkaloid Extractions. *Large Scale.* Plant material (*L. leucophyllus*, Ritzville, WA; bulk collection) was air-dried and ground to pass a 2 mm screen. The plant material (200 g) was extracted three successive times by steeping at room temperature for 16 h in methanol (3 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 H_2SO_4 (225 mL) and $CHCl_3$ (2 × 225 mL). The $CHCl_3$ layer was discarded. The aqueous portion was made basic to pH 9, with the addition of NH_4OH (14.8 N), and then extracted with $CHCl_3$ (2 × 225 mL). The $CHCl_3$ extracts from the basic aqueous portion were combined with each other, dried with anhydrous Na_2SO_4, filtered, and rotary evaporated, resulting in a gummy brown residue (2.4 g).

Small Scale. Alkaloid analysis of plant material from the *L. leucophyllus* collections made in July 2006 and the *L. sulfureus* and *L. sericeus* collections made in May 2006 was determined using a previously reported procedure (*18*) with minor modifications. Aerial plant material was frozen on dry ice immediately after collection, freeze-dried, and ground to pass a 2 mm screen. For each sample, 100 mg was weighed into a 16 mL screw-top glass test tube. The plant material was extracted by mechanical rotation with a mixture of 1 N HCl (4.0 mL) and $CHCl_3$ (4.0 mL) for 15 min. The samples were centrifuged (5 min), and the acidic aqueous layer was removed. An additional 2.0 mL of 1 N HCl was added to the test tube containing plant material and $CHCl_3$, and this mixture was extracted again by mechanical rotation (15 min), centrifugation, and acidic aqueous layer removal. The acidic aqueous portions were combined into a clean 16 mL screw-top glass test tube. The pH of the aqueous layer was adjusted to 9.0–9.5 with concentrated NH_4OH (14.8 N). The basic solution was extracted twice with $CHCl_3$: first with 4.0 mL and then with 2.0 mL. The $CHCl_3$ solutions were combined and filtered through anhydrous Na_2SO_4 into clean 16 mL screw-top glass test tubes, and the solvent was evaporated under N_2 at 60 °C. The alkaloid fraction extracted was reconstituted in 4 mL of methanol containing 1.3 μg/mL of caffeine (internal standard). A portion (~1 mL) was transferred to 1.5 mL autosampler vials for GC/MS analysis.

GC/MS Analysis. Samples (2 μL) were analyzed by GC/MS using a Finnigan MAT GCQ equipped with a split/splitless injector and a 30 m × 0.25 mm i.d. DB-5MS (J&W Scientific, Folsom, CA) column. The injection port temperature was 250 °C, and the instrument was operated in the splitless mode. The split vent flow rate was 50 mL/min, and the column was purged after 0.80 min. The oven temperature was 100 °C for 1 min, was increased to 100–200 at 40 °C/min, was increased to 200–275 at 5 °C/min, and was held at 275 °C for 1.5 min. Electron impact ionization (EI) at 70 eV was used with an ion source temperature of 200 °C. Chemical ionization mass spectra were collected using methane gas to confirm molecular ions when necessary. The detector scanned the mass range *m/z* 50–650.

High Performance Liquid Chromatography. *Semi-Preparative HPLC.* Select alkaloid peaks were separated and isolated using a Shimadzu LC-20AT high performance liquid chromatograghy (HPLC) system equipped with a Shimaduzu-HTA autosampler and a SPD-M20A diode array detector (λ 200–400 nm) and using a 250 mm × 10 mm

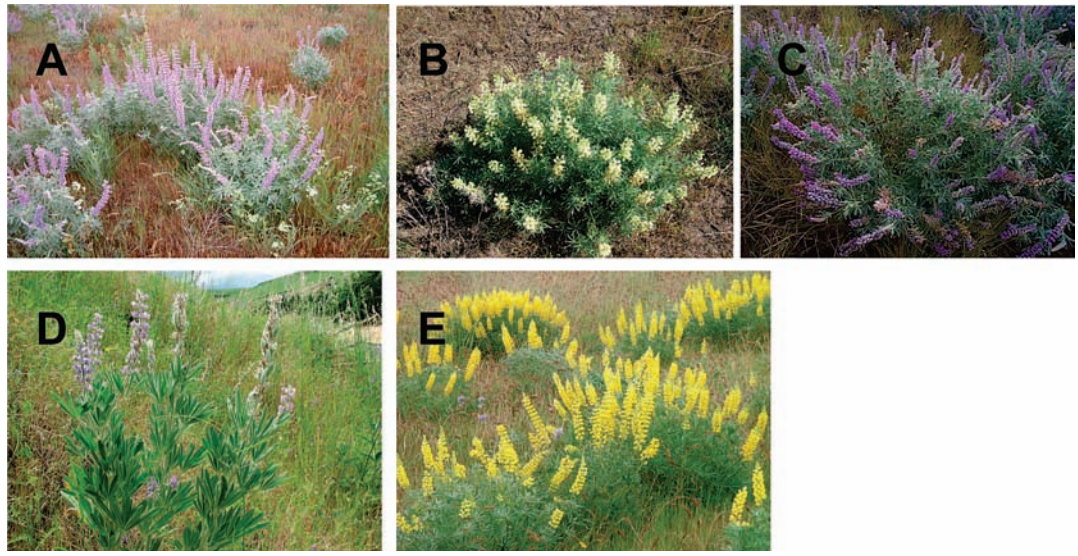

Figure 2. Photographs of (**A**) *L. leucophyllus*, (**B**) *L. sulfureus*, and (**C**) *L. sericeus* from Adams County, WA, and (**D**) *L. leucophyllus* and (**E**) *L. sulfureus* from Umatilla County, OR.

i.d., 5 μm semipreparative scale Microsorb Dynamax C_{18} HPLC column with a 50 mm × 10 mm i.d. guard column containing the same stationary phase (Rainin Instument Co., Woburn, MA). Alkaloids were eluted from the column with a mobile phase of 20 mM ammonium acetate in water (solvent A) and methanol (solvent B). The mobile phase program (3.00 mL/min) was started at a composition of 50% A for 15 min, followed by a linear gradient to 20% A from 15 to 30 min, and then it was held at 20% A from 30 to 35 min. The HPLC system was re-equilibrated for 10 min at the initial mobile phase composition of 50% A prior to the next injection. Using these HPLC conditions, six peaks eluted, were collected, and were combined with the corresponding peaks in subsequent runs. These fractions could then be directly analyzed by analytical scale reversed phase HPLC/MS.

Analytical HPLC/MS. Analysis of the peaks isolated from semipreparative HPLC alkaloids was accomplished using analytical scale reversed phase HPLC/MS. A 10 μL aliquot was injected onto a 100 mm × 2.1 mm i.d. Betasil C8 reversed phase column (Thermo Electron Corporation, Waltham, MA) protected by a guard column of the same stationary phase. The alkaloids were eluted from the column with a gradient flow (0.250 mL/min) of 20 mM ammonium acetate in water (solvent A) and methanol (solvent B) delivered by a Hewlett-Packard 1100 series binary HPLC pump. The mobile phase program was started at a composition of 70% A and then changed by linear gradient to 30% A over 15 min. The HPLC system was re-equilibrated for 15 min at the initial mobile phase composition of 70% A prior to the next injection. Detection was performed using a ThermoFinnigan (San Jose, CA) LCQ ion trap mass spectrometer. Ionization was achieved using an atmospheric pressure chemical ionization (APCI) source with a vaporizer temperature of 450 °C and a corona discharge current of 5 μamps. The capillary inlet temperature and voltage were 200 °C and 15 V.

Alkaloid Identifcations. Five individual alkaloids were identified from commercially obtained standards (sparteine, lupanine, and D-α-isolupanine) and authenticated (MS, NMR) samples of ammodendrine and anagyrine from the alkaloid collection of the Poisonous Plants Research Laboratory, USDA, ARS, Logan, UT. The remaining alkaloids were determined from correlation of measured retention times to retention indices (RI) calculated by linear extrapolation from RI values generated from known standards and assigned RI numbers from the literature and their CI and EI mass spectra (**Figure 3**) (*19*). Alkaloids were also determined by correlation of measured relative retention times (RR_t) to those of lupanine and by correlation of EI mass spectra to those reported in the literature (*20*).

RESULTS AND DISCUSSION

Identification of Alkaloids. *L. leucophyllus.* GC/MS analysis of *L. leucophyllus* plants collected near Ritzville, WA, resulted in six major peaks (**Figure 4A**). Peak 2 was determined to be 5,6-dehydrolupanine (**2**) based on RI, RR_t, and EI mass spectra data (**Table 1**). Peak 1 is unknown at this time; however, it is suspected to be an isomer of 5,6-dehydrolupanine (**2**) based on mass spectra data and the close retention time to that of 5,6-dehydrolupanine (**2**). Peaks 3 and 6 were confirmed as lupanine (**3**) and anagyrine (**6**), respectively, by comparison with standards. Peaks 4 and 5 were identified by extracting 200 g of dry *L. leucophyllus* plant material collected near Ritzville, WA, and isolating the total alkaloid fraction. Semipreparative reversed phase HPLC was used to isolate peaks 4 and 5 from the other alkaloids in the extract. These were each analyzed by GC/MS, which resulted in chromatograms of single peaks eluting at the same retention times and with the same mass spectra as those of peaks 4 and 5 in the total alkaloid fraction. However, when peak 4 was analyzed by analytical scale reversed phase HPLC/APCI/MS, two separate peaks were observed with the same base ion at *m/z* 279 (MH^+). Similarly, when peak 5 was analyzed by reversed phase HPLC/APCI/MS, two separate peaks were again observed with the same base ion (MH^+ at *m/z* 263). Peak 4 was the same molecular weight and exhibited the same behavior as a mixture of the interconvertible epimeric carbinolamides (2 *R*)-hydroxyaphyllidine (**4**) (also known as (–)-argyrolobine) and (2*S*)-hydroxyaphyllidine (**4**), while peak 5 was also the same molecular weight and exhibited the same behavior as a mixture of the interconvertible epimeric carbinolamides of (2*R*,9*R*)-dihydroxyaphyllidine (**5**) and (2*S*,9*R*)-dihydroxyaphyllidine (**5**) that have been previously reported in *L. argenteus* (*21*). The GC/MS analysis of *L. argenteus* collected from the same site described by Arslanian et al. (*21*) resulted in the presence of peaks 4 and 5 and confirmed the identities of peak 4 as a mixture of (2*R*)-hydroxyaphyllidine (**4**) and (2*S*)-hydroxyaphyllidine (**4**) and peak 5 as a mixture of (2*R*,9*R*)-dihydroxyaphyllidine (**5**) and (2*S*,9*R*)-dihydroxyaphyllidine (**5**).

L. leucophyllus collected near Pendleton, OR, contained nine major GC/MS peaks (**Figure 4B**; **Table 1**). Peaks 3 and 9 were determined to be lupanine (**3**) and α-isolupanine (**9**), respectively, by comparison with standards. The remaining peaks 7,

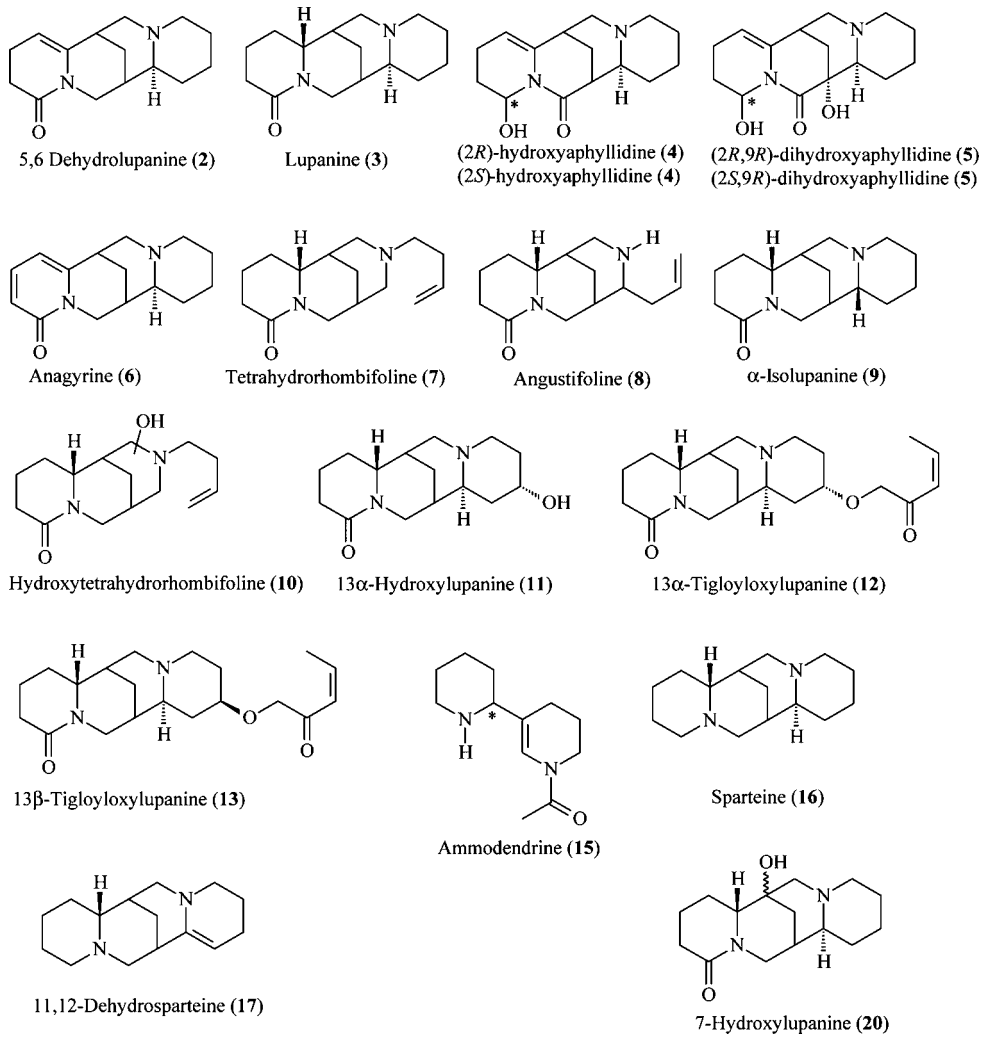

Figure 3. Structures of alkaloids identified by GC/MS in lupine species.

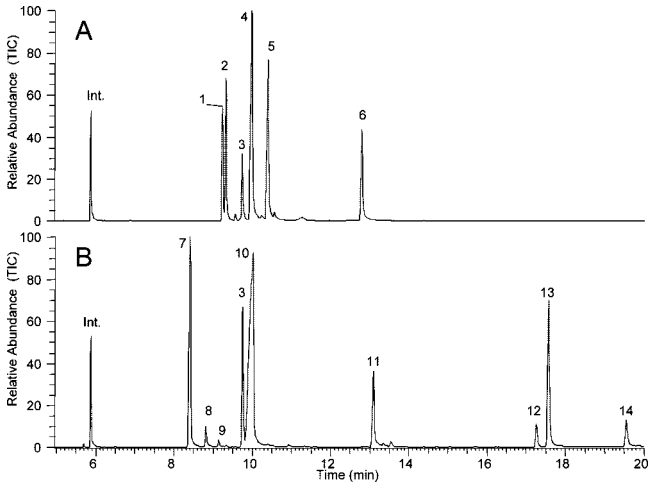

Figure 4. GC/MS total ion chromatograms of alkaloids from (**A**) *L. leucophyllus* collected near Ritzville, WA, and (**B**) *L. leucophyllus* collected near Pendleton, OR.

8, 10, 11, 12, and 13 were determined to be tetrahydrorhombofoline (**7**), angustifoline (**8**), hydroxytetrahydrorhombofoline (**10**), 13α-hydroxylupine (**11**), 13α-tigloyloxylupanine (**12**), and 13*β*-tigloyloxylupanine (**13**), respectively, based on RI, RR_t, and EI and CI/MS data. Low molecular ion abundances for tetrahydrorhombofoline (**7**), angustifoline (**8**), hydroxytetrahydrorhombofoline (**10**), 13α-tigloyloxylupanine (**12**), and 13*β*-tigloyloxylupanine (**13**) with GC/EI/MS conditions necessitated the use of GC/CI/MS to positively confirm the molecular ions for these compounds. One major peak, 14, remained unknown; however, its retention time, molecular ion, and fragmentation pattern suggested that it is tigloyloxylupanine with a hydroxyl moiety.

L. sulfureus. GC/MS analysis of *L. sulfureus* plants collected near Ritzville, WA, resulted in a single peak (**Figure 5A**; **Table 1**). This peak (peak 15) was confirmed to be ammodendrine (**15**) based on comparison with a standard.

L. sulfureus collected near Pendleton, OR, contained three major GC/MS peaks (**Figure 5B**; **Table 1**). Peak 2 was determined to be 5,6-dehydrolupanine (**2**) based on RI, RR_t, and EI mass spectra. Peaks 3 and 6 were determined to be lupanine (**3**) and anagyrine (**6**), respectively, by comparison with standards.

L. sericeus. GC/MS analysis of *L. sericeus* collected near Ritzville, WA, had six major peaks (**Figure 6**; **Table 1**). Peaks 16 and 3 were determined to be sparteine (**16**) and lupanine (**3**), respectively, based on comparison with standards. Peaks 17 and 20 were determined to be 11,12-dehydrosparteine (**17**) and 7-hydroxylupanine (**20**), respectively, based on RI, RR_t, and EI mass spectra. Peaks 18 and 19 remain unknown.

Three major lupine species, *L. leucophyllus*, *L. sulfureus*, and *L. sericeus*, are present in Adams County, WA, where losses of calves due to "crooked calf disease" have occurred in the past and continue to occur (*14*, *15*). *L. leucophyllus* and *L.*

Table 1. Identification of Lupine Alkaloids by GC/MS

peak	RI DB-5	CI/MS $[M + H]^+$	M^+	other fragments (relative abundance) (*m/z*)
5,6-dehydrolupanine isomer (**1**)	2102		246 (100)	245 (98), 218 (44), 217 (20), 190 (20), 189 (34), 163 (21), 162 (18), 148 (24), 136 (22), 135 (37), 134 (49), 120 (19), 108 (18), 98 (84), 97 (67), 96 (43)
5,6-dehydrolupanine (**2**)	2109		246 (76)	245 (29), 189 (11), 163 (9), 162 (12), 148 (17), 136 (11), 135 (18), 134 (28), 120 (16), 98 (100), 97 (53)
lupanine (**3**)	2165	249	248 (34)	247 (30), 219 (6), 150 (33), 149 (60), 148 (34), 136 (100), 134 (33), 112 (10), 110 (17), 108 (11), 98 (16), 97 (13)
(2*R*)-hydroxyaphyllidine (**4**) (2*S*)-hydroxyaphyllidine (**4**)	2185		262 (100)	261 (44), 244 (53), 243 (43), 219 (23), 216 (59), 205 (61), 136 (32), 134 (38), 122 (23), 110 (26), 106 (25), 98 (99), 97 (83)
(2*R*,9*R*)-dihydroxyaphyllidine (**5**) (2*S*,9*R*)-dihydroxyaphyllidine (**5**)	2215		278 (29)	278 (29), 277 (6), 148 (6), 134 (6), 120 (6), 106 (7), 99 (8), 98 (100), 97 (19), 96 (73)
anagyrine (**6**)	2390		244 (75)	243 (18), 229 (17), 161 (9), 160 (19), 147 (10), 146 (34), 145 (8), 136 (33), 134 (12), 122 (13), 99 (9), 98 (100), 96 (14)
tetrahydrorhombifoline (**7**)	2032	249		208 (15), 207 (100), 112 (51), 108 (36), 94 (8), 84 (9)
angustifoline (**8**)	2065	235		207 (6), 194 (14), 193 (100), 151 (7), 150 (56), 148 (5), 113 (7), 112 (87), 94 (19), 84 (24)
α-isolupanine (**9**)	2092	249	248 (48)	247 (39), 150 (25), 149 (59), 148 (30), 137 (13), 136 (100), 134 (29), 122 (12), 120 (8), 110 (14), 108 (10), 98 (18), 96 (11)
hydroxytetrahydrorhombifoline (**10**)	2158	265		224 (14), 223 (100), 124 (26), 112 (44), 110 (6), 98 (7), 96 (4)
13α-hydroxylupanine (**11**)	2429	265	264 (21)	247 (25), 246 (67), 245 (31), 231 (18), 166 (16), 165 (35), 164 (16), 152 (83), 150 (21), 149 (14), 148 (42), 135 (14), 134 (100), 132 (14), 122 (14), 112 (21), 108 (24), 94 (18)
13α-tigloyloxylupanine (**12**)	2779	347	346 (2)	247 (35), 246 (100), 245 (24), 231 (29), 148 (33), 147 (12), 146 (11), 135 (14), 134 (97), 132 (10), 112 (15), 98 (11)
13β-tigloyloxylupanine (**13**)	2806	347	346 (1)	247 (29), 246 (100), 245 (22), 231 (29), 148 (32), 147 (12), 146 (10), 135 (14), 134 (98), 132 (9), 112 (17), 98 (9)
unknown (**14**) hydroxytigloyloxylupanine	2974	363	362 (2)	344 (3), 263 (27), 246 (18), 245 (100), 244 (19), 233 (17), 193 (16), 150 (16), 149 (11), 146 (11), 132 (17), 112 (47), 94 (8), 84 (12)
ammodendrine (**15**)	1865		208 (10)	207 (20), 192 (15), 191 (100), 179 (15), 165 (49), 137 (16), 136 (35), 123 (28), 122 (28), 120 (20), 110 (32), 109 (34), 108 (19), 95 (16), 94 (44)
sparteine (**16**)	1785		234 (20)	233 (11), 193 (39), 191 (10), 176 (12), 150 (11), 148 (7), 138 (13), 137 (100), 136 (41), 134 (16), 122 (27), 110 (12), 108 (7), 98 (35), 96 (10), 94 (8)
11,12-dehydrosparteine (**17**)	1842		232 (58)	176 (12), 175 (65), 163 (17), 161 (12), 149 (11), 148 (27), 136 (14), 135 (26), 134 (100), 122 (10), 120 (14), 98 (16), 97 (17), 96 (23), 94 (12)
unknown (**18**)	2057		248 (35)	247 (15), 221 (16), 220 (100), 219 (24), 191 (39), 150 (20), 138 (18), 137 (26), 136 (43), 134 (26), 123 (20), 122 (26), 111 (14), 110 (44), 98 (44), 97 (23), 96 (26)
unknown (**19**)	2212		246 (7)	206 (10), 205 (65), 192 (8), 149 (8), 134 (8), 110 (24), 95 (8), 94 (100), 82 (10), 80 (11)
7-hydroxylupanine (**20**)	2280		264 (66)	263 (44), 262 (26), 247 (28), 246 (69), 246 (69), 166 (28), 165 (25), 164 (29), 152 (100), 150 (66), 136 (29), 134 (42), 124 (57), 122 (55), 110 (42), 98 (48), 97 (37), 96 (32)

sericeus are both purple flowered lupines that can be misidentified in the field, especially where they overlap in habitat. *L. leucophyllus* from Adams County, WA contains exclusively quinolizidine alkaloids, including the teratogenic alkaloid anagyrine (**6**) (**Figure 4A**; **Table 1**). *L. sulfureus*, a yellow lupine, is present on isolated pastures in Adams County, WA, and contains a single piperidine alkaloid, ammodendrine (**15**), a reported teratogen (**Figure 5A**, **Table 1**) (*10*). "Crooked calves" were reported on pastures where *L. sulfureus* grows; however, *L. leucophyllus* was also present and the impact of *L. sulfureus* on the incidence of crooked calves is speculative. *L. leucophyllus* is known to be the primary cause of "crooked calf disease" in Adams County because it contains anagyrine and is available to pregnant cows during the critical period of gestation (*14*, *15*, *22*).

L. leucophyllus and *L. sulfureus* are present on a ranch in Umatilla County, OR, where a "crooked calf disease" outbreak occurred in 1992 (*17*). Similar to the lupines in Adams County, *L. leucophyllus* is a purple flowered lupine, while *L. sulfureus* is a yellow flowered lupine. *L. leucophyllus* and *L. sulfureus* from this site in Umatilla County both contain exclusively quinolizidine alkaloids (**Figures 4B** and **5B**; **Table 1**). Contrary to the lupines identified as *L. leucophyllus* and *L. sulfureus* in Adams County, WA, at this site *L. sulfureus* contains anagyrine whereas *L. leucophyllus* does not. *L. sulfureus* was determined to be the cause of the "crooked calf" outbreak in Umatilla County because only *L. sulfureus* at this site contains anagyrine (**6**) and breeding records showed pregnant cows were grazed on a pasture containing *L. sulfureus* in the seedpod stage during the critical gestational period (*17*).

Consistent with previous information on "crooked calf disease", the teratogenic alkaloid anagyrine (**6**) is present in both of the lupine species responsible for "crooked calf disease" in these incidents (*9*, *17*). However, the alkaloid profiles of the two lupines identified as *L. leucophyllus* are very different (**Figure 4**); likewise, the alkaloid profiles of the two lupines identified as *L. sulfureus* are also different (**Figure 5**). Analysis of the *L. leucophyllus* from Adams and Umatilla counties shows six major quinolizidine alkaloid peaks from the Adams County collection and nine major quinolizidine alkaloid peaks from the Umatilla County sample (**Figure 4**). While both of these *L. leucophyllus* plants exclusively synthesize the same class (quinolizidine) of alkaloids, only one major alkaloid, lupanine (**3**), is common between the two. The comparison of the analysis of the *L. sulfureus* alkaloids from Adams and Umatilla counties

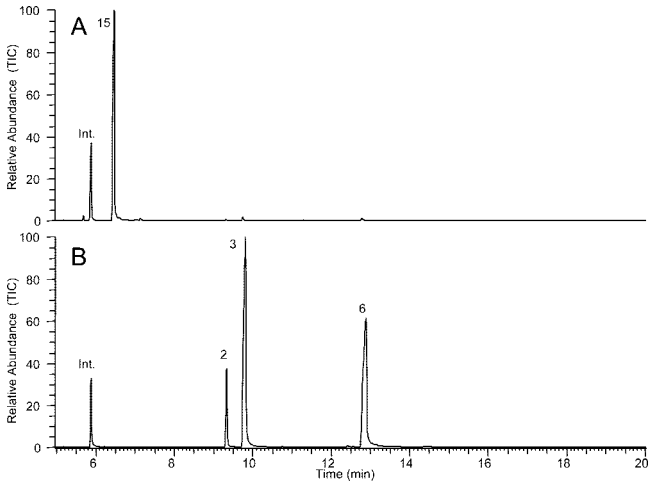

Figure 5. GC/MS total ion chromatograms of alkaloids from (**A**) *L. sulfureus* collected near Ritzville, WA, and (**B**) *L. sulfureus* collected near Pendleton, OR.

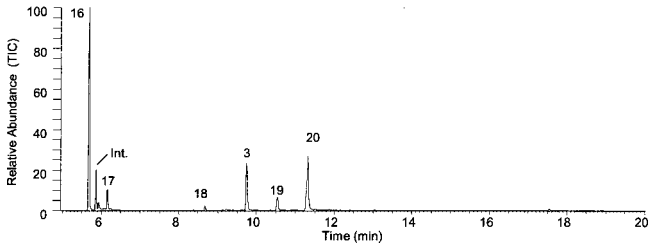

Figure 6. GC/MS total ion chromatograms of alkaloids from *L. sericeus* collected near Ritzville, WA.

was even more unexpected, in that these plants did not even contain the same class of alkaloids as each other. The *L. sulfureus* from Adams County sample contained a single piperidine alkaloid, ammodendrine, while the *L. sulfureus* from Umatilla County contained three quinolizidine alkaloids, 5,6-dehydrolupanine (**2**), lupanine (**3**), and anagyrine (**6**) (**Figure 5**). In fact, the two lupine plants that have the most similar alkaloid profiles are the *L. leucophyllus* from Adams County (**Figure 4A**) and the *L. sulfureus* from Umatilla County (**Figure 5B**). Both of these lupines contain exclusively quinolizidine alkaloids with three common peaks, 5,6-dehydrolupanine (**2**), lupanine (**3**), and the teratogen anagyrine (**6**).

At the current time we cannot explain the inconsistencies in alkaloid composition that have been demonstrated experimentally, although some possibilities merit consideration. The first distinct possibility is that these plants do not belong to the same species of lupine. It has been shown that *L. polyphyllus* from North America has a similar alkaloid profile in multiple populations (*19*). On the other hand, *L. argenteus* and *L. formosus* have been shown to have multiple chemotypes (*23*, *24*). A second possibility influencing their chemical variability could be the result of localized environmental pressures, such as herbivores. It has been proposed that the evolution of the various chemical phenotypes is connected to the frequency dependent selection of various herbivores (*25*). A third possibility is the fact that the individual populations may be the result of hybridization with another species, thus creating this variability. To address these possibilities, we are currently pursuing chemotaxonomic, phylogenetic, and taxonomic studies with these and other select lupines to address these observations and to provide experimental evidence explaining this variation in chemical phenotypes.

The inconsistency between lupines characterized as the same species yet having very different toxicological and teratogenic potential can be confusing in preparing and disseminating management recommendations for rangelands in which lupine is a component. This study demonstrates that taxonomic identification of lupine plant species is not sufficient to prevent crooked calf disease and that alkaloid analysis must be performed on each individual lupine population to establish the presence or absence of teratogenic alkaloids.

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