Alkaloids from *Lupinus argenteus* var. *stenophyllus*

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Received May 5, 1977, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.* Accepted for publication June 14, 1977.

Abstract □ TLC and GLC of an alkaloid extract of the aboveground portions of *Lupinus argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis (Leguminosae) suggested the presence of sparteine, β-isosparteine, Δ^5-dehydrolupanine, α-isolupanine, lupanine, thermopsine, and anagyrine. GLC–mass spectrometry confirmed these preliminary findings. Preparative TLC was used to isolate sparteine, and this alkaloid was further characterized by IR spectral analysis and derivatization.

Keyphrases ◘ Alkaloids, various—isolated from aboveground portions of *Lupinus argenteus* var. *stenophyllus* □ *Lupinus argenteus* var. *stenophyllus*—various alkaloids isolated from aboveground portions

The legume genus *Lupinus* is native to the Rocky Mountain states, and several of its species have been reported as being toxic to grazing livestock (1). The age and species of *Lupinus* together with the species of animal contribute to a variation in the symptoms and severity of lupine poisoning (2, 3). It is generally acknowledged that the quinolizidine alkaloid content of the lupine is responsible for acute toxicoses and death in livestock (1–4). It recently was shown that the ingestion of certain lupine species by pregnant cows causes crooked calf disease in the offspring (5). One study (6) suggested that the quinolizidine alkaloid anagyrine is the teratogen responsible for the congenital deformities.

The silvery lupine, *L. argenteus* Pursh., has long been considered to be highly toxic to grazing sheep (7, 8). While investigating possible causes of crooked calf disease, Shupe *et al.* (5) found that this species exhibited marked toxicity in cows. In a later study, Keeler (9) suggested *L. argenteus* to be a nonteratogenic lupine because it did not contain anagyrine and grew in range areas where crooked calf disease was unknown. A search of the chemical literature revealed no other investigation of *L. argenteus* or its variety *stenophyllus*.

The present study was directed at characterizing alkaloids from the aboveground portions of mature, flowering *L. argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis. Data from this investigation explained the acute toxicity associated with this plant but provided questions as to its nonteratogenic nature.

EXPERIMENTAL[1]

Plant Material—The flowering aboveground portions of *L. argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis (Leguminosae) were used[2].

Extraction and Fractionation—The air-dried powdered plant material (235 g) was homogenized with ethanol in a blender[3]. After filtration, the ethanolic extract was concentrated to 20 ml *in vacuo*, acidified with 10% acetic acid, and extracted with two successive 100-ml portions of ether, ethyl acetate, and chloroform. The acidic aqueous solution was made basic with 28% ammonium hydroxide and extracted with four 100-ml portions of chloroform. The combined chloroform extracts were filtered through anhydrous magnesium sulfate and evaporated to give a brown syrup (3 g), which solidified on standing.

TLC—Chloroform–methanol–28% ammonium hydroxide (100:10:1) and cyclohexane–diethylamine (7:3) were the developing solvents during chromatography of the alkaloid fraction over 0.25-mm layers of silica gel G. Dragendorff spray reagent revealed the presence of six alkaloidal substances. Cochromatography with reference standards suggested the presence of α-isolupanine, lupanine, thermopsine, and anagyrine. An Ehrlich-positive chromophore indicated the presence of Δ^5-dehydrolupanine (10). Both TLC systems revealed the presence of an alkaloid that could have been sparteine, β-isosparteine, or genisteine (α-isosparteine) based on identical R_f values.

GLC—With 3% OV-17 on Gas Chrom Q (2-m × 2-mm i.d. glass column) and a program of 4°/min from 125 to 265°, the alkaloid fraction was partially resolved to give peaks corresponding with reference β-isosparteine, Δ^5-dehydrolupanine, α-isolupanine, lupanine, thermopsine, and anagyrine. This GLC system failed to separate sparteine from genisteine.

Combined GLC–Mass Spectrometry—The alkaloid fraction was injected directly into a 2-m × 2-mm i.d. glass column containing 3% OV-17 on Gas Chrom Q. The column temperature was programmed from 160 to 265° at 2°/min. The injection temperature was 230°, and the carrier gas was helium at a flow of 28 ml/min. The effluent from the column entered the mass spectrometer through a glass jet separator maintained at 220°. The ion source temperature was maintained at 220° with the ionizing voltage at 70 ev and accelerating voltages of 12,600–900 ev. Spectra were recorded every 6 sec on the low-resolution mass spectrometer interfaced with a data reduction system.

Identification of β-Isosparteine—The GLC retention time relative to sparteine was 1.29 (standard 1.29); mass spectrum: M^+ *m/e* 234 (29%), 193 (26), 150 (26), 137 (92), 122 (19), 110 (35), and 98 (100) [standard M^+ *m/e* 234 (7%), 193 (9), 150 (13), 137 (96), 122 (17), 110 (25), and 98 (100)].

Identification of Δ^5-Dehydrolupanine—The GLC retention time relative to sparteine was 3.20 (standard 3.20); mass spectrum: M^+ *m/e* 246 (4%), 245 (3), 205 (3), 163 (9), 148 (14), 134 (21), 120 (14), and 98 (100) [standard M^+ *m/e* 246 (34%), 245 (10), 205 (2), 163 (5), 148 (5), 134 (8), 120 (5), and 98 (100)].

Identification of α-Isolupanine—The GLC retention time relative to sparteine was 3.36 (standard 3.36); mass spectrum: M^+ *m/e* 248 (16%), 247 (10), 219 (14), 150 (86), 149 (100), 148 (43), 137 (75), 136 (90), 134 (64), 124 (52), 112 (77), 110 (91), and 96 (37) [standard M^+ *m/e* 248 (89%), 247 (46), 219 (77), 150 (28), 149 (56), 148 (13), 137 (16), 136 (100), 134 (13), 124 (9.7), 112 (10), 110 (20), and 96 (11)].

Identification of Lupanine—The GLC retention time relative to sparteine was 3.76 (standard 3.76); mass spectrum: M^+ *m/e* 248 (3.8%), 247 (3.4), 149 (51), 136 (100), 134 (21), 112 (17), 110 (20), and 98 (36) [standard M^+ *m/e* 248 (7%), 247 (5), 149 (99), 136 (100), 134 (50), 112 (39), 110 (60), and 98 (56)].

Identification of Thermopsine—The GLC retention time relative to sparteine was 5.06 (standard 5.06); mass spectrum: M^+ *m/e* 244 (17%), 243 (6), 229 (6), 160 (47), 146 (84), 136 (46), 134 (30), 122 (47), and 98 (100) [standard M^+ *m/e* 244 (44%), 243 (10), 229 (3), 160 (11), 146 (16), 136 (10), 134 (4), 122 (7), and 98 (100)].

Identification of Anagyrine—The GLC retention time relative to sparteine was 5.61 (standard 5.61); mass spectrum: M^+ *m/e* 244 (1.8%), 161 (6), 160 (5), 146 (15), 136 (10), 122 (7), 117 (5), and 98 (100) [standard M^+ *m/e* 244 (60%), 161 (7), 160 (8), 146 (12), 136 (9), 122 (7), 117 (3), and 98 (100)].

The GLC peak corresponding with both sparteine and genisteine gave the following mass spectrum: M^+ *m/e* 234 (18%), 233 (13), 193 (21), 150 (21), 137 (100), 122 (19), 110 (32), and 98 (94). Under the conditions of

[1] IR spectra were recorded neat using a Perkin-Elmer model 257 spectrophotometer. GLC was conducted using a Hewlett-Packard model 5720A. Combined GLC–mass spectrometry was carried out with a DuPont 321 Dimaspec and 320 data system. The melting-point determinations were made using a Thomas-Hoover capillary melting-point apparatus and are uncorrected.

[2] Collected in Boulder Basin, Blaine County, Idaho, on August 6, 1973, and identified by Dr. Karl Holte, Botany Department, Idaho State University. A voucher specimen (No. 51553) is on deposit at the Idaho State University Herbarium, Pocatello, ID 83201.

[3] Waring.

combined GLC–mass spectrometry, reference sparteine and genisteine gave essentially the same mass spectrum.

Isolation of Sparteine—The entire alkaloid fraction (3 g) was dissolved in ethanol–chloroform (1:1) and streaked onto 12 silica gel PF_{254} plates (1 mm) using a previously described technique (11). The band corresponding with sparteine and genisteine was scraped from the plates, and the alkaloid material was eluted from the silica gel scrapings with three 30-ml portions of methanol. The eluates were combined and processed in the usual fashion (12) to give a light-brown oil. The IR spectrum of this material was the same as that recorded for reference sparteine. The oil was dissolved in benzene and treated with methyl iodide to give sparteine methiodide, mp 235–236° [lit. (13) mp 237–238°].

Quantitation of Alkaloids—The quantity of each identified alkaloid in the extract was determined as previously described (10). Based on the dry weight of the plant, the following levels of alkaloid were found: sparteine, 0.05%; β-isosparteine, 0.005%; Δ^5-dehydrolupanine, 0.04%; α-isolupanine, 0.60%; lupanine, 0.01%; thermopsine, 0.36%; and anagyrine, 0.01%.

DISCUSSION

Crooked calf disease is characterized by congenital malformations in calves born to cows that ingested certain *Lupinus* species during early gestation (9). Symptoms include arthrogryposis or torticollis and scoliosis and, occasionally, cleft palate (5). Keeler (6) suggested that the quinolizidine alkaloid anagyrine may be the teratogen responsible for crooked calf disease because of its high concentration in those species of *Lupinus* shown to induce the disease. In another report, Keeler (9) pointed out that *L. argenteus* is probably nonteratogenic because of a total lack of anagyrine; this report revealed lupanine as the only alkaloid identified in extracts of *L. argenteus*.

The present study of *L. argenteus* var. *stenophyllus* verifies the presence of lupanine in the species but also reveals low concentrations of anagyrine. According to Keeler (9), this situation would suggest nonteratogenicity. However, several alkaloids present in the title plant were apparently not studied by Keeler (6, 9). For instance, the epimer of anagyrine, thermopsine, may also be a potential teratogen.

Furthermore, Keeler (6) stated that the GLC peak associated with extracts of teratogenic lupines is really a mixture of anagyrine and α-isolupanine. Perhaps α-isolupanine is also teratogenic. In view of the large concentrations of α-isolupanine and thermopsine in *L. argenteus* var. *stenophyllus*, further biological testing with whole extracts and individual alkaloids appears necessary before concluding that this plant is nonteratogenic.

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ACKNOWLEDGMENTS

The authors thank Dr. G. M. Hatfield for help in collection of plant material and Dr. F. R. Cole for technical assistance. The authors are deeply indebted to Dr. S. I. Goldberg for supplying samples of β-isosparteine, α-isolupanine, and anagyrine.

Simultaneous Salt and Ethanol Removal from Human Serum Albumin

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Received April 8, 1977, from the *Department of Biochemical Development, Cutter Laboratories, Inc., Berkeley, CA 94710.* Accepted for publication June 28, 1977.

Abstract □ Diafiltration of salt and ethanol from human serum albumin was shown to be a competitive alternative to freeze drying. At least 99% of the original alcohol content could be removed in approximately five volume changes. Data on changes in ionic strength, bacteriological buildup, permeate flux, and dimer contents are presented.

Keyphrases □ Albumin, human serum—simultaneous salt and ethanol removal by diafiltration □ Diafiltration—simultaneous salt and ethanol removal from human serum albumin

Albumin is prepared commercially by variation of the low temperature–alcohol methods (1). Method 6 yields albumin as Fraction V upon lowering the pH to 4.7–4.9 at 40% ethanol. Salts are removed by redissolving Fraction V in 10% ethanol and reprecipitating at 40% ethanol.

Most pharmaceutical companies use freeze drying for the removal of ethanol from the albumin paste, but other routes such as gel filtration (2) and thin-layer evaporations under vacuum (3) also are feasible. An alternative process, diafiltration, is proposed for the simultaneous removal of salt and alcohol. The data obtained provide the basis for utilizing this method in the large-scale production of albumin preparations for clinical use.

EXPERIMENTAL

Fraction V is the starting material. An ultrafiltration thin-channel system[1] was employed in the preliminary study. This system streams the process fluid over the membrane (10,000 mol. wt. retentivity) in shallow channels, and smooth laminar flow is produced. The accompanying shear force is regulated by adjusting the fluid velocity with a reversible peristaltic pump. The system is connected with a fluid reservoir pressurized to 25 psig. A hollow fiber unit[2] also was studied for operations greater than

[1] Model TCF10, Amicon, Lexington, Mass.
[2] Model DC-30, Amicon, Lexington, Mass.