(−)-α-Isosparteine from *Lupinus argenteus* var. *stenophyllus*

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Abstract □ Combined GLC–mass spectrometry revealed that an unidentified sparteine isomer was the major component of an alkaloid extract of the aboveground portions of *Lupinus argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis (Leguminosae). After isolation, this alkaloid was characterized as the least common of the known sparteine isomers, (−)-α-isosparteine. A preliminary pharmacological study showed (−)-α-isosparteine to have a more rapid onset and a shorter duration of action when compared with (−)-sparteine on rat myocardium.

Keyphrases ◻ *Lupinus argenteus* var. *stenophyllus*—(−)-α-isosparteine, GLC-mass spectrometry, sparteine isomer ◻ (−)-α-Isosparteine—*Lupinus argenteus* var. *stenophyllus*, GLC-mass spectrometry, sparteine isomer ◻ GLC-mass spectrometry—(−)-α-isosparteine from *Lupinus argenteus* var. *stenophyllus*, sparteine isomer

The legume genus *Lupinus* is a rich source of a wide variety of quinolizidine alkaloids. Due to high concentrations of these quinolizidine bases, lupines have been implicated in acute toxicoses and death in grazing livestock (1–3). Additionally, certain lupines have been shown to cause crooked calf disease (4), and the quinolizidine alkaloid anagyrine appears to be the teratogen responsible for congenital deformities (5).

The silvery lupine, *L. argenteus* Pursh., has long been considered to be highly toxic to grazing sheep (6, 7). Recently this species has been found to exhibit marked toxicity in cattle as well (4). A chemical study of the previously uninvestigated *stenophyllus* variety of the silvery lupine revealed the presence of α-isolupanine (0.60% of dry weight), thermopsine (0.36%), sparteine (0.05%), Δ^5-dehydrolupanine (0.04%), anagyrine (0.01%), lupanine (0.01%), and β-isosparteine (0.005%) (8). The concentrations of these quinolizidine alkaloids provided an explanation for the acute toxicity associated with this plant and suggested that it may be teratogenic.

The present study was directed at characterizing the major component of the alkaloid fraction from the aboveground portions of mature, flowering *L. argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis. At the time of the initial investigation (8), the most abundant alkaloid in this plant was thought to be an unknown sparteine isomer. However, the data presented here show that this compound is (−)-α-isosparteine.

Sparteine was originally isolated in 1851 from *Cytisus scoparius* (L.) Link. (9) and has since been found to occur in many other members of the Leguminosae as well as in species belonging to the Monimiaceae, Papaveraceae, and Scrophulariaceae (10). The known isomers of sparteine, α-isosparteine and β-isosparteine, have a much more restricted distribution and were isolated at a later date (11). Genisteine, for instance, was first isolated in 1918 from *C. scoparius* (12), and its identity as (−)-α-isosparteine was confirmed in 1951 (13). Since that time (−)-α-isosparteine has been isolated from only two other legumes, *Lupinus caudatus* Kell. (14) and *Genista tinctoria* L. (15).

EXPERIMENTAL[1]

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

Extraction and Fractionation—The air-dried powdered plant material (500 g) was homogenized with ethanol in a blender[3]. After filtration, the ethanolic extract was concentrated to 30 ml *in vacuo*, acidified with 10% aqueous acetic acid, and extracted with two successive 200-ml portions of ether, ethyl acetate, and chloroform. The acidic aqueous solution was made basic with 58% ammonium hydroxide and extracted with four 200-ml portions of chloroform. The combined chloroform extracts were filtered through anhydrous magnesium sulfate and evaporated to give a brown syrup (6 g), which solidified on standing.

Chromatographic Systems—The following TLC systems utilized 0.25-mm silica gel G layers: system A, chloroform–methanol–58% ammonium hydroxide (100:10:1); system B, cyclohexane–diethylamine (9:1). Alkaloids were detected using Dragendorff's reagent, and both systems provided a good separation of the major alkaloid from the available sparteine isomers and the other components of the extract.

The GLC system consisted of 3% OV-17 on Gas Chrom Q(2-m × 2 mm-i.d. glass column) and a program of 4°/min from 140 to 265°. This system produced a good resolution of the alkaloid mixture with the major component being eluted first.

Mass Spectral Analysis—The major component of the alkaloid fraction was analyzed using combined GLC-MS. The effluent from the 3% OV-17 GLC column entered the mass spectrometer through a glass jet separator maintained at 220°. The ion source temperature was 220° with an ionizing voltage of 70 eV. The mass spectrum of the major alkaloid component in the extract was basically the same as that recorded for the available sparteine isomers (8).

Isolation of the Major Alkaloid—A portion (2.5 g) of the alkaloid fraction was dissolved in ethanol–chloroform (1:1) and chromatographed (preparative TLC, 36 silica gel PF 254 plates, 1 mm thickness, TLC system B). The large band at R_f 0.61 was scraped from the plates, and the alkaloid material was eluted from the silica gel scrapings with three 100-ml portions of methanol. The eluates were combined and processed in the usual fashion (16) to give a light-brown oil which crystallized immediately upon exposure to air. Recrystallization of the free base from dry acetone gave 745 mg of colorless needles, mp 60–62°. The melting point remained constant after sublimation. Portions of the isolated free base were converted to the following derivatives using standard procedures: bisulfate, mp 244–245°; picrate, mp 205–207°; perchlorate, mp 302–304°; methiodide, mp 217–218°. The free base was used to determine the specific rotation, $[\alpha]_D^{26} = -48.1°$ (c = 0.006 g/ml in methanol).

Quantitation of the Major Alkaloid—Using a previously described GLC method (17), the major alkaloid was found to be present in the dry plant material at a level of 0.72%. This represents 40% of the total alkaloid content in the plant.

Synthesis of α-Isosparteine—Starting with sparteine sulfate[4], the method of Leonard and Beyler (18) was used to produce α-didehydros-

[1] IR spectra were determined neat using a Beckman IR-33 spectrophotometer. ^{1}H-NMR spectra were recorded on a Perkin-Elmer model R-24 spectrometer. ^{13}C-NMR spectra were obtained on a Varian XL-200 spectrometer. GLC was conducted using a Hewlett-Packard model 5720A gas chromatograph. Combined GLC–mass spectrometry was carried out with a Du Pont 321 Dimaspec low-resolution mass spectrometer interfaced with a 320 data reduction system. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter.

[2] Collected in Boulder Basin, Blaine County, Idaho, on August 19, 1977 and identified by Dr. Karl Holte, Department of Botany, Idaho State University. A voucher specimen (No. 51553) is on deposit at the Idaho State University Herbarium, Pocatello, ID 83201.

[3] Waring.

[4] Merck and Co., lot. no. 52421.

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parteinium bisulfate. A portion of this compound (122 mg) was converted to the free base and hydrogenated over palladium-on-carbon catalyst for 15 hr at room temperature and 45 lbs of pressure. This reaction mixture was filtered, concentrated *in vacuo* to 1 ml, and chromatographed over a small (4 g) silica gel column. The free base (26 mg) was eluted with ether containing 10% of a mixture of methanol–58% ammonium hydroxide (7:3) and was found to have mp 59–60° [lit. (19) mp 60–62°] after sublimation.

RESULTS AND DISCUSSION

During the course of a chemical investigation of *L. argenteus* var. *stenophyllus*, large quantities of the major alkaloid were isolated in chromatographically pure form. Spectral data (IR, ^{1}H-NMR, and MS) suggested the isolate to be an isomer of sparteine. Initially, the alkaloid was thought to be (−)-α-isosparteine, since the two known isomers of this compound, sparteine and β-isosparteine, were shown to be present in the plant (8). However, chromatographic (TLC and GLC) comparisons with a commercial sample[5] of (−)-α-isosparteine refuted this original postulate. Differences in observed and literature melting points for both the free base and numerous derivatives also supported the view that the major alkaloid from *L. argenteus* var. *stenophyllus* was an unknown sparteine isomer.

An X-ray crystallographic study was performed on the perchlorate salt of the isolated alkaloid in an effort to establish its molecular structure. Data from this investigation revealed the compound to be α-isosparteine perchlorate[6] and corresponded very closely with a previous X-ray diffraction study of α-isosparteine (20). Synthesis of α-isosparteine supported the information from the X-ray diffraction study with the synthetic material corresponding in all respects (TLC, GLC, IR, ^{1}H-NMR, ^{13}C-NMR, MS, and mp of the free base) with the isolated alkaloid. This evidence together with an observed $[\alpha]_D^{26} = -48.1°$ [lit. (14) $[\alpha]_D^{22} = -51.3°$] established the major alkaloid from *L. argenteus* var. *stenophyllus* as (−)-α-isosparteine. A reference sample of 1-α-isosparteine, purchased from a second commercial supplier[7], was also identical with the isolated and synthesized products.

The initial anomalous observations involving chromatographic differences between isolated and reference materials were resolved by determining that the initial commercial sample, labeled α-isosparteine, was misbranded. The mislabeled commercial sample[5] is, from TLC and ^{13}C-NMR evidence, actually a mixture of sparteine: (−)-α-isosparteine, 4:1. Further, there have been confusing discrepancies in the literature regarding the melting points of α-isosparteine and some of its derivatives. For example, the degree of crystal hydration greatly affects the melting point of the free base α-isosparteine. A previous study (14) reported the monohydrate as mp 108–110°, while another study (19) found the anhydrous free base to be mp 60–62°. The α-isosparteine isolated in this study apparently crystallized in the anhydrous form. The same situation probably exists with the picrate, mp 205–207° [lit. (21) mp 221–222°] and the perchlorate, mp 302–304° [lit. (14) mp 262–263°]. The bisulfate mp 244–245° [lit. (22) mp 244–245°] and the methiodide, mp 217–218° [lit. (19) mp 217–218°] correlated well with literature values.

The present study establishes *L. argenteus* var. *stenophyllus* as the richest source of (−)-α-isosparteine yet reported. For example, less than 0.01% of (−)-α-isosparteine was found (14) in *L. caudatus* while this work demonstrated a concentration of 0.72% in the title plant. Additionally, *L. argenteus* var. *stenophyllus* is now the only plant known to produce and accumulate all three sparteine isomers.

Sparteine has been used for many years in the management of various cardiac arrhythmias (23). In isolated atria, sparteine exhibits a positive inotropic action and prolongs the refractory period (24). Preliminary pharmacologic studies in this laboratory have revealed that (−)-α-isosparteine exhibits basically the same action as does (−)-sparteine but possesses a more rapid onset and a shorter duration when compared using rat myocardial tissue[8]. A more complete pharmacologic characterization of (−)-α-isosparteine is presently in progress.

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