ALKALOID VARIATION IN *LUPINUS HOLOSERICEUS*

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Several species of the legume genus *Lupinus* have been reported as being toxic to grazing livestock in the Rocky Mountain region of the United States (1). The Kellogg's spurred lupine, *L. caudatus* Kell., is one toxic lupine which has caused cattle loss in Nevada and Utah (2). Presently, *L. holosericeus* Nutt. and *L. caudatus* are viewed as being taxonomically distinct (3–5), although they have been considered as being synonymous in the past (6). Because of an apparent close relationship to the toxic *caudatus* species, the above-ground parts of the previously uninvestigated *L. holosericeus* have been extracted and found to contain the unusual quinolizidine base lamprolobine [1-(glutarimidomethyl)-quinolizidine] (0.40% of dry weight) together with lupanine (0.19%), α-isolupanine (0.02%) and anagyrine (0.02%) (7). The occurrence of lamprolobine as the major alkaloid in *L. holosericeus* was considered unusual since it had been isolated only one other time from the Australian legume *Lamprolobium fruiticosum* Benth. (8), a member of the alkaloid-poor Galegeae tribe.

The *L. holosericeus* material was collected in Blaine County, Idaho, on August 22, 1977, (7) and was identified by Dr. David B. Dunn, a recognized expert on the taxonomy of *Lupinus*. On August 23, 1977, another lupine collection was secured from a topographically similar site 10 miles from the area where *L. holosericeus* had been collected. Based largely on plant size, the two collections appeared to involve different species. However, a voucher specimen from the August 23 collection was positively identified as *L. holosericeus* after careful examination of leaf and flower morphology (5). Initially, this identification was surprising since a preliminary screen indicated that the alkaloids from the two collections differed both qualitatively and quantitatively. A detailed study of the August 23 collection revealed the presence of sparteine (0.43% of dry weight), lamprolobine (0.14%), Δ^5-dehydrolupanine (0.12%), lupanine (0.10%), anagyrine (0.09%), and β-isosparteine (0.02%).

The presence of sparteine as the major alkaloid in one population of *L. holosericeus* together with its apparent absence in another population of the same species was particularly striking. The qualitative difference involving Δ^5-dehydrolupanine was also noteworthy. Since seasonal variation cannot explain these differences, it may be that there are geographically separate gene pools within the same taxon. Nowacki and Dunn (9) have demonstrated that the formation and accumulation of sparteine is a recessive trait. Apparently the recessive gene for sparteine production was not expressed in the *L. holosericeus* polulation collected on August 22. Experiments involving California shrubby lupines have revealed that all crosses between sparteine-rich and lupanine-rich plants resulted in F_1 progeny where sparteine either disappeared completely or its concentration was very much reduced (9). Introgression involving an adjacent lupine population having the dominant trait of lupanine production may partially explain the apparent absence of sparteine and the elevated levels of lupanine in the August 22 collection

as compared to the population collected on August 23. An analogous situation may exist in the case of Δ^5-dehydrolupanine.

Quinolizidine alkaloid biosynthesis has generally centered on sparteine being involved in a series of oxidations to give lupanine, Δ^5-dehydrolupanine, and anagyrine in succession (10,11). This theory is quite acceptable in view of the alkaloids identified as being present in the August 23 collection of *L. holosericeus*. However, sparteine and Δ^5-dehydrolupanine were not detected in *L. holosericeus* collected on August 22. This may be due to a turnover rate so rapid that these compounds do not accumulate to give detectable concentrations. On the other hand, sparteine and Δ^5-dehydrolupanine may indeed be completely absent thereby necessitating the development of an alternative biosynthetic scheme. Cho *et al.* (12) have demonstrated that in certain species of *Lupinus* sparteine and lupanine may be synthesized independently of one another from a yet unidentified precursor. It has been speculated that this common precursor is $\Delta^{1(2)}$-dehydrosparteine, which may give rise to sparteine or lupanine or both. Similarly, the postulated intermediate between lupanine and α-isolupanine, $\Delta^{11(16)}$-dehydrolupanine (13), may also function as the precursor to anagyrine thereby obviating the biosynthetic need for Δ^5-dehydrolupanine. The presence of $\Delta^{1(2)}$-dehydrosparteine and $\Delta^{11(16)}$-dehydrolupanine in the August 22 collection of *L. holosericeus* would have escaped detection because of the relatively polar character of these potential biosynthetic intermediates. Lamprolobine, present in both *L. holosericeus* collections, probably arises prior to the formation of lupanine by way of an independent biosynthetic pathway (8).

Variation in quinolizidine alkaloid composition within a species has been documented in previous studies. Cranmer and Turner (14) were unable to use alkaloid data for taxonomic purposes at the infrageneric level because of qualitative and quantitative differences within a single *Baptisia* species collected at different locations. Dolinger *et al.* (15) found considerable variation in the alkaloid composition of Colorado lupine populations and used this information to explain patterns of predation by the flower-feeding lycaenid butterfly. Both qualitative and quantitative alkaloid differences within individual *Lupinus* species were noted by Keeler (16) during a screen designed to distinguish teratogenic and nonteratogenic lupines. These observations raise a number of genetic and biosynthetic questions which have been alluded to in this paper. The general concept of variation of the secondary metabolite composition within a single taxon is significant and warrants extensive investigation. In this laboratory, biosynthetic studies involving young *L. holosericeus* derived from seeds collected at several Idaho locations may provide some insight.

EXPERIMENTAL

PLANT MATERIAL.—The *Lupinus holosericeus* analyzed in this study was collected 3 miles southwest of the Bellevue city limits in Blaine County, Idaho, on August 23, 1977. The plant was identified by Dr. David B. Dunn, and a voucher specimen (SBLY-77) is on deposit at the University of Missouri Herbarium, Columbia, Missouri 65201.

EXTRACTION AND FRACTIONATION.—The dried, powdered (40 mesh) above-ground plant parts (100 g) were homogenized with 95% ethanol and processed as usual (17) to give a crude alkaloid fraction.

CHROMATOGRAPHY.—All analytical thin layer chromatography (tlc) was performed with chloroform-methanol-ammonium hydroxide (100:10:1) as the solvent, while preparative tlc involved developing 1 mm silica gel plates two times with cyclohexane-diethylamine (8:2). Gas chromatography (gc) was carried out over 3% OV-17 on Gas

Chrom Q with an initial temperature of 140° and programming to 265° at 4° per minute. The same gc system was combined with a DuPont 321 Dimaspec low-resolution mass spectrometer interfaced with a 320 data reduction system.

ALKALOID ISOLATION AND IDENTIFICATION.—Anagyrine, Δ^5-dehydrolupanine, lamprolobine, lupanine, sparteine, and β-isosparteine were all identified as being present in the crude alkaloid fraction by tlc, gc, and mass spectral comparisons with reference standards. Lamprolobine and sparteine were isolated by preparative tlc and chemically characterized by derivatization. Lamprolobine was converted to the picrate, mp 152–153° [lit. (8) mp 153–154°], while the isolated sparteine was treated to give the methiodide derivative, mp 235–236° [lit. (18) mp 237–238°].

QUANTITATION OF ALKALOIDS.—An internal standard (20 mg of *N,N*-dimethyl-3,4-dimethoxyphenethylamine hydrochloride) was added to 10 g of air-dried powdered plant material prior to homogenization with 95% ethanol. After extraction and partitioning, the alkaloid fraction was assayed quantitatively by gc as previously described (19).

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