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Alkaloid Variation in the *Lupinus pusillus* group (Fabaceae: tribe Genisteae)

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Key Word Index—Fabaceae; *Lupinus*; Genisteae; quinolizidine alkaloids; α-pyridone alkaloids; chemotaxonomy.

Abstract—The major alkaloids of *Lupinus pusillus* and related species were studied by GC and GC–MS. More than 25 alkaloids were detected in 20 extracts from *L. flavoculatus*, *L. kingii*, *L. odoratus*, *L. pusillus* and *L. shockleyi*. Esters of alkaloids appear to be absent, and sparteine, β-isosparteine, isolupanine, 5,6-dehydrolupanine, lupanine and anagyrine are the major alkaloids. The presence of the α-pyridone pathway is thus confirmed, with anagyrine as the major seed alkaloid in four of the five species. *Lupinus* seems to differ from other α-pyridone-bearing genera of the tribe Genisteae, which accumulate *N*-methylcytisine or cytisine in the seeds and not anagyrine. Despite the small number of samples and populations studied, the remarkable differences between the species showed that the alkaloids of *Lupinus* may provide valuable taxonomic information even at the species level.

Introduction

Lupinus pusillus and related species are a distinctive group of highly derived ephemerals from dry areas of North America and Canada (Dunn, 1959; Dunn and Gillett, 1966). Our interest in these species is based on the reported occurrence of α-pyridone alkaloids in *L. pusillus*, together with the expected enantiomer pair comprising (+)-sparteine and (−)-lupanine (Mears and Mabry, 1971). Kinghorn *et al.* (1980), Meissner and Wink (1992) and Wink *et al.* (1995) have shown that anagyrine and thermopsine are relatively common in New World *Lupinus* species. Other species, for which data is available, have (−)-sparteine and (+)-lupanine, often with the expected hydroxylated lupanines and their esters (Mühlbauer *et al.*, 1988; Meissner and Wink, 1992; Wink *et al.*, 1995). The reported distribution of compounds thus agrees with the idea of two separate and independent biosynthetic pathways in the Fabaceae (Van Wyk and Verdoorn, in press). With the exception of *L. kingii*, none of the species of the *L. pusillus* group were included in a recent survey of 56 *Lupinus* species (Wink *et al.*, 1995). As part of an ongoing study of alkaloids as chemotaxonomic characters in quinolizidine-bearing tribes of the Fabaceae, we were interested to see if the α-pyridone alkaloids of the *L. pusillus* group are similar to the typical pattern found in other *Lupinus* species and also in other genera of the tribe Genisteae.

Materials and Methods

Plant materials. Small samples of aerial parts were obtained from the following herbarium specimens [sample numbers (see Table 1) in bold print]: *Lupinus flavoculatus* Heller: **1**, **2** and **3**, Duran 3229 (K), California, Darwin, Inyo County (leaves, pods and seeds from the same plant). *Lupinus kingii* S. Wats.: **4**,

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Babcock & Goddard 524 (K), Arizona; **5**, Bethel *et al.* 4193 (K), Colorado; **6** and **7**, Moseley s.n. (K), Wingate to Zuni, S.E. Mexico (leaves and seeds from the same plant). *Lupinus odoratus* Heller: **8**, Barneby 237/37 (K), Mohave Desert, Victorville and Little Rock; **9**, Lennon s.n. (K), Mohave Desert; **10**, Munz 12426 (K), locality not recorded; **11**, Lennon s.n. (K), Mohave Desert (leaves and seeds from the same plant). *Lupinus pusillus* Pursh ssp. *pusillus*: **12** and **13**, Gordon 225 (K), locality not recorded (leaves and seeds from the same plant); **14** and **15**, Nelson & Nelson 2143 (K), Arizona, between Flagstaff and Holbrook. *Lupinus pusillus* Pursh ssp. rubens (Rydb.) Dunn: **16**, Clokey 8711 (K), Nevada, Clark County. *Lupinus shockleyi* Wats.: **17**, Epling *et al.* 32 (K), Mohave Desert; **18** and **19**, Parish 96 (K), Colorado Desert, Whitewater (leaves and seeds from the same plant); **20**, Spencer 650 (K), Colorado Desert, Palm Springs. Most of these specimens were determined by D.B. Dunn (see Dunn, 1959; Dunn & Gillett, 1966). Since *Lupinus* species are notoriously difficult to identify, the use of authentic herbarium specimens was an important consideration to ensure the reliability of the data.

Procedures. The powdered material (*ca* 20–100 mg dry wt.) was mixed with 15 ml 0.05 M H_2SO_4 and extracted at room temp. for 20 min. After filtration, the remaining solids were re-extracted with 5 ml 0.05M H_2SO_4. The aqueous phases were combined, applied to glass columns with celite (24 g), alkalinized with ammonia and extracted (1 ×) with 100 ml CH_2Cl_2. The CH_2Cl_2 extracts were dried with anhydrous Na_2SO_4, the solvent evaporated under reduced pressure and then dissolved in minimum MeOH for study by comparative GC and GC–MS. Due to the exceptionally small quantities of plant material, the total yields of alkaloids could only be estimated, using 4 mg ml^{-1} sparteine as external standard. These estimates are given here in mg g^{-1} dry wt. **1**: 0.1, **2**: 2.0, **3**: 5.6, **4**: 2.5, **5**: 5.7, **6**: 1.6, **7**: 20.9, **8**: 3.7, **9**: 2.9, **10**: 5.8, **11**: 5.3, **12**: 18.9, **13**: 17.3, **14**: 14.3, **15**: 20.7, **16**: 1.6, **17**: 2.0, **18**: 4.9, **19**: 4.2, **20**: 8.5. The GC and GC–MS systems are described in detail elsewhere (Van Wyk *et al.*, 1993). To confirm preliminary identifications, six samples (numbers **3**, **7**, **10**, **11**, **15** and **16**) were studied by GC–MS. Authentic reference samples from several previous studies were available to us (e.g. Greinwald *et al.*, 1990a,b, 1992a,b; van Wyk *et al.*, 1988a,b; Van Wyk and Verdoorn, 1989) and comparisons with literature data (Neuner-Jehle *et al.*, 1964; Schumann *et al.*, 1968) allowed the positive identification of all the major and virtually all the minor compounds by their retention indices (*RI*) and mass spectra. Retention indices were calculated according to Kovats, using co-chromatographed standard hydrocarbons. Mass spectral data of all the major alkaloids detected in the *L. pusillus* group are recorded below (for *RI* values see Table 1). More than 81 different alkaloids were detected, but those not recorded below occurred only as trace amounts in some of the samples and did not contribute to the overall pattern.

Epilupinine: 169 (61), 152 (100); lupinine: 169 (70), 152 (100); α-isosparteine: 234 (39), 137 (62), 98 (100); sparteine: 234 (20), 137 (100), 98 (85); β-isosparteine: 234 (18), 193 (15), 137 (100), 98 (63); 11,12-dehydrosparteine: 232 (47), 134 (100), 97 (79); ammodendrine: 208 (62), 165 (100); **X1** (tetrahydrocytisine isomer?): 194 (63), 151 (15), 136 (30), 112 (22), 110 (19), 96 (38), 97 (100), 95 (67), 84 (38), 83 (50), 55 (33); *N*-methylcytisine: 204 (28), 58 (100); cytisine: 190 (76), 146 (100); **X2** (13-hydroxy-β-isosparteine?): 250 (20), 233 (8), 209 (16), 152 (18), 137 (100), 98 (74); 17-oxosparteine: 248 (65), 220 (35), 191 (10), 150 (18), 136 (31), 123 (22), 110 (58), 97 (100); angustifoline: 234 (-), 193 (100), 150 (7), 112 (31), 94 (15); α-isolupanine: 248 (38), 149 (52), 136 (100), 98 (30); 5,6-dehydrolupanine: 246 (35), 98 (100); rhombifoline: 203 (100), 58 (55); lupanine 248 (65), 149 (75), 136 (100); *N*-formylcytisine: 218 (65), 146 (100); *N*-acetylcytisine: 232 (28), 146 (100); 17-oxolupanine: 262 (66), 234 (20), 150 (100); anagyrine: 244 (38), 98 (100); 13α-hydroxylupanine: 264 (45), 247 (22), 246 (26), 166 (29), 165 (50), 152 (100); baptifoline: 260 (35), 114 (100); epibaptifoline: 260 (58), 114 (100).

Results

The distribution of major alkaloids in the *L. pusillus* group is given in Table 1. Sparteine, β-isosparteine, ammodendrine, α-isolupanine, 5,6-dehydrolupanine, lupanine, anagyrine and 13α-hydroxylupanine were the major alkaloids in at least some of the species. Most of the extracts also had a large number of minor alkaloids: epilupinine, lupinine, 11,12-dehydrosparteine, lusitanine, *N*-methylcytisine, cytisine, 17-oxosparteine, angustifoline, rhombifoline, *N*-formylcytisine, *N*-acetylcytisine, 17-oxolupanine, baptifoline and epibaptifoline. Most of these alkaloids were reported by Kinghorn *et al.* (1980) in their survey of 21 other New World *Lupinus* species and also more recently by Meissner and Wink (1992). **X1** and **X2** could not be unambiguously identified by their mass spectral data alone (see Material and Methods).

Discussion

The presence of the α-pyridone pathway in the *L. pusillus* group is confirmed, with anagyrine as major seed alkaloid in most of the seed samples. This is not typical of

TABLE 1. MAJOR ALKALOIDS IN 20 SAMPLES FROM FIVE SPECIES OF THE *LUPINUS PUSILLUS* GROUP. Alkaloid distributions are given as percentages of total yield (see Materials and Methods), as estimated from GC results using peak area. (– = not detected; + = present in trace amounts only, i.e. less than 0.5% of total yield; L = leaves, P = pods, S = seeds; all seed samples are listed immediately after the leaf sample from the same plant—see Materials and Methods)

		L. flavoculatus			*L. kingii*				*L. odoratus*				*L. pusillus*					*L. shockleyi*			
	Retention	L	P	S	L	L	L	S	L	L	L	S	L	S	L	S	L	L	L	L	S
Alkaloids	index (*RI*)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Epilupinine	1418	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+
Lupinine	1420	+	+	+	+	+	+	+	+	1	+	+	+	+	+	+	+	+	+	+	+
α-isosparteine	1706	4	+	+	–	–	–	+	5	4	3	+	+	+	+	+	+	3	6	3	+
Sparteine	1780	–	1	+	+	+	+	+	92	88	92	12	7	+	12	4	4	79	84	83	1
β-isosparteine	1830	8	+	+	+	–	–	–	+	+	+	+	38	1	25	1	21	+	1	1	+
11,12-dehydrosparteine	1837	1	+	+	–	–	–	–	1	1	+	+	1	+	1	+	2	2	1	1	+
Ammodendrine	1863	34	17	3	+	+	1	+	2	1	1	+	+	+	1	+	1	7	2	5	+
Lusitanine	1880	–	–	–	+	–	+	+	+	–	1	+	–	+	–	–	–	3	+	1	–
X1	1907	4	1	+	+	–	1	–	–	1	+	1	+	+	+	+	+	–	–	–	–
N-methylcytisine	1953	–	+	+	–	–	–	–	+	+	+	+	–	+	–	–	+	+	+	+	2
Cytisine	1987	–	+	7	–	–	–	–	+	+	+	+	+	+	–	+	+	+	+	+	3
X2	2055	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	17	–	–	–	–
17-oxosparteine	2075	–	–	–	–	–	–	–	1	2	1	+	+	+	+	+	+	2	1	1	+
Angustifoline	2083	–	–	–	1	+	+	1	–	–	–	–	–	–	–	–	–	–	–	–	–
α-isolupanine	2100	+	1	+	86	19	2	2	+	+	+	+	+	+	+	+	+	+	+	+	+
5,6-dehydrolupanine	2127	1	6	10	+	1	1	+	–	+	+	6	2	20	5	23	2	+	+	+	9
Rhombifoline	2150	2	1	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Lupanine	2160	5	39	43	10	79	93	76	+	1	1	3	45	25	47	19	15	4	3	3	51
N-formylcytisine	2315	–	+	2	–	–	–	–	–	+	–	+	–	+	+	+	–	+	+	+	3
N-acetylcytisine	2323	–	+	+	–	–	–	–	–	+	–	2	–	+	+	+	–	+	+	+	+
17-oxolupanine	2345	–	–	–	1	–	1	+	+	+	–	–	+	–	+	+	–	2	1	1	+
Anagyrine	2387	34	34	32	+	–	–	+	–	+	–	71	5	52	8	53	36	+	1	+	29
13α-hydroxylupanine	2400	–	–	–	1	1	1	21	–	+	–	+	+	+	+	+	+	+	+	+	+
Baptifoline	2630	6	+	1	+	+	+	–	–	+	–	+	–	–	–	–	–	+	+	+	+
Epibaptifoline	2650	1	+	2	+	+	–	–	+	+	–	1	–	–	+	–	–	+	–	1	+

other α-pyridone-accumulating genera of the tribe Genisteae, where anagyrine is often a major leaf alkaloid but where high yields of *N*-methylcytisine and/or cytisine are found in the seeds (e.g. Greinwald *et al.*, 1990a, 1990b). As in other genera, there are also large quantitative differences between leaves and seeds in the *L. pusillus* group, but *N*-methylcytisine and cytisine are here only minor compounds. Esters of alkaloids, which occur in several species of *Lupinus* (Mühlbauer *et al.*, 1988; Wink *et al.*, 1995) appear to be absent in the *L. pusillus* group. Leaves from different populations of the same species were unexpectedly uniform, except for a distinct α-lupanine/lupanine dichotomy in *L. kingii* and, to a lesser extent, a lupanine/anagyrine dichotomy in *L. pusillus*.

Remarkable differences were found between the species and all of them except *L. odoratus* and *L. shockleyi* can be identified by a diagnostically different combination of major and minor alkaloids. For example, sparteine was the major leaf alkaloid of *L. odoratus* and *L. shockleyi*; *L. pusillus* had β-isosparteine and lupanine; *L. kingii* had α-isolupanine or lupanine, and *L. flavoculatus* had ammodendrine and anagyrine. *Lupinus kingii* is the only species where anagyrine is replaced by lupanine and 13α-hydroxylupanine as the major seed alkaloids. In their survey of a large number of *Lupinus* species, Meissner and Wink (1992) and Wink *et al.* (1995) also found only α-isolupanine and lupanine in the leaves of *L. kingii* (the only species of this group included in their study). Table 1 shows an exceptionally uniform distribution of minor compounds. For example, α-isosparteine was present in all the leaves of *L. flavoculatus, L. odoratus* and *L. shockleyi* (invariably between 3 and 6% of total alkaloids). Another example is the distribution of 11,12-dehydrosparteine (1 or 2% in all the leaves and only trace amounts in seeds).

The sample size does not allow definite taxonomic conclusions, but the results indicate that different populations of the same species are remarkably similar and that reliable data can be obtained from only a few milligrams of herbarium material (even old material). The use of herbarium specimens shows that sample limitations are no longer a serious problem in detailed chemotaxonomic studies of quinolizidine alkaloids. Furthermore, it is now clear that the alkaloids of *Lupinus* are of diagnostic value even amongst closely related species [as was tentatively suggested by Kinghorn *et al.* (1980) and Wink *et al.* (1995)]. In contrast, several studies of other genera of the Genisteae and Thermopsideae (e.g. Cranmer and Turner, 1967; van Wyk *et al.*, 1988a,b, 1993; Van Wyk and Verdoorn, 1989; Greinwald *et al.*, 1992a,b) have indicated that alkaloids are more valuable at the generic and tribal levels, largely because of infraspecific variation.

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