



## PATTERNS OF QUINOLIZIDINE ALKALOIDS IN 56 SPECIES OF THE GENUS *LUPINUS*

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**Key Word Index**—*Lupinus*; Leguminosae; quinolizidine alkaloids; GLC-MS; alkaloid profiles; chemotaxonomy.

**Abstract**—The alkaloid composition of 56 species (90 taxa if all subspecies and chemotypes are included) of the genus *Lupinus* was studied by capillary gas-liquid chromatography and GLC-mass spectrometry (GC-EIMS). The distribution of 100 alkaloids (quinolizidines, piperidines, dipiperidines and simple indoles) and their relative abundances in leaves and seeds (if available) are recorded.

### INTRODUCTION

Quinolizidine alkaloids (QA) are characteristic secondary metabolites of the Fabaceae and are especially abundant in the tribes Genisteae, Sophoreae and Thermopsideae [1, 2]. The genus *Lupinus* forms a distinct subtribe within the Genisteae [3, 4]. More than 500 lupin species have been described. Whereas *ca* 12 species occur in Europe and Africa, roughly 200–300 species inhabit North and South America. It has been speculated that lupins originated from South America [1, 2, 5, 6]. Within the Genisteae, members of the genus *Lupinus* rely substantially on quinolizidine alkaloids for chemical defence against herbivores [7–12]. Especially rich in QA are the lupin seeds which also contain up to 40% protein and 20% lipids. Since many lupins might be of potential use in agriculture as a protein crop or are toxic to cattle and other domestic wild ranging animals, the knowledge of the alkaloid patterns of lupins is of importance.

In this communication we report on the alkaloid profiles of 56 lupin species (seeds or leaves) which were studied by capillary GLC and GLC-mass spectrometry. This study covers all species of Old World lupins, six taxa from South America and 36 from North America and provides the most comprehensive alkaloid survey of lupins which exists so far. Some of the data shown are updates of previously published alkaloid studies from our laboratories [5, 6, 13–24].

### RESULTS AND DISCUSSION

Capillary GLC in combination with mass spectrometry (mostly GC-EIMS; for *ca* 40% of the samples also GC-CIMS, if alkaloids were present which did not produce significant molecular ions) was found to be the method of choice for the analysis of complex natural mixtures of QA [1, 2, 5, 6, 13–23, 25–37]. We have studied the QA patterns of 56 lupin species (altogether 90 taxa, if chemotypes and subspecies are considered) by this method and could determine the structure of 100 alkaloids according to their mass fragmentation patterns [2, 5, 6, 13–23, 25–33, 38–60], in combination with their indicative Kovat's retention indices (Tables 1 and 2) and in comparison with authentic alkaloids. In many lupin samples we could detect unknown minor alkaloids (abundance < 1% of total alkaloids), such as derivatives of gramine, lupinine, oxosparteine, dehydrosparteine, dehydrolupanine, hydroxysparteine, aphylline, hydroxylupanine, tetrahydrorhombifoline, angustifoline and multiflorine. The amount of the material available to us did not allow an unambiguous structure elucidation in these instances. However, almost all major alkaloids (abundance > 1%) could be identified unequivocally (Tables 1 and 2) although the stereochemistry of the alkaloids concerned needs to be determined in some instances.

Lupin alkaloids are produced by leaf chloroplasts, are then distributed all over the plant via the phloem and stored in epidermal cells and in seeds [11–13, 16]. As a consequence, alkaloid profiles are more diverse in leaves than in seeds [2, 10, 11] which can also be seen from Table 2; e.g. esters of 3 $\beta$ - and 13 $\alpha$ -hydroxylupanine

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Table 1. Identification of lupin alkaloids by capillary GLC and mass spectrometry

Compound	RI	M <sup>+</sup>	EI-MS				
<i>N</i> -Acetylammmodendrine	2220	250	189 (100)	232 (70)	207 (46)	165 (38)	250 (6)
<i>N</i> -Acetylytisine	2325	232	146 (100)	147 (75)	232 (40)	160 (20)	190 (15)
Acetylepilupinine	1520	211	152 (100)	211 (20)	168 (10)	98 (10)	83 (10)
<i>N</i> -Acetylhystrin/Dehydroammmodendrine	1932	206	163 (100)	206 (48)	108 (23)	122 (12)	191 (5)
13 $\alpha$ -Acetyloxylupanine	2450	306	246 (100)	134 (70)	148 (30)	112 (39)	55 (30)
Albine	1900	232	191 (100)	110 (50)	149 (40)	122 (35)	232 (20)
11-Allylytisine	2240	230	189 (100)	146 (30)	160 (10)	134 (5)	230 (8)
Ammodendrine	1865	208	165 (100)	136 (60)	123 (60)	208 (55)	191 (50)
Anagyrene	2390	244	98 (100)	244 (40)	146 (20)	160 (15)	136 (15)
3 $\beta$ -Angeloyloxylupanine	2755	346	134 (100)	246 (20)	148 (20)	346 (1)	
13 $\alpha$ -Angeloyloxylupanine	2733	346	246 (100)	134 (30)	148 (15)	112 (12)	55 (10)
13-Angeloyloxymultiflorine	2935	344	244 (69)	344 (19)	132 (100)	149 (48)	
13-Angeloyloxy-17-oxolupanine	2885	360	260 (100)	232 (5)	162 (10)	148 (35)	133 (15)
Angustifoline	2083	234	193 (100)	112 (85)	150 (15)	55 (20)	94 (11)
Aphyllidine	2120	246	98 (100)	246 (28)	136 (18)	134 (16)	97 (57)
Aphylline	2180	248	136 (100)	220 (35)	124 (40)	248 (35)	191 (20)
Argyrolobine	2185	262	98 (100)	262 (30)	84 (28)	205 (18)	134 (6)
Baptifoline	2625	260	114 (100)	260 (27)	160 (13)	146 (27)	96 (31)
13 $\alpha$ -Benzoyloxylupanine	3100	368	246 (100)	134 (20)	148 (15)	112 (12)	55 (10)
13-Benzoyloxy-17-oxolupanine	3255	382	260 (100)	232 (5)	162 (10)	148 (35)	133 (15)
13 $\alpha$ -Butyryloxylupanine	2620	334	246 (100)	134 (60)	148 (30)	112 (37)	334 (10)
Camoensidine	2080	234	122 (100)	135 (56)	234 (52)	233 (44)	84 (52)
<i>N</i> -Carboxyethylangustifoline	2305	306	112 (100)	265 (79)	193 (23)	150 (13)	306 (1)
<i>N</i> -Carboxymethylangustifoline	2265	292	112 (100)	251 (71)	102 (24)	152 (3)	292 (1)
13-Cinnamoyloxymultiflorine	3250	392	244 (60)	149 (10)	131 (15)	96 (100)	392 (1)
13 $\alpha$ - <i>cis</i> -Cinnamoyloxylupanine	3260	394	246 (100)	134 (20)	148 (15)	112 (12)	55 (10)
13 $\alpha$ - <i>trans</i> -Cinnamoyloxylupanine	3390	394	246 (100)	134 (20)	149 (15)	112 (10)	55 (10)
13- <i>cis</i> -Cinnamoyloxy-17-oxolupanine	3418	408	260 (100)	232 (5)	162 (10)	148 (35)	408 (1)
13- <i>trans</i> -Cinnamoyloxy-17-oxolupanine	3590	408	260 (100)	232 (5)	162 (10)	148 (35)	133 (15)
Cytisine	1990	190	146 (100)	147 (80)	190 (65)	160 (25)	134 (25)
Dehydroangustifoline	2070	232	232 (60)	191 (60)	120 (100)	149 (94)	94 (83)
Dehydrocytisine	1977	188	134 (100)	188 (99)	148 (70)	146 (60)	160 (35)
Dehydroepilupinine	1440	167	97 (100)	167 (75)	136 (70)	150 (30)	108 (25)
Dehydrolupanine	2133	246	150 (100)	246 (80)	136 (80)	134 (40)	217 (10)
5,6-Dehydrolupanine	2132	246	98 (100)	246 (15)	134 (10)	97 (30)	163 (5)
5,6,-Dehydro- $\alpha$ -isolupanine	2075	246	98 (100)	97 (49)	246 (30)	134 (7)	84 (12)
11,12-Dehydrolupanine	2190	246	134 (100)	246 (67)	148 (39)	231 (18)	112 (18)
Dehydromultiflorine	2110	244	244 (100)	134 (75)	160 (20)	146 (20)	110 (20)
11,12-Dehydrosparteine	1840	232	134 (100)	97 (90)	232 (50)	148 (20)	175 (16)
11,12- <i>seco</i> -12,13-Didehydromultiflorine (formerly <i>N</i> -methylalbine)	2215	246	205 (50)	58 (100)	110 (15)	94 (20)	246 (10)
Dihydromultiflorine	2100	248	134 (100)	136 (80)	150 (20)	248 (15)	219 (15)
Dihydroxyaphyllidine	2215	278	98 (100)	96 (58)	278 (11)	136 (2)	221 (2)
Dihydroxyaphylline	2282	280	98 (100)	84 (9)	280 (6)	206 (2)	124 (7)
Dihydroxylupanine	2371	280	98 (100)	84 (15)	111 (10)	280 (6)	150 (2)
3 $\beta$ ,13 $\alpha$ -Dihydroxylupanine	2508	280	152 (100)	280 (96)	165 (43)	134 (35)	262 (34)
10,17-Dioxo- $\beta$ -sparteine	2340	262	150 (100)	84 (66)	262 (40)	124 (27)	234 (6)
10,17-Dioxosparteine-isomer	2325	262	84 (100)	150 (58)	152 (47)	262 (47)	110 (30)
Epiaphyllidine	2020	246	98 (100)	246 (35)	134 (7)	107 (18)	97 (67)
Epiaphylline	2055	248	136 (100)	220 (45)	248 (35)	191 (20)	97 (40)
Epibaptifoline	2655	260	96 (100)	146 (30)	243 (30)	160 (20)	114 (60)
13-Epihydroxyisolupanine	2345	264	152 (100)	165 (61)	264 (55)	247 (23)	150 (21)
Epilupinine	1416	169	83 (100)	138 (75)	97 (77)	110 (43)	169 (57)
Feruloyllupinine	2960	345	152 (100)	136 (6)	345 (20)	168 (40)	
<i>N</i> -Formylammmodendrine	2210	236	218 (100)	175 (50)	207 (10)	193 (10)	236 (5)
<i>N</i> -Formylangustifoline	2363	262	112 (100)	193 (98)	221 (41)	262 (2)	150 (19)
<i>N</i> -Formylcytisine	2315	218	146 (100)	218 (75)	147 (45)	160 (20)	134 (15)
Gramine	1622	174	130 (100)	174 (30)	131 (21)	77 (10)	103 (7)
Hydroxyammmodendrine	2092	224	207 (100)	109 (48)	165 (46)	224 (40)	152 (35)
<i>p</i> -Hydroxycinnamoyllupinine	2860	315	152 (100)	136 (10)	315 (7)	168 (5)	
3- $\beta$ -Hydroxylupanine	2250	264	136 (100)	264 (97)	150 (61)	134 (61)	247 (38)

Table 1. *Continued*

Compound	RI	M <sup>+</sup>	EI-MS				
7-Hydroxylupanine	2275	264	98 (100)	152 (80)	84 (51)	264 (42)	150 (33)
13 $\alpha$ -Hydroxylupanine	2400	264	152 (100)	165 (40)	264 (40)	246 (40)	134 (30)
13-Hydroxymultiflorine	2558	262	150 (100)	262 (50)	132 (10)	110 (20)	163 (20)
13-Hydroxy-17-oxolupanine	2615	278	278 (38)	260 (100)	232 (15)	166 (100)	148 (90)
Hydroxysparteine	2000	250	153 (100)	114 (51)	98 (25)	136 (36)	250 (17)
7-Hydroxysparteine	1918	250	98 (100)	166 (31)	84 (28)	153 (27)	250 (9)
7-Hydroxy- $\beta$ -isosparteine	1966	250	98 (100)	166 (31)	84 (30)	153 (26)	250 (21)
Hydroxytetrahydrohombifoline	2138	264	223 (100)	58 (35)	108 (33)	128 (18)	264 (< 1)
Hystrine	1663	164	163 (100)	164 (91)	108 (35)	149 (32)	107 (22)
Isoangustifoline	2033	234	193 (100)	112 (70)	150 (10)	55 (30)	94 (20)
13 $\alpha$ -Isobutyryloxyupanine	2570	334	246 (100)	134 (60)	148 (30)	112 (40)	334 (10)
13 $\alpha$ -Isovaleroxyloxyupanine	2680	348	246 (100)	134 (50)	148 (25)	112 (30)	348 (5)
Lupanine	2165	248	136 (100)	149 (60)	248 (40)	150 (34)	219 (8)
$\alpha$ -Isolupanine	2105	248	136 (100)	248 (50)	149 (50)	98 (30)	219 (5)
Lupinine	1420	169	152 (81)	169 (84)	138 (76)	97 (70)	83 (100)
Lusitanine	1875	208	136 (100)	166 (88)	110 (72)	208 (45)	179 (30)
13-Methoxylupanine	2370	278	166 (100)	247 (61)	263 (44)	278 (34)	179 (47)
13-Methoxymultiflorine	2470	276	276 (25)	261 (1)	245 (100)	164 (25)	134 (40)
<i>N</i> -Methylammodendrine	1835	222	98 (100)	222 (72)	150 (57)	207 (12)	136 (47)
<i>N</i> -Methylcytisine	1955	204	58 (100)	204 (30)	146 (10)	160 (10)	
Multiflorine	2310	246	134 (100)	246 (65)	148 (20)	110 (15)	217 (5)
17-Oxolupanine	2350	262	150 (100)	262 (40)	110 (30)	234 (10)	55 (20)
17-Oxosparteine	2070	248	97 (100)	98 (90)	110 (80)	248 (59)	220 (30)
17-Oxo- $\beta$ -isosparteine	2182	248	97 (100)	110 (65)	136 (50)	248 (33)	220 (15)
3 $\beta$ -Propyloxyupanine	2590	320	134 (100)	246 (20)	148 (20)	319 (8)	320 (4)
13 $\alpha$ -Propyloxyupanine	2530	320	246 (100)	134 (60)	148 (30)	112 (37)	320 (10)
Retamine	1980	250	98 (100)	134 (24)	232 (10)	207 (15)	250 (10)
Rhombifoline	2155	244	58 (100)	203 (80)	160 (15)	146 (10)	244 (2)
Smipine	1580	180	109 (100)	96 (74)	151 (14)	112 (11)	163 (2)
Sparteine	1785	234	137 (100)	98 (90)	234 (44)	193 (25)	84 (10)
$\alpha$ -Isosparteine	1710	234	98 (100)	137 (57)	193 (22)	234 (40)	150 (15)
$\beta$ -Isosparteine	1830	234	98 (62)	137 (100)	193 (16)	234 (20)	150 (13)
Tetrahydrocytisine	1843	194	95 (100)	82 (42)	194 (17)	113 (15)	150 (6)
Tetrahydrohombifoline	2050	248	207 (100)	58 (80)	112 (15)	108 (10)	248 (1)
Thermopsine	2310	244	98 (100)	244 (30)	136 (15)	146 (10)	160 (5)
3 $\beta$ -Tigloyloxyupanine	2850	346	134 (100)	246 (20)	148 (20)	346 (1)	
13 $\alpha$ -Tigloyloxyupanine	2753	346	246 (100)	134 (30)	148 (15)	112 (12)	55 (10)
13-Tigloyloxy-17-oxolupanine	2905	360	260 (100)	232 (5)	162 (10)	148 (35)	133 (15)
13-Tigloyloxy-multiflorine	2955	344	244 (56)	344 (13)		132 (100)	149 (40)
13 $\alpha$ -Valeroxyloxyupanine	2745	348	246 (100)	134 (60)	148 (30)	112 (30)	348 (5)

RI, Kovat's retention index; M<sup>+</sup>, molecular ion; GC-EIMS, five significant fragments and their relative abundance (%). For several quinolizidine alkaloids, stereochemical details are still unresolved or could not be determined by our GLC-MS technique. In cases where the differentiation of epimers was possible, the appropriate names are given.

and of multiflorine are abundant in leaves whereas the hydroxylated alkaloids predominate in seeds [15, 16]. During germination these ester alkaloids are generated by specific acyltransferases [2, 12].

Tetracyclic quinolizidine alkaloids with a sparteine, lupanine and hydroxylupanine skeleton can be detected in almost all lupins (Table 2). The bicyclic lupinine and derivatives occur in 19 species and are especially abundant in Old World lupins such as *L. luteus*, *L. hispanicus*, *L. cosentinii*, *L. digitatus*, *L. pilosus*, *L. princei* and *L. varius* (the synonyms *L. pilosus* L. and *L. digitatus* Forsk. are included in *L. varius*). New World species with lupinine and epilupinine are *L. albifrons*, *L. bakeri* and *L. holosericeus*.

Multiflorine and derivatives have a more restricted distribution and were found in Old World lupins such as *L. albus*, *L. atlanticus*, *L. cosentinii*, *L. micranthus*, *L. palaestinus*, *L. princei* and *L. varius* but also in South American taxa, such as *L. albescens*, *L. aureonitens*, whereas North American lupins do not accumulate these alkaloids in substantial amounts (Table 2). Our recent studies on sequence comparisons of the chloroplast gene *rbcL* indicate that the lupins of the lupinine and of the multiflorine complex are genetically closely related and probably share a common ancestry [61].

Aphylline and derivatives are 10-oxosparteine-type QA, which occur in a limited number of North American lupins as major alkaloids, such as *L. argenteus*, *L.*



















*caudatus*, *L. cruckshanksii*, *L. hartwegii*, *L. holosericeus* and *L. leucophyllus*.

QA of the  $\alpha$ -pyridone skeleton, such as anagryne and cytisine, are typical for many genera of the Papilionoideae [1–3]. They are usually absent from members of the genus *Lupinus*. However, a few lupin taxa were found that do accumulate  $\alpha$ -pyridone alkaloids, such as *L. microcarpus*, *L. densiflorus*, *L. ruber*, *L. arbustus*, *L. argenteus*, *L. bicolor*, *L. caudatus* and *L. nanus*. Whereas cytisine and *N*-methylcytisine are common in *Genista*, *Baptisia* and *Thermopsis* [1–3], anagryne and thermopsine dominate in these lupin species.

Besides QA, most lupins produce bipiperidine alkaloids, such as ammodendrine, at least as trace compounds. Ammodendrine and derivatives are abundant however in some North American lupins, such as *L. andersonii*, *L. bakeri*, *L. caudatus*, *L. elegans*, *L. excubitus*, *L. sericeus* and even constitute the main alkaloid as in *L. sulphureus* (Table 2). A few lupins produce simple indole alkaloids [35], such as gramine: *L. hispanicus*, *L. argenteus*, *L. bakeri*, *L. caudatus*, *L. hartwegii*, *L. holosericeus*, *L. sericeus* and *L. texensis*.

Normally, the alkaloid profiles are typical and characteristic for a given lupin species (thus producing an 'alkaloid fingerprint'). Whereas Old World and South American lupins have profiles without much intraspecific variation [5, 6], a number of lupins exist in North America which show large intraspecific variations, e.g. *L. argenteus*, *L. bakeri*, *L. caudatus*, *L. holosericeus*, *L. leucophyllus* and *L. sericeus* (Table 2). In the case of *L. argenteus*, these chemotypes coexist within a close geographical area, whereas in most other cases differences were population-specific [24, 62].

It would be interesting to discuss the alkaloid data of Table 2 under chemosystematic aspects. Such an analysis of the alkaloid data reported here and of those published already [3, 24–60], together with a comparison of molecular data (*rbcL* nucleotide sequences [4]) is in preparation in our laboratory and will be published elsewhere [61].

As far as the toxicity of lupin alkaloids is concerned, lupanine- and sparteine-type alkaloids display a medium herbivore toxicity, whereas  $\alpha$ -pyridone alkaloids, such as cytisine, are almost 10–100 times more toxic [1, 7]. Common molecular targets are nicotinic and muscarinic acetylcholine receptors [2, 63] and  $\text{Na}^+$ ,  $\text{K}^+$ -ion channels, besides protein biosynthesis [2]. It has been shown by Keeler and coworkers [64, 65] that anagryne and ammodendrine have mutagenic properties and produce malformations ('crooked calf disease') in early foetal stages. As can be seen from Table 2, these alkaloids are widely distributed among lupins, especially in those of North America. Thus lupins produce potent defence chemicals affecting a wide array of targets. It has been shown experimentally that QA provide efficient defence against most invertebrate and vertebrate herbivores [7–10]. Variation in structural diversity (which is evident from Table 2) is thought to enhance toxicity (i.e. enabling the attack at several targets) and to reduce the chance that a herbivore will develop resistance towards alkaloids

(because usually, insensitivity would be needed for each target [63, 66]).

## EXPERIMENTAL

*Plant material.* Lupin seeds or leaves were from seed-banks, botanical gardens, herbariums or in a few cases collected in the wild. The following seeds were from the Institut für Saatgutforschung, FAL (Braunschweig): *L. albus* L., *L. angustifolius* L., *L. atlanticus* Gladst., *L. cosentinii* Guss., *L. hispanicus* Boiss. et Reuter, *L. hispanicus* var. *bicolor* Boiss. et Reuter, *L. luteus* L., *L. micranthus* Guss., *L. palaestinus* Boiss., *L. varius* L. The Institut für Genetik und Kulturpflanzenforschung, Gatersleben provided: *L. angustifolius* L., *L. hartwegii* Lindl., *L. nootkatensis* Donn ex Sims, *L. succulentus* Dougl., *L. varius* L., *L. mutabilis* Sweet, *L. pilosus* Murr., *L. nanus* Dougl.; *L. pubescens* Benth., *L. benthamii* Heller, *L. leucophyllus* Dougl., *L. polycarpus* Greene, *L. subcarnosus* Hook. and *L. densiflorus* Benth. Seeds came from the following Botanical Gardens: Braunschweig, London (Royal Botanical Garden, Kew and Chelsea Physick garden), Glasgow, Oslo, Heidelberg, München, Santa Barbara (CA), Claremont (Rancho Santa Ana Botanic Garden) and Perth: *L. digitatus* Forsk., *L. albifrons* Benth., *L. princei* Harms, *L. mutabilis* Sweet, *L. cruckshanksii* Sweet, *L. elegans* H.B.K., *L. floribundus* Benth., *L. formosus* Greene, *L. perennis* L., *L. sulphureus* Dougl. ex. Hook, *L. texensis* Hook., *L. latifolius* Agardh and *L. aureonitens* Gill. A few North American lupins are naturalized in Europe and were collected in the wild by the author (M.W.): *L. polyphyllus* Lindl. (Braunschweig, München, Mainz) and *L. arboreus* Sims (Wexford, Ireland). South American lupins were collected by A. Planchuelo-Ravelo in Argentina: *L. albescens* Hook., *L. giberianus* C.P.S.M., *L. linearis* Desr.; *L. microcarpus* Sims by the author (M.W.) near Temuco (Chile). Many lupins from North America which had been analysed for flavonoid profiles [51] before came from the herbarium of K. W. Nicholls [number of specimens (*n*) and sites of collection in parenthesis]: Although many years old, reliable non-degraded QAs were obtained from 1–2 leaves of nearly all species [24]: *L. andersonii* Wats. (Mono Co., California), *L. arboreus* Sims (Mendocino Co., California), *L. arbustus* ssp. *silvicola* Dunn (Modoc Co., California), *L. arcticus* ssp. *subalpinus* Wats. (Lake Penask, British Columbia), *L. argenteus argenteus* Pursh. (*n* = 3, Oregon, California, Montana), *L. argenteus* ssp. *rubricaulis* (Utah Co., Utah), *L. argenteus* var. *tenellus* Dunn (Uinta Co., Utah), *L. bicolor* Lindl. (Benton Co., Oregon), *L. bicolor* ssp. *bicolor* Lindl. (Victoria Co., British Columbia), *L. burkei* Wats. (Route 3, British Columbia), *L. caudatus* Kell. (*n* = 6: Nevada, Idaho, California), *L. caudatus* × *hillii* (Coconino, Arizona), *L. densiflorus* (Benth.) (*n* = 2; Obispo and Mendocino, California), *L. elatus* Jtn. (Los Angeles, California), *L. excubitus* var. *austromontanus* (Heller) C.P.S.M. (Kern Co., California), *L. hillii* Heller (Maricopa, Arizona), *L. holosericeus* (*n* = 3; Nevada, Idaho), *L. kingii* (Coconino Co., Arizona), *L. latifolius* Agardh (Lane Co., Oregon),

*L. leucophyllus* Dougl. ( $n = 6$ ; Idaho, Washington, Oregon, British Columbia), *L. longifolius* (Wats.) Abrams (Los Angeles, California), *L. parviflorus* Nutt. (Elko Co., Nevada), *L. polycarpus* Greene (Sonoma Co., California), *L. polyphyllus* Lindl. (Klamath Co., Oregon), *L. ruber* Heller (Modoc Co., California), *L. sericeus* var. *eggles-tonianus* Pursh. ( $n = 4$ ; Idaho, Montana, Washington, British Columbia), *L. sericeus* ssp. *huffmannii* Pursh. ( $n = 5$ ; Arizona, Utah), *L. sericeus* ssp. *sericeus* Pursh. ( $n = 7$ ; Oregon, Montana, Washington), *L. sericeus* ssp. *flexuosus* Pursh. ( $n = 13$ ; Idaho, Washington, Montana, British Columbia). A few North American lupins were collected by the author or coworkers (D. Carey, D. Dreyer): *L. arboreus* Sims (San Francisco, California), *L. argenteus* Pursh. (Colorado Rocky Mountains, Colorado), *L. bakeri* Greene (Colorado Rocky Mountains, Colorado), *L. nanus* Dougl. (San Francisco, California) and *L. sulphureus* Dougl. ex. Hook (Utah). If seeds were obtained seedlings and plants were cultivated either in the greenhouse (seedlings) or in an experimental garden in Germany. Plant material harvested was stored at  $-20^{\circ}$  until further processing.

**Alkaloid extraction.** Plant material was homogenized in 0.5 M HCl. After 30 min at room temperature, the homogenate was centrifuged for 10 min at  $10000 \times g$ . The supernatant was made alkaline by adding ammonia or 2 M NaOH and was applied to Extrelut columns (Merck, Darmstadt). Alkaloids were eluted with  $\text{CH}_2\text{Cl}_2$  and the solvent evaporated *in vacuo* [13–24].

**Alkaloid analysis.** Alkaloid extracts were separated on fused-silica capillary columns (0.3 mm  $\times$  30 m) with covalently bound liquid phases (DB 1, or DB 5 or equivalents; J & W Scientific) as described in refs [5, 6, 13–23]. For GLC–MS measurements a Kratos instrument (MS 30) or Finnigan MAT 4515 was used in combination with the INCOS data system [for details see refs [5, 6, 13–24].

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