Alkaloid Profile of Leaves and Seeds of *Lupinus hintonii* C. P. Smith

Kalina Bermúdez Torres[a,*], Norma Robledo Quintos[a], Laura L. Barrera Necha[a] and Michael Wink[b]

[a] Centro de Desarrollo de Productos Bióticos- Instituto Politécnico Nacional, P. O. Box 24, Yautepec, Morelos 62731, Mexico. Fax: 0527353941896. E-mail: kbermud@hotmail.com
[b] Institut für Pharmazeutische Biologie, Ruprecht-Karls Universität, D-69120 Heidelberg, Germany

* Author for correspondence and reprints request

Z. Naturforsch. **57c,** 243–247 (2002); received October 9/November 11, 2001

Lupinus hintonii, Quinolizidine Alkaloids, Alkaloid Profile

L. hintonii C. P. Smith grows in the Central Highland forests of Mexico at altitudes between 2800 m to 3200 m above see level. Members of the genus *Lupinus* produce quinolizidine alkaloids as main chemical defensive compounds against herbivores. Surprisingly alkaloid profiles are rather constant within this species, while substantial variation was found when compared to morphologically closely related other taxa. As part of a phytochemical project on Mexican wild lupins, we report on the alkaloid profiles of seeds and leaves *of L. hintonii*. 19 alkaloids could be identified by capillary GLC-MS. Six major alkaloids occurred in leaves and seeds: 13-hydroxylupanine (28% and 45% respectively), tetrahydrorhombifoline (31% and 23% respectively), angustifoline (2% and 4% respectively), lupanine (7% and 5% respectively), 13α-tigloyloxylupanine (19% and 5% respectively) and 4α-angeloyl-3β-hydroxylupanine (9% and 2%). This chemical pattern resembles that of the North American lupin *L. floribundus.*

Introduction

Lupinus (Leguminosae) is a large and diverse genus comprising about 500 annual, biannual and perennial herbaceous, prostrate and shrubby plants characterized by palmate-compound leaves, and regular "papilionaceous" flowers allocated in a terminal raceme. While only 12 taxa are known from the Mediterranean and North African region the majority of lupins is indigenous of Americas.

Mexico belongs to the Mountain Range (MAC) subregion (Planchuelo, 1994), where the majority of *Lupinus* species occurs (Dunn, 1984). This region is important as a germplasm resource (Planchuelo, 1994). More than 110 wild species have been reported in Mexico (Bermúdez Torres, 1998), comprising 39 endemic species (Sousa and Delgado, 1998). In other words, Mexico harbours ca 22% of all lupin taxa known world-wide of which 7.8% are endemic to Mexico, indicating that Central America is a hot spot of global biodiversity for this genus.

However, the genus *Lupinus* is notorious for being a very complex and difficult group because of its diversity, the great ability of numerous species to hybridise, and often the lack of clear diagnostic features. Only the use of more than one criteria such as morphology, phytochemistry and DNA allows a deeper understanding of this genus and can contribute to clarify its taxonomy and systematics.

Quinolizidine alkaloids, which confer resistance to herbivores and pathogen microorganisms (Wink, 1988; Wink, 1992), are characteristic secondary metabolites of the Leguminosae and are especially abundant in the tribes Genisteae, Sophoreae and Thermopsideae (Mears and Mabry, 1971; Kinghorn and Balandrin, 1984). The genus *Lupinus* forms a distinct and monophyletic subtribe within the Genisteae (Mears and Mabry, 1971; Käss and Wink, 1997).

There are a considerable number of comparative studies on the alkaloid patterns of *Lupinus* species (Kinghorn *et al.*, 1980; Wink *et al.*, 1993; Múzquiz *et al.*, 1994,; Planchuelo and Wink, 1993). Chemical studies can help or refute proposed taxonomic classifications of uncertain taxa. However, the use of the quinolizidine alkaloids as taxonomical markers is controversial because they repre-

0939–5075/2002/0300–0243 $ 06.00

sent adaptive traits which are liable to convergence and differential gene expression (Wink and Waterman, 1999).

The goal of this study is to analyze quinolizidine alkaloid profiles of a Mexican wild and unstudied lupin species *L. hintonii* by GLC-MS. Furthermore, we tried to compare the chemical pattern with morphologically very close species in order to test the usefulness of QA composition as taxonomical marker.

Results and Discussion

Capillary GLC-MS analysis of *L. hintonii* revealed the presence of 28 alkaloids. By comparing Kovats retention index and mass spectral data to those of reference data stored in our data library (Wink, 1993; Wink *et al.*, 1995) 19 alkaloids could be identified unequivocally (Table I). The profile is characterized by six major alkaloids. Alkaloid composition of 19 specimens: Tetrahydrorhombifoline: average alkaloid contents in seeds (S): 23± 9%, in leaves (L): 31± 11%; angustifoline: S 4± 7%, L 2± 2%; lupanine: S 5± 8%, L 7± 5%; 13α-hydroxylupanine: S: 45± 12%, L 28± 18%; 13α-tigloyloxylupanine: S 5± 3%, L 19± 16%; and 4α-angeloyl-3β-hydroxylupanine: S 12± 8%, L 9± 17%.

Other compounds were identified as minor alkaloids: sparteine, ammodendrine, dihydrocytisine, 11,12-seco-12,13-dehydromultiflorine, isoangustifoline, α-isolupanine, aphyllidine, N-formylammondendrine, 17-oxolupanine, 11-oxotetrahydrorhombifoline, 13α-butyryloxylupanine, 13α-isovaleroyloxylupanine and 13-tigloyloxy-17-oxolupanine (Table I). Further minor alkaloids

Table I. Identification of quinolizidine alkaloids from *L. hintonii* Smith by GLC-MS; M+: molecular ion; RI: Kovats retention index; F1 to F5; characteristic fragment ions.

Nr.	Alkaloid	RI	M+	Mass spectral data (*m/z* (intensity%) EI-MS				
				F1	F2	F3	F4	F5
1	Sparteine	1734	234 (30)	137 (100)	98 (80)	55 (55)	193 (47)	110 (30)
2	Ammodendrine	1791	208 (47)	165 (100)	110 (83)	94 (57)	123 (57)	80 (54)
3	Dihydrocytisine	1799	192 (100)	192 (100)	55 (62)	94 (60)	68 (48)	80 (44)
4	11,12-seco-12,13-didehydromultiflorine	1980	246 (2)	205 (100)	58 (66)	162 (27)	120 (18)	106 (14)
5	Isoangustifoline	2051	234 (2)	193 (100)	112 (55)	152 (42)	55 (41)	94 (29)
6	Tetrahydrorhombifoline	2080	248 (1)	207 (100)	58 (77)	112 (22)	81 (13)	95 (7)
7	Angustifoline	2104	234 (1)	193 (100)	150 (15)	112 (76)	55 (57)	74 (32)
8	RI 2109	2109	280 (5)	207 (100)	55 (78)	67 (62)	81 (44)	95 (27)
9	RI 2116	2116	248 (21)	55 (100)	208 (83)	112 /75)	96 (54)	68 (52)
10	α-Isolupanine	2131	248 (20)	136 (100)	55 (45)	207 (35)	94 (26)	149 (23)
11	Aphyllidine	2145	246 (43)	98 (100)	246 (43)	55 (32)	136 (22)	67 (20)
12	Lupanine	2185	248 (50)	136 (100)	55 (64)	149 (56)	248 (52)	98 (29)
13	N-Formylammodendrine	2222	236 (5)	218 (100)	175 (68)	55 (57)	136 (44)	112 (37)
14	RI 2334	2334	266 (7)	207 (100)	55 (53)	150 (32)	112 (23)	94 (20)
15	17-Oxolupanine	2364	262 (48)	150 (100)	262 (65)	112 (55)	207 (47)	94 (45)
16	RI 2371	2371	340 (1)	55 (100)	245 (79)	112 (76)	163 (65)	94 (55)
17	RI 2380	2380	262 (70)	55 (100)	150 (74)	262 (76)	245 (66)	112 (42)
18	RI 2392	2392	278 (15)	193 (100)	112 (100)	55 (86)	207 (42)	150 (36)
19	RI 2403	2403	262 (35)	55 (100)	114 (75)	193 (75)	150 (66)	96 (63)
20	11-Oxotetrahydrorhombifoline	2451	262 (13)	221 (100)	55 (53)	150 (27)	112 (20)	67 (13)
21	13α-Hydroxylupanine	2478	264 (35)	246 (45)	55 (77)	152 (100)	165 (40)	134 (35)
22	13α-Butyryloxylupanine	2620	334 (7)	246 (100)	55 (73)	134 (65)	112 (39)	148 (37)
23	RI 2622	2622	262 (33)	245 (100)	55 (40)	112 (38)	262 (30)	94 (17)
24	13α-Isovaleroyloxylupanine	2714	348 (4)	246 (100)	134 (46)	207 (40)	55 (37)	148 (22)
25	13α-Tigloyloxylupanine	2749	346 (1)	246 (100)	55 (32)	134 (30)	112 (17)	148 (13)
26	13-Tigloyloxy-17-oxolupanine	2971	360 (1)	260 (100)	55 (60)	148 (36)	112 (20)	82 (16)
27	4α-Angeloyl-3β-hydroxylupanine	2972	362 (3)	245 (100)	263 (78)	112 (68)	55 (66)	149 (22)
28	RI 3034	3034	340 (89)	148 (100)	277 (91)	340 (89)	55 (87)	120 (61)

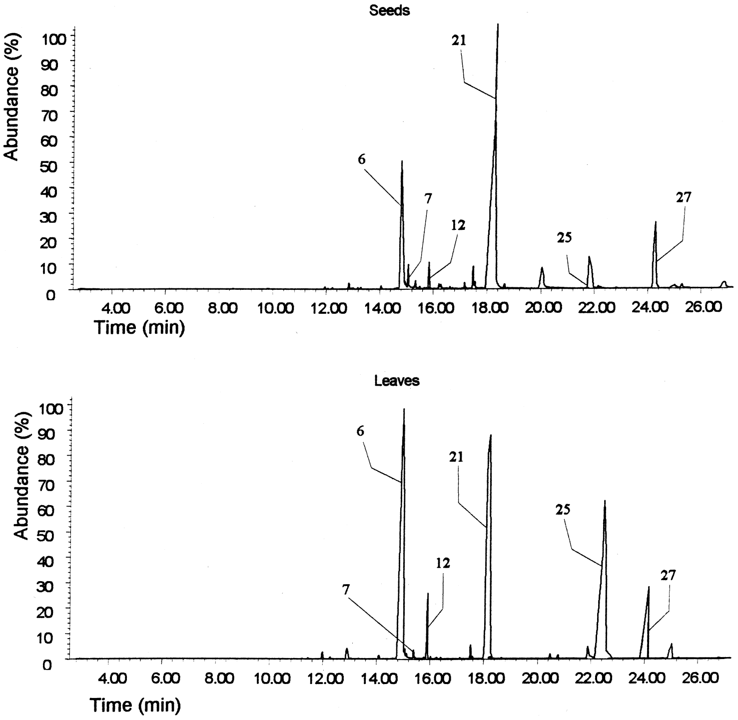

Fig. 1. Separation of quinolizidine alkaloids from *L. hintonii* by GC. Conditions: injector: 250 °C, mass-specific detector: 280 °C, oven: 120 °C, 3 min isothermal, then 10 °C/min to 260 °C. Numbers refer to alkaloids of Table I.

were present which could not be identified because their amounts were too small to allow isolation and characterization by NMR.

Alkaloid profiles of leaves exhibit a higher diversity and esters of 4β- and 13α-hydroxylupanine, like 13α-tigloyloxylupanine are commonly observed. This observations follows a general scheme in that hydroxylated alkaloids, like 13-hydroxylupanine, are more abundant in the seeds and during germination an active acyl *trans*ferase produces a variety of hydroxylupanine esters (Wink, 1983).

Whilst tetracyclic alkaloids like sparteine, lupanine, 3- and 13-hydroxylupanine and their derivatives are very common in most lupin species, tetrahydrorhombifoline and their derivatives show a restricted distribution. They were found in traces of New World lupins such as the North American species *L. burkei, L. elatus, L. hillii, L. leucophyllus, L. polyphyllus, L. sericeus, L. subcarnosus* and *L. texensis* and in South American species such as *L. giberteanus and L. mutabilis* (Wink *et al.*, 1995).

The alkaloid profile of *L. hintonii* is very similar to that of the North American lupin *L. floribundus,* whose leaves contain tetrahydrorhombifoline (38%), angustifoline (4%), lupanine (5%), 13α-hydroxylupanine (22%), 13-angeloyloxylupanine (3%), 13-tigloyloxylupanine (12%), 13-benzoyloxylupanine (1%), 13-*cis*-cinnamoyloxylupanine (9%), 13-*trans*-cinnamoyloxylupanine (2%), and 13-methoxylupanine (1%) (Wink *et al.*, 1995). This similarity suggests that both species, *L. hintonii* and *L. floribundus*, are closely related although their distribution ranges differ substantially. Further comparative analyses of morphological and molecular characters are necessary to test their relatedness.

Also *L. aschenbornii* shows a similiar alkaloid profile. This Mexican wild species contains 13-hydroxylupanine (55% and 57% respectively), tetrahydrorhombifoline (8% and traces respectively), 13α-tigloyloxylupanine (4% and traces respectively), lupanine (4% and 0.3% respectively) and angustifoline (traces and 19%) in both, leaves and seeds (Bermúdez Torres *et al.*, 1999). *L. hintonii* and *L. aschenbornii* are morphologically very different and their habitats are also distinct. Whereas

Table II. Alkaloid composition and alkaloid content of leaves and seeds of *L. hintonii* Smith.

Nr.	Alkaloid	Seeds		Leaves	
		%	Amount [μg/g]	%	Amount [μg/g dry wt]
1	Sparteine	0.21	47.60	0.53	0.00153
2	Ammodendrine	0.33	74.60	0.6	0.00174
3	Dihydrocytisine	0.15	33.80	– –	–
4	11,12-seco-12,13-didehydromultiflorine	0.19	43.20	0.3	0.00087
5	Isoangustifoline	0.49	109.20	0.32	0.00093
6	Tetrahydrorhombifoline	23.15	4666.80	31.34	0.07600
7	Angustifoline	4.03	1322.40	1.53	0.00372
8	RI 2109	–	–	1.28	0.00370
9	RI 2116	0.23	51.80	–	–
10	α-Isolupanine	0.4	90.80	0.57	0.00165
11	Aphyllidine	0.09	20.80	0.42	0.00122
12	Lupanine	4.81	853.60	7.26	0.01762
13	N-Formylammodendrine	–	–	0.33	0.00095
14	RI 2334	0.19	42.80	–	–
15	17-Oxolupanine	0.21	48.00	0.65	0.00188
16	RI 2371	0.33	75.20	–	–
17	RI 2380	0.12	27.40	–	–
18	RI 2392	0.1	22.00	–	–
19	RI 2403	0.25	56.80	–	–
20	11-Oxotetrahydrorhombifoline	–	–	1.01	0.00292
21	13α-Hydroxylupanine	44.8	9736.80	28.03	0.06800
22	13α-Butyryloxylupanine	–	–	0.08	0.00023
23	RI 2622	1.2	270.40	1.6	0.00464
24	13α-Isovaleroyloxylupanine	–	–	0.61	0.00176
25	13α-Tigloyloxylupanine	4.64	932.40	19.24	0.04680
26	13-Tigloyloxy-17-oxolupanine	0.87	195.60	1.02	0.00296
27	4α-Angeloyl-3β-hydroxylupanine	11.84	3261.00	9.29	0.02260
28	RI 3034	0.11	24.00	–	–

L. hintonii is growing in the *Pinus-Quercus* forest at altitudes of 2800–3200 m above see level, *L. aschenbornii* is part of the subalpine vegetation of the Central Mexican highlands and grows at altitudes between 3500–4300 m above see level. In contrast, the morphology of *L. hintonii* is closer to that of *L. montanus* but their alkaloid profiles differ significantly: sparteine 77%, lupanine 17%, multiflorine 4% and 3β-hydroxylupanine 3% (unpublished data). These comparisons imply that similar alkaloid pattern do not necessarily correlate with morphological relatedness.

Among the major alkaloids found in *L. hintonii* some inhibit bacterial growth (angustifoline (ED_{50} 50 mM), lupanine (ED_{50} <5 mM), 13α-hydroxylupanine (ED_{50} 175 mg/kg), are toxic to insects (lupanine (ED_{50} >0.1%, 13α-tigloyloxylupanine (<0.1 mM)) and other animals (Wink, 1992). Considering the alkaloid profile of *L. hintonii* it is likely that QA serve as chemical defence compounds as was demonstrated for other lupin species (Wink, 1992).

Experimental

Samples of *Lupinus hintonii* were collected between May 1996 and August 1999 from Tres Marías and Fierro del Toro in State Morelos, and San Rafael, Huisquilucan and Hacienda el Mayorazgo in State of Mexico at altitudes between 2800–3200 m above see level. Leaves and seeds were analyzed separately in order to compare the respective alkaloid profiles. Alkaloid extraction was carried out as described in Wink *et al.* (1995): 0.5 g of leaves or seeds were homogenized in 20 ml 1 N hydrochloric acid. The homogenate was adjusted to pH 12 with 6 N aqueous sodium hydroxide. Alkaloids were extracted by solid phase extraction using Extrelut-columns® (Merck) and CH_2Cl_2 as eluent. The alkaloid extracts were separated and analyzed by GLC-MS with the equipment Mod. HP 6890/5972. The column type used was DB 1; 30 m; 0.25 mm i. d.; 0.25 μm film thickness; split ratio 1:20; carrier gas: He; flow 1 ml / min; injector temperature: 250 °C; oven temperature program: 120 °C; 3 min isothermal; 120–260 °C with a rate of 10 °C/min; then 10 min isothermal; auxiliary temperature was 280 °C. The electron impact mass spectra were recorded at 70 eV ionisation energy, scan (50–550 VMA). The Kovats index was determined by co-chromatography with a mixture of linear alkanes. Pure lupanine and sparteine were used as external standard for quantification.

Acknowledgements

We appreciate the financial support from: Deutscher Akademischer Austauschdienst (DAAD), CGPI-IPN (Project number: 978038) and CONACyT (26411-N). We thank Dr. Mario Rodríguez, Dr. Carlos Cespedes and Dr. Luc Legal for helpful discussion and comments on the manuscript.

Bermúdez Torres K., Robledo Quintos N. R., Martínez Herrera J., Tei, A. and Wink M. (1999), Patrón de acumulación de alcaloides en hojas y semillas de *Lupinus aschenbornii* crecidos en México. Revista Latinoamericana de Química **27** (3), 101–105.

Bermúdez Torres K. (1998), Vorkommen und Verbreitung der Lupinen in Mexiko. In: Lupinen in Forschung und Praxis (Wink, M., ed.). Universität Heidelberg, pp. 27–40.

Dunn D. B. (1984), Genetic resources: Cytotaxonomy and distribution of new world lupin species. In: Proceedings of the third International Lupin Conference. International Lupin Association, La Rochelle, France, pp. 68–85.

Käss E. and Wink M. (1997), Molecular phylogeny and phylogeography of *Lupinus* (Leguminosae) inferred from nucleotide sequences of the rbcL gene and ITS 1+2 regions of rDNA, Pl. Syst. Evol. **208**, 139–167.

Kinghorn A. D. and Balandrin M. F. (1984), Quinolizidine alkaloids of the leguminosae: structural types, analysis, chemotaxonomy, and biological activities. In: Alkaloids: Chemical and Biological Perspectives (Pelletier, E. S. ed.). Wiley, New York, pp. 105–148.

Kinghorn A. D., Selim M. A. and Smolenski S. J. (1980), Alkaloid distribution in some new world *Lupinus* species. Phytochemistry **19**, 1705–1710.

Mears J. A. and Mabry T. J. (1971), Alkaloid in the Leguminosae. In: Chemotaxonomy of the Leguminosae (Harborne, J. B., Boulter, D. and Turner, B. L., eds.). Academic Press, London, pp. 83–168.

Muzquiz M., Cuadrado C., Ayet G., de la Cuadra C., Burbano C. and Osagie A. (1994), Variation of alkaloid components of lupin seeds in 49 genotypes of *Lupinus albus* L. from different countries and locations. J. Agric. Food Chem. **42**, 1447–1450.

Planchuelo A. M. (1994), Wild lupin distribution and its implications as germplasm resources. In: Advances in Lupin Research (Neves Martins, J. M. and Beirao da Costa, M. L.; eds.), pp. 65–69.

Planchuelo-Ravelo A. M. and Wink M. (1993), Alkaloid composition of *Lupinus albescens* (Fabaceae) from South America. Z. Naturforsch. **48c**, 414–416.

Sousa S. M. and Delgado A. (1998), Leguminosas mexicanas: fitogeografía, endemismo y orígenes. In: Diversidad biológica de México: Orígenes y Distribución. (Instituto de Biología, UNAM. ed.). pp. 449–500.

Wink M. and Waterman P. (1999), Chemotaxonomy in relation to molecular phylogeny of plants. In: Biochemistry of Plant Secondary Metabolism (M. Wink, ed.). Sheffield Academic Press and CRC Press, Annual Plant Reviews Vol. **2**, 300–341.

Wink M. (1988), Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivors. Theor. Appl. Gen. **75**, 225–233.

Wink M. (1992), The role of quinolizidine alkaloids in plants insect interactions. In: Insect Plant Interactions, IV (Bernays, E. A., eds.). IRC Press, Boca Raton, pp. 131–166.

Wink M. (1993), Quinolizidine alkaloids. In: Methods in Plant Biochemistry. Vol **8**. Academic Press, London, pp. 197–239.

Wink M. and Witte L. (1983), Evidence for a widespread occurrence of the genes of quinolizidine alkaloid biosynthesis. FEBS Lett. **159**, 196–200.

Wink M., Meißner C. and Witte L. (1995), Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. Phytochemistry **38**, 139–153.