Quinolizidine Alkaloid Profiles of South American Lupins: *Lupinus linearis* and the *Lupinus gibertianus* Complex

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Z. Naturforsch. **48 c**, 702–706 (1993); received June 28, 1993

Lupinus, Leguminosae, Quinolizidine Alkaloids, Chemotaxonomy, Alkaloid Profile

The alkaloid composition of leaves of *Lupinus linearis* Desr. (3 ecotypes) and *L. gibertianus* Smith (6 ecotypes) from Argentina and Brazil were studied by capillary gas-liquid chromatography and GLC-mass spectrometry (EI-MS). Both species are closely related according to morphological criteria. This view is supported by the alkaloid profiles which are very similar and share a series of new and uncommon alkaloids. Main alkaloids are lupanine, 13-hydroxylupanine, esters of 13-hydroxylupanine (*e.g.* 13-angeloyloxylupanine, 13-tigloyloxylupanine, 13-benzoyloxylupanine, 13-*cis/trans*-cinnamoyloxylupanine). Minor alkaloids are: sparteine, 11,12-dehydrosparteine, ammodendrine, tetrahydrorhombifoline, angustifoline, α-isolupanine, 5,6-dehydrolupanine, 11,12-dehydrolupanine, N-formylangustifoline, 13-*cis*-cinnamoyloxymultiflorine. New minor alkaloids which have been tentatively identified by GLC-MS are 13-hydroxy-17-oxolupanine and corresponding esters (13-angeloyloxy-17-oxolupanine, 13-tigloyloxy-17-oxolupanine, 13-benzoyloxy-17-oxolupanine, 13-*cis*-cinnamoyloxy-17-oxolupanine, and 13-*trans*-cinnamoyloxy-17-oxolupanine).

Introduction

The *Lupinus gibertianus* C. P. Smith complex represents a group of annual lupins occurring in Brazil, Uruguay and Argentina. Based on leaf and flower morphology several taxa have been described which seem to represent adaptations to different ecological conditions [1]. *L. linearis* Desr. grows in Argentina and Uruguay and shares many morphological similarities with the members of the *L. gibertianus* complex.

Quinolizidine alkaloids (QA) are characteristic features of the Fabaceae and are especially abundant in the genus *Lupinus* [2, 3]. Lupins rely substantially on quinolizidine alkaloids for chemical defence against herbivores and to a minor degree against microorganisms and competing plants [3–6]. About 500 taxa are known of the genus *Lupinus*. Whereas only few (12 species) occur in the Old World, lupins are abundant in North and South America. Alkaloid profiles have been studied for most Old World and a substantial number of North American lupins [2, 3, 6–8], whereas South American taxa have hardly been analyzed yet. Because it has been suggested that lupins originated from South America [9, 10], it would be interesting to know whether South American lupins have evolved similar alkaloid profiles as their North American or European counterparts.

In this study we have analyzed the alkaloid patterns of 3 ecotypes of *L. linearis* and 6 ecotypes from the *L. gibertianus* complex. As a method of choice we employed capillary GLC and GLC-MS for the analysis of the complex alkaloid profiles present (for review [2, 3]).

Results and Discussion

Because quinolizidine alkaloids are mainly synthesized in leaves, alkaloid profiles from this organ usually show the highest diversity in terms of structures present [3, 11]. Another advantage of analyzing leaves is the possibility of using herbarium specimens since lupin alkaloids do not deteriorate under these conditions at least as long as the material is dried quickly and stored away from moisture [7, 8]. For this study we have analyzed leaves of herbarium specimens which had been evaluated morphologically before [12, 13]. One or two dried leaves contain sufficient amounts of alkaloids to allow an analysis by capillary GLC and GLC-MS. This procedure does not allow however, to isolate any new alkaloid and to determine its structure by other means, such as NMR.

Reprint requests to Prof. M. Wink.

Verlag der Zeitschrift für Naturforschung,
D-72072 Tübingen
0939–5075/93/0900–0702 $ 01.30/0

Employing our large library of mass spectra and GLC retention indexes [3, 6, 7, 11] we were able to identify most quinolizidine alkaloids unambiguously (Table I, Fig. 1). The alkaloid profiles from both species and respective ecotypes were quite similar as far as the qualitative and quantitative composition was concerned. This confirms the view that both species are closely related [14] and that the members of the *L. gibertianus* complex are not genetically differentiated but represent different phenotypes.

Main alkaloids were lupanine, 13-hydroxylupanine and esters of 13-hydroxylupanine such as 13-acetyloxylupanine, 13-angeloyloxylupanine, 13-tigloyloxylupanine, 13-benzoyloxylupanine, and 13-*cis/trans*-cinnamoyloxylupanine. They were ac-

Table I. Alkaloid profiles of different ecotypes of the *L. gibertianus* complex.

Origin of samples:
L. linearis 1 = sample from Rio Grande do Sul, 1961, Pereira; RB 115710 (Brazil);
L. linearis 2[a] = sample from Dpto. Apóstoles, 1977, Crabrera 28596;
L. linearis 3 = sample from Dpto. Capital, 1950, Spegazzini 10810 (Argentina);
L. gibertianus 1 = sample from Dpto. San Cosme, 1978, Ferrucci 38;
L. gibertianus 2 = sample from Calle Maipú, 1975, Schinini 12213;
L. gibertianus 3 = sample from Isla Apigé Grande, 1978, Schinini 15736;
L. gibertianus 4 = sample from Dpto. Ituzaingo, Isla Apigé Chico, 1978, Schinini 15472;
L. gibertianus 5 = sample from Riachuelo, 1973, Cristobal 1083;
L. gibertianus 6 = sample from Dpto. Capital, 1976, Martinez Croveto 10611.

		Alkaloid profile (total alkaloids = 100%)								
		L. linearis			*L. gibertianus*					
Alkaloid	RI	1	2	3	1	2	3	4	5	6
Sparteine	(1785)		*	*						
N-methyltetrahydrocytisine	(1800)	*	*	*						
11,12-Dehydrosparteine	(1840)	*	*	*						
Ammodendrine	(1865)	1	2	1	*	1	2	*	1	*
Isoangustifoline	(2030)	*		*	*	2	1	*	*	1
Tetrahydrorhombifoline	(2050)	*	*	*	*	2	1	1	*	*
17-oxosparteine	(2070)		*	*						
Angustifoline	(2078)	*	*	*	3	2	1	1	1	1
α-Isolupanine	(2105)	1	1	*	1	1	*	*	*	*
5,6-Dehydrolupanine	(2128)	*	*		*	*				
Lupanine	(2165)	19	29	31	19	17	31	15	4	9
11,12-Dehydrolupanine	(2190)	*	*	*	*	*	*	*	*	*
Multiflorine	(2310)	*	*	*	*	*				
"11,12-Dehydro-13-hydroxylupanine"	(2348)	*	*	*	*	*	*	*	*	*
17-Oxolupanine	(2330)	*	*	*	*	1	*	*	*	*
13 α-Hydroxylupanine	(2402)	27	42	55	30	64	46	49	52	15
13 α-Acetyloxylupanine	(2450)	*	*	*	*	*	*	*	*	*
13-Hydroxymultiflorine	(2558)	*	*	1	*	*	*	*	*	*
13-Angeloyloxylupanine	(2733)	1	1	2	1	4	1	3	2	23
13-Tigloyloxylupanine	(2753)	9	12	1	7	3	4	14	10	36
13-Benzoyloxylupanine	(3110)	*	*	*	*	*	*	*	*	*
13-*cis*-Cinnamoyloxylupanine	(3260)	16	8	*	15	*	*	*	*	*
13-*trans*-Cinnamoyloxylupanine	(3390)	1	1	*	1	*	*	*	*	*
13-Methoxymultiflorine	(2472)	1	*	*	*	1	*	*	*	*
N-Formylangustifoline	(2363)	1	*	*	*	*	*	*	*	*
"13-Hydroxy-17-oxolupanine"	(2615)	1	*	*	*	*	*	*	*	*
"13-Angeloyloxy-17-oxolupanine"	(2885)	*	*	*	*	*	*	*	*	*
"13-Tigloyloxy-17-oxolupanine"	(2905)	2	*	*	*	*	*	*	*	*
"13-Benzoyloxy-17-oxolupanine"	(3255)	*	*	*	*	*	*	*	*	*
"13-*cis*-Cinnamoyloxy-17-oxolupanine"	(3418)	4	2	*	1	*	*	*	*	*
"13-*trans*-Cinnamoyloxy-17-oxolupanine"	(3590)	1	1	*	*	*	*	*	*	*
13-Cinnamoyloxy-multiflorine	(3250)				*	*				

[a] Possibly an intermediate between *L. gibertianus* and *L. linearis*.
RI = Kovats retention index; * = traces.

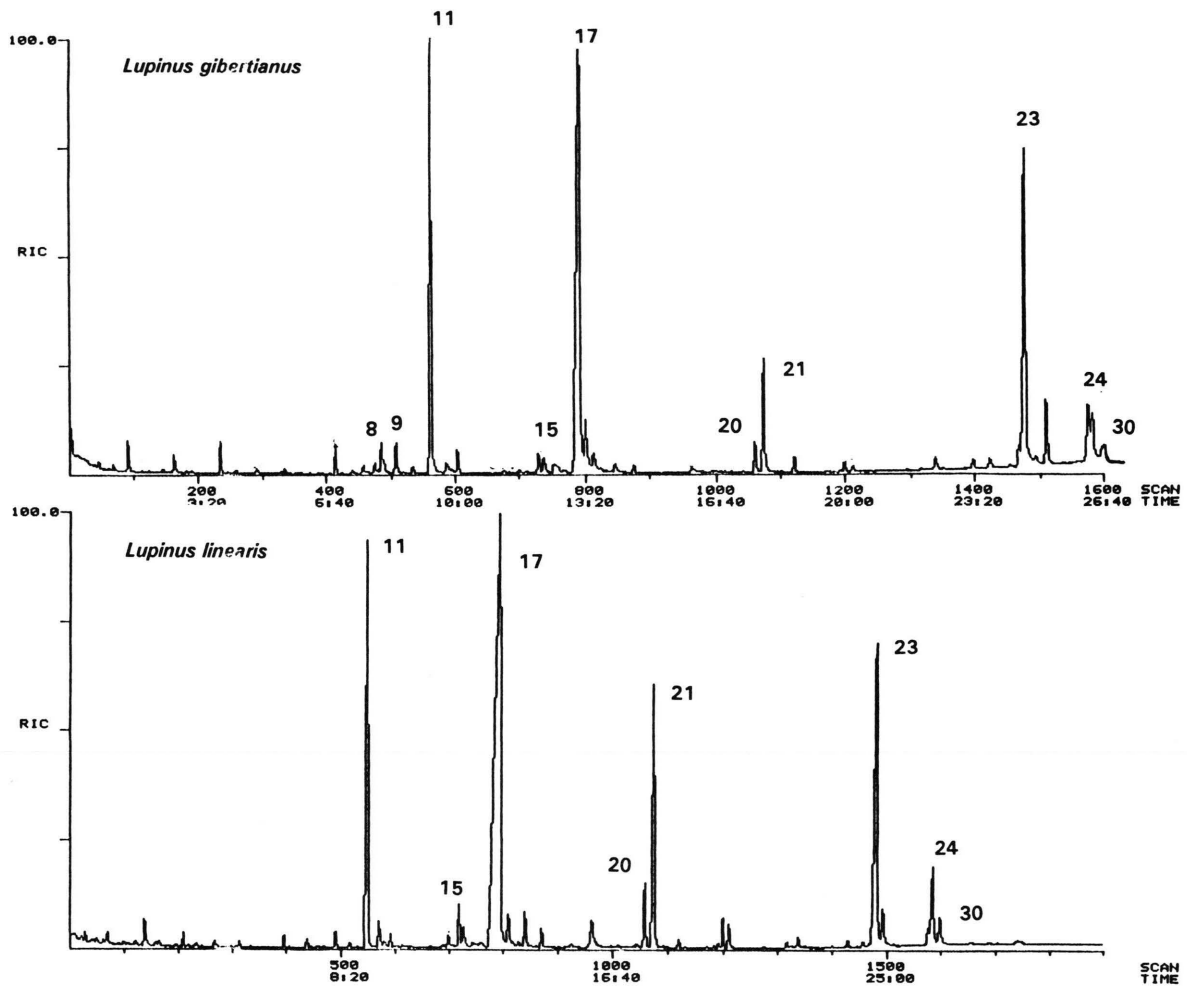

Fig. 1. Analysis of quinolizidine alkaloids of *Lupinus linearis* and *Lupinus gibertianus* by capillary GLC-MS. Numbers are identical with those in Table II.

companied by minor, but typical lupin alkaloids, such as sparteine, angustifoline, tetrahydrorhombifoline, α-isolupanine, 5,6-dehydrolupanine, 11,12-dehydrolupanine, N-formylangustifoline and the bipiperidylalkaloid ammodendrine [3, 6, 7].

A few minor alkaloids were unknown to us and scarcity of the material did not allow any further characterization. However, a series of new alkaloids could be tentatively identified according to their informative MS fragmentation patterns [3]: The mass spectrum of 17-oxolupanine (M^+ = 262) is characterized by a significant *m/z* at 234 which arises from the elimination of CO (= 28). In both lupin species, a hydroxy derivative of 17-oxolupanine is present with a M^+ of 278 (abundance 34%). A significant fragment ion at *m/z* 260 (100%) can be explained by loss of a hydroxyl group in form of H_2O (−18 mass units, mu) in analogy to the fragmentation pattern of other hydroxylated QA [2, 3]. Indicative for a 17-oxolupanine derivative is a fragment ion at *m/z* 232 (11%), *i.e.* loss of a CO group (260-28 = 232). Because all other hydroxylated QA of the species studied had the hydroxyl group in position 13 (Table I), we assume that this new alkaloid is a 13-hydroxy-17-oxolupanine. In addition, we found a series of ester alkaloids, which had a significant fragment ion at *m/z* 260 instead of the typical *m/z* 246 of 13-hydroxylupanine esters. According to the molecular ions recorded (M^+ 360, 374, 408), the organic acids were of the typical series, involving

Table II. Mass spectral identification of quinolizidine alkaloids from *L. linearis* and *L. gibertianus*.

Alkaloid	M^+	Significant fragment ions (abundance)				
1. Sparteine	234	234(20)	193(30)	137(100)	98(100)	84(20)
2. 11,12-Dehydrosparteine	232	232(40)	164(20)	148(30)	134(100)	97(100)
3. Ammodendrine	208	208(60)	191(45)	165(100)	123(70)	109(85)
4. N-methyltetrahydrocytisine	208	208(40)	148(12)	109(80)	96(100)	58(45)
5. Isoangustifoline	234	193(100)	150(20)	136(5)	112(65)	55(20)
6. Tetrahydrorhombifoline	248	248(0.5)	207(100)	112(25)	84(12)	58(80)
7. 17-Oxosparteine	248	248(10)	191(15)	136(20)	111(50)	97(100)
8. Angustifoline	234	234(0.5)	193(100)	150(20)	112(85)	55(20)
9. α-Isolupanine	248	248(40)	149(45)	136(100)	98(40)	84(25)
10. 5,6-Dehydrolupanine	246	246(50)	134(10)	98(100)	84(10)	
11. Lupanine	248	248(35)	149(50)	136(100)	98(30)	84(20)
12. 11,12-Dehydrolupanine	246	246(95)	231(20)	148(35)	134(100)	55(25)
13. Multiflorine	246	246(40)	148(40)	134(100)	110(30)	82(20)
14. "11,12-Dehydro-13-hydroxylupanine"	262	262(80)	245(10)	163(80)	150(100)	108(60)
15. 17-Oxolupanine	262	262(40)	234(10)	150(100)	136(15)	84(30)
16. N-Formylangustifoline	262	262(0.5)	221(40)	193(100)	150(15)	112(90)
17. 13 α-Hydroxylupanine	264	264(30)	246(30)	165(45)	152(100)	134(40)
18. 13 α-Acetyloxylupanine	306	306(6)	246(80)	149(100)	134(60)	112(30)
19. 13-Hydroxymultiflorine	262	262(40)	164(15)	150(100)	134(10)	94(20)
20. 13-Angeloyloxylupanine	346	346(2)	246(100)	148(20)	134(30)	112(25)
21. 13-Tigloyloxylupanine	346	346(2)	246(100)	148(20)	134(30)	112(25)
22. 13-Benzoyloxylupanine	360	360(1)	246(100)	148(20)	134(30)	112(25)
23. 13-*cis*-Cinnamoyloxylupanine	394	394(1)	246(100)	148(20)	134(30)	112(25)
24. 13-*trans*-Cinnamoyloxylupanine	394	394(1)	246(100)	148(20)	134(30)	112(25)
25. 13-Methoxymultiflorine	276	276(25)	261(1)	245(100)	164(25)	134(40)
26. "13-Hydroxy-17-oxolupanine"	278	278(38)	260(100)	232(15)	166(100)	148(90)
27. "13-Angeloyloxy-17-oxolupanine"	360	260(100)	232(5)	162(10)	148(35)	133(15)
28. "13-Tigloyloxy-17-oxolupanine"	360	260(100)	232(5)	162(10)	148(35)	133(15)
29. "13-Benzoyloxy-17-oxolupanine"	374	260(100)	232(5)	162(10)	148(35)	133(15)
30. "13-*cis*-Cinnamoyloxy-17-oxolupanine"	408	260(100)	232(5)	162(10)	148(35)	133(15)
31. "13-*trans*-Cinnamoyloxy-17-oxolupanine"	408	260(100)	232(5)	162(10)	148(35)	133(15)
32. 13-Cinnamoyloxy-multiflorine	392	244(60)	149(10)	134(30)	96(100)	

" = tentative identification.

angelic, tiglic, benzoic, *cis*- and *trans*-cinnamic acid. If we assume that the corresponding alcohol is 13-hydroxy-17-oxolupanine, both fragmentation patterns and retention data would be in agreement. Thus it is likely, that the following new ester alkaloids are present: 13-angeloyloxy-17-oxolupanine, 13-tigloyloxy-17-oxolupanine, 13-benzoyloxy-17-xolupanine, 13-*cis*-cinnamoyloxy-17-oxolupanine, and 13-*trans*-cinnamoyloxy-17-oxolupanine (Fig. 1). Mass spectra of 13-tigloyloxy-17-oxolupanine had been recorded but not been identified before in *L. polyphyllus, L. albus* [15] and *L. hillii* [7].

Experimental

Plant material

L. gibertianus and *L. linearis* were collected in Argentina or Brazil and kept as herbarium specimens. 1 or 2 leaves were taken for alkaloid analysis.

Alkaloid extraction

Plant material was homogenized in 0.5 M HCl in a mortar. After 30 min at room temperature, the supernatant was made alkaline by adding ammonia and was applied onto Extrelute columns (Merck, Darmstadt). Alkaloids were eluted with CH_2Cl_2 and the solvent evaporated *in vacuo* [3, 6, 7].

Alkaloid analysis

Alkaloid extracts were separated on fused silica capillary columns (0.3 mm × 30 m) with covalently bound liquid phases (DB 1, DB 5 and equivalents; J & W Scientific) as described in [3, 6, 7, 11, 16]. For GLC-MS measurements a Finnigan MAT 4515 was

used in combination with the INCOS data system (for details [3, 6, 7, 16]).

Alkaloids were identified according to their specific Kovats retention indexes and their characteristic mass spectra (EI-MS) in comparison to standard alkaloids described in previous studies. An extensive list of the relevant RI and MS data is tabulated in [3, 6, 7, 16].

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