Quinolizidine Alkaloid Profiles of *Lupinus varius orientalis, L. albus albus, L. hartwegii,* and *L. densiflorus*

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Quinolizidine Alkaloids, Biological Activity

Alkaloid profiles of two *Lupinus* species growing naturally in Egypt (*L. albus albus [synonym L. termis], L. varius orientalis*) in addition to two New World species (*L. hartwegii, L. densiflorus*) which were cultivated in Egypt were studied by capillary GLC and GLC-mass spectrometry with respect to quinolizidine alkaloids. Altogether 44 quinolizidine, bipiperidyl and proto-indole alkaloids were identified; 29 in *L. albus*, 13 in *L. varius orientalis*, 15 in *L. hartwegii*, 6 in *L. densiflorus.* Some of these alkaloids were identified for the first time in these plants. The alkaloidal patterns of various plant organs (leaves, flowers, stems, roots, pods and seeds) are documented. Screening for antimicrobial activity of these plant extracts demonstrated substantial activity against *Candida albicans, Aspergillus flavus* and *Bacillus subtilis.*

Introduction

Lupins represent a monophyletic subtribe of the Genisteae (Leguminosae) (Käss and Wink, 1996, 1997a, b). Whereas more than 300 species have been reported from the New World (North and South America) only 12 species are known from Europe (Mediterranean region) and North Africa.

A characteristic trait of all lupins is the production of quinolizidine and bipiperidine alkaloids (reviews, Kinghorn and Balandrin, 1984; Wink, 1993) which constitute essential defence compounds against herbivores, but are also acting against microorganisms and competing plants (reviews in Wink, 1984, 1987, 1988, 1992, 1993). Most lupin alkaloids are toxic to animals (symptoms are cramps, vomiting and even death due to respiratory paralysis). The pharmacological responses include oxytocic, uteretonic, hypoglycemic, hypotensive and antiarrhythmic activities (Kinghorn and Balandrin, 1984). Lupins which accumulate anagyrine and the bipiperidine alkaloid ammodendrine exhibit mutagenic properties and produce crooked calf disease in grazing animal especially when consumed in early foetal stages (Keeler, 1969, 1976; Keeler *et al.*, 1977).

The main molecular targets which are affected by quinolizidine alkaloids (QAs) are nicotinic and muscarinic acetylcholine receptors (Schmeller *et al.*, 1994) as well as Na^+ and K^+ channels (Körper *et al.*, 1998). In addition, protein biosynthesis and membrane permeability are modulated at higher doses (Wink and Twardowski, 1992).

In the course of our study on the Egyptian legumes containing quinolizidine alkaloids (El-Shazly *et al.*, 1996a, b) we have investigated the active constituents and antimicrobial activity of four *Lupinus* species growing and/or being cultivated in Egypt. These species have been analysed before in our laboratory, but material had mainly derived from plants grown in the greenhouse, in the experimental garden in Germany or from seeds (Wink *et al.*, 1995; Wink and Witte, 1991, 1993). About 8 alkaloids were identified already in the seeds of *L. varius*, 31 in *L. albus*, 13 in *L. hartwegii* (Wink *et al.,* 1995). Recently, 14 alkaloids were isolated from the seeds of *L. varius* and 13 from *L. hartwegii* growing in Egypt (Mohamed and Hassanean, 1997). Egyptian termis (*L. albus*) has been subjected to intensive investigation and 15 alkaloids have been isolated and identified by GLC-MS (Mohamed *et al.*, 1990; 1991; 1992; 1994; Mohamed and Shorbagi, 1993).

Capillary gas-liquid chromatography (GLC) in combination with mass spectrometry (GLC-MS) is a rapid and powerful technique for the analysis of

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complex QA mixtures (Wink *et al.*, 1991; Wink, 1993; Wink *et al.*, 1993, 1995). Since a large library of mass spectral and GLC retention index (RI) data of authentic QAs is available in our laboratory, we were able to unambiguously identify most QAs in this study and have found a number of alkaloids which were new for the species studied.

Materials and Methods

Plant material

Plants of *L. albus* L. ssp. *albus* (*L. termis* Forssk.) were collected from the fields near Zagazig during the flowering season or when mature fruits were formed in March to May 1997. Seeds of *L. varius* ssp. *orientalis* and *L. densiflorus*, were obtained from the local market in Cairo and then cultivated in the Botanical Garden of the Faculty of Pharmacy, Zagazig University and were collected during the flowering and fruiting stages in March and May 1997, respectively. Flowering *L. hartwegii* plants were collected from Botanical Garden of the Faculty of Agriculture, El- Minufiya University, Egypt in March 1997. Voucher specimens have been deposited both in the Institut für Pharmazeutische Biologie, Universität Heidelberg and in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

Extraction

About 20 g plant material was macerated in 90% ethanol for 24 h. After filtering off the insoluble material, the filtrate was evaporated under reduced pressure. About 680 mg residue was obtained from *L. albus* seeds (3.4%) and 370 mg from aerial parts (1.85%); from *L. varius* 384 mg (1.92%) were extracted from seeds and 242 mg from aerial parts (1.21%). Half of the residue was used for antimicrobial tests, the other half for alkaloid extraction.

About 100 to 340 mg of the residue was dissolved in 15 ml 0.5 N HCl and defatted with 100 ml dichloromethane. The homogenate was then adjusted to pH 10 with ammonia (25%). Alkaloids were extracted by solid phase extraction using Extrelut columns (Merck, Darmstadt) and dichloromethane as an eluent. Different plant parts were analysed by GLC in order to compare the respective alkaloid profiles. The alkaloid extracts were subjected to GLC-mass spectrometry.

Capillary GLC

Separation of quinolizidine alkaloid mixtures was carried out with a Carlo Erba ICU 600 gas chromatograph equipped with FID and spectra physics integrator. Column: DB1–30 W (15 m, 0.317 mm ID). Conditions: carrier gas He (2 ml / min.); detector temp. 300° C; injector temp. 250° C; oven temp. program: initial 150° C 2 min. isothermal, 150–250° C at 15 min^{-1}, 250–300° C at 25 min^{-1}, 300° C, 5 min isothermal. Retention indices (RI): Kovats indices (Kovats, 1958) were calculated with respect to a set of co-injected even numbered hydrocarbons (C_{10} – C_{28}). Each RI was subjected to a library search by comparison with reference RI values previously stored in a data base in the Institut für Pharmazeutische Biologie, Universität Heidelberg (Table I).

GLC-MS analysis

A Carlo Erba HRGC 4160 gas chromatograph equipped with a fused silica column (OV1; 30 m, 0.3 mm ID). The capillary column was directly coupled to a quadrupole mass spectrometer Finnigan MAT 4500. EI-mass spectra were recorded at 40 eV. Condition: injector 250 °C; temp. program 70–300° C, 6° C /min or 150–300° C, 6° C /min. Split ratio 1 : 20; carrier gas He 0.5 bar.

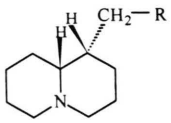
Screening for antimicrobial activity

Microorganisms were *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive bacteria); *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative bacteria); *Candida albicans* and *Aspergillus flavus* (fungi). The microorganisms were obtained from the stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. Antimicrobial activity was assayed via the agar diffusion method. Small cups were taken out of the agar which could take approx. 60 µl of extracts. Each cup was filled accurately with 50 µl extracts (25 mg residue of total or alkaloidal extracts were dissolved in 1 ml dimethylformamide, DMF) as well as DMF as a control. The plates were incubated overnight at 37° C for bacteria and 30° C for fungi.

Table I. Mass spectral data (M^+, base peak) of quinolizidine alkaloids from Egyptian species of *L. a. albus, L. varius orientalis, L. hartwegii* and *L. densiflorus.*

No.	Alkaloid	RI	M+	Base peak	Ref.
1	Lupinine	1422	169	83	1, 2
2	Tryptophol	1580	161	130	3
3	Gramine	1620	174	130	3
4	Sparteine	1785	234	137	4, 2
5	β-Isosparteine	1830	234	137	5, 6
6	11,12-Dehydrosparteine	1840	232	134	2, 7
7	Ammodendrine	1865	208	165	8, 9
8	Albine	1900	232	191	10, 11
9	Dihydroalbine	1915	234	193	
10	N-Methylcytisine	1955	204	58	9, 12
11	Epiaphyllidine	2020	246	98	21
12	Isoangustifoline	2033	234	193	12, 13
13	Tetrahydrorhombifoline	2050	248	207	12, 14
14	Epiaphylline	2055	248	136	15, 16
15	Dehydroangustifoline	2070	232	120	12
16	Angustifoline	2083	234	193	12, 16
17	Dihydromultiflorine	2100	248	134	12
18	α-Isolupanine	2105	248	136	17
19	$\Delta^{5,6}$-Dehydromultiflorine	2110	244	134	18
20	$\Delta^{5,6}$-Dehydrolupanine	2132	246	98	2, 19
21	Rhombifoline	2155	244	58	16
22	Lupanine	2165	248	136	2, 15
23	Aphylline	2180	248	136	15, 16
24	11, 12-Dehydrolupanine	2190	246	134	7
25	11, 12-seco-12,13-Didehydromultiflorine	2215	246	58	20, 21
26	3β-Hydroxylupanine	2260	264	136	7
27	Multiflorine	2310	246	134	12
28	17-Oxolupanine	2340	262	150	12, 15
29	N-Formylangustifoline	2363	262	193	13, 21
30	13α-Hydroxylupanine	2402	264	152	13, 22
31	13α-Acetyloxylupanine	2450	306	246	12, 13
32	13α-Propyloxylupanine	2530	320	246	21
33	N-Formylalbine	2530	260	219	11
34	13α-Hydroxymultiflorine	2566	262	150	11, 20
35	Baptifoline	2625	260	98	21
36	15β-Hydroxy-17-oxolupanine	2646	278	260	23
37	Epibaptifoline	2655	260	114	3
38	13-(2-methylbutanoyl oxy) lupanine	2660	348	246	12
39	13α-Angeloyloxylupanine	2733	346	246	2, 13
40	13α-Tigloyloxylupanine	2753	246	246	13
41	3β-Tigloyloxylupanine	2850	346	134	21
42	*p*-Hydroxycinnamoyllupinine	2860	315	152	21
43	Feruloyllupinine	2960	345	152	2
44	13α-Tigloyloxymultiflorine	2955	344	132	11

1 = Neuner-Jehle *et al.* (1964); 2 = Wink *et al.* (1983); 3 = Meissner and Wink (1992); 4 = Neuner-Jehle *et al.* (1967); 5 = Greinwald *et al.* (1991a); 6 = Greinwald *et al.* (1991b); 7 = Wink and Carey (1994); 8 = Fitch and Djerassi (1974); 9 = El-Shazly *et al.* (1996b); 10 = Mohamed *et al.* (1992); 11 = Planchuelo-Ravelo and Wink (1993); 12 = Wink and Witte (1991); 13 = Planchuelo-Ravelo *et al.* (1993); 14 = Balandrin and Kinghorn (1981); 15 = Schumann *et al.* (1968); 16 = Wink *et al.* (1991); 17 = Wink and Witte (1993); 18 = Mohamed *et al.* (1990); 19 = Murakoshi *et al.* (1982); 20 = Mohamed *et al.* (1991); 21 = Wink *et al.* (1995); 22 = Mühlbauer *et al.* (1988); 23 = Mohamed *et al.* (1994).



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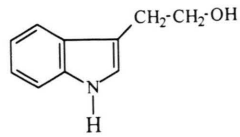

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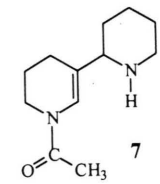

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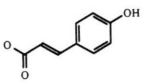
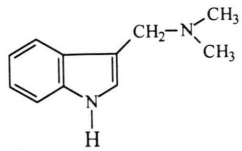
42: R = 



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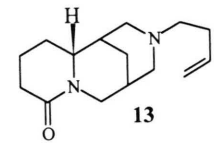

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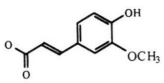
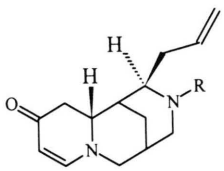
43: R = 



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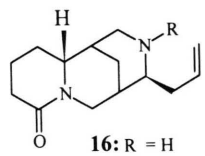

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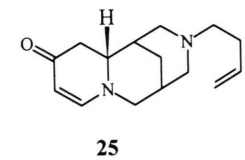

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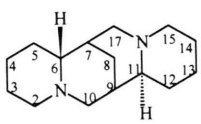

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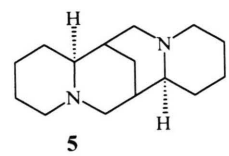

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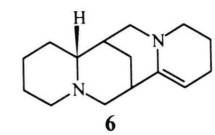

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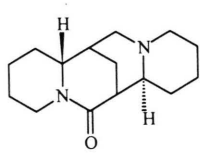

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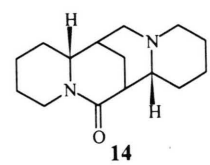

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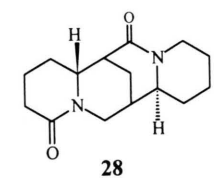

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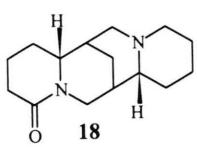

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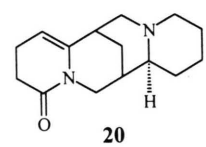

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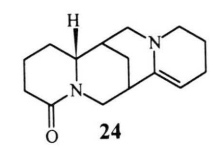

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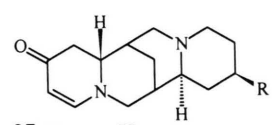

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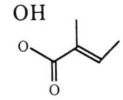
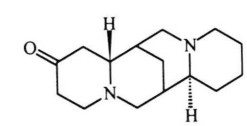
44: R = 



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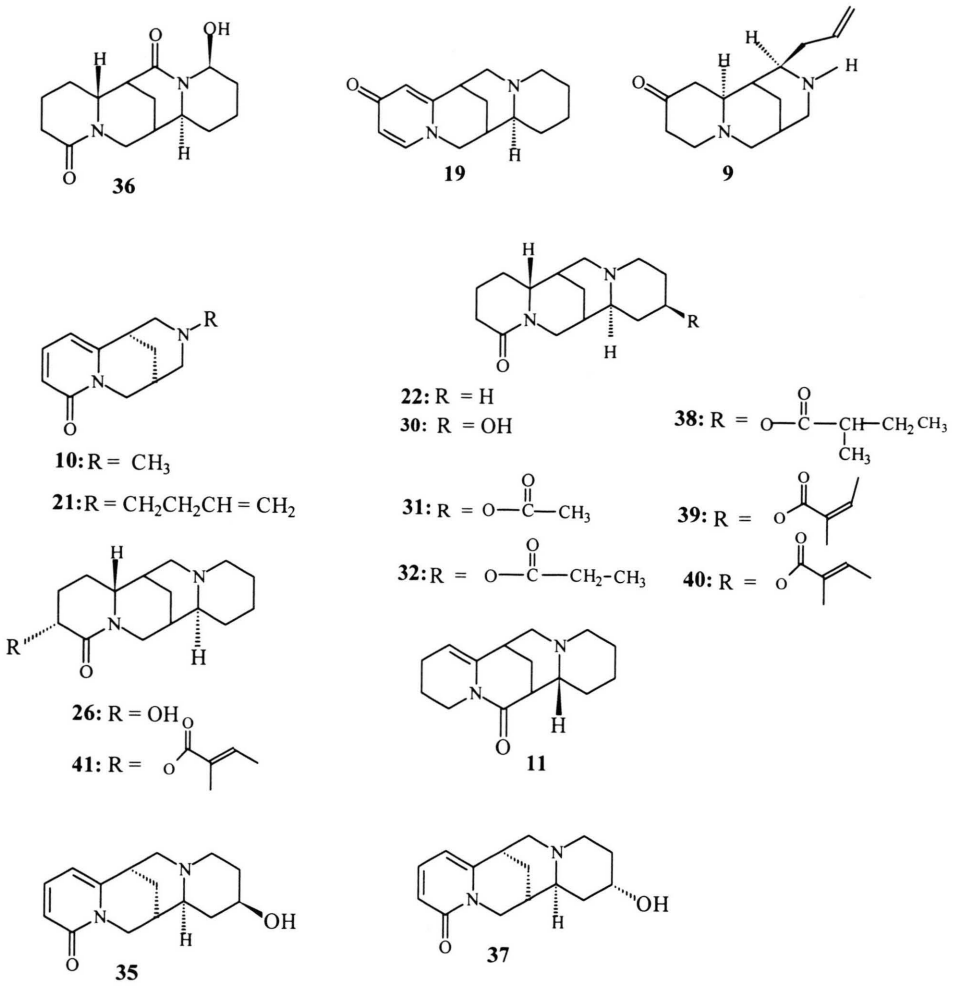

Fig. 1. Structures of quinolizidine alkaloids. Numbers are identical with those given in Table I.

Results and Discussion

Alkaloid profiles

Our GLC-MS analysis of the Egyptian *L. a. albus* (syn. *L. termis*) plants (Table II) showed the presence of previously reported (Mohamed *et al.*, 1990; 1991; 1992; 1994; Mohamed and Shorbagi, 1993) alkaloids (albine, tetrahydrorhombifoline, angustifoline, $\Delta^{5,6}$-dehydromultiflorine, lupanine, 11,12-seco-12,13-didehydromultiflorine, multiflorine, 17-oxolupanine, 13α-hydroxylupanine, 13-hydroxymultiflorine, 15β-hydroxy-17-oxolupanine, 13α-angeloyloxylupanine, 13α-tigloyloxylupanine). Our analysis revealed the presence of additional 15 alkaloids (Table II) for Egyptian Termis lupins, e. g. sparteine, isoangustifoline, dehydroangustifoline, dihydromultiflorine, α-isolupanine, $\Delta^{5,6}$-dehydrolupanine, 11, 12-dehydrolupanie, 3β-hydroxylupanine, N-formylangustifoline, 13α-acetyloxylupanine, 13α-propyloxylupanine,13α-tigloyloxymultiflorine, 13-(2-methylbutanoyloxy)lupanine, N-formylalbine and the bipiperidine alkaloid ammodendrine. A new alkaloid (RI 1916) which has not been reported before was identified tentatively according to its mass fragmentation pattern (GC-EIMS): RI 1916 shows a molecular ion at *m/z* 234 and base peak at m/z 193 (2 mass units more than that of albine; *m/z* 232 and 191, respectively). Since RI value and fragmentation pattern [194 (11), 193 (100), 136 (16), 134 (7), 123

Table II. GLC and GLC-MS analysis of alkaloid content and composition of Egyptian *Lupinus a. albus* (*L. termis*).

	Alkaloid	Alkaloid content (total alkaloid = 100%) Seeds	Pods	Flowers	Leaves	Stems
1	Sparteine*	+	–	–	–	+
2	Ammodendrine*	0.8	2.0	+	+	0.5
3	Albine•	+	–	–	–	–
4	Dihydroalbine*	+	–	–	–	–
5	Isoangustifoline*	+	–	–	–	–
6	Tetrahydrorhombifoline•	+	–	–	–	–
7	Dehydroangustifoline*	+	–	–	–	–
8	Angustifoline•	7.2	2.8	1.2	+	1.3
9	Dihydromultiflorine*	+	–	–	–	–
10	α-Isolupanine*	+	–	–	–	–
11	$\Delta^{5,6}$-Dehydromultiflorine•	+	–	–	–	–
12	$\Delta^{5,6}$-Dehydrolupanine*	+	–	–	–	–
13	Lupanine•	59.6	58	72.3	64.4	77.8
14	11, 12-seco-12, 13-Didehydromultiflorine•	6.6	+	+	+	+
15	11, 12-Dehydrolupanine*	+	–	–	–	–
16	3β-Hydroxylupanine*	+	–	–	–	–
17	Multiflorine•	4.2	5.5	11.4	13.2	6.9
18	17-Oxolupanine•	+	–	–	–	–
19	N-Formylangustifoline*	+	–	–	–	–
20	13α-Hydroxylupanine•	19.8	30.0	5.4	12.3	10.9
21	13α-Acetyloxylupanine*	+	–	–	–	–
22	13α-Propyloxylupanine*	+	–	–	–	–
23	N-Formylalbine*	+	–	–	–	–
24	13-Hydroxymultiflorine•	+	+	1.3	1.0	+
25	15β-Hydroxy-17-Oxolupanine•	+	–	–	–	–
26	13-(2-methylbutanoyloxy)lupanine*	+	–	–	–	–
27	13α-Angeloyloxylupanine•	0.4	0.6	1.6	1.1	0.5
28	13α-Tigloyloxylupanine•	1.4	1.0	6.8	7.8	2.1
29	13α-Tigloyloxymultiflorine*	+	+	–	–	–
Total alkaloid (% dry weight)		1.56	0.67	1.78	0.73	0.57

+ = Trace amounts < 0.1%.
– = not detectable; * = alkaloids new for *L. a. albus* from Egypt.
• = known alkaloid of *L. a. albus* from Egypt (Mohamed *et al.*, 1990, 1991, 1992, 1994).

(5), 110 (23), 94 (8), 84 (23), 82 (13), 68 (9), 55 (15)] are not identical with that of angustifoline (which also exhibits a base peak at *m/z* 193), we suggest that this alkaloid is a derivative of albine. A dihydroalbine would agree with the observed fragmentation pattern. There was not enough material for a structure elucidation by NMR methods. No evidence was obtained for the presence of $\Delta^{5,6}$-dehydroalbine and termisine, which had been reported for this plant (Mohamed *et al.*, 1990; 1991; 1992; 1994; Mohamed and Shorbagi, 1993).

GLC-MS analysis of two cultivars of *Lupinus varius orientalis*, (a pinkish and a blue flowered cultivar) indicated that lupinine and multiflorine were the main alkaloids, whereas 11, 12-seco-12, 13-didehydromultiflorine and 13α-tigloyloxymultiflorine amount to 10% each (Table III). Sparteine, 11,12-dehydrosparteine, dihydromultiflorine, $\Delta^{5,6}$-dehydromultiflorine, α-isolupanine, 13α-hydroxymultiflorine, 4′-hydroxy-cinnamoyllupinine, feruloyllupinine and ammodendrine have been also unambiguously identified. The alkaloid profiles from both cultivars were quite similar in qualitative and quantitative terms. Tetrahydrorhombifoline, lupanine, epilupinine, albine, 13β-hydroxymultiflorine and the N-oxide of epilupinine and multiflorine previously reported in the seeds of *L. varius* (Mohamed and and Hassanean 1997) were absent from the plants investigated here. To our knowledge six alkaloids were reported for the first time in the plant cultivated in Egypt e.g. lupinine, 11, 12-dehydrosparteine, dihydromultiflorine, α-isolupanine, 4-hydroxycinnamoyllupinine and feruloyllupinine.

Table III. GLC and GLC-MS analysis of alkaloid content and composition of Egyptian *Lupinus varius* var. *orientalis* (pinkish and blue flowers cultivar).

	Alkaloid	Alkaloid content (total alkaloids = 100%) Seeds		Pods		Flowers		Leaves		Stems		Roots	
		pinkish	blue	pinkish	blue	pinkish	blue	pinkish	blue	pinkish	blue	pinkish	blue
1	Lupinine◆ƒ	26.9	54.8	49.2	48.1	28.5	14.2	43.6	33.1	20.0	59.8	36.7	70.1
2	Sparetine*◆	+	+	+	0.8	–	+	–	+	–	+	–	–
3	11,12-Dehydrosparteineƒ	+	+	+	+	–	–	–	–	–	–	–	–
4	Ammodendrine*◆	+	+	+	+	+	+	2.1	+	–	+	–	–
5	Dihydromultiflorineƒ	+	+	+	+	–	–	+	–	–	–	–	–
6	$\Delta^{5,6}$-Dehydromultiflorine	+	+	+	+	–	–	–	–	–	–	–	–
7	α-Isolupanineƒ	+	+	+	+	–	–	+	–	–	–	–	–
8	11, 12-seco-12, 13-Didehydromultiflorine*◆	1.8	1.3	8.9	8.5	6.5	9.9	4.0	5.6	7.5	10.0	14.9	+
9	Multiflorine*◆	71.3	43.9	38.8	34.2	59.6	61.8	28.3	32.2	69.0	30.2	30.0	29.5
10	13α-Hydroxymultiflorine*	+	+	+	+	–	–	–	–	–	–	–	–
11	4-Hydroxycinnamoyllupinineƒ	+	+	+	+	+	1.4	10.4	2.6	–	–	–	–
12	Feruloyllupinineƒ	+	+	–	+	+	+	8.3	20.0	–	–	–	–
13	13α-Tigloyloxymultiflorine*	+	+	3.1	8.4	5.4	12.7	3.3	6.5	3.5	+	12.4	+
Total alkaloid (% dry weight)		0.53	0.77	0.43	0.48	0.42	0.62	0.66	0.58	0.07	0.15	0.10	0.33

+ = Trace amounts < 0.5; – = not detectable.
◆ = known alkaloid for *L. varius* (Wink *et al.*, 1995).
* = known alkaloids for *L. varius* growing in Egypt (Mohamed and Hassanean, 1997).
ƒ = alkaloid new for *L. varius* growing in Egypt.

Table IV. GLC and GLC-MS analysis of alkaloid content and composition of *Lupinus hartwegii* cultivated in Egypt.

	Alkaloid	Alkaloid content (total alkaloids = 100%) Leaves	Stems	Flowers	Roots
1	Tryptopholφ	8.1	–	2.9	–
2	Gramine*◆	5.6	–	1.6	–
3	Sparteineφ	+	–	–	–
4	β-Isosparteineφ	+	–	–	–
5	Epiaphyllidine*	+	–	–	–
6	Tetrahydrorhombifolineφ	+	+	–	–
7	Epiaphylline*	25.4	3.6	18.4	3.9
8	α-Isolupanine*◆	+	+	+	+
9	$\Delta^{5,6}$-Dehydrolupanine*◆	+	+	+	+
10	Lupanine*◆	24.3	80.2	52.7	60.6
11	Aphylline*◆	36.1	9.4	20.9	6.4
12	3β-Hydroxylupanine*◆	0.5	6.8	3.5	29.1
13	17-Oxolupanine*	+	–	–	–
14	13α-Hydroxylupanine*	+	–	–	–
15	13α-Tigloyloxylupanineφ	+	+	+	+
Total alkaloid (% dry weight)		0.6	0.38	0.8	0.34

+ = Trace amounts < 0.5; – = not detectable.
* = known alkaloids for *L. hartwegii* (Wink *et al.*, 1995, Kinghorn *et al.*, 1980).
◆ = alkaloids reported for *L. hartwegii* cultivated in Egypt (Mohamed and Hassanean, 1997).
φ = alkaloids new for *L. hartwegii* cultivated in Egypt.

The New World lupin *Lupinus hartwegii* cultivated in Egypt, revealed the presence of lupanine, aphylline, epiaphylline and 3β-hydroxylupanine as a major alkaloids (Table IV). Leaves and flowers have been found to contain simple indole alkaloids: gramine and tryptophol. Traces of epiaphyllidine, β-isosparteine, sparteine, tetrahydrorhombifoline, α-isolupanine, $\Delta^{5,6}$-dehydrolupanine, 17-oxolupanine, 13α-hydroxylupanine and 13α-tigloyloxylupanine were also identified. We did not find

aphyllidine, 11,12-seco-12,13-didehydromultiflorine, virgiline, 2β-hydroxyaphylline and 13-hydroxyaphyllidine previously reported from Egyptian *L. hartwegii* (Mohamed and Hassanean, 1997) were not confirmed. Tryptophol, sparteine, β-isosparteine, tetrahydrorhombifoline, 13α-tigloyloxylupanine were reported here for the first time in *L. hartwegii*.

The α-pyridone alkaloid N-methylcytisine was detected as the main component in the New Wolrd lupin *L. densiflorus*. In addition other α-pyridones, such as rhombifoline, baptifoline and epibaptifoline and traces of lupanine and $\Delta^{5,6}$-dehydrolupanine were detected (Table V). The occurrence of α-pyridone alkaloids in genus *Lupinus* is quite unusual and have been detected in a few species only, which are genetically closely related (Käss and Wink, 1997) e.g. *L. microcarpus*. They contain N-methylcytisine as major component and rhombifoline and baptifoline as minors (Wink *et al.*, 1995). The tetracyclic α-pyridone alkaloid anagyrine which is responsible for teratogenic effect has been reported in *L. argenteus*, *L. densiflorus*, *L. sparsiflorus*, *L. nanus*, *L. polycarpus*, *L. longifolius* and *L. latifolius*, *L. argenteus* var. *stenophyllus*, *L. sericeus* and *L. caudatus* (Kinghorn *et al.*, 1980; Keller *et al.*, 1977; Keller and Zelenski, 1978; Wink and Carey, 1994; Wink *et al.*, 1995).

Antimicrobial activity

The antimicrobial activity of alkaloid extracts was determined by the agar diffusion (cup plate)

Table V. GLC and GLC-MS analysis of alkaloid contents and composition of *L. densiflorus* cultivated in Egypt.

		Alkaloid content% (total alkaloids = 100%)					
	Alkaloid	Seeds	Pods	Flowers	Leaves	Stems	Roots
1	N-Methylcytisine	98	98.2	95.1	78.4	46.8	94.9
2	$\Delta^{5,6}$-Dehydrolupanine	tr	tr	1.0	4.1	6.0	0.4
3	Rhombifoline	tr	tr	tr	tr	tr	tr
4	Lupanine	1.2	0.3	1.5	2.7	3.4	tr
5	Baptifoline	0.8	1.5	2.4	14.8	43.8	4.7
6	Epibaptifoline	tr	tr	tr	tr	tr	tr
Total alkaloid% dry weight		1.7	1.3	1.5	0.6	0.7	0.4

tr = traces < 0.1%.

Table VI. Results of antimicrobial screening of different extracts of *L. albus* and *L. varius*, and *L. densiflorus*.

	Extracts and controls	Diameter of inhibition zone in mm					
		Gram $^-$ bacteria		Gram $^+$ bacteria		Fungi	
		E. coli	*K. pneumoniae*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. flavus*
1	Tetracyclin 30 μg /disc	–	9	8	16	–	–
2	Chloramphenicol 30 μg /disc	15	15	20	15	–	–
3	Penicillin 10 μg /disc	–	–	5	–	–	–
4	Gramicidin 10 μg /disc	–	18	12	25	–	–
5	Alkaloid extract of *L. albus* seeds	10	15	2	–	17	14
6	Alkaloid extract of *L. albus* aerial parts	8	4	–	–	12	10
7	Total extract of *L. albus* seeds	6	12	–	11	22	26
8	Total extract of *L. albus* aerial parts	–	10	–	9	13	25
9	Alkaloid extract of *L. varius* seeds	–	5	10	10	10	10
10	Alkaloid extract of *L. varius* aerial parts	2	–	9	10	15	32
11	Total extract of *L.varius* seeds	7	10	–	10	10	33
12	Total extract of *L. varius* aerial parts	11	12	–	5	17	18
13	Alkaloid extract of *L.densiflorus*	5	3	10	10	10	24

Total extract = residue from evaporated ethanolic extract (see Experimental)
Alkaloid extract = residue from evaporated dichloromethane extracts (See Experimental)
All assays consisted of 50 μl of a test solution, containing 25 mg residue in 1 ml DMF; – = no inhibition.

method. Results are listed in Table VI. The diameters of inhibition zones (in mm) was measured using tetracyclin, gramicidin and chloramphenicol as positive controls. The seed alkaloids as well as the ethanol extracts exhibited significant antifungal activity. In *Lupinus albus* the alkaloidal extract showed half the antifungal activity when compared with that of the crude plant extract, indicating that other antifungal components, such as isoflavones, may be present in the non-alkaloid fraction of the plant (Tahara *et al.*, 1984; 1994). The seed alkaloids of *L. albus* as well as the total ethanolic extracts of *L. varius* showed substantial effect on gram negative bacteria, whereas the alkaloid extracts of *L. varius* (both seeds and aerial part) exhibited an inhibition of gram positive bacteria. The alkaloid extracts of *L. densiflorus* exhibited a marked inhibition activity against Gram positive bacteria, whereas the activity against Gram negative bacteria is considered to be moderate. Antimicrobial activities of sparteine and lupanine have already been described by Wink (1984) and Tyski *et al.*, (1988) and it was assumed that QA play a role in antimicrobial defence (besides flavonoids and isoflavones) of lupins.

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