Quinolizidine Alkaloid Profiles of *Retama raetam, R. sphaerocarpa* and *R. monosperma*

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Z. Naturforsch. **51c,** 301–308 (1996); received January 3/January 24, 1996

Quinolizidine Alkaloids, Capillary GLC, Alkaloid profiles, Leguminosae, *Retama spp.*

About 28 quinolizidine alkaloids and the dipiperidine alkaloids ammodendrine, dehydroammodendrine and N-formylammodendrine were identified in the alkaloidal extracts of *Retama raetam, Retama sphaerocarpa* and *Retama monosperma* by capillary GLC and GLC-MS. Alkaloid profiles of the three *Retama* species with a Mediterranean distribution are more similar than alkaloid patterns of different organs of the same plant. Whereas sparteine and retamine are the major components of stems, lupanine, retamine, N-methylcytisine and cytisine dominate in flowers and pods, and cytisine in seeds indicating a high degree of organ specificity of alkaloid storage.

Introduction

A wide range of pharmacological and toxicological activities as oxytocic, uterotonic, antiarrhythmic, diuretic, hypoglycemic, hypotensive, respiratory stimulant and depressent, antipyretic, hallucinogenic properties are associated with quinolizidine alkaloids (Kinghorn and Balandrin, 1984; Wink, 1993a). At the moleculer level quinolizidine alkaloids affect nicotinic and muscarinic acetylcholine receptors (Schmeller *et al.*, 1994) and Na^+/K^+ channels (Wink, 1993b). In addition, anagyrine and ammodendrine are mutagenic and cause the crooked calf disease if pregnant animals feed on plants rich in these alkaloids (Keeler, 1969; 1972; 1973; 1976). These pharmacological activities are important requisites for the role of quinolizidine alkaloids as chemical defence compounds against herbivores, and to a lesser degree against microorganisms and competing plants (Wink, 1985; 1987; 1988; 1992; 1993a,b).

The genus *Retama* (synonym *Lygos*) includes four species with a distribution in the Mediterranean area, North Africa and on the Canary islands. *Retama* spp. represent shrubs of broom-like appearance which have been classified as belonging to the *Genista*-complex within the subtribe Genisteae (Bisby, 1981). Recent molecular studies based on nucleotide sequences of the rbcL gene and ITS regions of rDNA have confirmed the close relatedness of *Retama* with *Genista* (Käss and Wink, 1995).

Although some alkaloids were previously isolated from *Retama raetam* (Abdel-Halim, 1995; Abdel-Halim *et al.*, 1992; Ahmed *et al.*, 1972), *R. sphaerocarpa* (Balandrin *et al.*, 1982; Cordero *et al.*, 1989; 1991; 1993; Neuner-Jehle *et al.*, 1964; Vazquez *et al.*, 1966) and *R. monosperma* (Morales Mendez *et al.*, 1971) our phytochemical studies employing the sensitive capillary GLC-mass spectrometry, which is the method of choice for analysis and convenient identification of quinolizidine alkaloids in complex mixtures (Greinwald *et al.*, 1991; Kinghorn *et al.*, 1980; Wink, 1993b; Wink *et al.*, 1980; 1981; 1982; 1983; 1991; 1995; Wink and Witte, 1991) showed the presence of much more alkaloids than previously reported. In this communication we report on the occurrence 31 alkaloids in three *Retama* species with special emphasis on interspecific and the organspecific alkaloid variation.

Materials and Methods

Plant material

Stems, flowers, mature pods, seeds, and roots of *Retama raetam* (Forsskål) Webb [syn. *Lygos rae-*

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0939–5075/96/0500–0301 $ 06.00

tam (Forskål) Heywood] were collected in Wadi Feiran on Sinai (Egypt) in May 1994. *Retama sphaerocarpa* (L.) Boiss. [syn. *Lygos sphaerocarpa* (L.) Heywood] and *Retama monosperma* (L.) Boiss (syn. *Lygos monosperma*) were collected in the Alentejo region, (Portugal) in April 1993. Voucher specimens are stored at the Institut für Pharmazeutische Biologie der Universität Heidelberg.

Alkaloid extraction

The plant material (10 g) was homogenized by Ultra-turrax in 200 ml CH_2Cl_2 – MeOH – NH_4OH (25%), (15 : 5 : 1) and left to stand at least for one hour. The extracts were concentrated under reduced pressure in a RotaVapor and taken up in 20 ml 0.5 N HCl. Basification of the aqueous acid solution with NH_4OH, was followed by extraction with methylene chloride (in a shaking funnel), drying over anhydrous Na_2SO_4 and evaporation of the solvent in a RotaVapor. Preparative TLC [silica gel Merck F_{254}, CH_2Cl_2 – MeOH – NH_4OH (25%), 85 : 15 : 2] yielded about 30 mg of the alkaloids **12** and **14** with *Rf* 0.51 and 0.58, respectively.

Capillary GLC and GLC-MS

Capillary GLC: A Carlo Erba ICU 600 gas chromatograph was used equipped with FID or nitrogen detector and a Spectra Physics integrator. Column: DB1–30W (J & W Scientific); 30 m (0.317 mm inner diameter). Conditions: carrier gas He; detector temp. 300 °C; injector temp. 250 °C; oven temp. program: initial temp. 150 °C, 2 min isothermal, 150–300 °C with 10 °C / min to 300 °C, then 10 min isothermal. Retention indices (RI): Kovats indices (Kovats, 1958) were calculated with respect to a set of co-injected even numbered hydrocarbons (C14-C28). Each RI was subjected to a library search by comparison with reference RI values stored in a data base of the institute.

GLC-MS Analysis: A Carlo Erba Mega 5160 gas chromatograph equipped with a fused silica column (DB1, J&W Scientific) was employed. The capillary column was directly coupled to a quadrupole mass spectrometer (Finnigan MAT 4515). EI-mass spectra were recorded at 40 eV. Conditions: injector 250 °C; temperature program 150–300 °C, 6 °C/ min; split ratio 1 : 20; carrier gas He 0.5 bar.

NMR measurements

^1H and ^{13}C NMR spectra were recorded with a AC 300 Bruker instrument in $CDCl_3$, at 300 and 75 MHz, respectively.

Results and Discussion

Altogether 31 alkaloids were recorded in the extracts of different plant parts of the three *Retama* species (Table I): At least 21 alkaloids were detected in *R. raetam*, 20 alkaloids in *R. sphaerocarpa* and 27 alkaloids in *R. monosperma*. Shoots and stems of these species showed the most complex alkaloidal profiles; therefore extracts of these plant parts were examined in detail by GLC-MS (Table II).

Since many quinolizidine alkaloids have been analysed before in our laboratories (Wink 1993b; Wink *et al.* 1980; 1981; 1982; 1983; 1991; 1995; Wink and Witte, 1991) the following *Retama* alkaloids could be identified unambiguously by direct comparison (mass fragmentation, retention index) with authentic material or by comparison of their mass spectra with published data (Table II): epilupinine, α-isosparteine, sparteine, α-isosparteine, 11,12-dehydrosparteine, ammodendrine, dehydroammodendrine, N-methylcytisine, dehydrocytisine I, retamine, dehydrocytisine II, cytisine, 17-oxosparteine, α-isolupanine, 5,6-dehydrolupanine, rhombifoline, lupanine, aphylline, N-formylammodendrine, N-carbomethoxycytisine, 11-allylcytisine, 17-oxoretamine, N-formylcytisine, N-acetylcytisine, 12-α-hydroxylupanine, anagyrine and baptifoline. Alkaloids **12** and **14** were isolated by prep. TLC from extracts of the roots and seeds of *R. raetam*, respectively. The structure of **12** was determined by MS and ^{13}C NMR (Tables II and III) as (+) retamine (Bohlmann and Zeisberg, 1975; Neuner-Jehle *et al.*, 1967). Alkaloid **14** was identified by MS, ^1H- and ^{13}C NMR (Tables II to IV) as (–) cytisine (Bohlmann and Zeisberg, 1975; Neuner-Jehle *et al.*, 1964; 1965). Both isolated alkaloids have been employed in pharmacological assays (Schmeller *et al.*, 1994).

The remaining four alkaloids, dehydrosparteine I (RI 1810), dehydrosparteine II (RI 1825), dehydroretamine and dehydrobaptifoline were only tentatively identified (Table II), since their amounts were too limited for a further spectroscopic analysis. Alkaloid **15** with M^+ 248 (RI 2017)

Table I. Alkaloid profile and contents (total alkaloids = 100%) of *Retama raetam*, *R. sphaerocarpa* and *R. monosperma*.

Alkaloid	*R. raetam*					*R. sphaerocarpa*		*R. monosperma*	
	Stems	Roots	Pods	Seeds	Flowers	Stems	Flowers	Stems	Fruits
1: Epilupinine	–	–	–	–	–	tr	–	–	–
2: α-Isosparteine	tr	–	–	–	–	tr	–	tr	–
3: Sparteine[a,b,c]	35.65	9.09	0.77	tr	tr	20.16	4.34	24.79	tr
4: Dehydrosparteine	tr	–	–	–	–	tr	–	tr	–
5: Dehydrosparteine	tr	–	–	–	–	tr	–	tr	–
6: β-Isosparteine	0.80	0.98	0.63	tr	tr	–	–	tr	–
7: 11,12-Dehydrosparteine	0.81	–	–	–	tr	tr	–	tr	–
8: Ammodendrine[b]	0.99	0.87	0.75	tr	tr	13.79	25.18	11.59	2.75
9: Dehydroammodenderine	–	–	–	–	–	–	–	tr	–
10: N-Methylcytisine[a,c]	9.91	20.13	4.46	5.63	10.81	tr	6.87	4.90	12.72
11: Dehydrocytisine I	–	–	–	–	–	–	–	tr	–
12: Retamine[a,b]	24.86	45.44	20.10	tr	tr	37.20	12.35	1.50	–
13: Dehydrocytisine II	–	–	–	–	–	–	–	tr	–
14: Cytisine[a,b,c]	0.91	6.00	36.44	94.37	54.25	5.04	24.16	7.95	58.69
15: Dehydroretamine	tr	–	–	–	tr	tr	–	tr	–
16: 17-Oxosparteine[c]	tr	–	–	–	tr	tr	–	2.57	–
17: α-Isolupanine	–	–	–	–	–	tr	–	tr	–
18: 5,6-Dehydrolupanine[b]	3.91	0.50	4.50	tr	3.62	5.32	11.78	9.90	tr
19: Rhombifoline	–	0.60	tr	–	–	–	–	tr	tr
20: Lupanine[a,b]	8.73	2.15	14.32	tr	2.26	15.40	15.32	4.24	tr
21: Aphylline	–	–	–	–	–	–	–	tr	–
22: N-Formylammodendrine	–	–	–	–	–	–	–	tr	–
23: N-Carbomethoxycytisine	tr	–	–	–	–	–	–	–	–
24: 11-Allylcytisine	0.58	1.87	4.95	tr	6.61	–	tr	0.50	tr
25: 17-Oxoretamine	0.50	–	–	–	–	0.50	tr	–	–
26: N-Formylcytisine	tr	–	–	–	–	tr	–	tr	–
27: N-Acetylcytisine	–	–	–	–	–	–	–	tr	–
28: 12α-Hydroxylupanine	tr	–	–	–	–	tr	–	–	–
29: Anagyrine[a,b]	12.35	11.92	13.08	tr	22.55	2.59	tr	32.06	25.84
30: Dehydrobaptifoline	–	–	–	–	–	–	–	tr	–
31: Baptifoline[b]	tr	–	–	–	tr	tr	–	tr	–
Alkaloid content*	2.56	1.36	0.55	2.00	0.57	0.82	0.13	0.70	0.21

tr = Trace amounts; – = not detected; [a] = known alkaloid of *R. raetam*; [b] = known alkaloid of *R. sphaerocarpa*; [c] = known alkaloid of *R. monosperma*; * = mg/100 mg by weight of dry material for *R. raetam*; * = mg/100 mg by weight of fresh material for *R. sphaerocarpa* and *R. monosperma*.

has not been described and was suggested to be dehydroretamine because of the close similarities to the respective fragmentation pattern of retamine; the MS spectrum of **15** differed from retamine (M^+ 250) by two mass units. The MS of compound **30** (RI 2605) displayed a M^+ at *m/z* 258 (two mass units less than baptifoline) together with the fragments at *m/z* 160, 147 and 146 that are characteristic for α-pyridone alkaloids. Thus, compound **30** could be tentatively identified as dehydrobaptifoline. The fragmentation pattern in compound **7** was similar to those of compounds **4** and **5**, differing only in the relative intensities in some fragment ions; this indicates that **4** and **5** are stereoisomers of **7**.

So far, only a restricted number of alkaloids had been found in *Retama* spp, such as 12-α-hydroxylupanine, retamine, sparteine, lupanine, anagyrine, cytisine, N-methylcytisine, ammodendrine, 17-oxosparteine, 5,6-dehydrolupanine (Abdel-Halim *et al.*, 1992; Balandrin *et al.*, 1982; Cordero *et al.*, 1991, 1993) which could been confirmed in our study. In addition, our study indicates the presence of 21 further alkaloids in *Retama* spp..The occurrence of thermopsine, sophoramine and sophochrysine (Ahmed *et al.*, 1972; Morales Mendez *et al.*, 1971) is doubtful, since these compounds could be discovered neither in our nor in other recent studies.

Alkaloid profiles of the three *Retama* species are rather similar (Table I); typical for *Retama* is

Table II. GLC-MS data of quinolizidine alkaloids of *Retama raetam*, *R. sphaerocarpa* and *R. monosperma*.

Alkaloid	RI	Formula	M^+	Characteristic Ions (relative abundance in %)	Ref.
1: Epilupinine	1416	$C_{10}H_{19}NO$	169(70)	168(60), 152(100), 138(75), 98(25), 97(90), 96(16), 83(98), 82(35), 55(57)	1, 4
2: α-Isosparteine	1712	$C_{15}H_{26}N_2$	234(23)	193(18), 152(6), 150(5), 137(50), 136(35), 122(10), 110(18), 98(100), 97(18), 96(8), 84(15), 83(8), 67(9), 55(12)	1, 4
3: Sparteine	1785	$C_{15}H_{26}N_2$	234(15)	205(5), 193(27), 176(5), 150(16), 137(90), 136(45), 122(19), 110(30), 98(100), 97(50), 84(30), 70(12), 67(11), 55(24)	2, 4
4: Dehydrosparteine	1810	$C_{15}H_{24}N_2$	232(50)	203(6), 191(12), 189(13), 175(23), 148(30), 136(24), 135(42), 134(100), 122(18), 110(8), 98(89), 97(53), 96(20), 84(14), 67(10), 55(14)	3
5: Dehydrosparteine	1825	$C_{15}H_{24}N_2$	232(50)	203(11), 191(18), 189(30), 175(18), 148(32), 136(23), 135(45), 134(100), 122(19), 110(10), 98(90), 97(30), 96(15), 84(6), 67(9), 55(14)	3
6: β-Isosparteine	1830	$C_{15}H_{26}N_2$	234(15)	205(3), 193(15), 150(10), 137(100), 136(30), 122(12), 110(18), 98(70), 97(30), 84(12), 67(5), 55(10)	4, 5
7: 11,12-Dehydrosparteine	1838	$C_{15}H_{24}N_2$	232(33)	203(2), 191(4), 175(18), 148(20), 136(19), 135(37), 134(100), 122(11), 110(9), 98(35), 97(80), 96(45), 84(10), 68(10), 67(10), 55(12)	4, 5
8: Ammodendrine	1865	$C_{12}H_{20}N_2O$	208(48)	207(27), 191(45), 179(45), 165(100), 152(23), 137(45), 136(72), 123(80), 122(51), 110(80), 109(78), 108(52), 94(52), 84(31), 80(40), 55(21), 43(60)	4, 6
9: Dehydroammodendrine	1935	$C_{12}H_{18}N_2O$	206(47)	205(12), 163(100), 149(6), 135(12), 122(8), 108(20), 84(15), 80(5), 55(10), 43(20)	7, 8
10: N-Methylcytisine	1952	$C_{12}H_{16}N_2O$	204(15)	160(5), 146(6), 117(2), 109(3), 96(3), 58(100)	1, 4
11: Dehydrocytisine I	1970	$C_{11}H_{12}N_2O$	188(68)	160(53), 159(25), 148(62), 146(50), 136(10), 135(26), 134(100), 77(18), 57(14)	4, 9
12: Retamine	1973	$C_{15}H_{26}N_2O$	250(3)	233(6), 232(11), 207(15), 150(11), 134(30), 114(10), 98(100), 97(31), 96(15), 84(15), 70(10), 55(10)	4, 10
13: Dehydrocytisine II	1985	$C_{11}H_{12}N_2O$	188(73)	160(18), 159(5), 147(40), 146(100), 117(12), 78(9)	1, 4
14: Cytisine	1990	$C_{11}H_{14}N_2O$	190(56)	160(23), 148(30), 147(80), 146(100), 134(36), 117(11), 109(21), 104(9), 93(9), 82(20), 77(9), 68(10), 44(73)	1, 4
15: Dehydroretamine	2017	$C_{15}H_{24}N_2O$	248(22)	229(12), 205(5), 150(40), 135(30), 134(30), 122(16), 114(13), 98(58), 97(100), 96(60), 80(14), 68(13), 54(9)	
16: 17-Oxosparteine	2070	$C_{15}H_{24}N_2O$	248(38)	220(20), 219(11), 191(11), 150(20), 137(25), 136(50), 123(22), 110(75), 98(85), 97(100), 96(28), 84(27), 69(15), 55(28)	4, 11
17: α-Isolupanine	2105	$C_{15}H_{24}N_2O$	248(24)	247(8), 150(29), 149(40), 148(25), 136(100), 134(25), 124(55), 110(18), 98(48), 97(30), 84(25), 83(23), 67(20), 55(45)	2, 4
18: 5,6-Dehydrolupanine	2128	$C_{15}H_{22}N_2O$	246(20)	217(3), 203(3), 189(3), 148(6), 134(10), 110(5), 98(100), 97(35), 84(8), 68(7), 55(8)	3, 4, 12
19: Rhombifoline	2155	$C_{15}H_{20}N_2O$	244(0.1)	204(10), 203(98), 160(18), 146(12), 117(6), 98(16), 58(100), 55(15)	4, 13
20: Lupanine	2165	$C_{15}H_{24}N_2O$	248(28)	247(25), 219(5), 205(3), 191(2), 150(35), 149(51), 148(18), 136(100), 122(12), 110(23), 98(29), 97(24), 84(17), 67(10), 55(30)	4, 11

Table II. (Continued).

Alkaloid	RI	Formula	M^+	Characteristic Ions (relative abundance in %)	Ref.
21: Aphylline	2180	$C_{15}H_{24}N_2O$	248(20)	247(22), 220(20), 205(8), 192(10), 191(15), 164(10), 150(10), 149(15), 138(25), 137(48), 136(100), 124(22), 123(24), 122(18), 110(30), 98(40), 97(45), 96(32), 84(31), 55(26)	
22: N-Formylammodendrine	2207	$C_{13}H_{20}N_2O_2$	236(10)	218(100), 207(14), 193(10), 176(35), 175(55), 165(15), 150(25), 136(24), 122(30), 112(18), 108(19), 96(18), 84(18), 82(20), 67(13), 55(18), 43(40)	4, 14
23: N-Carbomethoxycytisine	2239	$C_{13}H_{16}N_2O_3$	248(34)	207(18), 160(15), 159(15), 146(95), 110(9), 102(100), 84(6), 68(9), 58(48), 57(34), 56(18)	4, 9
24: 11-Allylcytisine	2242	$C_{14}H_{18}N_2O$	230(5)	190(15), 189(100), 160(11), 148(10), 147(10), 146(30), 134(8), 122(7), 110(7), 84(12), 80(12), 55(6)	4, 15
25: 17-Oxoretamine	2255	$C_{15}H_{24}N_2O_2$	264(23)	248(13), 247(88), 236(3), 207(2), 164(6), 150(4), 136(25), 110(10), 98(100), 97(70), 96(28), 84(13), 69(14), 55(16)	11
26: N-Formylcytisine	2310	$C_{12}H_{14}N_2O_2$	218(42)	190(5), 189(8), 160(18), 148(22), 147(50), 146(100), 134(48), 122(10), 109(15), 82(11), 68(10), 55(9), 44(40)	4, 16
27: N-Acetylcytisine	2320	$C_{13}H_{16}N_2O_2$	232(30)	147(65), 146(84), 82(20), 68(23), 55(21), 44(100), 43(38), 42(53)	4, 17
28: 12α-Hydroxylupanine	2350	$C_{15}H_{24}NO_2$	264(0.5)	247(15), 246(100), 231(11), 207(35), 150(17), 149(20), 148(22), 134(60), 122(15), 121(15), 112(40), 108(23), 95(26), 94(26), 80(15), 68(15), 58(36), 55(35)	18
29: Anagyrine	2382	$C_{15}H_{20}N_2O$	244(19)	229(3), 215(2), 161(5), 160(8), 146(11), 136(10), 122(8), 98(100), 97(10), 70(10)	1, 4
30: Dehydrobaptifoline	2605	$C_{15}H_{18}N_2O_2$	258(78)	160(6), 148(20), 147(78), 146(100), 133(6), 132(6), 112(70), 84(50), 55(18)	13
31: Baptifoline	2630	$C_{15}H_{20}N_2O_2$	260(24)	243(4), 160(10), 146(17), 114(100), 96(17), 70(21)	4, 11

1 = Neuner-Jehle *et al.* (1964); 2 = Wink and Witte (1991); 3 = Wink and Witte (1993); 4 = Wink (1993); 5 = Wink *et al.* (1983); 6 = Fitch and Djerassi (1974); 7 = Fitch *et al.* (1974); 8 = Steinegger *et al.* (1968); 9 = Veen *et al.* (1992); 10 = Neuner-Jehle *et al.* (1967); 11 = Schumann *et al.* (1968); 12 = Murakoshi *et al.* (1982); 13 = Wink *et al.* (1991); 14 = Wyk van *et al.* (1991); 15 = Ohmiya *et al.* (1974); 16 = Ohmiya *et al.* (1974); 17 = Ohmiya *et al.* (1974); 18 = Abdel-Halim *et al.* (1992).

the occurrence of retamine and derivatives which are rather uncommon in other Leguminosae (Wink, 1993b). These phytochemical characters would support the finding from nucleotide sequences of the rbcL gene and the ITS regions of rDNA, that *Retama* constitutes a monophyletic taxon comprising closely related species (Käss and Wink, 1994).

Substantial differences were encountered in the alkaloid pattern of different plant organs: As can be seen from Table I α-pyridone alkaloids, such as cytisine and N-methylcytisine constitute the major component of flowers, pods and seeds. In contrast, sparteine, retamine and lupanine represent the major components of roots and aerial plant parts (except flowers and seeds). The dipiperidyl alkaloid ammodendrine, which shares part of the biosynthetic pathway with quinolizidine alkaloids (Brown *et al.*, 1991; Perrey and Wink, 1988) was detected in all plant organs, but was present in relative higher concentrations in *R. sphaerocarpa* and *R. monosperma* than in *R. raetam*.

Quinolizidine alkaloids are synthesized in chloroplasts (Wink and Hartmann, 1982) but are then translocated into all plant organs via the phloem (Wink and Witte, 1984). It has been demonstrated

Table III. ^{13}C NMR spectral data of compounds **12** *(+ retamine)** and **14** *(- cytisine)** (75 MHz, $CDCl_3$).

C	**12**	**14**
2	65.18	163.69
3	25.78	116.69
4	24.57	138.78
5	29.27	104.98
6	66.43	151.11
7	33.21	35.64
8	28.77	26.33
9	32.60	27.80
10	62.32	49.75
11	67.07	52.99
12	71.04	–
13	31.33	53.98
14	20.03	–
15	54.94	–
17	52.87	–

* Optical rotation was determined experimentally.

Table IV. ^1H NMR spectral data of compound **14** *(- cytisine)* (300 MHz, $CDCl_3$).

H	
3	6.42, 1H, *dd* (1.3, 7.7)
4	7.26, 1H. *dd* (6.3, 9)
5	5.97, 1H, *dd* (1.2, 5.7)
7	2.87, 1H, *br s*
8	1.92, 2H, *br s*
9	2.28, 1H, *m*
10α	3.86, 1H, *dd* (5.7, 9.8)
10β	4.08, 1H, *d* (15.6)
11	2.93–3.08, 2H, *m*
13	2.93–3.08, 2H, *m*
N–H	1.88, 1H, *br s*

Figures in parentheses are coupling constants in Hz.

(reviews in Wink, 1987, 1993b) that α-pyridone type alkaloids derive from the tetracyclic alkaloids of the lupanine-skeleton. The high abundance of the α-pyridone alkaloids in flowers and seeds can be explained in two ways: Either tetracyclic alkaloids such as lupanine or sparteine are transported to the flowers and are there transformed to the α-pyridone alkaloids or the α-pyridone alkaloids are also synthesized in the green aerial parts but are selectively transported and sequestered in flowers, fruits and seeds. Since α-pyridone alkaloids, such as anagyrine, N-methylcytisine and cytisine are present (albeit in small quantities) in the aerial parts alongside with the possible intermediate 5,6-dehydrolupanine, the selective transport hypothesis appears more likely. Ammodendrine, lupanine, retamine, cytisine and N-methylcytisine had been found in *Viscum cruciatum*, a hemiparasite living on *R. sphaerocarpa* (Cordero *et al.*, 1989; 1993). Since *Viscum* acquires the alkaloids via the phloem, this result would also agree with the selective transport hypothesis.

Acknowledgements

We thank Dr. G. Schilling (University of Heidelberg) for carrying out the NMR spectra and E. Käss for collecting *R. sphaerocarpa* and *R. monosperma*.

Abdel-Halim O. B. (1995), (–)-6α-Hydroxylupanine, a lupine alkaloid from *Lygos raetam var. sarcocarpa*. Phytochemistry **40,** 1323–5.

Abdel-Halim O. B., Sekine T., Saito K., Halim A. F., Abdel-Fattah H., and Murakoshi I. (1992), (+)-12α-Hydroxylupanine, a lupine alkaloid *from Lygos raetam*. Phytochemistry **31,** 3251–3.

Ahmed Z. F., Rizk A. M., and Hammouda F. M. (1972), Egyptian medicinal flora, (F. Kaczmarek, ed.: Inst. Przem. Zielarskiego, Poznan, Pol.), pp. Meeting Date 1970, 20–3.

Balandrin M. F., Robbins E. F., and Kinghorn A. D. (1982), Alkaloids of Papilionoideae. Biochem. Syst. Ecol. **10,** 307–11.

Bisby F. A. (1981), Genisteae (Adans.) Benth. (1865). In Advances in Legume Systematics (R. M. Polhill and P. H. Raven, eds.) Kew: Royal Botanical Gardens, pp. 409–425.

Bohlmann F., and Zeisberg R. (1975), ^{13}C- NMR-Spektren von Lupinen-Alkaloiden. Chem. Ber. **108,** 1043–1051.

Brown A. M., Robins D. J., Witte L., and Wink M. (1991), Stereochemistry of enzymic reactions involved in the formation of tetrahydroanabasine, ammodendrine, smipine and tripiperideine. Plant Physiol. (Life Science Adv.) **10,** 179–185.

Cordero C. M., Serrano A. M., and Gonzalez M. J. A. (1993), Transfer of bipiperidyl and quinolizidine alkaloids to *Viscum cruciatum* Sieber (Loranthaceae) hemiparasitic on *Retama sphaerocarpa* Boissier (Leguminosae). J. Chem. Ecol. **19,** 2389–2393.

Cordero M. C., Ayuso M. J., Richomme P., and Bruneton J. (1989), Quinolizidine alkaloids from *Viscum cruciatum,* hemiparasitic shrub of *Lygos sphaerocarpa*. Planta Med. **55,** 196.

Cordero M., C., Gil Serrano A. M., and Ayuso Gonzalez M. J. (1991), Variations of alkaloids from *Retama sphaerocarpa* Boissier. Plant. Med. Phytother. **25,** 157–60.

Fitch W. L., and Djerassi C. (1974), Mass spectrometry in structural and stereochemical problems. CCXLIII. Functional group interaction. Unusual fragmentations

of amides as exemplified by bipiperidyl alkaloids. J. Amer. Chem. Soc. **96,** 4917–4927.
Fitch W. L., Dolinger P. M., and Djerassi C. (1974), Alkaloid studies. LXVIII. Novel piperydyl alkaloids from *Lupinus formosus*. J. Org. Chem. **39,** 2974–2979.
Greinwald R., Witte L., Wray V., and Czygan F. C. (1991), The alkaloid pattern of *Cytisophyllum sessilifolium* (Fabaceae-Genisteae). Biochem. Syst.Ecol. **19,** 253–258.
Käss E., and Wink M. (1995), Molecular phylogeny of the Papilionoideae (family Leguminosae): RbcL gene sequences versus chemical taxonomy. Bot. Acta **108,** 149–62.
Keeler R. F. (1969), Toxic and teratogenic alkaloids of western range plants. J. Agric. Food Chem. **17,** 473–482.
Keeler R. (1972), Lupin alkaloids from teratogenic and nonteratogenic lupins. Teratology **7,** 23–30.
Keeler R. (1973), Lupin alkaloids from teratogenic and nonteratogenic lupins II. Teratology **7,** 31–35.
Keeler R. (1976), Lupin alkaloids from teratogenic and nonteratogenic lupins. III. Identification of anagyrine as the probable teratogen by feeding trials. J.Toxic.Environm. Health **1,** 887–898.
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 (W. S. Pelletier, 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.
Kovats E. (1958), Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkaloide, Aldehyde und Ketone. Helv. Chim. Acta **41,** 1915–1932.
Morales Mendez A., Gonzalez Gonzalez A., and Diaz Rodriguez F. (1971), Alkaloids from the bark of *Retama monosperma*. Rev. Fac. Farm., Univ. Los Andes **8,** 77–87.
Murakoshi I., Kidoguchi E., Kubota M., Haginiwa J., Ohmiya S., and Otomasu H. (1982), Lupin alkaloids from *Echinosophora koreensis*. Phytochemistry **21,** 2385–2388.
Neuner-Jehle N., Nesvadba H., and Spiteller G. (1964), Schlüsselbruchstücke in den Massenspektren von Alkaloiden. Mh.Chemie **95,** 687–709
Neuner-Jehle N., Nesvadba H., and Spiteller G. (1965), Anwendung der Massenspektroskopie zur Strukturaufklärung von Alkaloiden, 6. Mitt. Pyrrolizidinalkaloide aus dem Goldregen. Mh. Chemie **96,** 327–338.
Neuner-Jehle N., Schumann D., and Spiteller G. (1967), Schlüsselbruchstücke in den Massenspektren von Alkaloiden. 6. Mitt. Mh. Chemie **98,** 836–851.
Ohmiya S., Otomasu H., Murakoshi I., and Haginiwa J. (1974), N-Acetylcytisine from *Sophora tomentosa*. Phytochemistry **13,** 1016.
Ohmiya S., Otomasu H., Murakoshi I., and Haginiwa J. (1974), N-Formylcytisine: a new alkaloid from *Thermopsis chinensis*. Phytochemistry **13,** 643–644.
Perrey R., and Wink M. (1988), On the role of piperideine and tripiperideine in the biosynthesis of quinolizidine alkaloids. Z. Naturforsch. **43c,** 363–369.
Schmeller T., Sauerwein M., Sporer F., and Wink M. (1994), Binding of quinolizidine alkaloids to nicotinic and muscarinic acetylcholine receptors. J. Nat. Prod. **57,** 1316–1319.
Schumann D., Neuner-Jehle N., and Spiteller G. (1968), Schlüsselbruchstücke in den Massenspektren von Alkaloiden. Mh. Chemie **99,** 390–408.
Steinegger E., Moser C., and Weber P. (1968), Konstitution des neuen Alkaloids Hystrin aus *Genista hystrix*. Phytochemistry **7,** 849–853.
Vazquez M. D., Gonzalez G., Calvino J., and Ribas-Marques I. (1966), Papilionaceous alkaloids. XLIII. (–)-Epibaptifoline from *Retama sphaerocarpa*. An. R. Soc. Esp. Fis. Quim., Ser. B **62,** 837–44.
Veen G., Schmidt C., Witte L., Wray V., and Czygan F. C. (1992), Lupin Alkaloids from *Lupinus polyphyllus*. Phytochemistry **31,** 4343–4345.
Wink M. (1985), Chemische Verteidigung der Lupinen: Zur biologischen Bedeutung der Chinolizidinalalkaloide. Pl. Syst. Evol. **150,** 65–81.
Wink M. (1987), Chemical Ecology of quinolizidine Alkaloids. In Allelochemicals. Role in agriculture and forestry. G.R. Waler, ed., Amer. Chem. Soc. Symp Ser. 330, 525–533, Washington, DC.
Wink M. (1987), Quinolizidine alkaloids: biochemistry, metabolism, and function in plants and cell suspension cultures. Planta Med. **53,** 509–582.
Wink M. (1988), Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor. Appl. Genet **75,** 225–233.
Wink M. (1992), The role of quinolizidine alkaloids in plant-insect interactions. In Insect-plant interactions, E. A. Bernays, ed. (Boca Raton: CRC Press), pp. 131–166.
Wink M. (1993a), Allelochemical properties or the raison d'être of alkaloids. In The Alkaloids (G. A. Cordell, ed.) Academic Press, San Diego, pp. 1-118.
Wink M. (1993b), Quinolizidine alkaloids. In Methods in plant biochemistry (P. G. Waterman, ed.) Academic Press, London, vol. **8,** pp. 197–239.
Wink M., and Hartmann T. (1982), Localisation of the enzymes of quinolizidine alkaloid biosynthesis in leaf chloroplasts of *Lupinus polyphyllus*. Plant Physiol. **70,** 74–77.
Wink M., Meißner C., and Witte L. (1995), Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. Phytochemistry **38,** 139–153.
Wink M., Montllor C. B., Bernays E. A., and Witte L. (1991), *Uresiphita reversalis* (Lepidoptera: Pyralidae): carrier-mediated uptake and sequestration of quinolizidine alkaloids obtained from the host plant *Teline monspessulana*. Z. Naturforsch. **46c,** 1080–1088.
Wink M., Schiebel H. M., Witte L., and Hartmann T. (1982), Quinolizidine alkaloids from plants and their cell suspension cultures. Ester alkaloids of *Lupinus polyphyllus*. Planta Med. **44,** 15–20.
Wink M., and Witte L. (1984), Turnover and transport of quinolizidine alkaloids. Diurnal fluctuations of lupanine in the phloem sap,leaves and fruits of *Lupinus albus* L. Planta **161,** 519–524.
Wink M., and Witte L. (1991), Storage of quinolizidine alkaloids in *Macrosiphum albifrons* and *Aphis genistae* (Homoptera: Aphididae). Entomol. Gener. **15,** 237–254.
Wink M., and Witte L. (1993), Quinolizidine alkaloids in *Genista acanthoclada* and its holoparasite, *Cuscuta palaestina*. J. Chem. Ecol. **19,** 441–448.

Wink M., Witte L., and Hartmann T. (1981), Quinolizidine alkaloids of cell suspension cultures and plants of *Cytisus scoparius* and of its root parasite *Orobanche rapum-genistae*. Planta Med. **43,** 342–352.

Wink M., Witte L., Hartmann T., Theuring C., and Volz V. (1983), Accumulation of quinolizidine alkaloids in plants and plant suspension cultures: Genera *Lupinus*, *Cytisus*, *Baptisia*, *Genista*, *Laburnum*, and *Sophora*. Planta Med. **48,** 253–257.

Wink M., Witte L., Schiebel H. M., and Hartmann T. (1980), Alkaloids pattern of cell suspension cultures and differentiated plants of *Lupinus polyphyllus*. Planta Med. **38,,** 238–245.

Wyk van B. E., Verdoorn G. H., and Greinwald R. (1991). Taxonomic significance of alkaloids in the genus *Liparia* (Fabaceae – Liparieae). S. Afr. J. Bot. **57,** 344–347.