A Survey of Quinolizidine Alkaloids and Phenylethylamine Tyramine in *Cytisus scoparius* (Leguminosae) from Different Origins

Gabriele Gresser[a], Ludger Witte[b], Victor P. Dedkov[c], Franz-Christian Czygan[a]

[a] Julius-von-Sachs-Institut für Biowissenschaften mit Botanischem Garten der Universität Würzburg, Lehrstuhl Pharmazeutische Biologie, Mittlerer Dallenbergweg 64, D-97082 Würzburg

[b] Institut für Pharmazeutische Biologie der Universität Braunschweig, Mendelssohnstr. 1, D-38023 Braunschweig

[c] Kaliningrad State University, 236040 Kaliningrad, Russia

Z. Naturforsch. **51c,** 791–801 (1996); received April 23/July 8, 1996

Cytisus scoparius, Quinolizidine Alkaloids, Phenylethylamine, Tyramine, Capillary Gas Chromatography

Distribution and content of the quinolizidine alkaloids and phenylethylamine tyramine in various organs of *Cytisus scoparius* taken from different localities in Germany, Russia, Italy and France were analyzed by capillary GLC. Sparteine and sparteine-derivatives like 11,12-dehydrosparteine are predominant in shoots and flowers whereas lupanine-type alkaloids are mainly accumulated in seeds, pods and roots. During the year no changes within the alkaloid pattern of the young developing shoots could be observed, but the total alkaloid amount increases remarkably at the end of the vegetation period. With respect to the date of harvest only a limited variation of the alkaloid profile and content could be found in shoots, flowers and roots taken from different geographical origins. In comparison the alkaloid pattern of seeds and pods varied considerably which seemed to be correlated with the different states of maturity and not with the geographical origin. Phenylethylamine tyramine is accumulated in flowers, green pods and young developing shoots. In young developing shoots the tyramine content declined drastically during the vegetation period.

Preface

Dies ist unseres Wissens seit mehr als 50 Jahren die erste gemeinsame biologisch-chemische Publikation eines deutschen und Kaliningrader Universitätsinstituts. Wir widmen daher unsere Arbeit dem Gedenken an Prof. Dr. Kurt Mothes (1903–1983), dem international bekannten Pflanzenphysiologen und Arzneipflanzenforscher sowie letzten Direktor des Instituts für Botanik der Albertus-Universität zu Königsberg i. Pr.

Kurt Mothes, der zusammen mit seinen Mitarbeitern Wesentliches zur Biologie, Biochemie und Physiologie der Alkaloide beitrug, hatte sich nach dem 2. Weltkrieg um eine wissenschaftliche Zusammenarbeit mit russischen Forschern bemüht. Das war – wie er mir einmal sagte – sein Beitrag zur Verständigung der Menschen dieser beiden Völker, die immer wieder sowohl Opfer als auch Täter verfehlter Politik waren.

Franz-C. Czygan

Reprint requests to Prof. Dr. Franz-Christian Czygan.
Fax: 0931/888-6182.

Introduction

Cytisus scoparius (L.) Link [syn. *Sarothamnus scoparius* (L.) Wimmer ex Koch] is a multibranched shrub known to accumulate quinolizidine alkaloids like sparteine and p-hydroxy-phenylethylamine tyramine (Bisby *et al.*, 1994; Mears and Mabry, 1971; Wink *et al.*, 1981, 1982 and 1983; Wink and Witte, 1985). Herb, flowers and roots are used pharmaceutically because of their alkaloid content (Hänsel *et al.*, 1992; Wichtl, 1989).

The subatlantic species *Cytisus scoparius* is distributed in West,- South- and Central Europe, extending towards to South Scandinavia and eastwards to Central- and West-Ukraine (Hegi, 1906; Tutin *et al.*, 1968). East Prussia is one of the eastern distribution areas (Steffen, 1940) (its northern part today: Oblast Kaliningradskaja, Russia).

In cooperation with the Kaliningrad State University we have investigated the alkaloid- and phenylethylamine pattern of *Cytisus scoparius*. We report about the distribution and content of the compounds in the various organs of individuals taken from different sites in Germany, Russia, Italy and France. Furthermore the alkaloid- and phenylethylamine profile in young shoots during the vegetation period was analyzed. Since the distribution area of *Cytisus scoparius* is so wide, it was the objective of our studies to see whether there are differences in the quinolizidine alkaloid composition and phenylethylamine content of the plants.

Materials and Methods

Plant material

Plant material of *Cytisus scoparius* used for alkaloid analyses was harvested at different sites as listed below (with numbers of sites as noted in the tables). Voucher specimens are deposited at the Lehrstuhl für Pharmazeutische Biologie, University of Würzburg.

Franken (Germany): 1. Botanical Garden Würzburg (control plant); 2. Kienfeld (three different individuals); 3. Pretzdorf (twelf different individuals); 4. Lichtenau (two different individuals); 5. Uttrichshausen; 6. Gerchsheim (two different individuals). **Oblast Kaliningradskaja (Russia; formerly: Nord-Ostpreußen with the town Königsberg i.Pr.)**: 7. Laduschkin (formerly Ludwigsort) (two different individuals); 8. Swedlogorsk (formerly Rauschen) (two different individuals). **Tuskany (Central Italy)**: 9. Casa Civetta/ Prata; 10. Lucca; 11. Massa Marittima; 12. Niccioletta; 13. Poggibonsi; 14. Tati. **Massif Central (Southeast of France)**; 15. Ailleux; 16. Grouffe d'Enfer (two different individuals); 17. Firminy; 18. Mt. Pilat; 19. St. Victor s/Loire.

Extraction procedure

Air dried plant material was finely ground. Usually 200 mg of plant material was homogenized in 18 ml of 1 N H_2SO_4 and left standing at room temperature for 45 min. After filtration the homogenates were alkalinized with 25% ammonia to pH 10–11 and applied to Chemelut-columns (1.0 g Chemelut ml^{-1} extract; ICT, Frankfurt) for solid-phase extraction. The alkaloids were eluted with 100 ml dichloromethane and the solvent was concentrated *in vacuo*. Prior to GLC injection the extracts were redissolved in a defined volume of methanol (200 μl - 1500 μl) and subsequently analyzed by GLC.

Alkaloid analyses

Crude extracts were separated by GLC using a fused silica capillary column (0.25 mm x 15 m, film thickness 0.25 μm) with covalently bonded methylsilicon phases (DB 1, J & W Scientific) as described (Gresser *et al.*, 1993). Detection by a nitrogen specific detector (PND). The identity of the compounds was confirmed by capillary GLC-MS analyses. A detailed description of the GLC-MS system and the conditions are given by Gresser *et al.* (1993). The compounds were identified by comparing their mass spectral data with literature data.

Results

Alkaloid identification

Alkaloid extracts of various plant parts of *Cytisus scoparius* were analyzed by capillary GLC and GLC-MS. Altogether 34 quinolizidine alkaloids could be found, 20 of these could be identified unambiguously by comparing their mass spectrum and Kovàts-retention index with literature data (Murakoshi *et al.*, 1986; Wink, 1993; Wink *et al.*, 1995; Witte *et al.*, 1987): α-Isosparteine (**2**), sparteine (**3**), 11,12-dehydrosparteine (**6**), tetrahydrorhombifoline (**11**), 17-oxosparteine (**12**), angustifoline (**13**), α-isolupanine (**15**), 5,6-dehydrolupanine (**16**), lupanine (**18**), aphylline (**19**), 11,12-dehydrolupanine (**20**), 3β-hydroxylupanine (**23**), multiflorine (**24**), 10,17-dioxosparteine (**25**), 17-oxolupanine (**26**), anagyrine (**29**), 13α-hydroxylupanine (**30**), 3β,13α-dihydroxylupanine (**32**), 13α-angeloyloxylupanine (**35**) and 13α-tigloyloxylupanine (**36**) (Table **I**). Aphylline (**19**) and 10,17-dioxosparteine (**25**) are new for the species. The other quinolizidine alkaloids could only be identified tentatively and are not described for *Cytisus scoparius* so far. The concentration of these compounds was so low that it was not possible to isolate and to identify them by standard spectroscopic methods. According to their mass spectral data these quinolizidine alkaloids are derivatives of sparteine

Table I. Mass spectral identification of the compounds from *Cytisus scoparius*. The plant organs where the compounds were detected are shown.

Compound	RI	M^+	Significant fragment ions (abundance %)					Organ
1. Tyramine	1390	137	137(13)	108(81)	107(33)	77(16)	30(100)	T,F,P#
2. α-Isosparteine	1720	234	234(32)	193(18)	150(16)	137(51)	98(100)	T,S,R,P
3. Sparteine	1780	234	234(35)	193(50)	137(100)	98(92)	84(20)	T,F,S,P,R
4. "Dehydrosparteine"*	1810	232	232(65)	191(16)	148(31)	134(100)	98(96)	T,F,S,P,R
5. "Dehydrosparteine"*	1825	232	232(68)	189(28)	148(30)	134(97)	98(100)	T,F,S,P,R
6. 11,12-Dehydrosparteine	1835	232	232(27)	175(18)	148(20)	134(100)	97(88)	T,F,S,P,R
7. "Dehydrosparteine"*	1855	232	232(28)	191(5)	148(7)	134(25)	98(100)	T,F,S,P,R
8. Ammodendrine	1860	208	208(56)	191(47)	179(40)	165(100)	123(76)	T,F,S
9. "Dihydrorhombifoline"*	1980	246	205(100)	162(25)	120(11)	98(5)	58(69)	S
10. "Hydroxysparteine"*	2048	250	250(33)	209(100)	150(14)	136(37)	94(23)	S
11. Tetrahydrorhombifoline	2050	248	207(100)	112(18)	108(11)	98(5)	58(52)	S
12. 17-Oxosparteine	2070	248	248(35)	220(18)	136(39)	110(63)	97(100)	T,F,S,P,R
13. Angustifoline	2080	234	193(100)	150(19)	112(62)	94(8)	55(12)	S
14. "Hydroxysparteine"*	2090	250	250(13)	232(10)	149(51)	98(80)	97(100)	S
15. α-Isolupanine	2105	248	248(35)	149(55)	136(100)	110(5)	98(30)	T,S
16. 5,6-Dehydrolupanine	2130	246	246(23)	163(5)	134(12)	98(100)	97(35)	T,S,P,R
17. "Hydroxytetrahydrorhombifoline"*	2147	264	223(100)	128(11)	108(29)	96(12)	58(35)	S
18. Lupanine	2165	248	248(38)	149(50)	136(100)	110(18)	98(28)	T,F,S,P,R
19. Aphylline*	2180	248	248(22)	220(24)	191(18)	149(17)	136(100)	T
20. 11,12-Dehydrolupanine	2190	246	246(74)	231(17)	148(42)	134(100)	112(15)	S
21. "3β-Hydroxy-α-isolupanine"*	2210	264	264(77)	150(21)	136(100)	134(44)	98(22)	S
22. "11,12-Dehydro-α-isolupanine"*	2235	246	246(38)	231(5)	148(18)	134(100)	112(12)	S
23. 3β-Hydroxylupanine	2250	264	264(54)	247(8)	150(45)	136(100)	134(52)	T,S,P
24. Multiflorine	2305	246	246(68)	217(5)	148(18)	134(100)	110(15)	S
25. 10,17-Dioxosparteine*	2340	262	262(38)	234(7)	150(100)	124(6)	84(24)	T,S,R
26. 17-Oxolupanine	2350	262	262(72)	234(13)	150(100)	110(31)	55(20)	S
27. "13-Hydroxy-α-isolupanine"*	2355	264	264(50)	246(51)	165(34)	152(100)	134(31)	S
28. "Hydroxylupanine"*	2373	264	264(49)	150(29)	149(36)	136(100)	97(27)	S
29. Anagyrine	2380	244	244(50)	160(10)	146(18)	136(16)	98(100)	S
30. 13α-Hydroxylupanine	2410	264	264(25)	246(31)	165(41)	152(100)	134(28)	T,F,S,P,R
31. "13-Ketolupanine"*	2430	262	262(58)	245(100)	164(42)	150(69)	112(55)	S,R
32. 3β,13α-Dihydroxylupanine	2502	280	280(55)	262(12)	165(43)	152(100)	134(37)	S
33. "Hydroxyoxolupanine"*	2595	278	278(35)	260(93)	166(100)	148(76)	112(85)	S
34. "Dihydroxyoxolupanine"*	2720	294	294(36)	276(35)	166(100)	148(44)	126(34)	S
35. 13α-Angeloyloxylupanine	2740	346	246(100)	148(18)	134(46)	112(24)	55(19)	R
36. 13α-Tigloyloxylupanine	2755	346	246(100)	148(18)	134(46)	112(23)	55(19)	T,S

RI, retention index according to Kovàts; " " tentatively identified; T, shoots; F, flowers; S, seeds; P, pods; R, roots; *, new record for *Cytisus scoparius*; #, only in green pods.

(**4**,**5**,**7**,**10** and **14**), of lupanine (**21**,**22**,**27**,**28**,**31**,**33** and **34**) and of rhombifoline (**9** and **17**) (Table **I**).

Within the new sparteine derivatives three dehydrosparteine (**4**,**5** and **7**; Table **I**) could be found in all investigated organs of *Cytisus scoparius*. These alkaloids are also described for other sparteine accumulating species like *Adenocarpus hispanicus* or *A. decorticans* (Veen *et al.*, 1992). Dehydrosparteine (**4**) is of special interest as this compound was accumulated in all plant parts in higher amounts (Table **II**). The remaining quinolizidine alkaloids could only be detected in traces (< 5 $\mu g\ g^{-1}$ dry wt) mostly in the seeds.

In comparison to other studies (Wink *et al.*, 1981; Wink and Witte, 1985) the minor constituents and trace compounds N-methylangustifoline, 13-isohydroxylupanine, 13-*cis*-cinnamoyloxylupanine, 13-*trans*-cinnamoyloxylupanine and 13-benzoyloxylupanine could not be found in our analyses. 3β-Hydroxy-13α-tigloyloxylupanine, known to be accumulated in seedlings of *Cytisus scoparius* (Saito *et al.*, 1994) could not be detected in the organs studied. Furthermore the tetracyclic α-pyridone alkaloid anagyrine was only identified in seed extracts whereas this quinolizidine alkaloid is also described for leaves, stems, roots and pods of the species (Wink *et al.*, 1981; Wink and Witte, 1985).

Besides quinolizidine alkaloids phenylethylamine tyramine (**1**) and the dipiperidyl alkaloid ammodendrine (**8**) are accumulated in different organs of *Cytisus scoparius* (Table **I**). 3-Hydroxy-tyramine and 3-hydroxy-N-methyltyramine, known for flowers respectively green fruits and branches collected in autumn (Schmalfuss and Heider, 1931; Tocher and Tocher, 1972) could not be found by our analytical procedure.

Table II. Distribution and arithmetic means (μg g^{-1} dry wt) of phenylethylamine tyramine and quinolizidine alkaloids (QA) in **different plant parts** of *Cytisus scoparius* from the Botanical garden Würzburg (Franken, Germany; no. of site: 1), analyzed by capillary GLC. The numbers in ( ) represent the range of values found (μg g^{-1} dry wt). The range of values of quinolizidine alkaloids expressed as % of total alkaloids are shown in [ ]. Compound no. see Table **I**.

Organ	Shoot buds	Shoots with primary thickening	Flowers	Unripe (green) seeds	Ripe (brown) seeds	Unripe (green) pods	Ripe (brown) pods	Roots	Roots
Date of harvest	April	April	May	June	September	June	September	April	September
1. Tyramine	**531** (476–563)	+	**643** (267–1131)	–	–	**257** (10–503)	–	–	–
2. α-Isosparteine	–	**16** (11–20) [0.3–0.7.%]	–	–	–	**17** (6–28) [0.3–1%]	–	–	–
3. Sparteine	**434** (249–711) [58–96%]	**2867** (1275–3555) [75–98%]	**214** (123–369) [56–96%]	**515** [17%]	**329** (286–367) [26–27%]	**3038** (1330–4745) [77–84%]	**141** (116–166) [44–76%]	**83** [81%]	**5** [21%]
4. "Dehydrosparteine"	**27** (5–66) [0.5–15%]	**59** (23–114) [0.7–7%]	**35** (28–37) [9–12%]	+	+	**128** (82–173) [1–10%]	+	+	+
6. 11,12-Dehydrosparteine	**29** (11–76) [2–18%]	**66** (22–161) [0.7–9%]	**35** (10–60) [1–20%]	**137** [4%]	**34** (23–44) [2–4%]	**412** (163–660) [9–12%]	**19** (11–18) [2–14%]	**5** [5%]	+
12. 17-Oxosparteine	+	**28** (8–23) [0.2–4%]	**12** (9–13) [3–4%]	+	+	**62** (50–74) [1–3%]	+	+	+
18. Lupanine	+	+	+	**1022** [33%]	**289** (254–308) [21–42%]	**12** (5–18) [0.3%]	**39** (15–87) [2–11%]	+	**8** [33%]
24. 3β-Hydroxylupanine	–	–	–	**696** [23%]	**271** (221–349) [20–23%]	+	**15** (5–29) [2–11%]	–	+
31. 13α-Hydroxylupanine	–	+	–	**492** [16%]	**207** (180–227) [15–18%]	+	**9** (5–22) [1–8%]	**13** [13%]	**11** [46%]
32. 3β,13α-Dihydroxylupanine	–	–	–	**224** [7%]	**90** (57–125) [5–9%]	–	+	–	+
Total QA amount	**504** (408–797)	**3056** (1714–3693)	**299** (143–381)	**3090**	**1240** (1081–1492)	**3682** (1740–5623)	**227** (198–265)	**102**	**24**
No. of samples	4	4	9	1	3	2	3	1	1

–, not detected; +, arithmetic means < 5 μg g^{-1} dry wt.

Alkaloid pattern of Cytisus scoparius from Würzburg

Single samples of different organs were analyzed by capillary GLC. Table **II** shows the distribution and the amount of the quinolizidine alkaloids and phenylethylamine tyramine given as arithmetic means and range of values found (μg g^{-1} dry wt and% of total alkaloids, respectively). Quinolizidine alkaloids with arithmetic means < 5 μg g^{-1} dry wt in all plant parts are not given. Since the leaves of *Cytisus scoparius* are very small and inconspicuous in comparison to the stem axis, shoots were analyzed in this study.

The highest quinolizidine alkaloid content could be seen in unripe seeds and pods, furthermore in shoots with primary thickening (0.1% up to 0.37% dry wt). In comparison ripe seeds and pods, flowers and also shoot buds have a reduced alkaloid yield. The lowest alkaloid level (0.002% up to 0.01% dry wt) could be measured in roots.

Sparteine is the main quinolizidine alkaloid (abundance > 40% of total alkaloids) in shoots, flowers and pods as it is reported (Murakoshi *et al.*, 1986; Wink *et al.*, 1981 and 1982; Wink and Witte, 1985). In these organs 11,12-dehydrosparteine and dehydrosparteine (**4**) could also be found in higher amounts (11,12-dehydrosparteine: up to 20% of total alkaloids; dehydrosparteine (**4**) up to 15% of total alkaloids), whereas 17-oxosparteine represent a minor compound (abundance < 5% of total alkaloids). Seeds of *Cytisus scoparius* show a very complex alkaloid pattern of sparteine-, lupanine- and hydro-derivatives of rhombifoline (Table **I**). In ripe (brown) seeds lupanine, 3β-hydroxylupanine and sparteine could be found in approximate similar amounts, while 13α-hydroxylupanine and 3β,13α-dihydroxylupanine are accumulated in lower yields (% of total alkaloid content) (Table **II**). These findings differ from other studies (Murakoshi *et al.*, 1986; Wink *et al.*, 1981) which reported lupanine and the dihydroxylupanine-derivat (**32**) as the main constituents. The alkaloid profile of the unripe (green) seeds do not differ significantly from the ripe ones. The roots of *Cytisus scoparius* show a very simple alkaloid pattern. Depending on the date of harvest either sparteine (in April) or 13α-hydroxylupanine (in September) represent the major quinolizidine alkaloid.

Phenylethylamine tyramine could only be found in shoots, flowers and green (unripe) pods with the highest amount in flowers (0.06% dry wt) and shoot buds (0.05% dry wt). Ripe pods of *Cytisus scoparius* are not described as accumulation site of this compound so far. During the vegetation period the tyramine concentration in the young developing shoots decreases drastically (Table **III**).

Alkaloid profile of shoots from Cytisus scoparius from Würzburg over the year

Since annual fluctuations of the quinolizidine alkaloid accumulation are known e.g for *Spartium* (Greinwald *et al.*, 1990; Gresser, 1992) or *Laburnum* (Greinwald *et al.*, 1990a; Gresser, 1992) the alkaloid profile of young developing shoots of *Cytisus scoparius* and the corresponding elder shoots (with primary thickening) of one plant was studied over the year 1994/1995. For the sake of the plant only one sample was analyzed at the seven times of harvest.

Over the year no changes in the alkaloid pattern of the shoots could be seen (Table **III**). Sparteine is the major compound both in young and in the corresponding elder shoots, 11,12-dehydrosparteine is also accumulated in greater amounts (up to 20% of total alkaloid yield). At the beginning of the vegetation period (April) the minor compound 17-oxosparteine could only be found in the elder shoots and not in the young ones.

Table III. Distribution and content (μg g^{-1} dry wt) of phenylethylamine tyramine and quinolizidine alkaloids (QA) sparteine, 11,12-dehydrosparteine and 17-oxosparteine in **young developing shoots** and the corresponding **elder shoots** (with primary thickening) of *Cytisus scoparius* (Botanical Garden Würzburg; no. of site: 1) during the year 1994/1995. The values expressed as % of total alkaloids are shown in [ ].

Compound		Tyramine	Sparteine	11,12-Dehydro-sparteine	17-Oxo-sparteine	Total QA amount
Date of harvest (day/month)						
06. 04.	Young shoots	543	391 [93.5%]	17 [4.0%]	–	418
	Elder shoots	–	3225 [98.4%]	22 [0.7%]	18 [0.6%]	3277
27. 04.	Young shoots	132	548 [98.7%]	7 [1.3%]	–	555
	Elder shoots	–	3394 [98.5%]	24 [0.7%]	18 [0.5%]	3447
18. 05.	Young shoots (Fl)	95	434 [96.4%]	9 [2.0%]	+	453
	Elder shoots	–	1150 [97.3%]	14 [1.2%]	6 [0.5%]	1182
20. 07.	Young shoots (Fr)	–	2147 [98.8%]	17 [0.8%]	+	2174
	Elder shoots	–	2188 [95.6%]	74 [3.2%]	16 [0.7%]	2294
23. 11.	Young shoots (Fr)	–	3062 [84.0%]	491 [13.5%]	7 [0.2%]	3645
01. 02.	Young shoots (Fr)	–	3981 [96.6%]	47 [1.1%]	6 [0.2%]	4121
23. 02.	Young shoots (Fr)	–	3043 [77.3%]	776 [19.7%]	+	3939

–, not detected; +,< 5 μg g^{-1} dry wt; Fl, plant is flowering; Fr, plant bears fruits.

During the year an increase of the sparteine content in the young developing shoots is obvious: the sparteine concentration of samples harvested at the end of the vegetation period (fruiting state of the plant; July – Februar of the next year) is four to ten fold higher than those harvested at the beginning of the vegetation (April – May). An other special feature can be seen: in samples harvested in April and May the sparteine amount of the elder shoots is three to eight fold higher than in the corresponding young ones.

During the month phenylethylamine tyramine could only be identified in young shoots, not in the corresponding elder ones (Table **III**). The highest tyramine content (0.05% dry wt) could be measured at the beginning of the vegetation period (April) which then decreased remarkably to 0.01% dry wt in the flowering period of the plant (May). After bearing fruits tyramine could be detected not any more in the young shoots of *Cytisus scoparius*. For the sake of the plant it was not possible to continue the analysis of the elder shoots in the next month, but reproducible changes in the alkaloid- and tyramine content of the young shoots could also be demonstrated for the following vegetation period (data not shown).

Alkaloid pattern of Cytisus scoparius from other localities

The alkaloid pattern of young shoots of *Cytisus scoparius* from eighteen different sites in Franken (Germany), Oblast Kaliningradskaja (Russia), Tuskany (Italy) and Massif Central (France) was investigated at different states of development: at start of vegetation period, flowering period and fruiting period (Table **IV**). Considering the alkaloid analyses of the control plant from the Botanical Garden in Würzburg (Table **II**) a good correspondence between the samples taken from the different localities could be seen: phenylethylamine tyramine could only be found in young shoots harvested at the beginning of the vegetation period and during the flowering period. There are also no differences within the quinolizidine alkaloid profile: independent from the time of harvest sparteine is the main alkaloid of all samples studied, 11,12-dehydrosparteine is accumulated in higher yields (up to 29% of total alkaloids). The lowest total alkaloid content was determined in samples harvested at the beginning of the vegetation period, the highest alkaloid concentration at the fruiting state of the plants. But in comparison to the other corresponding samples the total alkaloid yield of shoots harvested in Tuskany (Italy) at the fruiting state is reduced. An other special feature has to be noted: the high relative yield of lupanine (up to 26% of total alkaloids) and compound (**5**) (up to 11% of total alkaloid content) found in three samples harvested in Massif Central (France; no. of the localities: 16 und 19) at the flowering period of the plants (data not shown).

The alkaloid profile of flowers, roots, seeds and pods of the individuals taken from different localities was analyzed too (Table **V** and **VI**). The quinolizidine alkaloid pattern of flowers (Table **V**) corresponded very well with sparteine as main constituent. There are only differences between the tyramine content which is drastically reduced in the samples from Oblast Kaliningradskaja (Russia). These samples were collected at a later time than the other ones. The alkaloid analyses of roots (Table **V**) are in agreement with the findings shown for the control plant (Table **II**): sparteine is the major constituent of roots harvested at the beginning of the vegetation period [samples of Franken (Germany), Massif Central (France) and Tuskany (Italy)]. In comparison the lupanine- and 13α-hydroxylupanine yield of roots collected in Oblast Kaliningradskaja (Russia) at the end of the vegetation period is increased (lupanine up to 62%, 13α-hydroxylupanine up to 46% of total alkaloid content). These results confirm the tendency of a preferred accumulation of lupanine-type alkaloids in roots of *Cytisus scoparius* during the year.

Considering the sparteine-, lupanine and hydroxylupanine- amounts of ripe and unripe seeds there are remarkable differences between the samples taken from different sites in Franken (Germany), Oblast Kaliningradskaja (Russia), Tuskany (Italy) (Table **VI**) and Würzburg (Table **II**). As shown for ripe seeds of Oblast Kaliningradskaja (Table **VI**) these alkaloid variations are present within one geographical area. The total quinolizidine alkaloid amount of the ripe seeds from the different origins (Table **VI**) is higher than those found in the samples of Würzburg (Table **II**) but in the same order as reported by Murakoshi *et al.* (1986). As demonstrated for the control plant in

Table IV. Distribution and arithmetic means (μg g^{-1} dry wt) of phenylethylamine tyramine and quinolizidine alkaloids (QA) in young **developing shoots** of *Cytisus scoparius* from different origins, analyzed by capillary GLC. The numbers in ( ) represent the range of values found (μg g^{-1} dry wt). The range of values of the quinolizidine alkaloids expressed as % of total alkaloids are shown in [ ]. Compound no. see Table **I**.

	Franken (Germany)			**Oblast Kaliningradskaja (Russia)**		
Time of harvest	Start of vegetation period	Flowering period	Fruiting period	Start of vegetation period	Flowering period	Fruiting period
1. Tyramine	**225** (103–395)	**376** (110–552)	–	**No samples available**	**45** (0–181)	–
2. α-Isosparteine	–	+	**35** (17–49) [0.5–1%]		**16** (5–44) [0.2–2%]	**26** (9–48) [0.5–2%]
3. Sparteine	**364** (85–881) [81–90%]	**1646** (511–3614) [76–96%]	**4816** (2002–7291) [77–97%]		**1925** (885–2540) [87–94%]	**3251** (1515–7347) [85–95%]
4. "Dehydrosparteine"	**11** (6–16) [2–9%]	**26** (7–37) [0.3–5%]	**22** (8–58) (0.2–1%]		**8** (6–11) [0.2–0.4%]	**39** (8–143) [0.2–2%]
6. 11,12-Dehydrosparteine	**17** (5–29) [3–16%]	**71** (21–125) [1–15%]	**160** (33–418) [1–16%]		**154** (34–347) [3–12%]	**193** (22–388) [0.5–11%]
12. 17-Oxosparteine	–	**8** (4–13) [0.3–2%]	**23** (10–33) [0.2–8%]		**8** (3–20) [0.1–0.8%]	**16** (6–33) [0.1–1%]
Total QA	**415** (102–1002)	**1785** (551–3858)	**5158** (2586–7890)		**2129** (958–2937)	**3610** (1682–7728)
No. of sites	2;3;6	2;3;5;6	2;3;4		7;8	7;8
No. of samples	5	6	9		4	12

	Tuskany (Italy)			**Massif Central (France)**		
Time of harvest	Start of vegetation period	Flowering period	Fruiting period	Start of vegetation period	Flowering period	Fruiting period
1. Tyramine	**49** (10–123)	**No samples available**	–	**519** (110–908)	**131** (50–264)	**No samples available**
2. α-Isosparteine	+		**20** (16–23) [1.2%]	+	+	
3. Sparteine	**783** (680–882) [70–97%]		**996** (808–1185) [58–62%]	**724** (143–1304) [70–95%]	**4330** (2448–6694) [47–98%]	
4. "Dehydrosparteine"	**50** (6–129) [0.7–13%]		**16** (12–20) [1%]	**8** (0–16) [0–8%]	+	
6. 11,12-Dehydrosparteine	**109** (19–126) [2–17%]		**457** (404–510) [27–29%]	**30** (20–39) [2–19%]	**127** (32–357) [1–7%]	
12. 17-Oxosparteine	**24** (7–44) [1–4%]		**42** (18–66) [1–4%]	–	**7** (0–14) [0.2–0.3%]	
Total QA	**980** (967–1809)		**1644** (1385–1903)	**788** (206–1370)	**5453** (4665–6810)	
No. of sites	10;12;13;14		9;11	16;18	16;17;18;19	
No. of samples	4		2	2	5	

–, not detected; +, arithmetic means < 5 μg g^{-1} dry wt.

Würzburg (Table **II**) sparteine is the main alkaloid in ripe pods collected at different sites in Franken (Germany) and in unripe pods harvested in Tuskany (Italy) (Table **VI**). But there are remarkable fluctuations within the sparteine- and lupanine yield (% of total alkaloids) of the ripe pods

Table V. Distribution and arithmetic means ($\mu g\ g^{-1}$ dry wt) of phenylethylamine tyramine and quinolizidine alkaloids (QA) in **flowers** and **roots** of *Cytisus scoparius* from different origins, analyzed by capillary GLC. The numbers in ( ) represent the range of values found ($\mu g\ g^{-1}$ dry wt). The range of values of the quinolizidine alkaloids expressed as % of total alkaloids are shown in [ ]. Compound no. see Table **I**.

	Flowers			Roots			
Locality	Franken (Germany)	Oblast Kalningrad-skaja (Russia)	Massif Central (France)	Franken (Germany)	Oblast Kalningrad-skaja (Russia)	Massif Central (France)	Tuskany (Italy)
Date of harvest	May	July-September	April-May	May	September-Dezember	June	June
1. Tyramine	**1657** (994–2564)	**68** (51–137)	**2148** (1240–2783)	–	–	–	–
3. Sparteine	**574** (301–755) [89–95%]	**394** (60–776) [91–98%]	**391** (267–484) [89–96%]	**219** (57–381) [59–87%]	**26** (10–47) [18–59%]	**109** [78%]	**180** (132–228) [68–81%]
4. "Dehydrosparteine"	+	+	+	+	+	+	+
6. 11,12-Dehydrosparteine	**18** (10–30) [1–4%]	**7** (5–12) [1–2%]	**10** (5–23) [1–5%]	+	+	+	**8** (6–10) [3–4%]
12. 17-Oxosparteine	+	+	+	–	–	–	+
18. Lupanine	+	+	+	**11** (10–11) [3–10%]	**12** (5–29) [5–62%]	**12** [9%]	**24** (6–41) [4–12%]
23. 3β-Hydroxylupanine	–	–	–	–	+	+	–
30. 13α-Hydroxylupanine	+	+	+	**31** (27–35) [8–28%]	**16** (5–29) [16–46%]	**18** [13%]	**37** (20–54) [12–16%]
32. 3β,13α-Dihydroxylupanine	–	–	–	–	+	–	–
Total QA amount	**610** (317–795)	**420** (71–801)	**422** (299–511)	**266** (96–436)	**59** (30–105)	**139**	**249** (163–335)
No. of sites	2;3;6	7;8	16;17;18;19	2;3	7;8	16	10;14
No. of samples	5	4	5	2	7	1	2

–, not detected; +, arithmetic means $< 5\ \mu g\ g^{-1}$ dry wt.

from Oblast Kaliningradskaja. The total alkaloid content of ripe pods (given as arithmetic mean) is up to four- and five fold higher than those of the control plant (Table **II**) and of literature data (Wink *et al.*, 1981). In contrast the total quinolizidine alkaloid amount (given as arithmetic mean) of unripe pods harvested in Tuskany (Italy) is up to five-fold lower than those from Würzburg.

Discussion

Distribution and concentration of quinolizidine alkaloids and phenylethylamine tyramine varies among the plant parts studied. There are distinct differences between shoot buds, shoots with primary thickening, flowers, seeds (ripe and unripe), pods (ripe and unripe) and roots.

Depending on the date of harvest a great correspondence between the alkaloid pattern and concentration of young shoots, flowers and roots taken from different geographical origins can be seen. As already demonstrated for *Spartium* (Greinwald *et al.*, 1990; Gresser, 1992) or *Virgilia* (Greinwald *et al.*, 1989) chemical varieties could not be found. The considerable variations within the alkaloid yields in seeds and pods [unripe (green) and ripe (brown)] seem to be independent of the geographical origin but may be in correlation with different states of maturity which could not be recognized by the colour (green or brown) of the organs.

In comparison to shoots seeds of *Cytisus scoparius* have a complex alkaloid profile. Green plant parts as leaves and stem axis are the main site of quinolizidine alkaloid biosynthesis (Wink and Hartmann, 1981; Wink, 1987) where they are transported over the phloem sap to the other target organs like roots and fruits (Wink *et al.*,

Table VI. Distribution and arithmetic means (μg g^{-1} dry wt) of phenylethylamine tyramine and quinolizidine alkaloids (QA) in **seeds** and **pods** of *Cytisus scoparius* from different origins, analyzed by capillary GLC. The numbers in ( ) represent the range of values found (μg g^{-1} dry wt). The range of values of the quinolizidine alkaloids expressed as % of total alkaloids are shown in [ ]. Compound no. see Table **I**.

	Seeds				**Pods**			
Locality	Franken (Germany)	Oblast Kaliningrad-skaja (Russia)	Oblast Kaliningrad-skaja (Russia)	Tuskany (Italy)	Franken (Germany)	Oblast Kaliningrad-skaja (Russia)	Oblast Kaliningrad-skaja (Russia)	Tuskany (Italy)
Date of harvest	July	September-Dezember	September	June	July	September-Dezember	September	June
Developing state	ripe (brown)	ripe (brown)	ripe (brown)	unripe(green)	ripe (brown)	ripe (brown)	ripe (brown)	unripe(green)
1. Tyramine	–	–	–	–	–	–	**19** (0–37)	**81** (10–200)
3. Sparteine	**244** (81–724) [2–14%]	**81** (31–183) [1–4%]	**1695** (1173–2217) [25–41%]	**1518** (911–2125) [62–69%]	**573** (117–768) [49–84%]	**45** (14–92) [12–58%]	**614** (401–827) [68–89%]	**642** (493–934) [81–93%]
4. "Dehydrosparteine"	+	+	+	+	+	+	+	+
6. 11,12-Dehydrosparteine	**161** (112–238) [3–8%]	**56** (18–147) [0.5–3%]	**52** (47–56) [0.9–1.2%]	**61** (58–64) [2–4%]	**67** (26–146) [5–12%]	**12** (7–31) [3–34%]	**22** (11–32) [2–3%]	**48** (24–68) [4–12%]
12. 17-Oxosparteine	+	+	**7** (5–9) [0.1–0.2%]	+	**9** (5–17) [0.5–1.5%]	+	**7** (4–9) [1%]	**13** (5–20) [1–4%]
18. Lupanine	**2300** (1125–3565) [65–81%]	**2371** (1882–3254) [52–88%]	**1928** (1707–2149) [31–46%]	**301** (278–324) [10–20%]	**172** (30–366) [4–38%]	**81** (7–214) [8–73%]	**89** (37–142) [4–24%]	**32** (6–65) [1–6%]
23. 3β-Hydroxylupanine	**115** (61–243) [2–5%]	**130** (43–357) [2–8%]	**138** (16–260) [0.3–6%]	**37** (5–69) [0.5–2%]	+	**6** (3–15) [1–6%]	**9** (5–12) [0.5–2%]	+
30. 13α-Hydroxylupanine	**114** (66–192) [1–8%]	**245** (24–808) [1–16%]	**797** (770–783) [15–17%]	**275** (176–373) [12%]	**8** (0–24) [0–3%]	**15** (0–62) [0–14%]	**16** (14–18) [1.5–3%]	**15** (0–46) [0–4%]
32. 3β,13α-Dihydroxylupanine	**231** (94–386) [3–10%]	**608** (97–1262) [4–35%]	**329** (212–446) [5–8%]	**48** (5–91) [0.3–3%]	+	+	–	+
Total QA amount	**3191** (1528–3486)	**3617** (2712–5028)	**5010** (4668–5352)	**2276** (1459–3094)	**865** (215–1213)	**175** (91–456)	**758** (588–928)	**747** (538–1151)
No. of sites	3	7;8	7;8	9;11	2;3	7;8	7;8	9;11
No. of samples	7	7	2	2	6	9	2	3

–, not detected; +, arithmetic means < 5 μg g^{-1} dry wt.

1982; Wink and Witte, 1984). Since several compounds, especially lupanine-derivatives, could only be detected in seeds and not in shoots of *Cytisus scoparius* it seems that green fruits are also able to biosynthesize quinolizidine alkaloids or to modify the lupanine structure.

In contrast to *Spartium* (Greinwald *et al.*, 1990; Gresser, 1992), a also multibranched shrub, no changes within the alkaloid pattern of shoots could be observed during the year. This fact may depend on different alkaloid profiles of both species. In stems of *Cytisus scoparius* the quinolizidine alkaloids are localized in the epidermis and probably the neighbouring one or two subepidermal cell layers (Wink *et al.*, 1984). The increase of the total alkaloid amount in the young developing shoots during the vegetation period may be correlated with the differentiation of the external stem tissues over the year. This possibility would also explain the high alkaloid content of the elder shoots (with primary thickening) in comparison to the young developing shoots demonstrated especially at the beginning of the vegetation period. The alkaloids stored in the shoots of *Cytisus scoparius* at the end of the vegetation period might be an alkaloid pool for the new developing twigs.

Flowers, young developing shoots and green pods differ from the other plant parts in storing phenylethylamine tyramine. Flowers are often described as the main site of tyramine accumulation (Bachmann, 1989; Murakoshi *et al.*, 1986; Witte *et al.*, 1987; Veen *et al.*, 1992). Although tyramine is widespread among higher plants (Wheaton and Stewart, 1970) the compound represent a special feature within the subtribus Genisteae which includes the genus *Cytisus* (Bisby, 1981; Bisby *et al.*, 1994; Gresser, 1992; Veen *et al.*, 1992). The decrease of tyramine in the young developing shoots up to the flowering period of the plant may be explained by transport mechanism from shoots to flowers. But until now it is not possible to distinguish experimentally between transport and degradation of natural compounds.

Acknowledgements

We thank Ministerialrat Günter Grote (München), the Präsident der Universität Würzburg, Prof. Dr. Dr. h.c. mult. Theodor Berchem and the Kanzler Bruno Forster for their personal help in establishing this German-Russian joint-venture. Dr. Willmar Schwabe GmbH (Karlsruhe) and Lichtwer Pharma GmbH (Berlin) are gratefully acknowledged for their financial support. Furthermore we wish to thank Dr. Elisabeth Krug for her assistance and Martin and Hille Czygan for collecting plant material.

Bachmann P. (1989), Untersuchungen zum Alkaloidstoffwechsel und zur genetischen Transformation von *Duboisia myoporoides* R.BR. (Solanaceae). Dissertation, Universität Würzburg.

Bisby F.A. (1981), Tribe 32. Genisteae (Adans.) Benth. (1865). In: Advances in Legume Systematics, Part **1** (Polhill R.M., Raven P.H., eds.). Royal Botanic Gardens, Kew, 409 pp.

Bisby F.A., Buckingham J. and Harborne J.B. (eds.) (1994), Phytochemical Dictionary of the Leguminosae, Chapman & Hall, London, 240 pp.

Greinwald R., Veen G., Van Wyk B.-E., Witte L.. and Czygan F.-C. (1989), Distribution and taxonomic significance of major alkaloids in the genus *Virgilia*. Biochem. Syst. Ecol. **17**, 231–238.

Greinwald R., Lurz G., Witte L. and Czygan F.-C. (1990), A survey of alkaloids in *Spartium junceum* L. (Genisteae-Fabaceae). Z. Naturforsch. **45c**, 1085–1089.

Greinwald R., Schultze W. and Czygan F.-C. (1990a), Über die Alkaloidzusammensetzung der oberirdischen Teile von *Laburnum watereri* (KIRCHN.) DIPP.. Biochem. Physiol. Pflanzen **186**, 1–10.

Gresser G. (1992), Aspekte zum Stoffwechsel und zur Chemotaxonomie der Chinolizidinalkaloide in Fabaceae and Berberidaceae. Dissertation, Universität Würzburg.

Gresser G., Bachmann P., Witte L. and Czygan F.-C. (1993), Distribution and taxonomic significance of quinolizidine alkaloids in *Leontice leontopetalum* and *L. ewersmannii* (Berberidaceae). Biochem.Syst. Ecol. **21**, 679–685.

Hänsel R., Keller K., Rimpler H. and Schneider G. (eds.) (1992), Hagers Handbuch der Pharmazeutischen Praxis, 5. Auflage, Bd. **4**. Springer Verlag, Berlin, 1124 pp.

Hegi G. (1906), Illustrierte Flora von Europa , Bd. **IV**, 3. J.F. Lehmanns Verlag, München, 167 pp.

Mears J.A. and Mabry T.J. (1971), Alkaloids in the Leguminosae. In: Chemotaxonomy of the Leguminosae (Harborne J.B., Boulter D., and Turner B., eds.). Academic Press, London, 73 pp.

Murakoshi I., Yamashita Y., Ohmiya S. and Otomasu H. (1986), (-)-3β,13α-Dihydroxylupanine from *Cytisus scoparius*. Phytochemistry **25**, 521–524.

Saito K., Suzuki H., Yamashita Y. and Murakoshi I. (1994), Isolation and enzymatic synthesis of an ester alkaloid, (-)-3β-hydroxy-13α-tigloyloxylupanine, from *Cytisus scoparius*. Phytochemistry **36**, 309–311.

Schmalfuss H. and Heider A. (1931), Tyramin und Oxytyramin, blutdrucksteigernde Schwarzvorstufen des Besenginsters *Sarothamnus scoparius* Wimm. Biochem. Z. **236**, 226–230.

Steffen H. (1940), Flora von Ostpreussen. Gräfe und Unzer Verlag, Königsberg, p.176.

Tocher S. and Tocher C.S. (1972), Dopa decarboxylase in *Cytisus scoparius*. Phytochemistry **11**, 1661–1667.

Tutin T., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H, Walters S.M. and Webb D.A. (eds.) (1968), Flora Europaea, Vol. **II**, Cambridge University Press, p.89.

Veen G., Greinwald R., Canto P., Witte L. and Czygan F.-C. (1992), Alkaloids of *Adenocarpus hispanicus* (Lam.) DC Varieties. Z. Naturforsch. **47c**, 341–345.

Wheaton T.A. and Stewart J. (1970), The distribution of tyramine, N-methyltyramine, hordenine, octopamine, and synephrine in higher plants. Llyodia **33**, 244–254.

Wichtl M. (ed.) (1989), Teedrogen. Wissenschaftliche Verlagsgesellschaft mbH, 2. Auflage, 91–93.

Wink M. and Hartmann T. (1981), Sites of enzymatic synthesis of quinolizidine alkaloids and their accumulation in *Lupinus polyphyllus*. Z. Pflanzenphysiol. **102**, 337–344.

Wink M., Witte L. and Hartmann T. (1981), Quinolizidine alkaloid composition of plants and photomixotrophic cell suspension cultures of *Sarothamnus scoparius* and *Orobanche rapum-genistae*. Planta Med. **43**, 342–352.

Wink M, Hartmann T., Witte L. and Rheinheimer J. (1982), Interrelationship between quinolizidine alkaloid producing legumes and infesting insects: exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cytisorum*. Z. Naturforsch. **37c**, 1081–1086.

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

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*. Planta **161**, 519–524.

Wink M., Heinen H.J., Vogt H. and Schiebel H.M. (1984), Cellular localization of quinolizidine alkaloids by laser desorption mass spectrometry (LAMMA 1000). Plant Cell Rep. **3**, 230–233.

Wink M. and Witte L. (1985), Quinolizidine alkaloids as nitrogen source for lupin seedlings and cell cultures. Z. Naturforsch. **40c**, 767–775.

Wink M. (1987), Quinolizidine alkaloids: biochemistry, metabolism, and function in plants and cell suspension cultures. Planta Med. **53**, 509–514.

Wink M. (1993), Chinolizidine alkaloids. In: Methods in Plant Chemistry, Alkaloids and Sulphur compounds, Vol. **8,** Academic Press, London/New York, 197–239.

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

Witte L., Müller K. and Arfmann H.-A. (1987), Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass-spectrometry. Planta Med. **53**, 192–197.