A Survey of Alkaloids in *Spartium junceum* L. (Genisteae-Fabaceae)

Roland Greinwald*, Gabriele Lurz*, Ludger Witte**, and Franz-Christian Czygan*

* Institut für Botanik und Pharmazeutische Biologie der Universität Würzburg, Mittlerer Dallenbergweg 64, D-8700 Würzburg, Bundesrepublik Deutschland

** Institut für Pharmazeutische Biologie der Universität Braunschweig, Mendelssohnstraße 1, D-3300 Braunschweig, Bundesrepublik Deutschland

Z. Naturforsch. **45c,** 1085–1089 (1990); received February 15/September 26, 1990

Spartium junceum, Fabaceae, Genisteae, Quinolizidine Alkaloids, Gas Chromatography/Mass Spectrometry, Chemotaxonomy

The alkaloid pattern of *Spartium junceum* has been investigated. Cytisine, N-methylcytisine, anagyrine, rhombifoline and epi-baptifoline occur as major compounds in most of the samples studied. Considerable quantitative differences were found between various parts of the plant. The alkaloid content changed strongly dependent on the seasons. Only a limited variation can be ascribed to geographical origin.

Introduction

As part of an evaluation of alkaloids as a generic character in the tribe Genisteae, we investigated the monotypic genus *Spartium* L. The virgate almost leafless shrubs of *Spartium junceum* L. are widely distributed around the Mediterranean. This species is known as a source of quinolizidine alkaloids with α-pyridone nucleus [1]. In this paper we report the distribution of alkaloids in nine individuals taken from different provenances.

Our aim also was to test the seasonal influence on the alkaloid content and pattern in the different organs of the plants. It was assumed that significant results could be obtained only, if several possible sources of variation are accounted for in the sampling. Only those data should provide important informations on the value of alkaloids as character for taxonomic research.

Experimental

Plant materials

Plants of *Spartium junceum* were collected from different localities (Table II).

Extraction procedure

Air dried plant material was extracted as previously described [2]. Two independent extractions were performed for each plant sample.

Reprint requests to Prof. Franz-C. Czygan.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/1100–1085 $ 01.30/0

Gas-chromatography

Capillary GC was performed according to published conditions [2]. For capillary GC/MS we used a Carlo Erba gas chromatograph (type 5160) equipped with a J & W DB1 capillary column (0.32 mm × 30 m), coupled directly to the quadrupole mass spectrometer Finnigan MAT 4515; carrier gas: He; split 1:20; injector 250 °C; temperature program: 150–300 °C 6 °C/min; electron energy 45 eV. Retention indices were calculated using co-chromatographed standard hydrocarbons.

Reference alkaloids

Authentic samples of the following alkaloids were available to us: ammodendrine, sparteine, cytisine, N-methylcytisine, anagyrine, lupanine, baptifoline, epi-baptifoline.

N-acetylcytisine and N-formylcytisine were prepared by refluxing cytisine with Ac_2O and 98% HCO_2H, respectively, according to published reaction conditions [3, 4].

Identification of the alkaloids

Crude alkaloid fractions were separated by capillary GC. The following compounds in the extracts were identified by direct comparison (retention index, MS) to authentic alkaloids or by comparison of obtained MS to literature MS data: Sparteine, RI: 1775; MS: *m/z,* 234 $[M]^+$ [5]. β-Isosparteine, RI: 1820; MS: *m/z,* 234 $[M]^+$ [6]. 11,12-Dehydrosparteine, RI: 1830; MS: *m/z,* 232 $[M]^+$ [7]. Ammodendrine, RI: 1865; MS: *m/z,* 208 $[M]^+$ [8]. N-Methylcytisine, RI: 1950; MS: *m/z,* 204

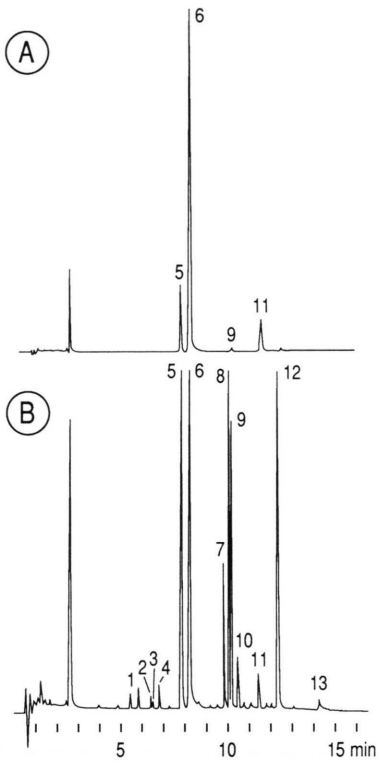

Fig. 1. Separation of alkaloids from *Spartium junceum* L. by capillary GC and detection by a nitrogen specific detector. A, Alkaloids from seeds; B, alkaloids from twigs; 1, sparteine; 2, β-isosparteine; 3, 11,12-dehydrosparteine; 4, ammodendrine; 5, N-methylcytisine; 6, cytisine; 7, 5.6-dehydrolupanine; 8, rhombifoline; 9, lupanine; 10, X1; 11, cytisine-12-carboxy-ethylester; 12, Anagyrine; 13, epi-baptifoline.

$[M]^+$ [1]. Cytisine, RI: 1990; MS: *m/z*, 190 $[M]^+$ [9]. 5,6-Dehydrolupanine, RI: 2125; MS: *m/z*, 246 $[M]^+$ [1]. Rhombifoline, RI: 2155; MS: *m/z*, 244 $[M]^+$ [7]. Lupanine, RI: 2160; MS: *m/z*, 248 $[M]^+$ [1]. X1, RI: 2210; MS: *m/z*, 248 $[M]^+$. Cytisine-12-carboxy-ethylester, RI: 2300; MS: *m/z*, 262 $[M]^+$ [10]. Anagyrine, RI: 2380; MS: *m/z*, 244 $[M]^+$ [1]. Epi-baptifoline, RI: 2655, MS: *m/z*, 260 $[M]^+$ [11].

Results

Alkaloid pattern of Spartium from Würzburg

Alkaloid extracts of buds, twigs (without wood, with primary and secondary thickening), flowerbuds, flowers, seeds (ripe and unripe), pods (ripe and unripe) and roots of *Spartium junceum* L. were analyzed. 15 alkaloids could be found in these extracts (Fig. 1/Table I). 9 of these compounds were new for this species (see Table I).

Table I. Alkaloid composition and yield [μg/g dry wt.] of different plant parts from *Spartium junceum* L. (Botanical Garden Würzburg) analyzed by capillary GC (Fig. 1).

Organ	Buds	Leaves	Twigs with primary thickening	Twigs with secondary thickening	Flower-buds	Flowers	Unripe pods	Ripe pods	Unripe seeds	Ripe seeds	Roots
Date of harvest	20.4.	26.5.	26.5.	26.5.	26.5.	26.5.	4.8.	29.10.	4.8.	29.10.	4.4.
Sparteine*	tr	–	tr	tr	tr	–	tr	tr	–	–	tr
β-Isosparteine*	–	–	tr	tr	–	–	–	–	–	–	tr
11,12-Dehydrosparteine*	–	–	tr	tr	–	–	–	–	–	–	tr
Ammodendrine	36	12	21	tr	9	tr	–	–	–	–	tr
N-Methylcytisine	1800	503	506	12	2937	1833	25	17	135	1022	198
Cytisine	1300	1366	3183	79	1945	1585	2941	222	4224	12637	266
5.6-Dehydrolupanine	167	63	66	13	34	20	–	tr	–	–	tr
Rhombifoline*	970	19	32	tr	7	7	–	–	–	–	tr
Lupanine	298	128	208	40	157	88	8	17	tr	tr	47
X1*	52	25	tr	–	42	25	–	–	–	–	–
Cytisine-12-carboxy-ethylester*	tr	59	85	23	81	109	tr	55	49	528	tr
N-Formylcytisine*	tr	tr	–	–	–	–	–	tr	–	–	–
N-Acetylcytisine*	tr	tr	–	–	–	–	–	tr	–	–	–
Anagyrine	2325	316	547	29	358	94	tr	10	–	–	29
Epi-Baptifoline*	107	125	52	10	45	30	tr	44	–	–	–
Total	7154	2762	4919	248	5835	3967	3012	370	4425	14198	552

* New alkaloid for the species. tr, traces.

Similar to other genera of the Genisteae, *e.g. Laburnum, Cytisophyllum* [11, 12], a great difference in the alkaloid content between various plant parts was observed, whereas the alkaloid pattern was quite constant.

The highest alkaloid concentration could be found in ripe seeds (1.42% dry wt.), compared to buds (0.72% dry wt.), flower buds (0.58% dry wt.), twigs with primary thickening (0.49% dry wt.), unripe seeds (0.44% dry wt.), flowers (0.40% dry wt.), unripe pods (0.30% dry wt.), leaves (0.28% dry wt.), ripe pods (0.04% dry wt.), roots (0.05% dry wt.) and finally twigs with secondary thickening (0.03% dry wt.). The seeds, pods and roots contained a very simple pattern, with cytisine as the main alkaloid. The twigs, buds, leaves and flowers however contained a more complex pattern of α-pyridone alkaloids, with cytisine, N-methylcytisine, anagyrine and rhombifoline as main compounds (Table I).

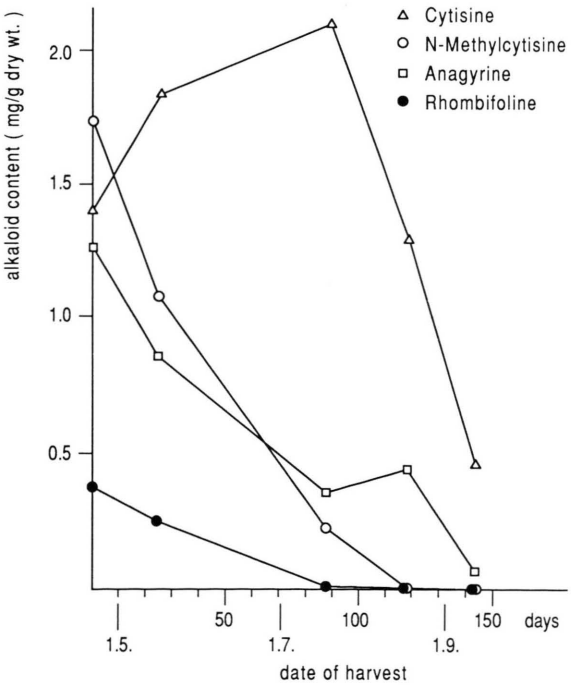

Fig. 2. Seasonal changes of the main alkaloids in twigs of *Spartium junceum* L. (Bot. Garden Würzburg) in 1987.

Seasonal variation of the alkaloid profile in twigs of Spartium from Würzburg

To test the influence of seasonal variation on the alkaloid profile, we harvested twigs from our control plant at five different dates in 1987 (Fig. 2) and at eight different dates in 1988 (data not shown). The alkaloid analyses of these twigs demonstrated reproducible changes of the alkaloid pattern in both years. Besides the main compounds cytisine and N-methylcytisine, the alkaloids rhombifoline and anagyrine are accumulated in greater amounts.

In spring, two special features can be seen in the alkaloid pattern of the young twigs. First of all, the concentration of rhombifoline was maximal in spring (7.0% of total alkaloids) and decreased further in the year. Second, in twigs harvested in april, N-methylcytisine occurred as main alkaloid of the plant. During the following months, the concentration of N-methylcytisine lowered, and cytisine becomes the main alkaloid of the plant. The high amount of N-methylcytisine in spring was already described for other α-pyridone-containing Leguminosae as *Laburnum* [11].

Alkaloid patterns of Spartium from eight other habitats

Samples from 8 different provenances and a control plant (Bot. Garden, Würzburg) were investigated for their alkaloid profile (Table II). The investigations were performed with twigs, as these organs revealed a complex alkaloid pattern.

The samples corresponded well, especially concerning the occurrence of the main constituents. This correspondence gets even clearer, if the seasonal variation, determined on a control plant from the Botanical Garden Würzburg, is considered. The highest total alkaloid content was found in samples harvested in spring (samples from Bonifacio and Lloret; see Table II).

The alkaloid pattern of *Spartium junceum* L. with its main constituents cytisine, N-methylcytisine, rhombifoline, anagyrine and epi-baptifoline proved to be independent from the locality of the plant (Table II). The specific accumulation of rhombifoline during spring time is a striking feature of *Spartium junceum* L.

Table II. Material of *Spartium junceum* used for alkaloid studies and yields of the main alkaloids obtained from twigs. Values in brackets represent the percentages of the total yields as estimated from GC results. Alkaloids: 1, cytisine; 2, N-methylcytisine; 3, rhombifoline; 4, anagyrine; 5, epi-baptifoline; 6, total alkaloids; tr, traces.

Locality	Date of harvest	Yield [µg/g dry wt.] 1	2	3	4	5	6
Würzburg (Germany)	21.04.87	1413 (27.1)	1730 (33.2)	280 (7.3)	1273 (24.4)	37 (0.7)	5213
Bonifacio (Corsica)	20.04.87	3298 (29.5)	2003 (17.9)	2718 (24.3)	2264 (25.6)	204 (1.8)	11178
Lloret (Spain)	1.05.87	3825 (50.9)	820 (10.9)	190 (2.5)	2437 (32.4)	166 (2.2)	7519
Alanya (Turkey)	25.05.87	1350 (78.4)	142 (8.3)	27 (1.6)	43 (2.5)	77 (4.5)	1722
Delphi (Greece)	5.06.87	1543 (76.1)	72 (3.6)	22 (1.1)	207 (10.2)	90 (4.4)	2028
Isola Brissago (Italy)	10.08.87	1758 (68.8)	39 (1.5)	15 (0.6)	326 (12.8)	194 (7.6)	2554
Troumbetta pass (Corfu, Greece)	23.08.87	2257 (91.2)	24 (1.0)	tr	35 (1.4)	181 (7.3)	2476
Montélimar (France)	28.08.89	744 (73.3)	tr	tr	tr	149 (14.7)	1015
Massif L'Esterel (France)	3.09.89	244 (52.4)	tr	tr	14 (3.0)	135 (29.0)	466

Discussion

Our results confirm that quinolizidine alkaloids may be of diagnostic value at the generic level. We found cytisine and other α-pyridone alkaloids, which are thought to be advanced characters [13], as main alkaloids of *Spartium junceum* L. As already shown for *Baptisia* [14] and *Virgilia* [2], we could not observe any qualitative differences between the individuals from different provenances.

The alkaloid pattern and concentration varies amongst the plant parts studied in this work. Table I reveals distinct differences between buds, twigs (without wood, with primary and secondary thickening), flower-buds, flowers, pods (ripe and unripe), seeds (ripe and unripe) and roots.

The high concentration of alkaloids and the very simple pattern in seeds seem to be of ecological importance. Cytisine, the main alkaloid of the seeds, has shown to be an effective repellent [9, 15] and thus can contribute to the protection of the reproductive organs.

The alkaloid pattern of *Spartium junceum* L. shows remarkable seasonal variations, which have to be taken into account, when alkaloids are used as phytochemical characters in taxonomic research. In the green parts of the plant, the highest yield of alkaloids was found in early spring and then strongly decreases during the further year. In summer and autumn the alkaloids appear to be translocated and to accumulate in the reproductive organs, as former shown for *Laburnum* [11] and other genera of the Genisteae [16].

Interesting changes in the alkaloid pattern can be observed during the seasonal variation of the alkaloid metabolism. N-methylcytisine and especially rhombifoline are present in greater amounts only in spring and disappear later in the year. The content of the tetracyclic alkaloid anagyrine decreases, too, during spring and early summer, but in the second part of the year this alkaloid increases from 0.04% dry wt. to 0.05% dry wt. in 1987 (Fig. 2) and from 0.01% dry wt. to 0.1% dry wt. in 1988 (data not shown).

For the biosynthesis of the cytisine-type alkaloids it has been postulated from pulse feeding experiments with $^{14}CO_2$ that lupanine is converted

into 5.6-dehydrolupanine, which is further oxidized to anagyrine, rhombifoline, cytisine and N-methylcytisine. In these experiments rhombifoline appeared to be the first of the tricyclic bases to be formed from the tetracyclic ones [17]. The occurrence of rhombifoline was already demonstrated in several other species of the Fabaceae with α-pyridone alkaloids [7, 18, 19]. The presence of the rhombifoline in young twigs of *Spartium junceum* could be a further hint that this compound is the likely intermediate between the tetracyclic anagyrine and the tricyclic compounds cytisine and N-methylcytisine. Cleavage of the C 11/C 12 bond of anagyrine would result in the formation of the tricyclic alkaloid rhombifoline and subsequent loss of the N 16 side chain would result in cytisine, the precursor of N-methylcytisine.

In further studies the alkaloid pattern of the genera *Calicotome, Erinacea* and *Gonocytisus,* which form together with *Spartium* a group of isolated genera within the tribe Genisteae, should be evaluated, to find out if cryptic characters like alkaloids can give some informations on the affinities of these genera.

[1] M. Wink and L. Witte, Z. Naturforsch. **42c,** 69 (1987).
[2] R. Greinwald, G. Veen, B.-E. Van Wyk, L. Witte, and F.-C. Czygan, Biochem. Syst. Ecol. **17,** 231 (1989).
[3] S. Ohmiya, H. Otomasu, I. Murakoshi, and J. Haginiwa, Phytochemistry **13,** 643 (1974).
[4] S. Ohmiya, H. Otomasu, I. Murakoshi, and J. Haginiwa, Phytochemistry **13,** 1016 (1974).
[5] N. Neuner-Jehle, D. Schumann, and G. Spiteller, Monatsh. Chem. **98,** 836 (1967).
[6] M. Wink, Habilitationsthesis, TU Braunschweig, 1984.
[7] M. Wink, L. Witte, T. Hartmann, C. Theuring, and V. Volz, Planta Med. **48,** 253 (1983).
[8] W. Fitch and C. Djerassi, J. Am. Chem. Soc. **96,** 4917 (1974).
[9] M. Wink, Z. Naturforsch. **39c,** 548 (1984).
[10] R. Greinwald, P. Bachmann, L. Witte, and F.-C. Czygan, Phytochemistry **29,** 3353 (1990).
[11] R. Greinwald, W. Schultze, and F.-C. Czygan, Biochem. Physiol. Pflanzen **186,** 1 (1990).
[12] R. Greinwald, L. Witte, V. Wray, and F.-C. Czygan, Biochem. Syst. Ecol. (in press).
[13] A. Salatino and O. Gottlieb, Biochem. Syst. Ecol. **8,** 133 (1980).
[14] M. Cranmer, Ph.D. Dissertation, University of Texas at Austin, 1965. In: J. Harborne, D. Boulter, and B. Turner, Chemotaxonomy of the Leguminosae. Academic Press, New York 1979.
[15] M. Wink, Z. Naturforsch. **39c,** 553 (1984).
[16] R. Greinwald, Untersuchungen zur chemotaxonomischen Bedeutung von Leguminosenalkaloiden und zum Alkaloidstoffwechsel in transformierten Geweben und Zellkulturen. Ph.D. Thesis, Universität Würzburg, 1988.
[17] Y. D. Cho and R. O. Martin, Can. J. Biochem. **49,** 971 (1971).
[18] D. Kinghorn, R. Hussain, E. Robbins, M. Balandrin, C. Stirton, and S. Evans, Phytochemistry **27,** 439 (1988).
[19] I. Murakoshi, M. Watanabe, T. Okuda, E. Kidoguchi, J. Haginiwa, S. Ohmiya, and H. Otomasu, Phytochemistry **24,** 2707 (1985).