Iridoid Patterns of Genus *Plantago* L. and Their Systematic Significance

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The distribution of 14 iridoid glucosides in 14 *Plantago* L. species (44 samples corresponding to 18 taxa) was shown. *P. tenuiflora* and *P. gentianoides* were studied for iridoids for the first time. The iridoid patterns showed a good correlation with morphological and other chemical features of the representatives of genus *Plantago*. The studied species are grouped together according to the iridoid patterns: species containing mainly aucubin (*P. major, P. cornuti, P. gentianoides*); species containing aucubin and aucubin derivatives (*P. subulata, P. media*); species containing aucubin and catalpol (*P. lanceolata, P. altissima, P. argentea, P. lagopus, P. atrata*); species containing aucubin and plantarenaloside (*P. afra, P. scabra*).

Introduction

The genus *Plantago* L. comprises 265 species and has a cosmopolitan distribution (Willis, 1980). According to Pilger (1937) the genus is divided into two subgenera – *Euplantago* Harms (subgenus *Plantago*) and *Psyllium* (Juss) Harms. Later Rahn (1978) subdivided the genus into three subgenera: subgenus *Plantago, Coronopus* (Lam. et DC.) Rahn (= sect. *Coronopus*, subgenus *Plantago* sensu Pilger, 1937) and *Psyllium* Rahn (here are included subgenus *Psyllium* and 5 sections of subgenus *Plantago* in the sense of Pilger, 1937). Recently, Rahn (1996) proposed a new original taxonomic scheme of the genus according to which genus *Plantago* includes 6 subgenera: subgenus *Plantago, Coronopus* (Lam. et DC.) Rahn, *Albicans* Rahn (includes different parts of subgenus *Plantago* sensu Pilger, 1937), subgenus *Psyllium* Juss. (sensu Pilger, 1937, not in Rahn, 1978), *Littorella* (Bergius) Rahn (= genus *Littorella* Bergius) and subgenus *Bougueria* (Decne.) Rahn (= genus *Bougueria* Decne.). Some authors as Sojak (1972), Holub (1973) and Dietrich (1980, 1982) accept the subgenus *Psyllium* (Juss.) Harms as a distinct genus.

The earlier studies on genus *Plantago* describe the macromorphological features of the species (Decaisne, 1852; Harms and Reiche, 1895; Pilger, 1937). The later works report data of embryological characters (Misra, 1964), pollen morphology (Dietrich, 1968; Clarke and Jones, 1977; Saad, 1986), seed structure (Rezk, 1980), chromosome numbers (Dietrich, 1975, 1980; Brullo *et al*., 1985; Kozhuharov *et al*., 1974), hair types (Rahn, 1992; Andrzejewska-Golec and Swietoslawski, 1987, 1988, 1989a,b; Andrzejewska-Golec, 1992) and chemical characters. As chemotaxonomic markers have been used sugars (Gorenflot and Bourdu, 1962), phenolcarboxylic acids (Andrzejewska-Golec and Swiatec, 1986), phenylethanoid glycosides (Andary *et al*., 1988; Ronsted *et al*., 2000), flavonoid glycosides (Tomas-Barberan *et al*., 1988, Kawashty *et al*., 1994) and iridoid glucosides (Rymkiewicz, 1979; Kuzmanov *et al*., 1984; Andrzejewska-Golec and Swiatek, 1984; Andrzejewska-Golec *et al*., 1993; Andrzejewska-Golec, 1997; Ronsted *et al*., 2000).

Iridoid glucosides are useful chemotaxonomic markers to genus *Plantago*. Rymkiewicz (1979) determined that some *Plantago* species differ in their aucubin content. Kuzmanov *et al.* (1984) showed a correlation between the caryological and phytochemical data of the subgenus *Plantago* and subgenus *Psyllium*. The authors hypothesized that the separation of these subgenera had taken place in the remote past and their evolutionary development had followed different patterns. According to Andrzejewska-Golec and Swiatek (1984), Andrzejewska-Golec *et al.* (1993), Andrzejewska-

Golec (1995, 1997) the iridoid glucosides with systematic value to genus *Plantago* are aucubin, catalpol, plantarenaloside, and bartsioside. Ronsted *et al.* (2000) confirmed that aucubin is typical for the whole genus and catalpol is characteristic only for the subgenus *Albicans* sensu Rahn (1996) and genus *Littorella*. Plantarenaloside, asperuloside and bartsioside were found in more than one subgenus.

In this paper we show the distribution of 14 iridoid glycosides in 14 *Plantago* species collected in Bulgaria and discuss the significance of iridoids to clarify the taxonomy and evolution of the genus *Plantago*.

Materials and Methods

Plant material

Samples of 44 Bulgarian natural populations of *Plantago* (Table 1) were collected at flowering and fruitification and the voucher specimens deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM). The taxa were identified according to Petrova (1995).

Isolation and identification of glucosides

P. major, P. cornuti: compounds **1**, **9** and **10** were isolated as it was described previously (Taskova *et al.*, 1999).

P. subulata: Dried ground aerial parts (70 g) were extracted with methanol (2 × 0.8 l for 24 h). The combined extracts were concentrated (8 g), water was added (200 ml) and extraction with dichloroethane was performed (3 × 70 ml). The water phase was treated with charcoal (40 g) and elution with water (1 l), water–methanol (20:1, v/v; 1 l), water–methanol (2:1, 1:1, v/v; 0.5 l), methanol (0.5 l), methanol–acetone (1:1, v/v; 0.5 l), and methanol–dichloroethane (1:1, v/v; 0.5 l) mixtures was carried out. The methanol–dichloroethane fr (0.6 g) after purification on silica gel (55 g) yielded pure **1** (81 mg), **3** (17 mg), **4** (51 mg), **5** (9 mg), and **6** (34 mg).

P. lagopus: Dried ground aerial parts (59 g) were extracted with methanol (2 × 0.6 l for 24 h). The combined extracts were concentrated (5 g), water was added (150 ml) and extraction with dichloroethane was performed (3 × 50 ml). The water phase was treated with charcoal (50 g) and elution with water (1 l), water–methanol (20:1, v/v; 1 l), water–methanol (2:1, 1:1, v/v; 0.5 l), methanol (0.5 l), methanol–acetone (1:1, v/v; 0.5 l), and methanol–dichloroethane (1:1, v/v; 0.5 l) mixtures was carried out. The combined methanol (0.1 g) and methanol–acetone (0.1 g) frs after purification on silica gel (22 g) yielded pure **1** (15 mg), **2** (18 mg), **8** (17 mg), **12** (9 mg), and **13** (7 mg). The methanol–dichloroethane fr (0.1 g) after purification on silica gel (11 g) yielded pure **7** (11 mg) and **8** (8 mg).

P. scabra: Dried ground aerial parts (47 g) were extracted with methanol (2 × 0.5 l for 24 h). The combined extracts were concentrated (5 g), water was added (150 ml) and extraction with dichloroethane was performed (3 × 50 ml). The water phase was treated with charcoal (50 g) and elution with water (1 l), water–methanol (20:1, v/v; 1 l), water–methanol (2:1, 1:1, v/v; 0.5 l), methanol (0.5 l), methanol–acetone (1:1, v/v; 0.5 l), and methanol–dichloroethane (1:1, v/v; 0.5 l) mixtures was carried out. The combined methanol (0.1 g) and methanol–acetone (0.2 g) frs after purification on silica gel (35 g) yielded pure **1** (13 mg) and **14** (42 mg).

The identification was achieved by ultraviolet, infrared and nuclear magnetic resonance spectroscopy (El-Naggar and Beal, 1980; Boros and Stermitz, 1990; Taskova *at al.*, 1999).

Thin layer chromatography-densitometry

Plant extracts preparation: Dried ground aerial parts from 44 *Plantago* samples (1 g of each) were extracted with methanol (2 × 20 ml) for 24 h. After the concentration of the combined extracts, water was added (3 ml) and a threefold extraction with dichlorethane was carried out. The water layer was filtered through 3 g neutral alumina 90 (Merck 1077) and after concentration dissolved in 2 ml methanol–water (1:1, v/v).

Stock solutions and calibration: About 50 mg of each standard (aucubin (**1**), catalpol (**2**), 10-O-acetylaucubin (**3**), 10-acetylmonomelittoside (**5**), 10-cinnamoylcatalpol (**7**), 10-benzoylcatalpol (**8**), asperuloside (**13**), and plantarenaloside (**14**)) was dissolved in 2 ml methanol. Reference solutions were prepared in the range 0.8–4 mg/ml from the stock solutions by dilution with methanol.

Thin layer chromatography: The sample solutions were applied to plates with silica gel GF_{254}

Table I. Samples of *Plantago* studied for iridoids, collection sites and voucher numbers.

Taxon	Sample No.	SOM[a]	Locality[b], basic rock[c], m a.s.l.[d]	Pheno-phase	Collection date
P. major L.	1	154168	t. Srednogorie, Si, 1000	F	15.06.1996
var. major	2	154169	Sofia region, v. Bunovo, Si, 1000	F	15.06.1996
	3	154076	Rhodopes Ms, l. Beglika, Si, 1200	Fr	28.06.1996
P. major L.	4	154170	Pirin M., Banderitza hut, Si, 1900	F	07.07.1996
var. intermedia	5	154171	Stara Planina M., Vezen hut, Si, 1650	F	08.07.1996
(DC.) Decne.	6	154172	Rila M., Maljovitza hut, Si, 2100	F	12.06.1996
	7	154173	Rhodopes Ms, v. Progled, Si, 1900	Fr	18.07.1996
P. tenuiflora Waldst. et Kit.	8	154174	Thacian plain, v. Belozem, Ca, 100	Fr	23.08.1996
P. cornuti Gouan	9	151024	Black Sea coast, t. Tzarevo, Si, 0	F	12.06.1991
P. coronopus L.	10	154175	Struma valley, l. Rupite, Ca, 100	F	17.05.1997
	11	154176	Black Sea coast, Kiten, Si, 20	Fr	24.06.1997
P. subulata L.	12	154177	t. Srednogorie, Si, 1000	F	15.06.1996
	13	154077	Pirin M., below Vihren peak, Ca, 2300	F	06.07.1996
	14	154178	Golo Bardo M., Ca, 1000	F	04.07.1996
	15	154179	Rila M., v. Madjare, Si, 1000	F	12.07.1996
P. media L.	16	154180	Rhodopes Ms, l. Rakovo Dere, Si, 1200	F	27.06.1996
var. media	17	154181	Pirin M., l. Pesterite, Ca, 1200	F	0507.1996
P. media L.	18	154182	Ljulin M., l. Bonsovi poljani, Si, 1000	F	19.06.1996
var. urvilleana Rapin	19	154183	Vitosha M., l. Tihia kat, Si, 1000	F	19.06.1996
P. atrata Hoppe	20	154184	Vitosha M., Tserni vrah peak, Si, 2200	Fr	28.06.1996
	21	154185	Pirin M., l. Dolen Kazan, Ca, 2200	F	07.07.1996
	22	154186	Rila M., l. Marichini Ezara, Si, 2300	Fr	28.07.1996
P. gentianoides	23	154187	Slavjanka M., v. Dobrotino, Ca, 600	F	18.05.1996
Sibth. et Sm.	24	154188	Rila M., l. Maljovitza, Si, 2100	F	12.07.1996
f. gentianoides	25	154189	Rila M., l. Maljovitza, Si, 2000	Fr	13.07.1996
P. gentianoides	26	154190	lPirin M., Vihren hut, Si, 2000	Fr	06.07.1996
f. stefanovii	27	154191	Pirin M., l. Kabata, Ca, 2500	F	06.07.1996
(Urum. et Jav.) Hayek	28	154192	Rila M., l. Marichini Ezara, Si, 2500	F	28.07.1996
P. lanceolata L.	29	154193	Pirin M., v. Dobriniste, Si, 600	F	17.05.1996
var. lanceolata	30	154194	Vitosha M., l. Zlatni Mostove, Si, 1400	F	09.06.1996
	31	154195	Struma valley, l. Rupite, Ca, 100	F	17.05.1997
P. lanceolata L.	32	154196	Black Sea coast, t. Varna, Ca, 50	F	24.05.1996
var. eriophylla Decne.	33	154197	Vitosha M., v. Vladaja, Si, 900	Fr	09.06.1996
	34	154198	Pirin M., l. Pesterite, Ca, 1200	F	05.07.1996
P. altissima L.	35	154199	t. Sofia, Si, 500	Fr	15.06.1996
	36	154200	Pirin M., l. Pesterite, Ca, 1200	Fr	05.07.1997
	37	154201	Struma valley, l. Rupite, Ca, 100	F	17.05.1997
P. argentea Chaix	38	154202	Golo Bardo M., Ca, 1000	Fr	10.07.1997
P. lagopus L.	39	154079	Struma valley, l. Rupite, Ca, 100	F	17.05.1997
	40	154203	Struma valley, l. Rupite, Ca, 100	F	12.04.1998
P. scabra Moench	41	154204	Black Sea coast, t. Kiten, Si, 0	Fr	24.06.1997
	42	154205	Black Sea coast, Zlatni pjasatsi, Si, 0	Fr	18.08.1996
	43	154080	Black Sea coast, t. Sozopol, Si, 0	Fr	14.08.1997
P. afra L.	44	154206	Mesta valley, t. Gotze Delchev, Si, 500	Fr	02.08.1996

[a] SOM – Herbarium of Institute of Botany, Bulgarian Academy of Sciences.
[b] M. – Mountain, l. – locality, t. – town, v. – village.
[c] Basic rock: Ca – limestone, Si – silicate.
[d] m a.s.l. – meters above sea level.
[e] Phenophase: F – at flowering; Fr – at fruitification.

(Merck Cat. 5554) for compounds **3**, **5**, **7–8**, **13–14** and neutral alumina $60F_{254}$ TypE (Merck 5550) for compounds **1** and **2**. Plates were developed with mobile phases chloroform–methanol–water (60:20:4, v/v/v, lower layer) and butanol–methanol–water (70:5:10, v/v/v) respectively, and left for 1 hour at room temperature for the solvents to evaporate.

Densitometry: Scanning was performed on a Shimadzu CS-930 densitometer in a zigzag reflection mode with a slit of 0.4×0.4 mm. Compounds **3**, **5**, **7–8**, **13–14** were determined by scanning at

230 nm. Compounds **1** and **2** were scanned at 450 nm after charring (the plates were placed over sulfuryl chloride vapours for 60 min and then heated at 120 °C for 30 min).

Results and Discussion

A total of 14 compounds (Fig. 1) were isolated and identified by spectral methods and comparison with authentic reference compounds.

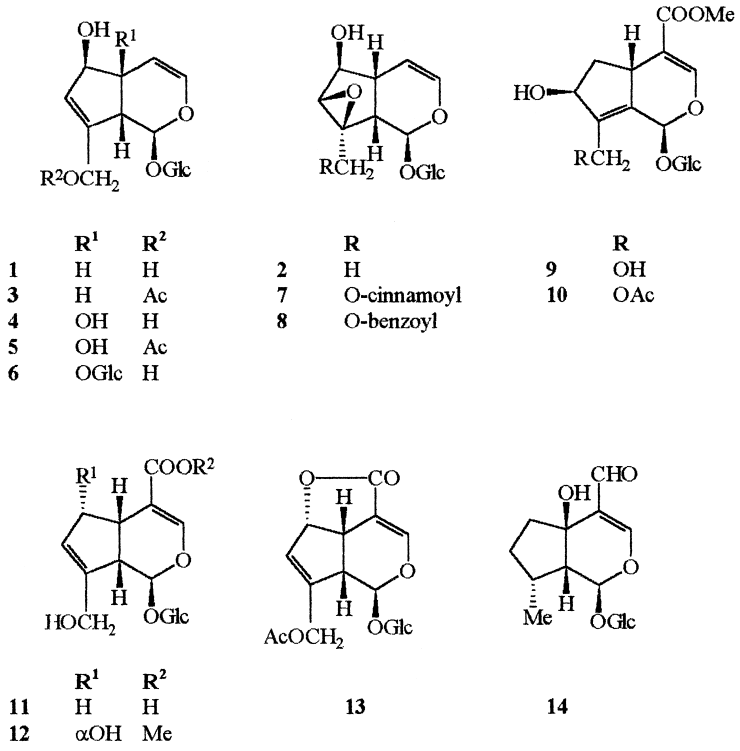
A thin layer chromatography-densitometry analysis was performed and the distribution of 8 iridoid glucosides (**1–3**, **5**, **7**, **8**, **13**, and **14**) in a total of 44 samples corresponding to 18 taxa was shown. *P. tenuiflora* and *P. gentianoides* were analyzed for iridoids for the first time. Plant samples from three localities of each taxon, when possible from habitats with different ambient conditions, were studied. The analysis showed qualitatively constant iridoid patterns of the studied species, which were not influenced by environmental conditions and phenophase.

For a better understanding of the phylogeny and the evolutionary processes in the genus the knowledge of the biosynthetic mechanisms of the isolated compounds was used. The probable biosynthetic routes of some of the *Plantago* iridoid glucosides, made in accordance with Inouye and Uesato (1986), Inouye (1991), Jensen (1991), Damtoft *et al.* (1993), Ronsted *et al.* (2000), are given by Fig. 2. In addition, the obtained data for the iridoid patterns in genus *Plantago* were interpreted in accordance with the known morphological, embryological, karyological and other chemical data.

Our studies were focused at higher taxonomic levels (section, subgeneric, and generic) based on a preliminary investigation, which had shown no systematic value of the iridoids at the infraspecific level. For example, the infraspecific taxa of *P. major* (*var. major* and *var. intermedia*), *P. media* (*var. media* and *var. urvilleana*), *P. gentianoides* (*f. gentianoides* and *f. stefanovii*), and *P. lanceolata* (*var. lanceolata* and *var. eriophylla*) showed no chemical differentiation concerning the iridoid pattern.

P. major, P. cornuti, P. media and *P. gentianoides*

A close iridoid pattern (iridoids **1**, **9**, **10**) for *P. major* and *P. cornuti* was found. This was in ac-



	R¹	R²
1	H	H
3	H	Ac
4	OH	H
5	OH	Ac
6	OGlc	H

	R
2	H
7	O-cinnamoyl
8	O-benzoyl

	R
9	OH
10	OAc

	R¹	R²
11	H	H
12	αOH	Me

13

14

Fig. 1. Iridoid glucosides isolated from the investigated *Plantago* species.

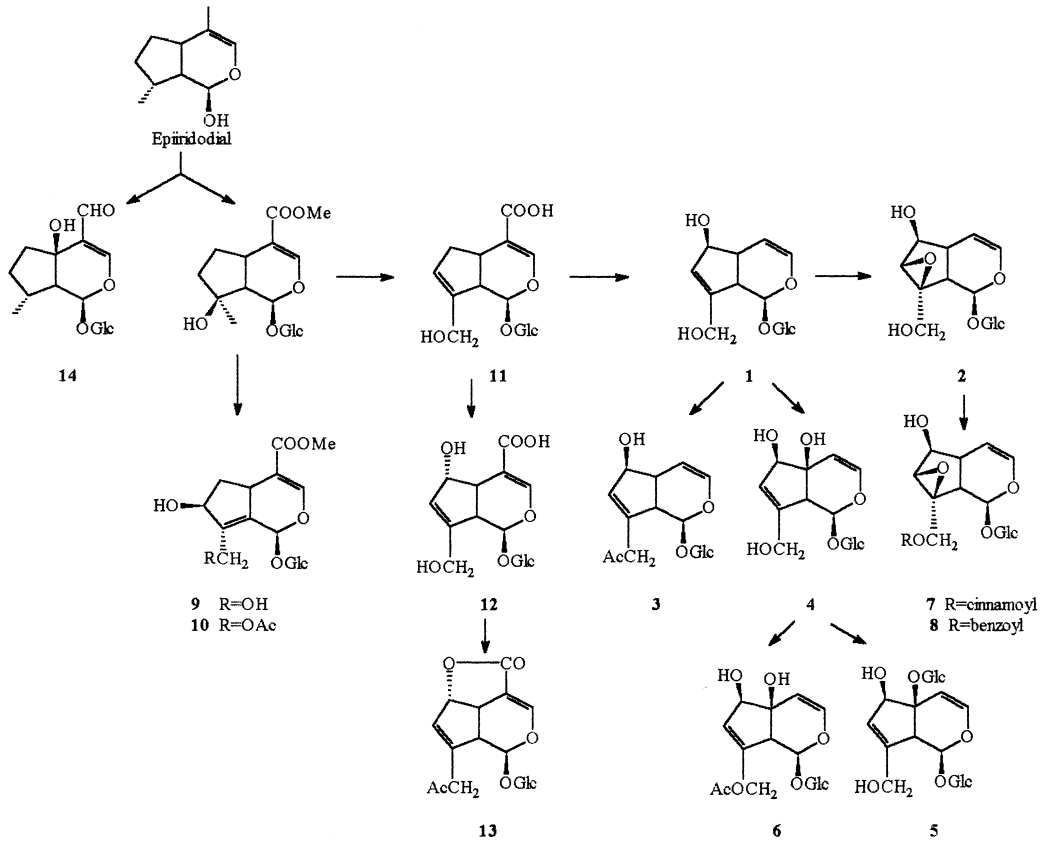


Fig. 2. Probable biosynthetic pathways to the *Plantago* iridoid glucosides.

cordance whit the resembling morphology and the same basic chromosome number (Table 2). Pilger includes these species into two separate sections, *Polyneuron* and *Palaeopsyllium,* which does not seem necessary. Rahn (1996) includes these two species into one section, section *Plantago* of subgenus *Plantago.* In this section the author includes also *P. media* and *P. gentianoides* which were studied here. In fact, *P. major, P. cornuti* and *P. gentianoides* possess similar iridoid patterns – the main component was aucubin (**1**) accompanied with traces of other iridoids. However, the iridoid profile of *P. media* was quite different. Besides aucubin (**1**) *P. media* contained high concentrations of other aucubin derivatives as 10-acetylaucubin (**3**), monomelittoside (**4**), 10-O-acetoxymonomelittoside (**5**) and melittoside (**6**). This makes us suggest that probably a more correct taxonomic decision is to include *P. media* in a separate section.

The iridoid pattern of *P. media* was very similar to those of *P. subulata* (Table 2). Chilikova (1997) proved the same phenylethanoid composition of both species. Consequently, the chemical characters suggest a relation between *P. media* and *P. subulata.*

P. coronopus and *P. subulata*

We established that the distributed in Bulgaria representatives of section *Coronopus* (sensu Pilger, 1937) – *P. coronopus* and *P. subulata,* differed considerably in their iridoid content. *P. coronopus* contained mainly aucubin (**1**), while *P. subulata* beside aucubin (**1**) possessed 10-O-acetylaucubin (**3**), monomellitoside (**4**), 10-O-acetoxymonomelittoside (**5**) and melittoside (**6**) (Table 2). These data are in good accordance with the results of previous morphological, caryological, and phytochemical investigations:

Table II. Occurrence of iridoid glucosides in the studied *Plantago* species.

Sample No.	Taxon[a]	x[b]	Iridoids[c]													
			C_9 iridoids								C_{10} iridoids					
			Aucubin type					Catalpol type			Majorozide type					
			1	**3**	**4**	**5**	**6**	**2**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Subgenus *Plantago* Sect. *Polyneuron* Decne.															
1–7	*P. major*	6	*								*	*				
	Sect. *Micropsyllium* Decne.															
8	*P. tenuiflora*	6	*								*	*	*			
	Sect. *Palaeopsyllium* Pilger															
9	*P. cornuti*	6	*													
	Sect. *Coronopus* DC.															
10–11	*P. coronopus*	5	*										*			
12–15	*P. subulata*	6	*	*	*	*	*									
	Sect. *Lamprosantha* Decne.															
16–19	*P. media*	6	*	*	*	*	*									
	Sect. *Oreades* Decne.															
20–22	*P. atrata*	6	*					*								
	Sect. *Gentianoides* Pilger															
23–28	*P. gentianoides*	6	*													
	Sect. *Arnoglossum* Decne.															
29–34	*P. lanceolata*	6	*					*	*				*		*	
35–37	*P. altissima*	6	*					*	*						*	
38	*P. argentea*	6	*					*	*						*	
39–40	*P. lagopus*	6	*					*	*	*				*	*	
	Subgenus *Psyllium* (Juss.) Harms Sect. *Psyllium*															
41–43	*P. scabra*	6	*													*
44	*P. afra*	6	*													*

[a] The species are grouped in sections and subgenera according to Pilger (1937).
[b] Basic chromosome number.
[c] Iridoids: aucubin (**1**), catalpol (**2**), 10-O-acetylaucubin (**3**), monomelittoside (**4**), 10-acetylmonomelittoside (**5**), melittoside (**6**), 10-cinnamoylcatalpol (**7**), 10-benzoylcatalpol (**8**), 10-hydroxymajoroside (**9**), 10-acetoxymajoroside (**10**), geniposidic acid (**11**), and deacetylasperulosidic acid methyl ester (**12**), asperuloside (**13**), and plantarenaloside (**14**).
Compounds **1–3**, **5**, **7**, **8**, **13**, **14** were analyzed by thin layer chromatography-densitometry and **4**, **6**, **9–12** were found only in purified iridoid fractions.

Pilger (1937) divides section *Coronopus* into groups A and B based on morphological features. Group A includes *P. coronopus* and group B – *P. subulata*. Gorenflot and Bourdu (1962) propose section *Coronopus* to be divided into two groups due to the content of ribose in the seeds and the different basic chromosome number. The *P. coronopus* group includes species with × = 5 and no ribose in the seeds, whereas the *P. maritima* goup – species with x = 6, which contain ribose in the seeds. Both groups are characterized with different pollen type (Clarke and Jones, 1977), structure of leaves and seeds (Rymkiewicz, 1979) and type of hairing (Andrzejewska-Golec and Swietoslawski, 1987). It is proved that *P. coronopus* and *P. subulata* differ in their iridoid (Rymkiewicz, 1979, Andrzejewska-Golec and Swiatek, 1984; Ronsted *et al.*, 2000), flavonoid (Tomas-Barberan *et al.*, 1988) and phenylethanoid composition (Andary *et al.*, 1988; Ronsted *et al.*, 2000).

Based on these data some authors changed the taxonomic scheme of Pilger (1937). Rahn (1978)

assumes the status subgenus for section *Coronopus* and divides the latter into section *Coronopus* Lam. et DC. and section *Maritima* Rahn. On the other hand, Dietrich (1980) distinguishes in the frame of subgenus *Plantago* section *Coronopus* DC and section *Maritima* Dietrich. We think that the species *P. coronopus* and *P. subulata* must be included into two separate sections of subgenus *Plantago* and consequently, we support the taxonomic scheme of Dietrich (1980).

P. lanceolata, P. altissima, P. argentea and *P. lagopus*

These species are well distinguishable in a morphological and chemical aspect from the other representatives of genus *Plantago.* We established similar iridoid patterns of the four studied species. Aucubin (**1**), catalpol (**2**), catalpol esters (**7**, **8**) and C_{10} iridoids (**11–13**) were found (Table 2). These facts confirm that it is correct to combine these four species into one section – section *Arnoglossum* of subgenus *Plantago* sensu Pilger (1937) or section *Lanceifolia* Barneoud of subgenus *Albicans* sensu Rahn (1996).

In the 14 studied *Plantago* species, catalpol (**2**) was present only in the representatives of sections *Arnoglossum* and *Oreades* (*P. atrata*) sensu Pilger (1937). Thus, we confirm the previous results of Andrzejewska-Golec and Swiatek (1984) and Ronsted *et al.* (2000) for the presence of catalpol only in the representatives of sections *Oreales, Arnoglossum* and *Bauphula* Decne (the latter section has no representatives in Bulgaria) and genus *Littorella*. The limited occurrence of catalpol in *Plantago* makes this character an important taxonomic marker proving a relation among the species, which possess the ability to synthesize this compound.

Rahn (1978) includes sections *Oreales, Arnoglossum* and *Bauphula* in subgenus *Psyllium* Rahn. The iridoid pattern of the species studied by us was not in support of this taxonomic decision. In the representatives of subgenus *Psyllium* (sensu Pilger, 1937) the main iridoid was plantarenaloside (**14**) and they lack catalpol (**2**). Contrary, in species of sections *Oreales, Arnoglossum* and *Bauphula* catalpol (**2**) was the main iridoid. The taxonomic proposal of Rahn (1978) has been criticized by Andrzejewska-Golec and Swiatek (1984), Andrzejewska-Golec and Swietoslawski (1988, 1989a), Andrzejewska-Golec (1992) and Andrzejewska-Golec *et al.* (1993) based on their chemical and micromorphological studies. Later Rahn (1996) changed his taxonomic conception and separated sections *Oreales, Arnoglossum, Bauphula, Lecopsyllum* and *Hymenopsyllum* from subgenus *Psyllum* including them into a new subgenus, *Albicans* Rahn.

P. afra and *P. scabra*

In these two species the main constituent was plantarenaloside (**14**) accompanied by aucubin (**1**) in lower concentrations (Table 2). Andrzejewska-Golec and Swiatek (1984), Andrzejewska-Golec *et al.* (1993) detected bartsioside in both species and considered it as an important chemosystematic marker for subgenus *Psyllium*. The presence of bartsioside in *P. afra* was confirmed by Ronsted *et al.* (2000) but this compound was also found in one representative of subgenus *Plantago* (Ronsted *et al.,* 2000).

The representatives of the subgenera *Plantago* and *Psyllium* (sensu Pilger, 1937) differ in morphological (Pilger, 1937; Saad, 1986; Andrzejewska-Golec, 1992), caryological (Kuzmanov *et al*., 1984) and phytochemical aspect (Kuzmanov *et al*., 1984; Andrzejewska-Golec and Swiatek, 1984, 1986; Andrzejewska-Golec *et al.*, 1993; Ronsted *et al*., 2000). This gives reason some authors (Sojak, 1972; Holub, 1973; Dietrich, 1980, 1982) to accept *Psyllium* as a distinct genus.

On the other hand, there is a hypothesis of a close relation between the representatives of subgenus *Psyllium* and the species *P. major* from subgenus *Plantago. P. major* is considered as an ancient species, from which have originated all other representatives of genus *Plantago* (Good, 1947; Croizat, 1952; Cox *et al*., 1977). Based on the structure and shape of the seeds Rezk (1980) considers *P. major* to stay at the base of all evolutionary lines of the seed types of genus *Plantago*. Saad (1986) shows that *P. major* combines features characteristic of both subgenera: it is stemless, with alternate leaves like the representatives of subgenus *Plantago* and simultaneously possesses pollen grains like the stemmed species of subgenus *Psyllium.* The author considers *P. major* as the most ancient species from which have originated and differentiated the representatives of both subgenera.

The obtained iridoid data support this hypothesis. The main constituent in *P. major* is aucubin (**1**) and this iridoid pattern probably is the primary one. The cases, when aucubin is accompanied with other iridoids could be regarded as secondary ones.

Based on the iridoid patterns of the studied species and taking into consideration the iridoid biosynthesis (Fig. 2) we could outline several evolutionary lines in genus *Plantago*:

– Species, in which the iridoid biosynthesis is limited to earlier stages, containing mainly aucubin (**1**): *P. major, P. cornuti, P. gentianoides.*
– Species, in which aucubin (**1**) is a precursor of aucubin derivatives as 10-O-acetylaucubin (**3**), monomelittoside (**4**), and monomelittoside derivatives (**5–6**): *P. subulata, P. media*.
– Species, which synthesize aucubin (**1**) and catalpol (**2**): *P. lanceolata, P. altissima, P. argentea, P. lagopus* (section *Arnoglossum*) and *P. atrata* (section *Oreades*). The representatives of section *Arnoglossum* have more advanced biosynthetic pathways and contain different catalpol derivatives (**7**, **8**) and C_{10} iridoids (**11–13**).
– Species, which synthesize aucubin (**1**) and plantarenaloside (**14**): *P. afra, P. scabra* (subgenus *Psyllium*).

The obtained iridoid data for *P. tenuiflora* and *P. coronopus* were insufficient. The presence of unidentified compounds, probably with an iridoid nature, makes determination the position of *P. tenuiflora* and *P. coronopus* in the above mentioned groups impossible.

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