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Flavonoids in the leaves of polish species of the genus *Betula* L.

I. The flavonoids of *B. pendula* Roth. and *B. obscura* Kot. leaves

LUCYNA PAWŁOWSKA

Department of Plant Variability, Institute of Botany, Polish Academy of Sciences, ul. Lubicz 46, 31-512 Kraków, Poland

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Abstract

Betula pendula Roth. leaves were found to contain, beside the flavonoids detected earlier by other researchers, isorhamnetin 3-galactoside, acacetin 7-glucoside, and perhaps scutellarein 7-glycoside and quercetin 3-glycoside-7,4′-dimethyl ether. The investigated specimens of this species can be divided into two groups on the basis of the presence or absence in them of 6-methoxykaempferide. Group I was characterized by larger leaf-blades containing this compound, whereas it was absent in group II with smaller leaves. The composition of the leaf flavonoids of *B. obscura* Kot. samples was identical with that of the specimens of the above-mentioned small-leaved *B. pendula*.

INTRODUCTION

The genus *Betula* grows in the whole of Europe, in northern and central Asia, and also in North America. The total number of species of this genus approximates 80, only seven of them occurre in Poland. All Polish species belong to the section *Eubetulae* (W i n k l e r, 1904). Five of them, i.e. *B. pendula* Roth. (= *B. verrucosa* Ehrh.), *B. pubescens* Ehr. (= *B. alba* L), *B. obscura* A. Kotula, *B. carpatica* W. K., and *B. oycoviensis* Bess. represent the subsection *Albae*, and two (*B. humilis* Schrk. and *B. nana* L.) the subsection *Nanae* including *fruticose* forms. According to another systematic classification (N a t h o, 1976), *B. nana* belongs to the subgenus *Chamaebetula* of the series *Nanae*, and the remaining species growing in Poland to the subgenus *Betula*, *B. pendula* and *B. oycoviensis* being grouped in the section *Verrucosae*, *B pubescens*, and *B. carpatica* in the section *Betula*, and *B. humilis* in the section *Fruticosae*. It should also be added that owing to their morpho-

logical similarity *B. pendula*, *B. pubescens*, *B. oycoviensis*, and the subendemic taxon *B. obscura* were sometimes included in the collective species *B. alba* L. (Jentys-Szaferowa, 1937, 1938; Johnsson, 1944).

B. pendula, which occurs in the whole of Europe, has already been the object of morphological, systematic, physiological, cytological and karyological studies (Winkler, 1904; Jentys-Szaferowa, l.c.; Woodworth, 1929; Białobrzeska, 1955; Białobrzeska, Truchanowicz, 1960; Clausen, 1960; Więckowska, 1965; Natho, 1976 and others). *B. pendula* aroused an even more vivid interest when it was found to be one of three segregants of *B. oycoviensis* × × *B. oycoviensis* (Jentys-Szaferowa, 1967; Więckowska, 1967; Korczyk, 1967; Szwabowicz, 1971, 1972; Jentys-Szaferowa et al., 1974). In order to obtain additional data on this species, chemotaxonomic examinations of the flavonoids present in its leaves were carried out.

The widespread occurrence and the great variety of flavonoids as well as a close relationship between their presence and the genotype of a given taxon have determined the utility of these secondary metabolites in chemotaxonomic investigations on vascular plants. They have been increasingly and successfully used as taxonomic markers, especially for taxa such as species, subspecies, or variety, and also for hybrids (Harborne 1967, 1975; Fahselt, Ownbey, 1968; Giannasi, 1975 and others).

Wollenweber's investigations (1974) on the leaf buds of *B. pendula* revealed in them the following flavonoids: scutellarein 6,4′-dimethyl ether, acacetin (apigenin 4′-methyl ether), kaempferol 3,4′-dimethyl ether, kaempferide (kaempferol 4′-methyl ether), isokaempferide (kaempferol 3-methyl ether), kaempferol 3,7-dimethyl ether, betuletol (6-methoxykaempferide), and isorhamnetin (quercetin 3′-methyl ether). On the other hand, in the *B. pendula* leaves collected in August, the main components of this group of compounds are flavonol glycosides: hyperoside, (quercetin 3-galactoside), quercitrin (quercetin 3-rhamnoside), and myricetin 3-digalactoside; besides, there occur acacetin, apigenin 7,4′-dimethyl ether, hesperidin (hesperetin 7-rutinoside), and polyphenol acids such as chlorogenic, caffeic, gallic and protocatechuic acids (Hörhammer et al., 1956, 1957; Hänsel, Hörhammer, 1954).

As already mentioned, *B. obscura* and *B. pendula* used to be included in the common species *B. alba* L. It is in a few traits only that *B. obscura* differs from *B. pendula*. The most essential of them is the dark colour of the bark, which, as has been demonstrated by Stecki and Ślósarz (1928) and Wiertelak. (1928), results from the

lack in its cork of thin-walled cells containing the white triterpene betulin (present name betulinol). Other differentiating traits of the two taxa, such as, e.g. the morphology of leaves, are considered by Jentys-Szaferowa (1958) to be of little importance. However, Stecki and Ślósarz (l.c.) and Hrynkiewicz-Sudnik (1962) show these characteristic to vary in their numerical values for either of the two taxa, and accordingly, postulate that the taxonomic class of *B. obscura* should be raised to a separate species. Therefore, for comparison, four specimens of *B. obscura* were included in the present investigations.

MATERIAL AND METHODS

Material

Examinations were carried out on fresh leaves of long shoots from the most insolated part of the head of the tree. A list of the specimens examined, together with a short description of each of them, is given in Table 1.

Complete comparative analysis was performed twice in 1972 on the material collected on 8th July and 4th September. The following year

Table 1

A list and short characteristic of *B. pendula* and *B. obscura* specimens under study

	Symbol of specimen	Locality	Size of leaves on a long shoot	Notes
B. pendula	"1956"	Botanical Garden Cracow	small	All specimens studied were mature
	"d"		small	
	"1959"		very large	
	"V-50"		very large	Cultivated, see: Korczyk, 1967; Jentys-Szaferowa, et al. 1974
	"R"	Park in Rostov on the Don — USSR	large	
	"V-1"	Sikornik — northern side of Kościuszko Mound in Cracow	large	
	"V-2"		large	
	"V-3"		small	
	"V-4"		small	
	"V-5"		small	
	"V-6"		very large	
B. obscura	"O-1"	Wola Justowska in Cracow	small	
	"O-2"		small	
	"O-3"		small	
	"O-4"		small	

examinations were repeated on the leaves gathered on 15th July and 27th August. Moreover, for comparison, on 10 th July, 1975 material was taken from specimen "R" (growing in the park in Rostov on the Don, USSR), which was dried, and on 14th September, 1978 from four *B. obscura* trees (which grow in Wola Justowska in Cracow). The material was collected invariably on sunny days, between 1^{00} p.m. and 2^{00} p.m., as according to the author's earlier findings (Pawłowska, 1976), these conditions ensure the highest flavonoid content of birch leaves.

Methods

1. Extraction

About 10 g of fresh leaves from each specimen were gathered according to Fahselt and Ownbey (1968). They were homogenized with petroleum ether, the extract being poured out after one hour and washed with petroleum ether until it became colourless. The remainder was infused with methanol and left to stand for 24 hours at about 4° C. Subsequently the material was centrifuged the methanol extract was decanted and methanol was poured over the leaves again. This procedure was repeated until the extract became colourless (Hänsel, Hörhammer, 1954). The methanol extracts from particular specimens were mixed and evaporated to the volume of about 50 ml, this being followed by treatment with an initial amount of chloroform. The material was next thoroughly shaken and put away for 15 minutes; afterwards it was separated. The bottom chloroform phase was discarded. The aqueous methanol phase was made up to a volume which ensured the proportion of 1 g of the initial material to 4 ml of this extract; 2 ml of this were poured off for comparative two-domensional thin-layer chromatography, whereas the remaining amount was subjected to preparative separation by paper chromatography.

2. Fractionation and purification of the flavonoids of birch leaves

The methanol-water extract was subjected to preparative separation on Whatman No. 3 paper by the ascending technique, in the following solvent system: formic acid : ethyl acetate : water (2 : 10 : 3 v/v). Fractions were eluted with methanol: I — from the bands with Rf 0.29-0.44; II — 0.45-0.53; III — 0.54-0.60; IV — 0.61-0.65; V — 0.66-0.72; VI — 0.73-0.77 and VII — 0.78-0.98. The fractions obtained were rechromatographed on Whatman No. 1 paper (by the ascending technique) in 30% acetic acid and the purified fractions were eluted with methanol again.

3. Identification methods

In order to identify the flavonoids, standards were used for comparison by means of ascending paper and thin-layer chromatography, by specific colour reactions, acid hydrolysis, and spectroscopy in visible and ultraviolet light.

Chromatography was performed in the following solvent systems:

A — n-butanol : water : acetic acid (12 : 5 : 3 v/v)
B — ethyl acetate : water : formic acid (10 : 3 : 2 v/v)
C — acetic acid : water (3 : 7 v/v)
D — amyl alcohol : isoamyl alcohol : 80% formic acid (1 : 1 : 1 v/v)
E — formic acid : water (1 : 9 v/v)
F — water : hydrochloric acid : acetic acid (5 : 1 : 5 v/v)
G — benzene : n-butanol : pyridine : water (1 : 5 : 3 : 3 v/v)
H — acetic acid : water (3 : 17 v/v)

a). Two-dimensional thin-layer chromatography (TLC)

This chromatography was applied with the aim to compare the chemical composition of the samples studied, to prepare eluates for hydrolysis, and to draw the spectra of the identified compounds.

The amount of 0.2 ml of the aqueous methanol extract from each specimen was mounted on a degreased 20 × 20 cm plate coated with a 2 mm layer of:

1. MN 300 cellulose, and developed in system C in the first dimension and system A in the other,
2. MN 300 cellulose : kieselguhr G (1 : 1 w/w), and developed in system D — in the first dimension, and in E in the other.

b). Paper chromatography

After preparative chromatography all fractions were chromatographed on Whatman No. 1 paper in the solvent systems A, B and C and were compared with appropriate standards.

c). Specific colour reactions

Chromatograms were developed with:

1. 3% aluminium chloride in ethanol (Hörhammer, Müller, 1955),
2. 2% zirconium oxychloride in methanol, and subsequently with a 5% aqueous solution of citric acid (Hörhammer, Müller, 1954 a),
3. diazobenzidine (Roux, Maihs, 1960).

Furthermore, after separation and rectification, the fractions were reduced with a magnesium strip and with 2 N HCl (Hörhammer, Müller, 1954b). The reduction products were chromatographed Whatman No. 1 paper in the system A, B and F, and were subjected to specific reactions with sodium acetate (NaOAc — crystals), 3% iron chloride in ethanol, and concentrated NH_4OH (Hörhammer, Müller 1954c).

d). Acid hydrolysis

After preparative chromatography the fractions were subjected to two-dimensional thin-layer chromatography, as described in item 3.a)., in the first or the second version, depending on which of them ensured better separation of a given fraction. Next the compounds which on some two-dimensional chromatograms showed identical localization were eluted with 0.1% HCl in methanol for 24 hours at about 4° C. After being strained, the eluates were subsequently heated with 2 N HCl in a boiling water bath for 10 minutes in the case of monoglycosides and partial hydrolysis of diglycosides, and in the case of complete hydrolysis of diglycosides for 20 minutes. The hydrolysates were strained through a Dowex 2 ionexchanger layer to remove the excess of HCl, this being followed by treatment with an equal amount of ethyl acetate, shaking, and separation after about 20 minutes.

Analysis of aglycones

The amount of 0.2 ml of acetate phase from each sample was mounted on an MN 300 cellulose-coated plate and developed in solvent systems A and F; as soon as it was dried, the phase was developed with zirconium oxychloride or with diazobenzidine.

Analysis of sugars

Besides standard sugars, 0.5 ml of the methanol-water phase was subjected to chromatographic separation on Whatman No. 1 paper. The development proceeded in systems A and G, and was then achieved with an ammonia solution of silver nitrate or with a saturated solution of aniline phthalane in butanol.

e). Spectroscopy in visible and UV light

Two-dimensional thin-layer chromatography was performed on separated and purified methanol fractions, exactly as in the case of

hydrolysis. The compounds demonstrating identical distribution on some two-dimensional chromatograms, were eluted with methanol. Subsequently the spectrum of the eluates was drawn on a Beckman Model 25 spectrophotometer in the UV range. Furthermore, the spectrum was drawn after application of the following tests (M a b r y et al., 1970):

1. methanol solution + 3 drops of saturated sodium methylate (CH_3ONa = NaOME) in methanol, per cuvette,
2. methanol solution + 3 drops of saturated aluminium chloride ($AlCl_3$) in methanol, per cuvette,
3. the same as item 2. + 2 drops of hydrochloric acid in methanol (concentrated HCl : HOMe 1 : 2 v/v) per cuvette,
4. 3 crystals of anhydrous sodium acetate (CH_3COONa = NaOAc), per cuvette,
5. the same as item 4. + 3 crystals of anhydrous boric acid (H_3BO_3), per cuvette,

and after adding 2 drops of methanol solution of 2% zirconium oxychloride ($ZrOCl_2$) to the cuvette with fresh methanol solution of the compound studies and, next, 2 drops of 5% methanol solution of citric acid ($C_6H_8O_7$).

As soon as the wavelengths (λ) for absorption maxima were read from the spectrograms, $\Delta\lambda$ was calculated for both bands of flavonols and of their glycosides:

$$\Delta\lambda_1 = \lambda_{NaOMe} - \lambda_{HOMe\ (methanol)}$$

$$\Delta\lambda_2 = \lambda_{AlCl_3} - \lambda_{HOMe}$$

$$\Delta\lambda_3 = \lambda_{HCl} - \lambda_{AlCl_3}$$

$$\Delta\lambda_4 = \lambda_{NaOAc} - \lambda_{HOMe}$$

$$\Delta\lambda_5 = \lambda_{H_3BO_3} - \lambda_{NaOAc}$$

$$\Delta\lambda_6 = \lambda_{ZrOCl_2} - \lambda_{HOMe}$$

$$\Delta\lambda_7 = \lambda_{C_6H_8O_7} - \lambda_{ZrOCl_2}$$

The reduction products in methanol-water solution of pH about 2 (after addition of hydrochloric acid) were examined in the range of 400-600 nm.

RESULTS

Betula pendula leaves were found to contain flavonol, flavone, dihydroflavonol, and flavanone derivatives as well as polyphenol acids.

Table 2

Chromatographic values and results of test reactions

Number of compound	Rf values in solvent systems					Colour in light		Results of test reaction			
	A	B	C	F	H	visible	UV	$ZrOCl_2$	$ZrOCl_2+C_6H_8O_7$	$AlCl_3$	diazo benzidine
1	0.70	0.70	0.60	0.35	0.50	y	d.y	y-o	—	y	p-ru
3	0.54	0.53	0.57	0.30	0.35	y	ru	l-y	—	y	p-ru
4	0.38	0.41	0.53	0.20	0.31	p-ru	d.ru	o-p	—	d.y-o	re-ru
6	0.48	0.50	0.80	0.41	0.77	—	fl.v-b	—	—	y-c	y-ru
7	0.59	0.60	0.59	0.32	0.37	y	d.y	y	—	y	p-ru
10	0.68		0.32					c-y	y-g	tr.y	ru
11	0.66		0.49					tr.y			tr.ru
13	0.53	0.52	0.74	0.59		—	ru	—	—	y-b	y-ru
15	0.66	0.76	0.51	0.28	0.34	y	fl.y	fl.y	fl.y	y	p-ru
16	0.52		0.50					tr.c	tr.c		tr.ru
17	0.70		0.56			—	fl.v-b	—	—	—	y/[a]
18	0.78		0.60			gr	fl.v-b	—	—	—	y/[a]
19	0.83		0.93			—	fl.v	—	—	—	y/[a]
20	0.69		0.52		0.32		tr.y	y	—	tr.y	p-ru
21	0.50		0.47					y-c	y-c	tr.y	ru
Standards											
Quercitrin	0.71	0.67	0.62		0.49	y	d.y	y-o	—	y	p-ru
Hyperosid	0.56	0.54	0.60		0.35	y	ru	l-y	—	y	p-ru
Hesperidin	0.50				0.78	—	fl.v-b	—	—	y-c	y-ru
Chlorogenic acid	0.87		0.90			—	fl.v	—	—	—	y/[a]
Gallic acid	0.80		0.61			gr	fl.v-b	—	—	—	y/[a]
Caffeic acid	0.73		0.61			—	fl.v-b	—	—	—	y/[a]

[a] — disappears after some minutes; d. — dark; c — celadon; b — blue; fl. — fluorescent; re — red; g — green; gr — grey; l — lemon-coloured; o — orange; p — pink; ru — russet; v — violet; y — yellow; tr. — trace amount

A. Glycosides and methoxy derivatives of flavonols

The chromatographic values and specific colour reactions (Tables 2 and 3) indicate that the compounds denoted with numbers 1, 3, 4, 7, 10, 11 and 15 are flavonols or their derivatives.

A comparison of the values obtained by chromatographic methods and by spectroscopy in UV and visible light point to the identity of compound "1" with standard quercitrin (Tables 2, 3, 4 and 5), and of compound "3" with standard hyperoside (Tables 2, 3, 4 and 5).

Table 3

Chromatographic and spectral values of reduction products

Number of compound	Rf values in solvent systems			Results of test reactions			Maximum of absorption in nm/[a]
	A	E	G	NaOAc	$FeCl_3$	NH_4OH	
1	0.44	0.49	0.56	g-b	y-g-b	g-b	513
3	0.36	0.47	0.52	o-b	ru	v-b	515
4	0.11	0.26	0.48	b-g	b	re-b	532
7	0.39	0.49	0.67	g-b	g-b		
Standards							
Quercitrin	0.47	0.51		g-b	y-g-b		513
Hyperoside	0.36	0.48		o-b	ru		515

[a] — in methanol solution of HCl, pH 2-3; b — blue; g — green; o — olive; re — red; ru — russet; v — violet; y — yellow

The spectrum of the methanol solution of compound "4" in UV and shift values (Table 5) suggest it to be myricetin 3-glycoside. Myricetin and galactose turned out to be products of hydrolysis of compound "4" (Table 4). Similar chromatographic and spectral values have been obtained by Hörhammer et al.(1957) for myricetin 3-digalactoside.

Isorhamnetin and galactose were detected in the hydrolysate of compound "7" (Table 4). The shift values for this compound, following spectral tests (Table 5), were very close to those reported by Mabry et al. (1970) for isorhamnetin 3-galactoside.

The chromatographic values of compound "10" (Table 2) and, above all, its distribution on two-dimensional chromatograms, and also specific colour reactions show it to be a free flavonol. Similar to the spectral data obtained for compound "10" (Table 5) were the shift values reported by Wollenweber (1974) for kaempferol 3,4′-dimethyl ether (kaempferide 3-methyl ether).

The results obtained by spectroscopy in UV for compound "11" (Table 5), and especially its shift values and localization on two-dimensional chromatograms suggest it to be quercetin 3-glycoside-7,4′-di-

Table 4

Chromatographic values of hydrolysates

Number of compound	Solvent system			
	A[a]	F[b]	A[c]	G[d]
1	0.72	0.26	0.26	0.49
3	0.73	0.29	0.09	0.18
4	0.50	0.12	0.11	0.15
6	0.91	—	0.08	0.10
			0.33	0.45
7	0.78	0.32	0.10	0.14
13	0.85	—	0.12	0.16
16	tr.0.93		tr.0.08	
21	tr.0.89	tr.0.58	tr.0.08	
Standards:				
Kaempferol	0.80	0.43		
Quercetin	0.73	0.30		
Myricetin	0.49	0.12		
Isorhamnetin	0.76	0.32		
Apigenin	0.86	0.55		
Luteolin	0.77	0.40		
Rhamnose			0.27	0.50
Glucose			0.08	0.12
Galactose			0.10	0.16
Arabinose			0.12	0.20
Xylose			0.15	0.30
Galacturonic acid			0.06	0.05

a — after development with diazo benzidine
b — after development with $ZrOCl_2$
c — after development with aniline phthalane
d — after development with an ammonia solution of $AgNO_3$
tr. — trace amounts

methyl ether. The author failed to achieve the definitive identification of compound "11" because of its insufficient amount.

The shift values of compound "15' (Table 5) observed after application of spectral tests encourage the assumption that this is a flavonol with free hydroxyl groups at positions C_3 and C_5 and C_7 and with two methoxyl groups at C_4' and C_6 or C_8. This is testified by the following facts: the addition of NaOMe to the methanol solution produces a bathochromic effect ($\Delta\lambda_1$ = 50 nm), while in the presence of $AlCl_3$ the maximum of band I of the methanol solutions shifted and $\Delta\lambda_2$ = 51 nm, but the hypsochromic effect observed in the $AlCl_3$/HCl test is insignificant and $\Delta\lambda_3$ = —9 nm; moreover, this is evidenced by shifts achieved in NaOAc/H_3BO_3, and by $\Delta\lambda_5$ values in particular, which are equal to 5 nm for band I and to —3 nm for band II. Similar spectral findings were noted by Wollenweber (1974) for kaempferol 6-methoxy-4'-methyl ether (6-methoxykaempferide = betuletol).

Table 5

UV spectra of identified flavonoids

Fraction	1 — quercitrin			3 — hyperosid			4 — quercetin 3-digalactoside		
Test	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II
MeOH	256, 267, 302, 350	n=1 43	18	257, 267, 299, 362	n=1 47	18	258, 270, 298, 359	n=1 56	14
NaOMe	274, 324, 393	n=2 80	20	275, 320, 409	n=2 76	18	272, 315, 415	n=2 80	15
$AlCl_3$	276, 305, 430	n=3 −21	−20	275, 306, 372, 438	n=3 −32	−16	273, 305, 439	n=3 −31	−17
$AlCl_3$/HCl	256, 306, 356, 409	n=4 5	4	259, 305, 364, 406	n=4 3	5	256, 268, 304, 360, 408	n=4 0	1
NaOAc	260, 273, 305, 355	n=5 17	2	262, 274, 307, 365	n=5 16	0	259, 271, 302, 359	n=5 20	5
NaOAc/H_3BO_3	262, 297, 372	n=6 77	18	262, 299, 381	n=6 57	19	264, 299, 379	n=6 63	14
$ZrOCl_2$	274, 308, 361, 427	n=7 −12	−17	276, 309, 419	n=7 −4	−13	272, 312, 422	n=7 −16	−14
$ZrOCl_2$/$C_6H_8O_7$	257, 267, 310, 355, 415			263, 306, 362, 415			258, 269, 302, 358, 406		

Fraction	7 — isorhamnetin 3-galactoside			10 — kaempferol 3,4′-dimethyl ether (?)			11 — quercetin 3-glycoside 7,4′-dimethyl ether (?)		
Test	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II
MeOH	255, 269, 303, 357	n=1 58	17	252, 258, 265, 288, 347	n=1 57	12	252, 256, 264, 298, 351	n=1 49	0
NaOMe	272, 320, 415	n=2 42	21	264, 272, 305, 404	n=2 53	24	252, 260, 265, 273, 308, 400	n=2 49	6
$AlCl_3$	276, 304, 332, 405	n=3 −3	−9	276, 301, 400	n=3 0	−19	258, 263, 273, 301, 400	n=3 −2	0
$AlCl_3$/HCl	267, 302, 360, 402	n=4 1	8	257, 264, 271, 298, 355, 400	n=4 25	9	258, 262, 298, 355, 398	n=4 −8	0
NaOAc	263, 272, 305, 358	n=5 21	2	261, 264, 290, 362	n=5 10	−2	252, 255, 262, 298, 343	n=5 21	5
NaOAc/H_3BO_3	265, 301, 361, 379	n=6 54	11	259, 265, 289, 372	n=6 68	6	257, 264, 294, 364	n=6 1	5
$ZrOCl_2$	266, 305, 361, 411	n=7 −3	−7	258, 265, 287, 307, 359, 415	n=7 −1	−1	252, 257, 264, 302, 352	n=7 +2	+1
$ZrOCl_2$/$C_6H_8O_7$	259, 268, 304, 357, 408			257, 265, 287, 308, 356, 414			253, 257, 265, 300, 354		

Fraction	15 — 6-methoxy-kaempferide (?)			6 — hesperidin	13 — dihydrohyperosid (?)	16 — scutellarein 7-glycoside (?)
Test	Maxima of absorption in nm	Values of shifts ($\Delta\lambda_n$) band I	band II	Maxima of absorption in nm	Maxima of absorption in nm	Maxima of absorption in nm
MeOH	268, 283, 357	n=1 50	6	284, 326	285, 326	272, 324
NaOMe	274, 316, 407	n=2 51	4	238, 318	262, 307, 374	284, 328
$AlCl_3$	272, 310, 408	n=3 −9	−1	224, 277	259, 301, 358	273, 410
$AlCl_3$/HCl	271, 301, 320, 399	n=4 13	2	275	253, 301, 328	265, 368, 399
NaOAc	270, 281, 319, 370	n=5 5	−3	284, 324	291, 328	260, 370
NaOAc/H_3BO_3	267, 283, 375	n=6 57	5	284	294, 331	268, 316, 368
$ZrOCl_2$	273, 414	n=7 0	−11	277	293, 348	
$ZrOCl_2$/$C_6H_8O_7$	262, 358, 414			272	285, 292, 337	

Fraction Test	21 — acacetin 7 -glucoside (?) Maxima of absorption in nm
MeOH	268, 324
NaOMe	289, 358
$AlCl_3$	275, 301, 343, 379
$AlCl_3$/HCl	275, 300, 338, 379

Polyphenol acids in MeOH Maxima of absorption in nm	
17 — caffeic acid	247, 297, 325
18 — gallic acid	276
19 — chlorogenic acid	249, 300, 331

Besides, compounds were found (Nos 20 and 24), whose products with zirconium oxychloride and diazobenzidine were characteristic of flavonol derivatives; however, their very low amount (in many specimens in microvestigial amounts) prevented their identification (Table 2).

B. Flavon glycosides

A trace amount of the compound marked No. 21, obtained by two-dimensional chromatography, permitted only the drawing of the spectra of its methanol solution and of the methanol solution in the presence of NaOMe, $AlCl_3$ and HCl (Table 5), and a hydrolytic test to be done. A comparison of the chromatographic data obtained for this compound (Tables 2 and 3) with those reported in the literature (S e i k e l, 1962) and of the spectral values with those obtained by M a b r y et al. (1970) indicates that compound "21" is probably acacetin 7-glucoside.

On the basis of the findings for compound "16" obtained by two-dimensional chromatography and spectroscopy in UV (Tables 2 and 5), it is supposed that this is scutellarein 7-glycoside. However, the author failed to ultimately identify compound "16" on account of its very small amount.

C. Flavanone and dihydroflavonol glycosides

The compound denoted No. 6 is identical with standard hesperidin (Tables 2, 4 and 5).

Chromatographic and UV spectroscopic findings indicate that compound "13" is probably dihydrohyperoside (Tables 2, 4 and 5).

D. Polyphenol acids

The compounds denoted Nos 17, 18 and 19 manifested their identity with standard polyphenol acids, i.e. "17" with caffeic acid, "18" with gallic acid, and "19" with standard chlorogenic acid (Tables 2 and 5).

The composition of compounds identified in the leaves of *B. pendula* specimens collected in July and at the end of August and the beginning of September, was qualitatively the same. A slightly augmented content of these compounds (evaluated from colour intensity and the size of spots in two-dimensional chromatography) could only be noted at the turn of August. Similar findings relating to the unaltered qualitative

Table 6

Composition of flavonoid compounds in *B. pendula* and *B. obscura* specimens under study

No of compound		1	3	4	6[a]	7	10	11	13[a]	15	16	17	18	19	20	21	24
Name / Specimen		quercitrin	hyperosid	myricetin 3--digalactoside	hespridin	isorhamnetin 3-galactoside	kaempferol 3,4'-dimethyl ether	quercetin 3-glycoside-7,4'-dimethyl ether (?)	dihydrohyperoside	6-methoxy--kaempferide	skutellarein 7-glycoside (?)	caffeic acid	gallic acid	chlorogenic acid	unidentified	acacetin 7-glucoside	unidentified
B. pendula	1956	++	++++	+	+	+	–	–	+	–	tr.	+	+	+	tr.	–	tr.
	d	++	++++	+	+	tr.	tr.	–	+	–	–	+	+	+	tr.	–	tr.
	V-3	++	++++	++	+	tr.	tr.	–	tr.	–	–	+	+	+	tr.	–	tr.
	V-4	++	++++	++	tr.	tr.	–	–	+	–	–	+	+	+	tr.	tr.	tr.
	V-5	+	+++	+	tr.	+	–	tr.	tr.	–	–	+	+	+	tr.	–	tr.
	V-2	++	++++	+	+	+	–	–	+	+	–	+	+	+	+	tr.	+
	V-1	++	++++	+	+	+	–	–	+	tr.	tr.	+	+	+	tr.	tr.	tr.
	R	+++	++	–	+	++	+	tr.	+	+	–	+	+	+	tr..	tr.	tr.
	V-50	++	++++	++	+	+	+	–	+	++	–	+	+	+	+	+	tr.
	V-6	++	++++	+	+	tr.	–	tr.	+	++	tr.	+	+	+	tr.	tr.	tr.
	1959	++	++++	+	+	+	+	tr.	+	++	tr.	+	+	+	+	+	+
B. obscura	O-1	++	++++	++	+	+	tr.	–	+	–	–	+	+	+	+	tr.	+
	O-2	++	++++	+	+	tr.	–	–	tr.	–	–	+	+	+	tr.	–	tr.
	O-3	++	++++	+	++	+	+	tr.	+	–	tr?	+	+	+	+	+	+
	O-4	++	++++	+	+	+	tr?	–	+	–	–	+	+	+	tr.	tr.	tr.

The number of symbols "+" corresponds to a relative amount in a given specimen; a — compound visible in UV;
tr. — trace amount.

composition of flavonoids in the leaves of this species, in full vegetation, were reported by Krawiarz (1972).

The presence of the compounds in each specimen studied is shown in Table 6.

DISCUSSION

The present chemotaxonomic investigations on leaves from ten *Betula pendula* specimens growing in Cracow, confirmed the results reported by Hänsel and Hörhammer (1954) and Hörhammer et al. (1956, 1957). The principal flavonoids present in the leaves of long shoots of this species are flavonol glycosides, i.e. hyperoside, quercitrin, and myricetin 3-digalactoside, and additionally, hesperidin and acacetin (the latter, however, probably as 7-glucoside), and also polyphenol acids. Moreover, the presence of 6-methoxykaempferide and kaempferol 3,4′-dimethyl ether was borne out, being earlier detected in leaf buds of this taxon by Wollenweber (1974).

As result of the present studies, the leaves gathered in full vegetation (July and the turn of August) were found to contain a compound which is most likely isorhamnetin 3-galactoside, whose aglycone has been detected by Wollenweber (1974) in leaf buds. This would indicate that isorhamnetin: UDP-galactose glycosyltransferase which catalyzes the reaction yielding isorhamnetin 3-galactoside, becomes activated only during vegetation.

The leaves of long shoots of *B. pendula* specimens from Cracow were found to lack scutellarein 6,4′-dimethyl ether, kaempferol 3,7-dimethyl ether, and isokaempferide, whose presence in leaf buds has been established by Wollenweber (l.c.). This probably results from a different physiological state of the leaves under study, though it cannot be ruled out that the absence of these three compounds reflects differences between individual specimens of *B. pendula*. It should also be added that instead of scutellarein 6,4′-dimethyl ether another scutellarein derivative, perhaps its 7-glycoside, was revealed in the samples studied. Besides, the present author failed to detect in them apigenin 7,4′-dimethyl ether, whereas the occurrence of this compound in the leaves collected in full vegetation has been ascertained by Hörhammer et al. (1956).

The flavonoid compounds vary in their composition from plant to plant, the specimen "R" from the USSR differing most from the others in this respect, as illustrated in Table 6. Thus the absence in the here studied samples of the above-mentioned compounds, contrary to the reports of other researchers concerning the leaves of this taxon, may

result from an intraspecific or geographical variability of *B. pendula* regarding its biochemical properties. It should be emphasized here that hyperoside, which is the most abundant of the flavonoids present in this species, as well as quercitrin are always found in *B. pendula* leaves collected in full vegetation.

The present investigations made possible the observation of a certain distinct correlation between the occurrence of 6-methoxykaempferide and the size of the leaf-blade. Taking the presence of this compound as basis, the *B. pendula* specimens under examination can be divided into two groups: one with large leaf-blades containing 6-methoxykaempferide, as e.g. "1959", and the other with smaller leaves deprived of this compound, as e.g. "V-3" (cf. Tables 1 and 6, and Fig. 1).

The composition of the flavonoids identified in *B. obscura* leaves does not exhibit any assential discrepancy as compared with that of *B. pendula*. The only differences to regarded the content of individual components of the two taxa (Table 6). Owing to the absence of 6-methoxykaempferide in both *B. obscura* leaves and the small-leaves of *B. pendula*, the set of flavonoids in these specimens is identical. It should be added that the smaller leaves of *B. obscura* have already attracted attention of Stecki and Ślósarz (1928), and Hrynkiewicz-Sudnik (1962). Thus the biochemical characteristics under study do not justify the distinction of *B. obscura* as a species.

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REFERENCES

Białobrzeska M., 1955. Właściwości morfologiczne i biologiczne brzozy ojcowskiej, brzozy brodawkowatej i ich mieszańca. Morphological and biological characters of *Betula oycoviensis* Bess., *Betula verrucosa* Ehrh. and their hybrid. Rocz. Dendr. 10: 165-189.

Białobrzeska M., Truchanowicz J. 1960. Zmienność kształtu owocków i łusek europejskich brzóz (*Betula* L.) oraz znaczenie ich w stanie kopalnym. Monogr. Bot. 9: 3-93.

Clausen K. E., 1960. A survey of variation in pollen size within individual plants and catkins of three taxa of *Betula*. Polen et Spores 2: 299-304.

Clausen K. E., 1962. Size variation in pollen of three taxa of *Betula* (2). Pollen et Spores 4: 169-174.

Fahselt D., Ownbey M., 1968. Chromatographic comparison of *Dicentra* species and hybrids. Am. J. Bot. 55: 334-345.

Giannasi D. E., 1975. The flavonoid systematics of the genus *Dahlia* (*Compositae*). Memoirs the New York Botanical Garden 26: 1-125.

PLATE I

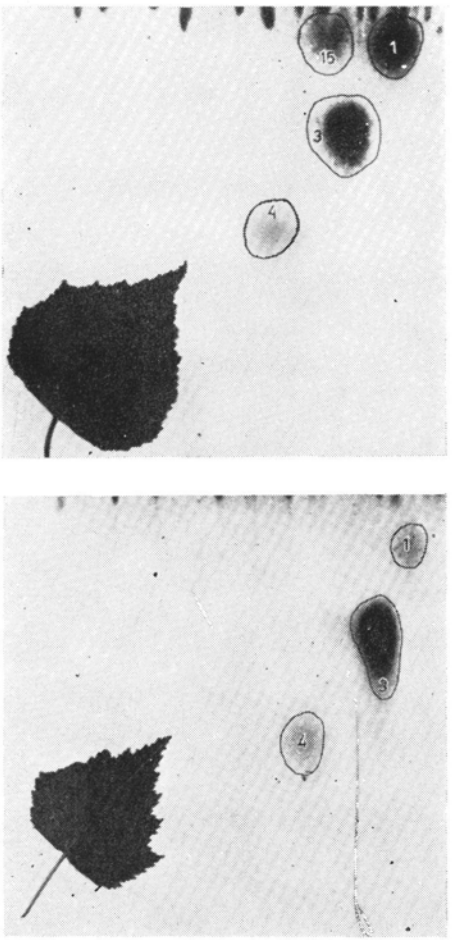

Fig. 1. Chromatograms and the leaves of the specimens "1959" and "V-3"

Harborne J. B., 1967. Comparative Biochemistry of the Flavonoids. Acad. Press: London, New York: 126-249.

Harborne J. B., 1975. Biochemical systematics of flavonoids. In: Harborne J. B., Mabry T. J., Mabry H. (ed.). The Flavonoids. Chapman and Hall, London: 1056-1095.

Hänsel R., Hörhammer L., 1954. Vergleichende Untersuchungen über die Flavon-glykoside der Betulaceen. Arch. Pharm. 287: 117-126.

Hörhammer L., Müller K. H., 1954a. Zur Analytic der Flavone. IV. Über die Anwendung des Zirkon-Zitronensäure-Tests als Sprüchreaktion in der Papierchromatographie. Arch. Pharm. 287: 310-313.

Hörhammer L., Müller K. H., 1954b. Zur Analytic der Flavone. V. Die Charakterisierung der Seitenphenylsubstitution durch einige Reaktionen der Reduktionsprodukte. Arch. Pharm. 287: 376-380.

Hörhammer L., Müller K. H., 1954c. Das optische Verhalten der Reduktionsprodukte einiger Polyoxy-flavone. Arch. Pharm. 287: 448-452.

Hörhammer L., Müller K. H., 1955. Zur Analytic der Flavone. VII. Das optische Verhalten der Reduktionsprodukte einiger Polyoxy-flavonol-glykoside und methyläther. Arch. Pharm. 288: 31-38.

Hörhammer L., Vorndran E., Wagner H., 1956. Zur Kenutnis der Flavon-glykoside aus Betulaceen. Arch. Pharm. 289: 316-323.

Hörhammer L., Wagner H., Luck R., 1957. Isolierung eines Myricetin-3--digalaktoside aus *Betula verrucosa* und *Betula pubescens*. Arch. Pharm. 290: 338-341.

Hrynkiewicz-Sudnik J., 1962. Zmienność i rozmieszczenie brzozy czarnej (*Betula obscura* A. Kotula) w Polsce. Arboretum Kórnickie 7: 5-98.

Jentys-Szaferowa J., 1937. Z badań biometrycznych nad zbiorowym gatunkiem *Betula alba* L. I. Wielopostaciowość liści brzóz. Biometrical studies on the collective species *Betula alba* L. I. Polymorphism of the leaves of birches. Inst. Bad. Las. Państw. ser. A 26: 1-57.

Jentys-Szaferowa J., 1938. Z badań biometrycznych nad zbiorowym gatunkiem *Betula alba* L. II. O możliwości krzyżowania się gatunków *Betula verrucosa* Ehrh. i *Betula pubescens* Ehrh. Biometrical studies on the collective species *Betula alba* L. II. The possibility of hybridization between species *Betula verrucosa* Ehrh. and *Betula pubescens* Ehrh. Inst. Bad. Las. Państw. ser. A 40: 1-84.

Jentys-Szaferowa J., 1959. Problematyka brzozy czarnej (*Betula obscura* Kotula). Rocz. Dendr. 13: 11-49.

Jentys-Szaferowa J., 1967. Badania systematyczno-doświadczalne nad *Betula oycoviensis* Bess. Rocz. Dendr. 21: 5-56.

Jentys-Szaferowa J., Białobrzeska M., Truchanowicz J., Więckowska I., 1974. Drugie 10 lat badań nad brzozą ojcowską. Fragm. Flor. et Geobot. 20: 203-242.

Johnsson H., 1944. Triploidy in *Betula alba* L. Bot. Notiser. 97: 85-96.

Korczyk A., 1967. Potomstwo brzozy ojcowskiej wyhodowane z nasion pochodzących z wolnego zapylenia. Rocz. Dendr. 21: 77-103.

Krawiarz K., 1972. Phenolic compounds in the Karelian birch (*Betula pendula* Roth. var. *carelica* (Merklin) Hejtmánek). Arboretum Kórnickie 17: 201-208.

Mabry T. J., Markham K. R., Thomas M. B., 1970. The Systematic Identification of Flavonoids. Springer-Verl. Berlin: pp 354.

Natho G., 1976. Zu Fruchtmorphologie und Gliederung der Gattung *Betula* L. Gleditschia 4: 9-21.

Pawłowska L., 1976. Quantitative daily changes of flavonol glycosides in the leaves of *Betula humilis* Schrk. Acta Soc. Bot. Pol. 45: 395-400.

Roux D. J., Maihs A. E., 1960. Selective spray reagents for the identification and estimation of flavonoid compounds associated with condensed tannins. J. Chrom. 4: 65-72.

Seikel M. K., 1962. Chromatographic methods of separation, isolation and identification of flavonoid compounds. In: Geissman T. A. (ed.). The Chemistry of Flavonoid Compounds. Pergamon Press, Oxford: 34-69.

Stecki K., Ślósarz Z., 1928. Studia nad brzozą czarną w Polsce. I. Studia nad rozmieszczeniem i morfologią brzozy czarnej. Rocz. Nauk Rol. i Leśn. 19: 323-376.

Szwabowicz A., 1971. Badania nad pyłkiem potomstwa brzozy ojcowskiej *Betula oycoviensis* Bess. Acta Soc. Bot. Pol. 40: 91-121.

Szwabowicz A., 1972. Badania kariologiczne brzozy ojcowskiej i jej potomstwa. Acta Soc. Bot. Pol. 41: 235-252.

Wiertelak M., 1928. II. Oznaczenie betuliny w korze brzozy czarnej *Betula obscura* Kot. In: Studia nad brzozą czarną w Polsce. Rocz. Nauk Rol. i Leśn. 19: 377-380.

Więckowska I., 1965. Wpływ kwiatostanu żeńskiego na kształt liści brzozy brodawkowatej. Acta Soc. Bot. Pol. 34: 273-286.

Więckowska I., 1967. Obserwacje morfologiczne i biologiczne nad siewkami brzozy ojcowskiej. Rocz. Dendr. 21: 57-76.

Winkler H., 1904. *Betulaceae*. In: Engler A. Das Pflanzenreich. *Regni vegetabilis conspectus*. IV. Weinheim: pp. 61.

Wollenweber E., 1974. Flavonoid-Exkretion bei *Betula* Arten. Biochem. Physiol. Pflan. 166: 425-428.

Woodworth R. H., 1929. Cytological studies in the *Betulaceae*. I. *Betula*. Bot. Gaz. 87: 331-363.

Flawonoidy liści polskich gatunków z rodzaju Betula L.

I. Flawonoidy B. pendula Roth. i B. obscura Kot.

Streszczenie

Na podstawie analizy chromatograficznej i spektralnej barwników flawonoidowych występujących w świeżych liściach *B. pendula* w pełni wegetacji stwierdzono, że we wszystkich jedenastu przebadanych osobnikach tego gatunku są obecne: 3-galaktozyd kwercetyny (hyperozyd), którego zawartość jest najwyższa, 3-ramnozyd kwercetyny (kwercytryna), 3-dwugalaktozyd myrycetyny, 3-galaktozyd izoramnetyny, 7-rutynozyd hesperetyny (hesperydyna), dwuhydrohyperozyd (?) oraz dwa związki niezidentyfikowane ze względu na zbyt niską zawartość. Poza tym u osobników o większych blaszkach liściowych znaleziono związek, którym jest najprawdopodobniej 6-metoksy-kempferyd. Ponadto w liściach niektórych osobników występują flawonoidy, które zostały zidentyfikowane jako prawdopodobne: 7-glikozyd skutelaryny, 7-glukozyd akacetyny, 3,4′-dwumetylo-kempferol i 3-glikozyd 7,4′-dwumetylo-kwercetyny. Skład liściowych flawonoidów czterech zbadanych osobników *B. obscura* był identyczny ze składem okazów *B. pendula* o mniejszych liściach i nie zawierających 6-metoksy-kempferydu.