Flavonoids in the leaves of Polish species of the genus *Betula* L. V. The taxonomic position on the basis of flavonoid com- position

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Abstract

A comparison of the qualitative composition of leaves from specimens of Polish taxa of the genus *Betula* L. points to a specific distinction of *B. pendula* Roth., *B. pubescens* Ehrh., *B. humilis* Schrk., *B. nana* L., and *B. oycoviensis* Bess., and also *B. "nova"* which requires description according to the International Code of Botanical Nomenclature. *B. obscura* Kot. can be recognized only as a subspecies of *B. pendula*, whereas *B. carpatica* Waldst. and *B. tortuosa* Ledeb. may be geographical varieties of one and the same subspecies distinguished within *B. pubescens*.

INTRODUCTION

In Poland, the genus *Betula* is represented by eight taxa: *B. humilis*, *B. nana*, *B. pendula*, *B. obscura*, *B. oycoviensis*, *B. "nova"*, *B. pubescens*, and *B. carpatica*. *B. humilis*, *B. nana*, *B. pendula*, and *B. pubescens* are generally regarded as established species, whereas the systematic identification of the remaining birches remains controversial.

The flavonoids which are found in the majority of lower taxa within the same higher systematic unit differ from one another only in the position or kind of substituent within the same structure: —OH, —OCH₃, or various sugar radicals (e.g. Harborne 1967, 1975). Such a differentiation of the flavonoids occurs not only between genera in one family but also between species of one genus (Harborne and Williams 1975). Hence the complexes of glycosides and methyl derivatives are of immense taxonomic value, especially in *Angiospermae*, and the identification of aglycones alone, similarly as of other intermediate metabolites, in biochemical-systematic investigations can only serve as indication.

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RESULTS

The qualitative composition of flavonol glycosides and luteolin glucoside in Polish birches is in general stable in one a species (Pawłowska 1980a, b, 1982a). A departure from this regularity is observed only in *B. oycoviensis* (of hybrid origin), and in presumable hybrids of *B. pubescens* × *B. humilis* (Pawłowska 1980c, 1982b). For this reason quercitrin, myricitrin, hyperoside, myricetin 3-digalactoside, rutin, isorhamnetin 3-galactoside, isoquercitrin, kaempferol 3-rhamno-7-glucoside, quercetin 3-arabino-side, kaempferol 3-glucoside, and luteolin 4'-glucoside have been adopted as diagnostic biochemical characters. Variability within the same taxon is connected with the occurrence of flavonol methyl ethers (quercetin-3,7,4'-trimethyl ether, kaempferol-3,4'-dimethyl ether, 6-methoxykaempferide, and, presumably, quercetin-7,3',4'-trimethyl ether and quercetin-3-glycoside-4'-methyl ether), besides flavone glycosides: acacetin 7-glucoside and perhaps scutellarein 7-glycoside (Table 1). This is why they have been considered as auxiliary characteristics only.

The occurrence of hesperidin and polyphenol (chlorogenic, caffeic and gallic) acids in all specimens of the studied species of the genus *Betula* has been ascertained by Hänsel and Hörhammer (1954) and by Pawłowska (1980a, b, c, 1982). Fresh leaves of all the specimens which contained hyperoside were also found to contain probably dihydrohyperoside, a presumable intermediate metabolite in the biosynthesis of which contained hyperoside were also found to contain probably dihydrohyperoside, a presumable intermediate metabolite in the biosynthesis of quercetin glycoside. Likewise kaempferol, present in most specimens of *B. pubescens* and related taxa, is a substrate in the biosynthesis of kaempferol glucoside in their leaves (Pawłowska 1982). Hence hesperidin and polyphenol acids have no taxonomic value, and dihydrohyperoside and kaempferol only an insignificant one. For this reason these compounds have not been included in the tables given in the present paper.

For some, in all probability, closely related taxa characterized by identical or very similar diagnostic biochemical traits, differences were found to occur between their components as regards quantitative ratios, because the amounts of the same compounds are different. The flavonoid content depends on exposure, weather, and the time (time of the day) of collecting material, thus, on the intensity of insolation (Pawłowska 1976). A *B. pendula* tree, growing in a more shaded place than all other specimens of the same population, exhibited a lower content of all flavonoid components (Pawłowska 1980a). A similar correlation was observed in *B. lenta* and *B. lutea* (Pawłowska 1983). Thus the total amount of flavonoids depends quite clearly on isolation, whereas relationships between particular components—final metabolites—do not depend on it so much. Therefore, in the author's opinion the ratio of the amounts of components may likewise serve as auxiliary taxonomic markers.

Table 1

Flavone and flavonol glycosides, and flavonol methyl ethers from leaves of Polish taxa of the genus *Betula* L.

Compound Taxon	Quercetin 7-rhamnoside	Kaempferol 3-glucoside	Rutin	Isoquercitrin	Hyperoside	Quercitrin	Quercetin 3-arabinoside	Myricetin 3-galactoside	Myricetin 3-digalactoside	Kaempferol 3-rhamno-7-glucoside	Myricitrin	Isorhamnetin 3-galactoside	Luteolin 4'-glucoside	Acacetin 7-glucoside	Scutellarein 7-glycoside	6-methoxy-kaempferide	Quercetin-3,7,4'-trimethyl ether	Kaempferol-3,4'-dimethyl ether	Quercetin-3-glycoside-7,4'-dimethyl ether	Quercetin-7,3',4'-tri-methyl ether
<i>B. pendula</i> s.s. (11)	—	po.	—	—	++++	++	po.	0	+	—	—	+	—	po./ ^b	tr./ ^c	+/ ^d	—	po./ ^b	tr./ ^b	—
<i>ssp. obscura</i> (4)	—	+	—	—	++++	++	po.	0	+	—	—	+/ ^a	—	po./ ^b	tr./ ^c	—	—	po.	tr./ ^c	—
<i>B. oycoviensis</i> (12)	—	po.	—	po./ ^a	+++	+	+	0	+	po./ ^a	++	po./ ^a	—	po./ ^b	tr./ ^c	+/ ^d	tr./ ^b	tr./ ^b	tr./ ^c	tr./ ^c
<i>B. nova</i> (3)	—	—	—	+	++	+	++	0	++	++	+++	—	—	—	—	—	tr./ ^a	—	—	tr./ ^c
<i>B. pubescens</i>																				
<i>ssp. pubescens</i> (7)	—	po.	—	+/ ^b	+++	+	po.	0	+	—	—	+	+	po./ ^a	tr./ ^c	+/ ^b	—	po./ ^b	tr./ ^c	—
<i>spp. tortuosa</i>																				
<i>var. tortuosa</i> (2)	po.	tr.	+	++	+++	+	+	po.	+	—	—	po.	+	po.	—	—?	—	—?	—	—
<i>var. carpatica</i> (5)	po./ ^b	tr.	po.	+	+++	++	po.	0	++	—	—	po.	+	po.	tr./ ^b	po./ ^b	—	tr./ ^b	—	—
<i>B. nana</i> (2)	+	po.	+	+	+++	+	po.	po.	—	—	—	—	—	tr./ ^b	—	—	—	po.	—	—
<i>B. humilis</i> (5)	+/ ^a	tr.	+++++	+/ ^a	po.	—	tr.	—	—	—	—	—	—	—	—	—	—	po.	tr./ ^a	—

()—number of specimens under study; ^a—in a higher number of specimens; ^b—in very few specimens; ^c—occasionally; ^d—in specimens with larger leaf-blades; tr. trace amounts; po.—poor amounts; +—++++ higher amounts, respectively.

DISCUSSION

Among 8 taxa of the genus *Betula* L. in Poland the following are regarded as species: *B. pendula*, *B. pubescens*, *B. nana*, and *B. humilis*. The systematic position of *B. obscura*, *B. oycoviensis*, *B. "nova"*, and *B. carpatica*, raises some doubts.

BETULA PENDULA s. l.*B. pendula* s. str.

In this species, when assuming as a criterion the presence of betuletol correlated directly with the size of a leaf-blade one can propose to distinguish two forms: 1) a specimen with larger leaves, containing this compound, and 2) a specimens with smaller leaf-blades, without betuletol (Pawłowska 1980a).

B. obscura

The biochemical characters studied in *B. obscura* are identical with those for the group of *B. pendula* with smaller leaf-blades, without betuletol (Pawłowska 1980a—Table 1). A more essential characteristic difference between the two taxa is the colour of bark. The whiteness of the *B. pendula* bark is caused by a layer of thin-walled cork cells with triterpene alcohol—correct name betulinol (Stecki and Ślósarz 1928, Wiertelak 1928, Seshardi and Vadantham 1971). Wiertelak (1928) gave it for at first a wrong name—betulin. Hence some botanists, give betulinol the wrong name of betulin, while it is an entirely different compound (Browning 1963). Seshardi and Vadantham (1971) found negligible amounts of betulinol also in the dark bark of *B. lenta*. Thus, considering what methods were available to Wiertelak, the absence of betulinol in the bark of *B. obscura* seems to require corroboration.

On the basis of a greater angle of inclination of branches and a uniform layer of betulinol-free thick-walled cork cells Stecki and Ślósarz (1928), Hryniewicz-Sudnik (1962), Browicz (1979) and Jentys-Szaferowa (1979), consider *B. obscura* as a separate species. Walters (1964) in *Flora Europea* has distinguished this birch as a separate but disputable taxon. Nevertheless, owing to so great an affinity of the biochemical characters of *B. pendula* to those of *B. obscura*, the distinction of the latter birch as a separate taxon is, in the author's opinion, unjustified. The best solution is to recognize it as a subspecies (*B. pendula* ssp. *obscura* Kot.), in accordance with the view of Kornaś (1957).

B. OYCOVIENSIS

According to Winkler (1904) and Rehder (1974), *B. oycoviensis* is a variety of *B. pendula* (*B. pendula* var. *oycoviensis* Bess.), but in

Flora Europea it has been classified as a separate species (Walters 1964).

For many years *B. oycoviensis* was the object of detailed studies in the Department of Plant Variability, Institute of Botany, Polish Academy of Sciences (Jentys-Szaferowa 1953, 1967, Korczyk 1967a, b, c. Więckowska 1967, Szwabowicz 1971, 1972, 1976, Jentys-Szaferowa et al. 1974). The results of these studies showed *B. oycoviensis* to be a hybrid of *B. pendula* and *B. "nova"*. Its hybrid character became even more pronounced in the analysis of flavonoids (Pawłowska 1980c). The specimens of this species contain compounds characteristic of both *B. "nova"* and *B. pendula* (Table 1). The findings of the present investigations demonstrate the ratio of similar traits to different ones in *B. oycoviensis* as compared with *B. "nova"* to be 12:5, and in *B. oycoviensis* compared with *B. pendula* 13:4 (Table 2). It follows from the comparison that flavonoids of *B. oycoviensis* resemble a little more *B. pendula* than *B. "nova"*.

Table 2

Ratio of similar to different biochemical characters in the species studied/^a

	<i>B. pendula</i> s.s	<i>B. oycoviensis</i>	<i>B. "nova"</i>	<i>B. pubescens</i> s.s	<i>B. nana</i>	<i>B. humilis</i>
<i>B. pendula</i> s.s.		13:4 0.765/ ^b	8:9 0.471	15:2 0.882	11:6 0.647	8:9 0.471
<i>B. oycoviensis</i>	13:4 0.765		12:5 0.706	12:5 0.706	8:9 0.471	6:11 0.353
<i>B. "nova"</i>	8:9 0.471	12:5 0.706		8:9 0.471	8:9 0.471	7:10 0.412
<i>B. pubescens</i> s.s	15:2 0.882	12:5 0.706	8:9 0.471		11:6 0.647	8:9 0.412
<i>B. nana</i>	11:6 0.647	8:9 0.471	8:9 0.471	11:6 0.647		14:3 0.824
<i>B. humilis</i>	8:9 0.471	6:11 0.353	7:10 0.412	8:9 0.471	14:3 0.824	

^a During calculation the following compounds were not taken into account: scutellarein 7-glycoside, quercetin 3-glycoside-7,4'-dimethyl ether, and quercetin- 7,3',4'-trimethyl ether, as their identification is highly questionable; ^b similarity coefficient

B. "NOVA"

B. "nova" was found in cultivation as one of three segregants of *B. oycoviensis* × *B. oycoviensis* (Jentys-Szaferowa 1967, Jentys-Szaferowa et al. 1974, Więckowska 1967).

This birch differs from *B. oycoviensis* not only in the ratio of the

myricitrin to hyperside content (Pawłowska 1980b, c) but also in the absence of kaempferol 3-glucoside and isorhamnetin 3-galactoside (Table 1). A comparison of the ratios of similar to different characteristics given in Table 2 indicates clearly that *B. "nova"* differs from *B. oycoviensis*. Furthermore, these two taxa differ morphologically e.g. the number of leaves on a fructifying dwarf shoot, and at the age at which they bloom for the first time. *B. "nova"* has slightly darker leaves and is more fertile in the first period of its life than *B. oycoviensis* (Więckowska 1967, Jentys-Szaferowa et al. 1974).

In the last study of Jentys-Szaferowa (1979) *B. "nova"* is regarded as a variety of *B. oycoviensis* and is called *B. oycoviensis* cf. "*szaferi*". However, owing to the indicated characters and its occurrence in a natural locality (Korczyk 1967c), in our opinion this birch deserves to be described as a new species.

B. PUBESCENS s. l.

B. pubescens s. str.

B. pubescens is probably a hybrid of *B. pendula* and some unknown species (Johnsson 1949, Klaehn 1950). According to Walters (1968), this unknown species may be *B. humilis*.

The leaves of *B. pubescens* specimens growing on sites occupied exclusively by this species, contained all flavonoid pigments detected in *B. pendula*. Besides, these trees were found to contain glycosides such as isoquercitrin and luteolin 4'-glucoside (Pawłowska 1982). This, the chemical characters studied point to relatively small discrepancies between these species. Likewise, the morphology *B. pendula* and *B. pubescens* is fairly similar, wherefore numerous researchers considered them as the collective species *B. alba* L. We assume, however, that they should still be distinguished as separate species; since they vary in the presence of isoquercitrin and luteolin 4'-glucoside (Table 1). These two traits occurring in *B. pubescens* have probably been inherited from the second parental form. It is, therefore, rather unlikely that *B. pubescens* should have descended from a hybrid *B. pendula* × *B. humilis*, as the latter species contains no luteolin glucoside and differs in the occurrence of rutin and quercetin 7-rhamnoside (Table 1).

B. tortuosa and *B. carpatica*

According to Regel (1868) *B. pubescens* and *B. tortuosa* are separate species, whereas *B. carpatica* is a variety of *B. pubescens*. Winkler (1904) regarded *B. tortuosa* and *B. carpatica* as varieties of *B. pubescens*. In Flora Europea Walters (1964) classified these three taxa as subspecies of one *B. pubescens* group, whereas Natho (1976) distinguished them as three species.

Jentys-Szaferowa (1950) was the first to put forward the assumption that *B. tortuosa* is a hybrid of *B. nana* $\times$ *B. pubescens*. It was corroborated by Vaarama and Valanne (1970, 1973). Jentys-Szaferowa (1950) demonstrated *B. carpatica* to be a form intermediate between *B. pubescens* and *B. tortuosa*. This was supported by Białobrzaska and Truchanowicz (1960) on the basis of biometric analysis of hulls and drupels. Examinations of the flavonoid compounds of the leaves would confirm the hypothesis of the hybrid origin of *B. tortuosa*. However, the qualitative composition of the *B. carpatica* specimens under study was identical with that of the desiccated material of *B. tortuosa* (Pawłowska 1982).

B. pubescens, *B. tortuosa*, and *B. carpatica* have identical chromosome numbers ($2n=56$) (Löve and Löve 1948, 1956, Natho 1959, Skałińska et al. 1959). *B. tortuosa* and *B. carpatica* grow in very similar habitats: the former species in the mountains of Scotland, Scandinavia and in the Ural; the latter in the Tatra Mountains.

If the composition of flavonol glycosides and luteolin glucoside finds confirmation in the fresh material of a larger number of *B. tortuosa* specimens, then it seems that both *B. tortuosa* and *B. carpatica* can be considered as geographically-conditioned varieties of one higher taxon. Taking into account the criteria of classification adopted by Winkler (1904) and Walters (1964), one can propose to distinguish two subspecies within *B. pubescens*: ssp. *pubescens* (purebred *B. pubescens* trees, with myricetin digalactoside only—Pawłowska 1982) and ssp. *tortuosa* (with rutin and myricetin digalactoside—Table 1). In the last taxon one could distinguish two varieties—var. *tortuosa* and var. *carpatica*.

B. HUMILIS

Though in some *B. humilis* specimens an additional compound—isoquercitrin—has been detected, this cannot indicate the occurrence of polymorphism, because the presence of isoquercitrin depends rather clearly on illumination conditions (Pawłowska 1980b). Likewise this glycoside is in all likelihood an intermediate metabolite in the synthesis of rutin (a constant component of *B. humilis* leaves).

B. NANA

In Poland this boreal species is a post-glacial relict (e.g. Środoń 1979). It now grows in the Sudety Mountains only (Browicz et al. 1971). The morphology of *B. nana* is most similar to *B. humilis* (Jentys-Szaferowa 1950, Białobrzaska and Truchanowicz 1960). These two taxa are also most alike in their chemical characteristics (Table 1). Nevertheless, they vary from each other in the presence of quercitrin and myricetin 3-monogalactoside, this pointing to their being separate spe-

cies. However, it should be emphasized that the composition of the leaf flavonoids in *B. nana* requires corroboration in a larger number of specimens from natural localities.

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Flawonoidy liści polskich gatunków z rodzaju Betula L. V. Pozycja taksonomiczna na podstawie składu flawonoidów

Streszczenie

W wyniku analizy flawonoidów z liści osobników reprezentujących osiem polskich taksónów rodzaju *Betula* L. określono charakterystyczny skład jakościowy gatunków *B. pendula*, *B. oycoviensis*, *B. "nova"*, *B. pubescens*, *B. humilis* i *B. nana*. Na podstawie występowania flawonoidów zaproponowano sklasyfikowanie *B. obscura* w randze podgatunku *B. pendula* (*B. pendula* ssp. *obscura*), natomiast *B. tortuosa* i *B. carpatica* jako odmiany geograficzne *B. pubescens* ssp. *tortuosa* (*B. pubescens* ssp. *tortuosa* i *B. pubescens* ssp. *tortuosa* var. *carpatica*).