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

## II. The flavonoids of *B. "nova"* and *B. humilis* Schrk. leaves

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Abstract

The leaves of the taxon *B. "nova"* were found to contain myricitrin, hyperoside, quercetin 3-glucoside, myricetin 3-digalactoside, quercitrin, quercetin 3-arabinoside, hesperidin, and probably also kaempferol 3-rhamno-7--glucoside, quercetin 3,7,4′-trimethyl ether, quercetin 7,3′,4′-trimethyl ether, and dihydrohyperoside. Jentys-Szaferowa has advanced a supposition that *B. "nova"* may descend from *B. humilis* Schrk., however, since the composition of the leaf flavonoids present in the two taxa compared is quite different, these biochemical traits preclude such a possibility.

## INTRODUCTION

*Betula "nova"* was revealed in breeding for the first time as one of three segregants of the hybrid, *B. oycoviensis* × *B. oycoviensis*, and was given this name by Jentys-Szaferowa (1967). Apart from this taxon, also *B. oycoviensis* and *B. pendula* were then obtained (Jentys-Szaferowa l.c.; Jentys-Szaferowa et al., 1974; Korczyk, 1967a; Więckowska, 1967). Soon after *B. "nova"* was found in a natural habitat in the Kobylańska Valley, near a solitary specimen of *B. oycoviensis*, which in all probability had become self-pollinated and its progeny split into the three above-mentioned segregants (Korczyk, 1967b).

Observations made during cultivation showed *B. "nova"* to be a highly photophilous and short-lived shrub — it lives about ten years (Więckowska, 1967; Jentys-Szaferowa, 1979). Therefore, it can be recognized as the eighth species of the genus *Betula* in the Polish flora. It is, however, to be emphasized that uptill now *B. "nova"* has not been described in accordance with the requirements of the In-

ternational Code of Botanic Nomenclature; this is why the name has been put in quotation marks by Jentys-Szaferowa and other investigators.

In order to obtain some information on this taxon, the flavonoid compounds present in its leaves were identified.

Taking as a basis the resemblance of some morphological traits of the "*nova*" birches to those of the birches of the subsection *Nanae*, Jentys-Szaferowa (1967) assumed that *B.* "*nova*" may have originated in the late glacial period as a result of crossing between a specimen representing a species similar to modern *B. humilis* and a tree of the subsection *Albae*. In connection with this assumption, five specimens of *B. humilis* and the hybrid from, *B.* "*nova*" × *B. humilis* were also included in the present studies.

## MATERIAL AND METHODS

### 1. Material

Investigations were conducted on fresh leaves from the most insolated part of the shrub. The material was collected from three specimens: one growing in natural habitat (Kobylańska Valley) and denoted with the symbol "DK", and the other two cultivated in the Botanical Garden of the Jagiellonian University in Cracov and denoted as "738" and "614". They belonged to the $F_2$ generation of the hybrid, *B. oycoviensis* × *B. oycoviensis* and were selected for the present study as the only ones that ensured the indispensable amount of leaf mass. The morphology of these three specimens had been analysed earlier by Jentys-Szaferowa (1967) and Więckowska (1967). Besides, the bred hybrid, *B.* "*nova*" × *B. humilis* and one of *B. humilis* specimens grew in the Botanical Garden.

Analysis of water-methanol extracts of *B.* "*nova*" leaves collected on 8th July and 4th September was performed twice in 1972 and was repeated in 1973 on the material gathered on 15th July and 27th August. Furthermore, for comparison, leaves of the hybrid, *B.* "*nova*" × *B. humilis* and of one *B. humilis* "OB" (Botanical Garden) were gathered on 27th August, 1973, and leaves from 4 specimens of *B. humilis* were collected in a natural locality in the Niepołomicka Forest, near Szarów, on 7th September, 1978.

### 2. Methods

Extraction, separation and purification of the flavonoid compounds were performed as described earlier (Pawłowska, 1980). Specimen

"614" yielded 5 to 6 g of the leaf material, and the other two about 10 g each.

### A. Obtention of standard myricitrin

Preliminary examination of water-methanol extracts of *B. "nova"* leaves revealed a rather large amount of a compound, whose Rf values indicated that it might be myricetin 3-rhamnoside (myricitrin). In view of the lack of a standard of this compound, it was isolated from the leaves of *Corylus avellana* L., from which it had been obtained earlier by Hörhammer et al., (1956). To this end about 30 g of mature leaves of this species of hazel were collected on to dry ice. Extraction, and separation on Whatman No. 3 paper in the system ethyl acetate : : water : formic acid (10 : 3 : 2 v/v) was carried out identically as for the leaves of *B. "nova"* plants studied, except that only the band of Rf 0.54-0.64 was eluted with methanol. The eluate was subjected to rechromatography in 30% acetic acid on Whatman No. 1 paper. The bands of Rf 0.48-0.55 were again eluted with methanol.

### B. Identification methods

In order to identify the flavonoids paper and thin-layer co-chromatography were applied, using the ascending two-dimensional technique, and also colour reactions characteristic of flavonoids, acid hydrolysis, and spectral analysis in the ultraviolet and visible light with the use of specific reagents. These methods are described in the first part of the author's study (Pawłowska, 1980). Moreover, identification of compounds "2" and "14" (flavonol glycoside so far undetected in birch leaves) was achieved by spectroscopy in infra-red. Besides, compounds "14" and "20" were analysed by mass spectroscopy.

#### a). Mass spectroscopy

The compounds "14" and "20" — containing fractions were subjected to thin-layer chromatography on 30 × 50 cm plates coated with a 2 mm layer of MN 300 cellulose and were developed in $H_2O$ for compound "14" or in 15% acetic acid for compound "20". The yellow bands of Rf 0.60-0.75 in $H_2O$ or of Rf 0.29-0.33 in 15% acetic acid were eluted with methanol. Subsequently, following crystallization of the substances from methanol, 1.5 mg of each was analysed by mass spectroscopy in a Jasiewo 12 apparatus in the range of 40 to 600 m/e. The temperature of decomposition was 70°-225° C.

Table 1

Chromatographic values and results of test reactions

| Number of compounds | Rf values in solvent systems | | | | | | | Colour in light | | Results of test reactions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | H | $H_2O$ | visible | UV | $ZrOCl_2$ | $ZrOCl_2+C_6H_8O_7$ | $AlCl_3$ | diazo benzidine |
| 1 | 0.71 | 0.70 | 0.60 | 0.72 | 0.35 | 0.52 | 0.15 | y | d.y | y-o | — | y | p-ru |
| 2 | 0.60 | 0.58 | 0.55 | 0.61 | 0.29 | 0.37 | 0.19 | p-y | p-ru | o | — | d.y | re-ru |
| 3 | 0.55 | 0.53 | 0.57 | 0.55 | 0.30 | 0.36 | 0.11 | y | ru | l-y | — | y | p-ru |
| 4 | 0.39 | 0.41 | 0.53 | 0.43 | 0.20 | 0.40 | 0.08 | p-ru | d.ru | o-p | — | d.y-o | re-ru |
| 6 | 0.48 | 0.50 | 0.85 | 0.49 | 0.41 | 0.80 | | — | fl.v-b | — | — | y-o | y-ru |
| 8 | 0.62 | 0.59 | 0.61 | 0.62 | 0.29 | 0.40 | 0.13 | y | ru | o-y | — | y | p-ru |
| 9 | 0.65 | | 0.49 | | | | | | tr.fl.y | tr.y | tr.y | | ru |
| 12 | 0.65 | | 0.55 | | | | | | tr.fl.y | tr.y | tr.y | | tr.ru |
| 13 | 0.53 | 0.52 | 0.74 | | 0.59 | | | — | ru | — | — | y-b | y-ru |
| 14 | 0.53 | 0.57 | 0.55 | 0.54 | 0.33 | 0.34 | 0.64 | y | o | o | — | y | re |
| 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 | 0.68 | | 0.54 | 0.66 | | 0.33 | 0.09 | y | d.y | y | — | y | p-ru |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. humilis | | | | | | | | | | | | | |
| 5 | 0.46 | 0.40 | 0.75 | 0.51 | 0.39 | 0.55 | 0.27 | o-y | re-ru | d.y | — | y | re-ru |
| 10 | 0.71 | | 0.29 | | | | | | | c-y | y-g | tr.y | ru |
| 11 | 0.68 | | 0.46 | | | | | | | tr.y | — | | tr.ru |
| Standards: | | | | | | | | | | | | | |
| Quercetin 3-glucoside | 0.60 | 0.57 | 0.61 | 0.62 | 0.30 | 0.38 | 0.12 | | | o-y | | | p-ru |
| Quercitrin | 0.72 | 0.69 | 0.62 | 0.72 | | 0.51 | 0.14 | | | y-o | | | p-ru |
| Myricitrin/[b] | 0.63 | 0.58 | 0.56 | 0.61 | | 0.38 | 0.17 | | | o | | | re-ru |
| Hyperosid | 0.56 | 0.54 | 0.56 | 0.55 | | 0.35 | 0.10 | | | l-y | | | p-ru |
| Rutin | 0.45 | 0.40 | 0.73 | 0.48 | | 0.53 | 0.25 | | | d.y | | | re-ru |
| Hesperidin | 0.50 | | 0.83 | | | 0.78 | | | | — | | | y-ru |
| Caffeic acid | 0.73 | | 0.60 | | | | | | | — | | | y/[a] |
| Gallic acid | 0.80 | | 0.61 | | | | | | | — | | | y/[a] |
| Chlorogenic acid | 0.87 | | 0.90 | | | | | | | — | | | y/[a] |

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

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 — n-amyl alcohol:isoamyl alcohol:formic acid:water (2:2:2:1 v/v)
E — formic acid:water (1:9 v/v)
H — acetic acid:water (3:17 v/v)

b). Spectroscopy in infra-red (IR)

After spectral analysis in UV, the pure froctions of compounds "2" and "14" were cystallized from methanol and 3 mg of each of them was pressed together with anhydrous potassium bromide (Voirin, 1970) and their spectra were drawn in the range from 550 to 4,000 $cm^{-1}$ in a UR-10 ZEISS spectrophotometer.

## RESULTS AND DISCUSSION

*Betula "nova"* leaves were found to contain flavonol, dihydroflavonol, and flavanone derivatives, and free polyphenol acids. A total of 13 compounds were detected and denoted by Arabic numerals.

As the flavonoid compounds marked with numbers 1, 3, 4, 6, and 13, and polyphenol acids "17", "18" and "19" occur in the leaves of both *Betula "nova"* and *B. pendula*, the results of their spectral analysis have already been presented in the author's earlier study concerned with the flavonoids of *B. pendula* leaves (P a w ł o w s k a, 1980).

### A. Glycosides and methoxy derivatives of flavonols

A comparison of the results of chromatography, specific colour reactions (Table 1), acid hydrolysis (Table 3), and spectral analysis shows compound "1" to be identical with standard quercitrin, and compound "3" with standard hyperoside.

The chromatographic values and the results of specific reactions for compound "2" suggested it to be flavonol 3-glycoside (Tables 1, 2 and 3). Spectral data which characterize this compound, i.e. the spectrum of the methanol solution in UV, the bathochromic shift $\Delta\lambda_2 = 80$ nm and the hypsochromic shift $\Delta\lambda_3 = -29$ nm in $AlCl_3/HCl$, besides the shifts obtained in the $NaOAc/H_3BO_3$ test and also the bathochromic ($\Delta\lambda_6 =$ $= 72$ nm) and hypsochromic ($\Delta\lambda_7 = -8$ nm) shifts with $ZrOCl_2/C_6H_8O_7$ (Table 5) as well as the spectrum in infra-red (peaks in the 1330—1380 $cm^{-1}$ band characteristic of ortho-hydroxyl groups and in the 1140—1180 $cm^{-1}$ band characteristic of meta-hydroxyl groups — Fig. 1) prove compound "2" to be the 3-derivative of 3,5,7,3′,4′,5′-hexahydroxylflavone. The hydrolysate of this compound was found to contain myricetin and rhamnose. Besides, compound "2" appeared to be identical with flavonol glycoside isolated from *Corylus avellana* leaves. It follows from the above data that compound "2" is myricetin 3-rhamnoside (myricitrin).

Table 2

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 | F | NaOAc | $FeCl_3$ | $NH_4OH$ | |
| 1 | 0.45 | 0.50 | 0.70 | g-b | y-g-b | g-b | 513 |
| 2 | 0.38 | 0.47 | 0.63 | g-b | re-b | re-b | 529 |
| 3 | 0.35 | 0.45 | 0.61 | o-b | ru | v-b | 515 |
| 4 | 0.11 | 0.26 | 0.48 | b-g | b | re-b | 532 |
| 8 | 0.36 | 0.45 | 0.61 | o-b | ru | p-b | 515 |
| 14 | 0.38 | 0.50 | 0.90 | | o | g-b | 508 |
| 20 | 0.42 | 0.44 | 0.59 | o-b | y-g-b | | 513 |
| Standards: | | | | | | | |
| Myricitrin | 0.40 | 0.47 | 0.65 | g-b | re-b | re-b | 530 |
| Quercetin 3-glucoside | 0.37 | 0.47 | 0.63 | o-b | ru | | 515 |

solvent systems: F — water:hydrochloric acid:acetic acid (5:1:5 v/v); A and E — see Table 1

[a]/ — in methanol solution of HCl, pH 2-3; myricitrin isolated from *Corylus avellana* leaves; b — blue; g — green; o — olive; re — red; ru — russet; v — violet; y — yellow

The results of analysis of compound "4" obtained by chromatographic methods, and especially the localization of this compound on two-dimensional chromatograms testify to its being flavonol glycoside. That compound "4" is myricetin 3-glycoside is evidenced by the UV spectrum of its methanol solution and by shift values (P a w ł o w s k a,

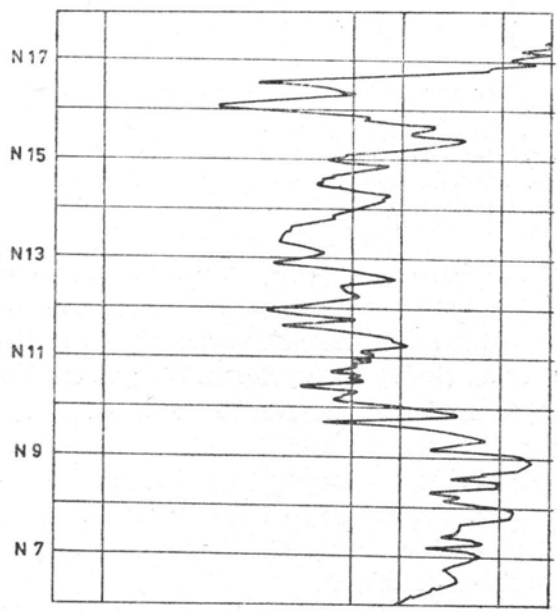


Fig. 1. IR spectrum of compound "2" (myricitrin).

Table 3

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 |
| 2 | 0.51 | 0.13 | 0.32 | 0.50 |
| 3 | 0.73 | 0.29 | 0.09 | 0.18 |
| 4 | 0.50 | 0.12 | 0.11 | 0.15 |
| 6 | 0.91 | — | 0.08<br>0.32 | 0.10<br>0.45 |
| 8 | 0.72 | 0.31 | 0.11 | 0.14 |
| 13 | 0.85 | — | 0.12 | 0.16 |
| 14 | 0.74 | 0.45 | 0.11<br>0.28 | 0.13<br>0.52 |
| 20 | 0.71 | 0.25 | 0.13 | 0.20 |
| Standards: | | | | |
| Myricitrin/[e] | 0.50 | 0.10 | 0.34 | 0.52 |
| Kaempferol | 0.80 | 0.43 | | |
| Quercetin | 0.73 | 0.30 | | |
| Myricetin | 0.49 | 0.12 | | |
| Isorhamnetin | 0.76 | 0.29 | | |
| Apigenin | 0.86 | 0.55 | | |
| Luteolin | 0.77 | 0.40 | | |
| Rhamnose | | | 0.27 | 0.50 |
| Glucose | | | 0.08 | 0.15 |
| Galactose | | | 0.10 | 0.14 |
| Arabinose | | | 0.12 | 0.20 |
| Xylose | | | 0.15 | 0.30 |

solvent systems: G — benzene:n-butanol:pyridine:water (1:5:3:3 v/v), A and F see Tables 1 and 2
/a — following development with diazo benzidine
/b — following development with $ZrOCl_2$
/c — following development with aniline phthalane
/d — following development with an ammonia solution of $AgNO_3$
/e — isolated from *Corylus avellana* leaves

1980). Myricetin and galactose were hydrolytic products of this flavonoid (Table 3). Chromatographic and spectral results similar to those obtained in the present study for compound "4" have been reported by Hörhammer et al. (1957) for myricetin 3-digalactoside.

Chromatographic data and the results of specific reactions for compound "8" (Tables 1, 2 and 3), and its shift values obtained with tests in spectral analysis (Table 4) indicate that this is 3,5,7,3′,4′-penthahydroxyflavone 3-glycoside. Comparison of Rf values of compound "8" with analogous ones for standard quercetin 3-glucoside suggests the identity of these two compounds (Table 1).

The negligible amount of compound "9" made possible only two-dimensional chromatography and spectral analysis in UV. The spectrum

of its methanol solution and of the methanol solution on addition of NaOMe is characteristic of flavonols (Table 4). Insignificant shift values on application of $AlCl_3/HCl$, $NaOAc/H_3BO_3$, and $ZrOCl_2/C_6H_8O_7$ show that this flavonol possesses two free hydroxyl groups: one in the $C_5$-position and the other probably at $C_3$. On the other hand, in the $C_3$-, $C_7$-, and $C_4'$-positions this compound is substituted by methoxyl groups. Similar spectral results have been obtained by Wollenweber (1974) for quercetin 3,7,4′-trimethyl ether.

There was a very low amount of a compound marked with No. 12. Therefore only spectral analysis of its eluate from two-dimensional chromatograms was performed. The findings of this analysis (very small shift values, especially in band II — Table 4) encourage the supposition that compound "12" is quercetin 7,3′,4′-trimethyl ether.

The results of chromatographic analysis and of specific colour reactions reveal compound "14" to be flavonol 3-glycoside (Tables 1 and 2). The shift values after spectral tests in UV and visible light and the character of the spectrum of the methanol solution (Table 4) shown the aglycone of compound "14" to possess free hydroxyl groups: one in the B-ring (this being evidenced by $\Delta\lambda_3 = -9$ nm), most probably at the $C_4'$-position, and the other in A-ring at $C_5$ (as evidenced by $\Delta\lambda_6 = 58$ nm and $\Delta\lambda_7 = -9$ nm). Its mass spectrum (Fig. 3) has its peak at 284 m/e corresponding to the fragment of aglycone, whose two hydroxyl groups at the $C_3$- and $C_7$-positions were involved in glycoside bindings (as evidenced by fragments of 153, 150 and 149 m/e corresponding to the A-ring). Likewise, the high Rf value in the $H_2O$ system of compound "14" testifies to the structure of flavonol 3,7-diglycoside. A two-minute acid hydrolysis of this compound yielded rhamnose and another compound, whose Rf value in the BAW system was 0.57. A similar Rf value in this system was obtained by Seikel (1962) for kaempferol 7-glucoside. After 10 minutes the hydrolysate of compound "14" was found to contain kaempferol, rhamnose and glucose (Table 3). Thus, compound "14" is probaly kaempferol 3-rhamno-7-glucoside (Fig. 2 and 3).

Besides, compound number 20 was found, whose products with zirconium oxychloride and diazobenzidine were characteristic of flavonol glycosides. This compound was identical with the unidentified substance present in the leaves of *B. pendula* (Pawłowska, 1980). Quercetin and arabinose were detected in the hydrolysate of compound "20" (Table 3). Its mass spectrum (Fig. 4) has its peak at 302 m/e corresponding to quercetin as aglycone of compound "20". Similar Rf values in BAW, 15% acetic acid, and $H_2O$ systems (Table 1) have been obtained by Seikel (1962) and by Harborne and Williams (1975) for quercetin 3-arabinoside.

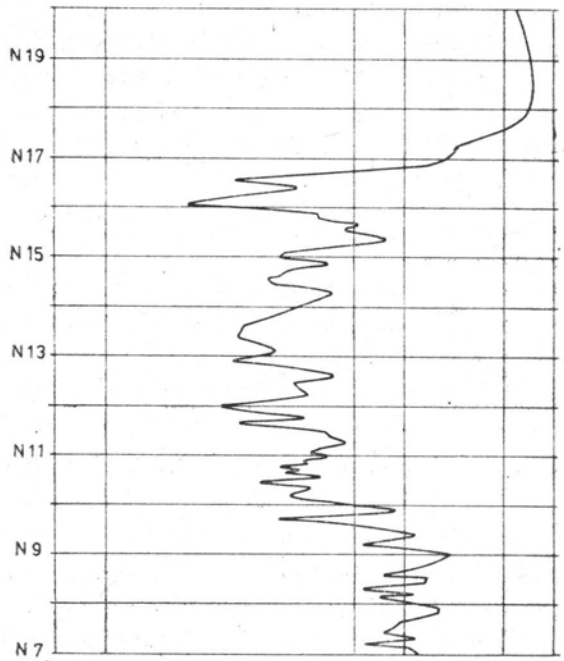


Fig. 2. IR spectrum of compound "14" (kaempferol 3-rhamno-7-glucoside).

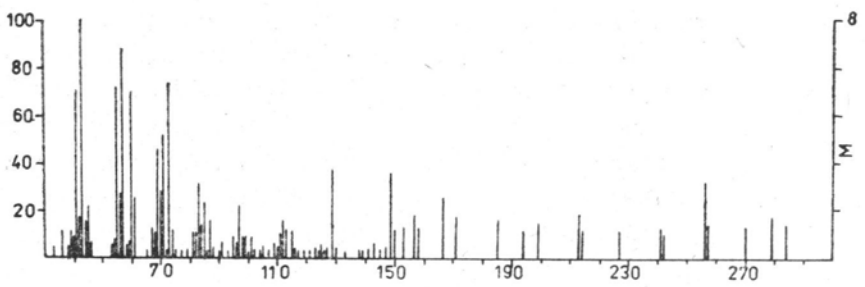


Fig. 3. Mass spectrum of compound "14" (kaempferol 3-rhamno-7-glucoside).

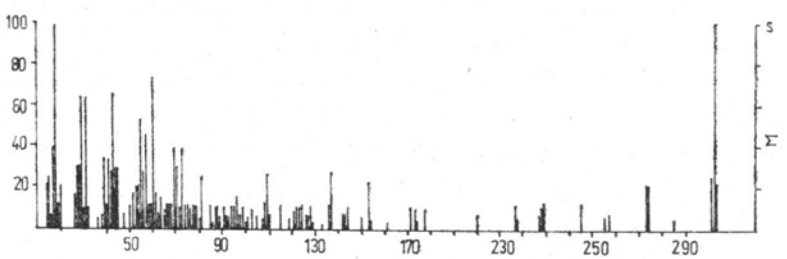


Fig. 4. Mass spectrum of compound "20" (quercetin 3-arabinoside).

Table 5

Flavonoid compounds in the leaves of *B.* "*nova*", *B. humilis* and their hybrid

| No. of compound | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 17 | 18 | 19 | 20 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name / Specimen / Date of collection | quercitrin | myricitrin | hyperoside | myricetin 3-digalactoside | rutin | hesperidin | quercetin 3-glucoside | quercetin 3,7,4′-trimethyl ether | kaempferol 3,4′-dimethyl ether | quercetin 3-glycoside--7,4′-dimethyl ether (?) | quercetin 7,3′,4′-trimethyl ether (?) | dihydrohyperoside | kaempferol 3-rhamno-7-glucoside | caffeic acid | gallic acid | chlorogenic acid | quercetin 3-arabinose | unidentified |
| *B.* "*nova*" | | | | | | | | | | | | | | | | | | |
| "DK" | | | | | | | | | | | | | | | | | | |
| 8.07.1972 | + | ++ | + | + | − | + | tr. | tr. | − | − | tr. | + | + | + | ++ | + | + | − |
| 4.09.1972 | ++ | ++++ | ++ | ++ | − | + | + | tr. | − | − | tr. | + | ++ | + | ++ | + | ++ | − |
| 15.07.1973 | + | ++ | + | + | − | + | tr. | tr. | − | − | tr. | + | + | + | + | + | + | − |
| 27.08.1973 | ++ | +++ | ++ | ++ | − | + | + | + | − | − | tr. | + | ++ | + | + | + | ++ | − |
| "738" | | | | | | | | | | | | | | | | | | |
| 8.07.1972 | + | ++ | + | + | − | + | tr. | tr. | − | − | − | + | tr. | + | + | + | + | − |
| 4.09.1972 | + | +++ | ++ | ++ | − | + | + | tr. | − | − | − | + | + | + | + | + | + | − |
| 15.07.1973 | + | +++ | + | + | − | + | tr. | tr. | − | − | − | + | + | + | + | + | + | − |
| 27.08.1973 | + | +++ | ++ | ++ | − | + | + | tr. | − | − | − | + | ++ | + | + | + | + | − |
| "614" | | | | | | | | | | | | | | | | | | |
| 8.07.1972 | + | ++ | + | + | − | tr. | −? | −? | − | − | − | tr. | tr. | + | + | + | tr. | − |
| 4.09.1972 | + | +++ | + | + | − | + | tr? | −? | − | − | − | + | + | + | + | + | tr. | − |
| 15.07.1973 | + | ++ | + | tr. | − | tr. | −? | −? | − | − | − | tr. | tr. | + | + | tr. | tr. | − |
| 27.08.1973 | + | ++ | + | + | − | + | −? | −? | − | − | − | + | + | + | + | + | tr. | − |
| hybrid *B.* "*nova*" × *B. humilis* | + | + | +++ | + | + | + | tr. | − | tr? | − | − | + | tr? | + | + | + | tr. | tr? |
| *B. humilis* | | | | | | | | | | | | | | | | | | |
| "OB" | − | − | + | − | +++++ | + | − | − | tr. | tr. | − | + | − | + | + | + | tr. | tr. |
| "1" | − | − | + | − | ++++ | + | ++ | − | + | tr. | − | − | − | + | + | + | + | − |
| "2" | − | − | + | − | +++++ | + | − | − | + | tr. | − | tr. | − | + | + | + | tr. | + |
| "3" | − | − | + | − | ++++ | tr. | tr. | − | tr. | − | − | tr. | − | + | + | + | tr. | tr. |
| "4" | − | − | + | − | +++ | + | + | − | tr. | − | − | tr. | − | + | + | + | tr. | + |

tr. — trace amount (compound visible in UV after development); "+" — observable in visible light after development; "++" to "+++++" — correspondingly more amount

## B. Flavanone and dihydroflavonol glycosides

Comparison of chromatographic and spectral values for compound "6" with those for standard hesperidin proves them to be identical (Tables 1, 3, Pawłowska, 1980).

On the basis of the results obtained it is supposed that compound "13" (Tables 1 and 3) is dihydrohyperoside.

## C. Polyphenol acids

The compounds denoted by numbers 17, 18 and 19 belong to the group of polyphenol acids. Compound "17" was identical with standard caffeic acid, compound "18" with standard gallic acid, and "19" with chlorogenic acid (Table 1).

The composition of the identified compounds present in *Betula "nova"* leaves collected in July and at the end of August and the beginning of September did not reveal any qualitative discrepancies (Table 5), similarly as in *B. pendula* (Krawiarz, 1972; Pawłowska, 1980). The compounds could only be observed to be more abundant at the turn of August. It should be stressed that quantitative assessment was based on the colour intensity and the size of the spot in two-dimensional chromatography and on the colour intensity and width of the band in preparative chromatography. Nonetheless, evaluation by the naked eye allows to ascertain that the main flavonoids to be found in *B. "nova"* leaves are myricitrin, hyperoside, myricetin 3-digalactoside, and quercitrin. They occur in greater amounts than do the remaining flavonoid components, the myricitrin content being the highest (marked in Table 5 by an appropriate number of plus sings). No quantitative analyses could be made on *B. "nova"* specimens as soon after indentification all the cultivated specimens perished.

Besides, predominant myricitrin, the *B. "nova"* leaves contain hyperoside, myricetin 3-digalactoside, quercitrin, quercetin 3-glucoside, kaempferol 3-rhamno-7-glucoside, quercetin 3-arabinoside, quercetin 3,7,4′-trimethyl ether, quercetin 7,3′,4′-trimethyl ether (?), hesperidin, dihydrohyperoside (?), and polyphenol acids (gallic, caffeic and chlorogenic — Tables 1 and 5).

The leaves of *B. humilis* contain rutin as predominant flavonol compound, marked no. 5 (Table 1 and 4), and also hyperoside. The presence of these two glycosides in *B. humilis* leaves had been ascertained earlier by Hörhammer et al. (1953), Hänsel and Hörhammer (1954) and Pawłowska (1976). In the specimens studied there also are negligible amounts of quercetin 3-glucoside, and probably kaempferol 3,4′-dimethyl ether (marked no. 10), quercetin 3-glycoside-7,4-dimethyl

ether ("11"), dihydrohyperoside as well as an unidentified compound denoted no. 23 (Tables 1, 5). These methyl derivatives of kempferol have been detected by Wollenweber (1975) in the leaf buds of this species. In addition, the specimens under investigation possessed hesperidin and polyphenol acids such as caffeic, gallic, and chlorogenic acid, the presence of which in *B. humilis* leaves gathered in August, had already been shown by Hörhammer et al. (1956).

It should be emphasized that the qualitative composition of all *B. humilis* plants was very much alike. The only differences to be found concerned the relations between flavonol glycosides, and, above all, between the amounts of rutin and the content of quercetin 3-glucoside — the more rutin the less quercetin 3-glucoside could be observed (Table 5). It may be assumed that this facts results from a different, for each specimen, catalytic activity of quercetin 3-glucoside:UDP--rhamnose (or TDP-rhamnose — Hahlbrock, Grisebach, 1975) glycosyltransferase, whose product is rutin.

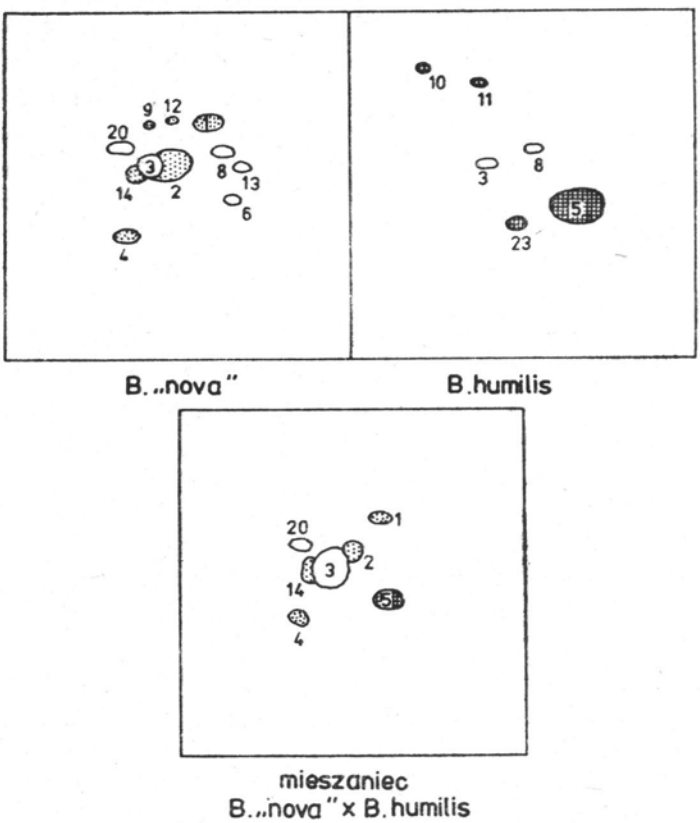


Fig. 5. Chromatograms of *B. "nova"*, *B. humilis* and their hybrid; characteristic spots for *B. "nova"* are dotted, and for *B. humilis* chequered.

The investigated hybrid, *B. "nova"* × *B. humilis* contains, besides prevailing hyperoside, also some compound characteristic of both parental taxa, i.e. rutin, myricitrin, and myricetin 3-digalactoside (Fig. 5).

The present investigations revealed that *B. "nova"* leaves differ conspicuously from those of *B. humilis* in the composition of their flavonoid compounds. Thus, the biochemical characteristics detected do not point to the possible origin of *B. "nova"* by hybridization of a plant which belonged to a species similar to modern *B. humilis* with a taxon of the subsection *Albae*, as was suggested by Jentys-Szaferowa (1967). Nevertheless, one cannot rule out a distant kinship of the taxa studied, which may be testified by the presence in *B. "nova"* of a very small amount of quercetin 3-glucoside.

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## *Flawonoidy liści polskich gatunków z rodzaju Betula L. II. Flawonoidy liści B. „nowa" i B. humilis Schrk.*

Streszczenie

W pełni wegetacji świeże liście *B. „nova"* zawierały: myrycytrynę, hyperozyd, kwercytrynę, 3-arabinozyd kwercetyny, izokwercytrynę, 3-dwugalaktozyd myrycetyny, 3-ramno-7-glukozyd kempferolu, hesperydynę, prawdopodobnie 3,7,4′-trójmetylo-kwercetynę i dwuhydrohyperozyd oraz być może 7,3′,4′-trójmetylo-kwercetynę. Badane cechy biochemiczne wykluczają wcześniej sugerowane pokrewieństwo *B. „nova"* z *B. humilis*.