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Flavonoids from the leaves of some American species of the genus *Betula* L.

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Abstract

On the basis of the qualitative composition of the leaf flavonoids it may be assumed that *B. lutea* Michx. is an intermediate form between *B. lenta* L. and *B. papyrifera* Marsh., whereas the taxa *B. neoalascana* Sarg. and *B. commutata* are rather different from *B. papyrifera*.

Key words: Betula species, Betulacae, flavonol and flavon glycosides.

INTRODUCTION

Among six American species covered by biochemical-systematic studies of the genus *Betula* L. (Pawłowska 1983), five were interesting from the point of view of taxonomy; these were *B. lenta*, *B. lutea*, *B. papyrifera*, *B. papyrifera* var. *neoalascana*, and *B. p.* var. *commutata*. The ranges of occurrence of *B. papyrifera*, *B. lutea* and *B. lenta* overlap on a considerable area (Preston 1961, Gleason and Cronquist 1963). The bark of *B. lenta* is tan-cherry-red, *B. papyrifere* has white bark, while *B. lutea* is characterized by the yellowish-grey colour of its bark. Clausen (1970) obtained by crossing *B. papyrifera* with *B. lenta* 60% of viable progeny. *B. lenta* has the chromosome number 2n = 28; *B. papyrifera* 2n = 56, 70, 84; and *B. lutea* 2n = 84.

MATERIAL AND METHODS

MATERIAL

Fresh leaves (ca 15 g from each specimen) of the specimens growing in the Botanical Gardens of the Jagiellonian and Warsaw Universities and in the Kórnik Arboretum were collected into ice. The gathering

proceeded in the period of full vegetation (end of August and beginning of September), from one to two o'clock p. m. Investigations covered also single specimens of *B. lenta* and *B. lutea* which grew in the Botanical Garden in Leningrad; this material, however, was available only in desiccated form, amounting to 2 g from each tree.

EXTRACTION

The homogenized leaves were treated with methanol, until a colourless extract was obtained. The extracts were then evaporated to 50 cm^3 and mixed with an equal volume of chloroform, thoroughly shaken and, after 15 minutes, separated. The upper aqueous-methanol phase was condensed again to the volume of about 10 cm^3.

ANALYSIS OF THE FLAVONOIDS COMPONENTS

The composition of the leaf flavonoids for each specimen was determined in 0.2 cm^3 of extract by two-dimensional thin-layer chromatography and cochromatography, on MN 300 cellulose, in five combinations of various systems (P a w ł o w s k a 1982).

ISOLATION AND IDENTIFICATION OF LUTEOLIN 7-RUTINOSIDE

The remaining amounts of the extracts from the leaves of all specimens of *B. lenta* and *B. lutea* were separated by two-dimensional chromatography in a FAWiA system (amyl alcohol-isoamyl alcohol-water-formic acid 1:1:1:1 v/v) in the first and in 10% HOF (formic acid-water 1:9 v/v) in the other dimension. After elution with methanol the fraction in question was identified with the use of one-dimensional chromatography, by 5- and 20-minute acid hydrolysis, reduction, and spectral analysis in UV, as described by P a w ł o w s k a (1980).

RESULTS ANL DISCUSSION

Although the present studies were conducted on one *B. papyrifera* specimen only, they coincided in a large measure with the results of investigations carried out by H ä n s e l and H ö r h a m m e r (1954) and by W o l l e n w e b e r (1975), thereby being more credible. This species was found to contain flavonol glycosides (quercitrin, myricitrin and myricetin 3-digalactoside), hesperidin, and also quercetin 3,7,4-trimethyl ether. Besides these, some new compounds were detected in the leaves

Table 1

Flavonoids from the leaves of *B. lenta*, *B, lutea*, *B. papyrifera*, *B. neoalascana* and *B. commutata*/[a]

Specimen	d	1	2/[b]	3	4	5	6	7	8	9	10	11	a	b	c
B. lenta															
BGW	++	++	+++	+	+++	+	po.	—	—	—	tr.	—	—	—	—
AK-1/[c]	+	+	++	po.	++	+	—	—	—	—	—	—	—	—	—
AK-2	+++	++	+++	+	+++	+	—	—	—	—	po.	—	—	—	—
BGL	—	+	+	++	+++	+	+	—	—	—	po.	—	—	—	—
B. lutea															
AK-1/[c]	+	+	+	++	+	+	+	+	—	—	—	tr?	po.	—	—
AK-2	++	+++	++	+++	++	+	—	—	tr.	—	—	—	—	—	—
AK-3	++	++	++	++	+	+	—	+	+	po.	+	tr?	po.	+	—
BGL	tr.	—	po.	++	+++	+	—	po.	—	tr.	+	—	—	—	+
B. papyrifera															
AK	—	—	—	—	—	—	po.	+	+	po.	po.	—	po.	+	+
B. neoalascana															
BGC	—	—	—	++	++	+	po.	+	tr.	tr.	po.	—	po.	tr.	—
AK	—	—	—	++	++	+	—	+	+	—	—	+	—	tr.	tr.
B. p. var. commutata															
BGC	—	—	—	po.	++	+	—	++	+	tr.	—	—	—	—	—

1 — probably luteolin 7-rutinoside; 2 — rutin; 3 — isoquercitrin; 4 — hyperoside; 5 — probably dihydrohyperoside; 6 — probably myricetin 3-galactoside; 7 — myricetin 3-digalactoside; 8 — isorhamnetin 3-galactoside; 9 — probably acacetin 7-glucoside; 10 — quercetin 3,7,4'-trimethyl ether; 11 — kaempferol 6-methoxy-4'-methyl ether; a, b, c, d — unidentified compounds; BGW — Botanical Garden in Warsaw; BGC — Botanical Garden in Cracow; BGL — Botanical Garden in Leningrad; AK — Arboretum Kórnik

/[a] — the table does not include compounds which occurred in all specimens examined (quercitrin, quercetin 3-arabinoside, myricitrin, kaempferol 3-rhamno-7-glucoside and luteolin 4'-glucoside) or compounds of lesser taxonomic importance e.g. flavone and flavonol aglycones, and scutellarein 7-glycoside; /[b] — the identification of compounds 2-11 was published in the earlier parts of this study; /[c] — specimens growing under unfavorable light conditions.

of this specimen, i.e. isorhamnetin 3-galactoside, kaempferol 3-rhamno--7-glucoside, and very small amounts of quercetin 3-arabinoside and myricetin 3-galactoside, as well as flavon glycosides (acacetin 7-glucoside, luteolin 4'-glucoside, and probably scutellarein 7-glycoside) and three unidentified flavonoids "a", "b", and "c" (Table 1).

In the leaves of the *B. lenta* specimens under study flavonol glycosides: quercitrin, myricitrin, hyperoside, rutin, isoquercitrin, kaempferol 3-rhamno-7-glucoside, and quercetin 3-arabinoside were found together with flavone glycosides (luteolin 4'-glucoside and luteolin 7-rutinoside), as well as hesperidin, dihydrohyperoside, and an unidentified compound "d" (Tables 1 and 2). All these compounds were present in *B. lutea*, but beside them flavonol glycosides: myricetin 3-digalactoside and isorhamnetin 3-galactoside; flavon glycosides i.e. acacetin 7-glucoside and scutellarein 7-glycoside (?); and unidentified compounds "a", "b" and "c" were revealed, the latter in *B. papyrifera* (Table 1). The absence of luteolin rutinoside in desiccated *B. lenta* and *B. lutea* material (specimens from Leningrad) may be explained as the result of its decomposition during the process of desiccation.

Biochemical characteristics and other data given above, showed *B. lutea* to be an intermediate form between *B. lenta* and *B. papyrifera*.

B. neoalascana and *B. papyrifera* var. *commutata* present an entirely different problem. *B. neoalascana* was recognized by Rehder (1974) and *B. papyrifera* var. *commutata* by Brittain and Grant (1966) as a variety of *B. papyrifera*. However, the composition of flavonol gly-

Table 2

Identifying analysis of luteolin 7-rutinoside

Rf values in system						Colour in light		Spectral maxima in methanol
BAW/[a]	A	FAWiA	10% HOF	15% HOAc	H_2O	visib.	UV	256; 270; 350
0.40	0.30	0.36	0.58	0.60	0.39	y	y—c	

Effects of test reactions			products of reduction				products of hydrolysis			
				Rf values in			Rf values in systems			
$ZrOCl_2$	$ZrOCl_2$ + $C_6H_8O_7$	$AlCl_3$	colour	BAW	10% HOF	B/[a]	BAW/[b]	B/[c]	BAW/[d]	C/[e]
y-c	l-y	y-c	o-p	0.42	0.67	0.90	0.76	0.43	0.09	0.16
									0.32	0.48

/[a] — BAW: n-butanol-water-acetic acid ⟨12:5:3 v/v⟩; A: ethyl acetate-water-formic acid ⟨10:3:2 v/v⟩; B: water--acetic acid-hydrochloric acid ⟨5:5:1 v/v⟩; C: benzene-n-butanol-pyridine-water (1:5:3:3 v/v), FAWiA: formic acid-amyl alcohol-water-isoamyl alcohol ⟨1:1:1:1 v/v;⟩ HOF — formic acid; HOAc-acetic acid; /[b] — developed with $ZrOCl_2$; /[c] — developed with diazobenzidine; /[d] — developed with aniline phthalane in butanol; /[e] — developed with ammonia solution of $AgNO_3$; l - lemon coloured, o — orange, p — pink, y — yellow.

cosides in the studied specimens of the two taxa demonstrates some essential differences as compared with *B. papyrifera*. *B. neoalascana* and *B. papyrifera* var. *commutata* were found to contain fairly large amounts of hyperoside and isoquercitrin, which were absent in the leaves of the *B. papyrifera* specimen studied (Table 1). The falvonoid components: quercitrin, myricitrin, myricitrin 3-digalactoside, isorhamnetin 3-galactoside, kaempferol 3-rhamno-7-glucoside, quercetin 3-arabinoside, acacetin 7-glucoside, and luteolin 4'-glucoside; were present in the investigated specimens of all three taxa, whereas quercetin 3,7,4'-trimethyl ether and kaempferol, and three unidentified ("a", "b" and "c") were detected in *B. neoalascana* and *B. papyrifera* only. Nevertheless, the above assumptions require corraboration by studies of a larger number of specimens of the afore-said species growing on natural sites. If the composition of flavonoids in the specimens of these taxa from natural localities will be confirmed they should be recognized as two species *(B. neoalascana* and *B. commutata)* separate from *B. papyrifera.*

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Flawonoidy liści kilku gatunków amerykańskich z rodzaju Betula L.

Streszczenie

W liściach *B. lenta* stwierdzono: myrycytrynę, hyperozyd, kwercytrynę, izokwercytrynę, rutynę, 3-arabinozyd kwercetyny, 3-ramno-7-glukozyd kempferolu, najprawdopodobniej 4'-glukozyd luteoliny i 7-rutynozyd luteoliny, hesperydynę oraz przypuszczalnie 3-galaktozyd myrycetyny, 3,7,4'-trójmetylo-kwercetynę i dwuhydrohyperozyd, a także niezidentyfikowany związek "d". U *B. papyrifera* znaleziono: myrycytrynę, kwercytrynę, 3-arabinozyd kwercetyny, 3-dwugalaktozyd myrycetyny, 3-galaktozyd izoramnetyny, 3-ramno-7-glukozyd kempferolu, 7-glukozyd akacetyny, hesperydynę oraz prawdopodobnie 3-galaktozyd myrycetyny, 7-glikozyd skutelaryny i 3,7,4'-trójmetylo-kwercetynę, a także niezidentyfikowane flawonoidy "a", "b" i "c". Na podstawie skadu jakościowego flawonoidów, liczby chromosomów, zasięgu występowania i barwy kory przypuszcza się, że *B. lutea* może być formą mieszańcową między *B. papyrifera* i *B. lenta*. Ponadto sugeruje się wydzielenie *B. neoalascana* i *B. commutata* jako odrębnych gatunków od *B. papyrifera*.