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NATURAL DRUG

FLAVONOIDS FROM *PRUNUS SEROTINA* EHRH.

MONIKA OLSZEWSKA*

Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Łódź,
1 Muszyński Str., 90-151 Łódź, Poland

Abstract: In the course of chemotaxonomic study of the genus *Prunus,* seven flavonol glycosides were isolated from the leaves of *Prunus serotina* Ehrh., characterized by UV and NMR spectroscopy, and identified finally as three quercetin monosides: hyperoside, avicularin, reynoutrin, three quercetin biosides: 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, 3-*O*-(2"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside and 3-*O*-(2"-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside as well isorhamnetin 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside. The presence of determined flavonoids in the flowers was confirmed by TLC.

Keywords: *Prunus serotina* Ehrh.; flavonoids; leaves; flowers; isolation; identification

This paper presents the results of phytochemical analysis of the leaves and flowers of *Prunus serotina* Ehrh. as a part of the chemotaxonomic survey of the genus *Prunus* L. (*Rosaceae*) (1-5).

Prunus serotina Ehrh. (American black cherry, bird cherry, virginian prune, rum cherry) represents the subgenus *Padus*, which contains only ca. 30 species, mainly native to continental climate areas (6). *P. serotina*, the largest of the native cherries, is a very fast growing tree indigenous to North America and planted, mainly in Europe, for timber and for ornamental purposes (handsome foliage) and locally naturalized (6, 7). In Poland it occurs commonly, especially in lowlands on dry soils and in many forest cover types (8).

Until the 90s of the 20th century the virginian prune bark was used in the USA and British official pharmacy in the treatment of irritating coughs, bronchitis and asthma (mainly as the principal ingredient of wild cherry syrup, a popular vehicle for cough syrups), due to the presence of cyanogenic glycosides (9-10). The fruits of *P. serotina* are used in the USA in food industry as a flavouring agent for rum and brandy. Interestingly, the extracts of *P. serotina* show a significant antioxidant activity and, which has been revealed recently (11), may be used for antioxidant cosmetics production. However, except several studies of cyanogenic glycosides and cyanogenesis during development stages (12, 13), phytochemical investigations of the taxon to date have been fragmentary and there has been no systematic exploration of its polyphenolic components, especially flavonoids, which can be connected with the mentioned antioxidant activity. From polyphenols only three flavonols (quercetin, serotrin – probably quercetin 3-glucoside and an unidentified compound with metoxyl group) from the leaves (14), two anthocyanins (cyanidin 3-glucoside and 3-rutinoside) (15, 16) accompanied by elagic acid (16) from the fruits and polymeric leucoanthocyanidins together with protocatechuic acid from the bark (17) were detected previously.

The partial lack of knowledge about the polyphenols of this widely occurring plant with potential use has led to the following examination of the flavonoid components of *P. serotina* leaves and flowers.

EXPERIMENTAL

Plant material

All samples of flowers (complete inflorescences: racemes) and leaves of *Prunus serotina* Ehrh. were collected in the Botanical Garden in Łódź, followed by air-drying in normal conditions and powdering. The leaves, collected in October 2001, were used for isolation, whereas the leaf and flower samples collected in Juni 2002 were the materials for cochromatographical analysis. Voucher specimens were deposited in the Department of Pharmacognosy.

* e-mail – molszewska@pharm.am.lodz.pl, tel.: (+42)6779169, fax: (+42)6788398

Equipment and methods

Melting points (uncorrected) were determined on a Boetius apparatus. UV spectra with usual shift reagents (according to the standard procedure (18)) were made with a Unicam 500 apparatus, ^{1}H and ^{13}C NMR were recorded on a Bruker spectometer DRX 300 and 500 MHz (in DMSO-d_6, TMS as int. standard).

Preparative column chromatography (CC) was performed on a polyamide SC6 (Roth), silica gel 60 (MN) and Sephadex LH-20 (Fluka); preparative TLC on polyamide 11 (Merck); analytical TLC on silica gel 60 precoated plates (Merck); PC on Whatman No 1. For TLC and PC the following solvent systems were employed:

S-1: n-BuOH / AcOH / H_2O (4:1:5, organic phase);

S-2: 15% AcOH;

S-3: EtOAc / HCOOH / H_2O (18:1:1);

S-4: n-BuOH / AcOH / HCOOH / H_2O (100:27:1:5, organic phase);

S-5: EtOH 96% / NH_4OH 25% / H_2O (20:1:4).

Flavonoids were visualized by UV light 366 nm, with NH_3 fumes and by spraying with 1% $AlCl_3$ in MeOH, proanthocyanidins by spraying with 1% vanillin in 36% HCl (19). Sugars were detected by spraying with aniline phthalate solution in n-BuOH and heating at 105°C.

Extraction and isolation

Powdered leaves of *P. serotina* (600 g) were preextracted with petrol followed by $CHCl_3$ in a Soxhlet extractor. Then, it was exhaustively extracted with boiling MeOH and 70% MeOH. Combined methanol extracts were evaporated, dissolved in water and partitioned between Et_2O, EtOAc and n-BuOH. The Et_2O extract (2.6 g) was submitted to CC on the polyamide (eluent: C_6H_6-MeOH with MeOH gradient) to yield five fractions: E-1 ÷ E-5. Fraction E-5 was rechromatographed in the same conditions and gave compounds **I** (150 mg) and **II** (85 mg), finally purified by crystallization from MeOH. The EtOAc (8.0 g) and n-BuOH (7.0 g) extracts were separately submitted to chromatographic gel filtration on Sephadex columns (using MeOH as eluent) to separate flavonoid and proanthocyanidins fractions. The EtOAc-flavonoid fraction was then first chromatographed on the polyamide (eluent: H_2O-MeOH with MeOH gradient). Fractions eluted with 70-80% MeOH were next rechromatographed on silica gel (eluent: EtOAc-MeOH 9: 1) to afford four fractions: EA-1 ÷ EA-4. The EA-4 fraction, after crystallization from MeOH, gave compound **III** (700 mg). The flavonoid fraction obtained from the n-BuOH extract was first separated on the polyamide (eluent: C_6H_6-MeOH with MeOH gradient) to afford two fractions: B-1 and B-2. Fraction B-1 was purified on Sephadex (MeOH as eluent) to obtain compound **IV** (25 mg), whereas fraction B-2 was rechromatographed on the polyamide (eluent: H_2O-MeOH with MeOH gradient 60-80%) and gave compounds **V** (52 mg), **VI** (with spuren of **V** and **VII**) and **VII** (48 mg). Compound **VI** (3.5 mg) was finally purified by preparative TLC on the polyamide in a two-step chromatographic procedure using first 60%, and next 80% MeOH as a solvent system.

Total acid hydrolysis

1-3 mg of a glycoside was refluxed with 5% HCl for 2 h. The hydrolysate was extracted with Et_2O and the obtained extract was washed with water, evaporated to dryness and resolved in MeOH. Identification of the aglycones was done by coPC (S-1) with standards of quercetin (R_f 0.78, isolated from *Prunus spinosa* (1)) and isorhamnetin (R_f 0.85, obtained from *Pyrus communis* (20)). The remaining aqueous solution was evaporated to dryness, resolved in MeOH and the sugars were identified by coPC (S-1) and coTLC (S-5) with authentic standards of L-rhamnose (R_fs: 0.31 (S-1), 0.57 (S-4)), D-glucose (R_fs: 0.15 (S-1), 0.44 (S-4)) and D-galactose (R_fs: 0.13 (S-1), 0.36 (S-4)).

Partial acid hydrolysis

1-2 mg of diglycosides **IV**, **V**, **VII** and 2 mg of a mixture of **V** and **VI** (because of lack of pure **VI**) were refluxed with 5 mL MeOH and 1 mL 15% AcOH for 15 min at 50°C. The obtained monosides were identified in the hydrolysate by coTLC (S-3, S-4) with standards of isorhamnetin 3-*O*-β--D-glucopyranoside (20), isoquercitrin (2) and hyperoside (**III**).

QUERCETIN 3-*O*-α-L-ARABINOFURANOSIDE, AVICULARIN (**I**)

Yellow prisms, m.p. 216-218°C (MeOH); PC R_fs: 0.82 (S-1), 0.36 (S-2); TLC R_f 0.63 (S-3), 0.78 (S-4). UV λ_{max}^{MeOH} nm: 256, 269sh, 300sh, 358; NaOMe 271, 325, 406; $AlCl_3$ 273, 307sh, 335sh, 434; $AlCl_3$-HCl 269, 303sh, 362, 401; NaOAc 274, 323sh, 390; NaOAc-H_3BO_3 262, 301sh, 376. ^{1}H NMR and ^{13}C NMR as in ref. (1).

QUERCETIN 3-*O*-β-D-XYLOPYRANOSIDE, REYNOUTRIN (**II**)

Yellow needles, m.p. 227-231°C (MeOH); PC R_fs: 0.75 (S-1), 0.35 (S-2); TLC R_f 0.44 (S-3),

0.60 (S-4); UV λ_{max}^{MeOH} nm: 257, 267sh, 300sh, 354; NaOMe 270, 326, 410; $AlCl_3$ 274, 305sh, 335, 436; $AlCl_3$-HCl 270, 301sh, 362, 404; NaOAc 268, 323sh, 394; NaOAc-H_3BO_3 261, 268sh, 303sh, 378. ^{1}H NMR (500 MHz) δ, ppm: 12.58 (1H, s, OH-5), 7.54 (1H, d, J=1.9 Hz, H-2'), 7.51 (1H, dd, J=1.9 and 8.5 Hz, H-6'), 6.82 (1H, d, J=8.5 Hz, H-5'), 6.37 (1H, d, J=1.9 Hz, H-8), 6.16 (1H, d, J= 1.9 Hz, H-6), 5.31 (1H, d, J=7.3 Hz, H-1"), 3.60 (1H, dd, J=5.2 and 11.5 Hz, H-5"$_a$), 3.20-3.45 (2H, m, H-3" and H-4"), 3.16 (1H, t, J=8.6 Hz, H-2"), 2.93 (1H, t, J=10.5 Hz, H-5"$_b$). ^{13}C NMR as in ref. (2).

QUERCETIN 3-*O*-B-D-GALACTOPYRANOSIDE, HYPEROSIDE **(III)**

Yellow needles, m.p. 222-226˚C (MeOH); PC R_fs: 0.64 (S-1), 0.41 (S-2); TLC R_f 0.27 (S-3), 0.41 (S-4); UV λ_{max}^{MeOH} nm: 258, 268sh, 300sh, 362; NaOMe 272, 327, 412; $AlCl_3$ 275, 307sh, 330, 435; $AlCl_3$-HCl 269, 303sh, 366, 407; NaOAc 274, 325sh, 410; NaOAc-H_3BO_3 259, 298sh, 370. ^{1}H NMR (500 MHz) δ, ppm: 12.60 (1H, s, OH-5), 7.63 (1H, dd, J=2.2 and 8.5 Hz, H-6'), 7.48 (1H, d, J=2.2 Hz, H-2'), 6.77 (1H, d, J=8.5 Hz, H-5'), 6.36 (1H, d, J=1.9 Hz, H-8), 6.15 (1H, d, J=1.9 Hz, H-6), 5.34 (1H, d, J=7.7 Hz, H-1"), 3.61 (1H, d, J=3.2 Hz, H-4"), 3.53 (1H, dd, J=8.0 and 9.3 Hz, H-2"), 3.42 (1H, dd, J=5.6 and 10.1 Hz, H-6"$_a$), 3.22-3.34 (3H, m, H-3", H-5" and H-6"$_b$). ^{13}C NMR (75.5 MHz) δ ppm: 177.56 (C-4), 164.11 (C-7), 161.23 (C-5), 156.32 (C-2 i C-9), 148.35 (C-4'), 144.73 (C-3'), 133.75 (C-3), 121.76 (C-6'), 121.28 (C-1'), 116.20 (C-5'), 115.30 (C-2'), 103.92 (C-10), 102.31 (C-1"), 98.65 (C-6), 93.53 (C-8), 75.74 (C-5"), 73.42 (C-3"), 71.28 (C-2"), 68.07 (C-4"), 60.56 (C-6").

ISORHAMNETIN 3-*O*-(6"-*O*-α-L-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDE, ISORHAMNETIN 3-*O*-RUTINOSIDE, NARCISSIN **(IV)**

Amorphous yellow powder, m.p. 194-199˚C; PC R_fs: 0.55 (S-1), 0.58 (S-2); TLC R_f 0.10 (S-3), 0.20 (S-4); UV λ_{max}^{MeOH} nm: 255, 271sh, 322sh, 367; NaOMe 272, 328, 418; $AlCl_3$ 268, 303sh, 362, 403; $AlCl_3$-HCl 270, 300sh, 358, 403; NaOAc 280, 328sh, 400; NaOAc-H_3BO_3 255, 270sh, 303sh, 360. ^{1}H NMR δ, ppm: 12.56 (1H, s, OH-5), 7.84 (1H, d, J=1.7 Hz, H-2'), 7.50 (1H, dd, J=1.7 and 8.4 Hz, H-6'), 6.90 (1H, d, J=8.4 Hz, H-5'), 6.40 (1H, d, J=1.6 Hz, H-8), 6.18 (1H, d, J=1.6 Hz, H-6), 5.42 (1H, d, J=7.2 Hz, H-1"), 4.40 (1H, s, H-1'"), 3.82 (3H, s, OMe-3'), 3.69 (1H, br d, J=11.0 Hz, H-6"$_a$), 0.96 (3H, d, J=6.2 Hz, Me-6'"), 3.15-3.40 and 2.97-3.07 (2×m, the remaining rutinosyl protons); ^{13}C NMR: see Table 1.

QUERCETIN 3-*O*-(6"-*O*-α-L-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDE, QUERCETIN 3-*O*-RUTINOSIDE, RUTIN **(V)**

Amorphous yellow powder, m.p. 189-193˚C; PC R_fs: 0.48 (S-1), 0.56 (S-2); TLC R_f 0.07 (S-3), 0.16 (S-4); UV λ_{max}^{MeOH} nm: 256, 266sh, 300sh, 360; NaOMe 272, 328, 410; $AlCl_3$ 273, 303sh, 433; $AlCl_3$-HCl 270, 300, 362, 401; NaOAc 275, 324sh, 392; NaOAc-H_3BO_3 257, 303sh, 380. ^{1}H NMR δ, ppm: 12.61 (1H, s, OH-5), 7.53 (2H, m, J=2.0 and 8.5 Hz, H-2' and H-6'), 6.84 (1H, d, J=8.2 Hz, H-5'), 6.38 (1H, d, J=2.0 Hz, H-8), 6.19 (1H, d, J=2.0 Hz, H-6), 5.34 (1H, d, J=7.3 Hz, H-1"), 4.38 (1H, s, H-1'"), 7.70 (1H, br d, J=10.6 Hz, H-"$_a$), 0.99 (3H, d, J=6.2 Hz, Me-6'"), 3.15-3.41 and 3.05-3.10 (2×m, the remaining rutinosyl protons). ^{13}C NMR: see Table 1.

QUERCETIN 3-*O*-(2"-*O*-α-L-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDE, QUERCETIN 3-*O*-NEOHESPERIDOSIDE **(VI)**

Amorphous yellow powder, m.p. 216-220˚C; PC R_fs: 0.65 (S-1), 0.67 (S-2); TLC R_f 0.11 (S-3), 0.24 (S-4); UV λ_{max}^{MeOH}nm: 255, 264sh, 360; NaOMe 274, 330, 414; $AlCl_3$ 270, 300sh, 364sh, 423; $AlCl_3$-HCl 270, 300, 358, 401; NaOAc 270, 393; NaOAc-H_3BO_3 261, 303sh, 379. ^{1}H NMR δ, ppm: 12.62 (1H, s, OH-5), 7.57 (1H, dd, J=1.8 and 8.5 Hz, H-6'), 7.53 (1H, d, J=1.8 Hz, H-2'), 6.85 (1H, d, J=8.5 Hz, H-5'), 6.42 (1H, d, J=1.1 Hz, H-8), 6.20 (1H, d, J=1.1 Hz, H-6), 5.50 (1H, d, J=7.3 Hz, H-1"), 5.08 (1H, s, H-1'"), 3.73 (1H, br s, H-2'"), 3.60 (1H, br d, J=11.8 Hz, H-6"$_a$), 0.90 (3H, d, J=7.0 Hz, Me-6'"), 2.90-3.54 (m, the remaining sugar protons). ^{13}C NMR: see Table 1.

QUERCETIN 3-*O*-(2-*O*-α-L-RHAMNOPYRANOSYL)-β-D-GALACTOPYRANOSIDE **(VII)**

Amorphous yellow powder, m.p. 209-214˚C; PC R_fs: 0.53 (S-1), 0.75 (S-2); TLC R_f 0.05 (S-3), 0.14 (S-4); UV λ_{max}^{MeOH} nm: 259, 268sh, 300sh, 360; NaOMe 272, 328, 410; $AlCl_3$ 274, 305sh, 332sh, 435; $AlCl_3$-HCl 269, 300sh, 364sh, 403; NaOAc 274, 324, 390; NaOAc-H_3BO_3 262, 300sh, 380. ^{1}H NMR δ, ppm: 12.70 (1H, s, OH-5), 7.72 (1H, dd, J=2.1 and 8.5 Hz, H-6'), 7.48 (1H, d, J=2.1 Hz, H-2'), 6.79 (1H, d, J=8.5 Hz, H-5'), 6.38 (1H, d, J=1.9 Hz, H-8), 6.18 (1H, d, J=1.9 Hz, H-6), 5.63 (1H, d, J=7.7 Hz, H-1"), 5.06 (1H, s, H-1'"), 0.77 (3H, d, J=6.2 Hz, Me-6'"), 3.1-3.80 (m, the remaining sugar protons). ^{13}C NMR: see Table 1.

Table 1. ^{13}C NMR data for compounds **IV**-**VII** (δ, ppm, in DMSO-d_6, at 125 MHz)

Carbon	**IV**	**V**	**VI**	**VII**
Aglycone				
2	157.30	156.55	156.52	156.56
3	133.58	133.28	133.34	133.28
4	177.92	177.32	177.40	177.64
5	161.66	161.19	161.25	161.61
6	99.39	98.69	98.84	99.04
7	164.65	164.21	164.25	164.58
8	94.51	93.57	93.68	93.79
9	157.12	156.41	156.48	156.28
10	104.63	103.89	104.12	104.21
1’	121.67	121.14	121.33	121.45
2’	113.75	115.21	115.36	115.49
3’	147.48	144.74	144.85	145.24
4’	149.85	148.42	148.53	148.74
5’	115.82	116.23	116.38	115.93
6’	122.99	121.56	121.33	122.52
3’-OMe	56.29	-	-	-
Sugar	3-glu	3-glu	3-glu	3-gal
1”	101.70	101.19	98.79	99.15
2”	74.77	74.06	77.42	76.03
3”	76.85	76.44	77.12	74.43
4”	70.69	69.98	70.48	68.91
5”	76.31	75.89	77.05	75.33
6”	67.55	66.97	60.85	60.49
1’”	6”-rha	6”-rha	2”-rha	2”-rha
	101.43	100.72	100.83	100.85
2’”	70.84	70.35	70.48	70.93
3’”	71.11	70.54	70.48	71.05
4’”	72.31	71.83	72.17	72.24
5’”	68.84	68.22	68.30	68.51
6’”	18.07	17.71	17.52	17.57

TLC analysis of proanthocyanidins

The proanthocyanidine fractions, isolated from EtOAc and n-BuOH extracts, were then analysed by TLC in S-3 solvent system (20) to reveal the presence of minimum twelve procyanidine compounds, characterized by the following R_f values: 0.84, 0.81, 0.71, 0.68, 0.65, 0.54, 0.43, 0.36, 0.30, 0.25, 0.17 and 0.07. The mentioned components were present only in traces, with the exception of the compounds exhibited the R_fs 0.84 and 0.71.

Cochromatographic analysis of the flowers

Samples (á 10 g) of the flowers and leaves (2002) were prepared similarly as described for the

isolation material to afford the MeOH extracts, fractionated between Et_2O, EtOAc and n-BuOH. The obtained fractions were finally analysed by coTLC (S-2, S-3) with seven isolated compounds **I-VII** and by TLC (S-3) as a test of the presence of proanthocyanidins.

RESULTS AND DISCUSSION

Seven flavonol glycosides **I-VII** were isolated from the fractionated methanolic leaf extract of *P. serotina* by applying a combination of column chromatography on the polyamide, silica gel and Sephadex LH-20, followed by preparative thin layer chromatography on the polyamide.

Compounds **I-III** showed chromatographic properties characteristic of monosides and upon acid hydrolysis released quercetin and sugars identified with L-arabinose, D-xylose and D-galactose, respectively. The UV spectra analysis indicated the site of glycosylation at position 3 in all cases (18). By the direct comparison (PC, TLC) with authentic standards of avicularin, reynoutrin (both isolated earlier from *P. spinosa* flowers (1, 2)) and hyperoside (isolated earlier from *V. vitis idaea* leaves (21)) and by the observed ^{1}H and ^{13}C NMR spectral data compound **I** was confirmed as quercetin 3-*O*-α-L-arabinofuranoside, avicularin, **II** as quercetin 3-*O*-β-D-xylopyranoside, reynoutrin and **III** as quercetin 3-*O*-β--D-galactopyranoside, hyperoside (1, 2, 22-24).

Compound **IV** was recognized as an isorhamnetin 3-*O*-diglycoside from hydrolysis experiments, its chromatographic behaviour and UV spectral analysis (18). Thus, acid hydrolysis gave isorhamnetin, D-glucose and L-rhamnose. The controlled partial acid hydrolysis gave an intermediate isorhamnetin 3-*O*-β-D-glucopyranoside, suggesting that **IV** is an isorhamnetin 3-*O*-rhamnosylglucoside. In the ^{1}H NMR spectrum, in addition to the signals of five aromatic protons and one metoxyl group of isorhamnetin, the presence of two sugar moieties was evidenced by the two proton signals: at d 5.42 ppm (doublet with diaxial coupling constant $J_{1,2}$=7.2 Hz), assignable to the anomeric β-glucopyranosyl proton and at δ 4.40 (singlet), assignable to the anomeric α--rhamnopyranosyl proton (22). In the ^{13}C NMR spectra, signals corresponding to the anomeric carbons were found at d 101.70 and 101.43 ppm, respectively. A downfield shift of Dd ca. 6.5 ppm of the C-6 glucosyl signal, accompanied by a minor (Δδ ca. 1.2 ppm) upfield shift of the C-5 glucosyl signal [in comparison to C-6 and C-5 glucosyl signals of quercetin 3-glucoside (2, 23)], proved that the terminal rhamnosyl moiety is attached to C-6 of the inner glucosyl moiety and indicated the sugar residue as rutinosyl (23, 25). The remaining signals in NMR spectra of **IV** were in close agreement with the proposed structure. Consequently, **IV** is identified as isorhamnetin 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β--D-glucopyranoside, narcissin (isorhamnetin 3-*O*--rutinoside).

Compound **V** was identified by chromatography (including direct comparison with the standard isolated earlier from *Forsythia viridissima* flowers (26)), hydrolysis results and spectral properties (UV, ^{1}H and ^{13}C NMR) as a quercetin analoque of **IV**, namely quercetin 3-*O*-rutinoside, rutin (18, 22, 23, 25, 26).

Compound **VI** exhibited chromatographic behaviour characteristic of a flavonol diglycoside and upon total and partial acid hydrolysis yielded the same components as **V** (quercetin, sugars and intermediate glycoside). The UV spectrum pointed to the glycosylation position at C-3 of quercetin only (18), and therefore indicated **VI** as a monodesmoside. The interglycosidic linkage in rhamnoglucosyl moiety was determined by NMR experiments. The ^{1}H NMR spectrum showed a singlet at δ 5.08 ppm, attributed to the anomeric α-rhamnopyranosyl proton and a doublet at δ 5.50 ppm (with diaxial coupling constant $J_{1,2}$=7.3 Hz), assignable to the anomeric β--glucopyranosyl proton (22) with chemical shift characteristic of glycosidation in a close position i. e., in C-2 position of the glucosyl residue [Δδ=0.5 ppm, downfield shift in comparing with chemical shift of the anomeric proton signal in quercetin 3-glucoside (2, 22)]. In the ^{13}C NMR spectrum of **VI** the C-1 and C-2 carbon signals of the glucosyl moiety were observed at δ 98.79 and 77.42 ppm, respectively, which proved the suggested interglycosidic bond (1→2) by the recognizable downfield shift of the C-2 carbon signal (Δδ=3.3 ppm) and upfield shift of the C-1 signal (Δδ=2.1 ppm) in comparison with quercetin 3-glucoside (2, 23). Finally, the remaining signals in NMR spectra of **VI** were in close agreement with the indicated structure, quercetin 3-*O*-(2"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, quercetin 3-*O*-neohesperidoside (27-29).

Compound **VII** showed a similar UV, chromatographic and hydrolytic behaviour to **VI**, but D-galactose was detected instead of glucose. Analogically, its ^{1}H NMR spectrum supported a quercetin 3-O--disaccharide with L-rhamnose as a terminal sugar. The anomeric protons resonances were observed at δ 5.06 (singlet), assignable to the α-rhamnopyranosyl and at δ 5.63 ppm (doublet, diaxial $J_{1,2}$=7.7 Hz), assignable to the β-galactopyranosyl (22) with chemical shift characteristic of glycosidation in C-2 po-

sition of the galactosyl moiety (Δδ=0.3 ppm, downfield shift in comparing with chemical shift of the anomeric proton signal in **III** (quercetin 3-galactoside)). In the ^{13}C NMR spectrum of **VII,** the C-1 and C-2 carbon signals of galactosyl moiety were observed at d 99.15 and 76.03 ppm, respectively, which proved the suggested interglycosidic bond (1→2) by the downfield shift of the C-2 carbon signal (Δδ=4.8 ppm) and upfield shift of the C-1 signal (Δδ=3.2 ppm) in comparison with reference compound **III**. The remaining signals in NMR spectra of **VII** were in close agreement with the indicated structure, namely quercetin 3-*O*-(2"-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside (30, 31).

The presence of isolated compounds **I**-**VII** was then determined chromatographically by the comparative TLC and PC analysis in the fractionated methanolic extract of *P. serotina* flowers (inflorescences). The composition of flavonoid complexes of the leaves and flowers appeared to be very similar, consisted mostly of monosides and were especially rich in quercetin 3-galactoside (hyperoside).

All the described flavonoids have been isolated and characterized in taxon *P. serotina* for the first time.

Flavonol 3-*O*-mono- and 3-*O*-diglycosides (mostly quercetin gluco-, galacto- and rhamnosides) occurs commonly in the genus *Prunus* (12). Interestingly, the leaves and flowers of the analysed taxon contain also quercetin pentosides (arabinoside and xyloside), similar to *P. spinosa* (1-3) and isorhamnetin 3-*O*-rutinoside, whose presence has been confirmed, to my knowledge, for the first time in the subgenus *Padus*.

Apart from flavonoid compounds, the proanthocyanidine fraction has been isolated from *P. serotina* leaves and exhibited TLC chromatographic properties characteristic of leucoanthocyanidine dimers and oligomers (19), which are known as biologically active antioxidants (32), but their concentration in the studied material was significantly lower in comparison to flavonoids. The analogous proanthocyanidine fraction was next determined cochromatographically in the flowers.

Finally, in the conducted study the flavonoids have been recognized as the main chemical components, which may be connected with expected antioxidant activity of *P. serotina* leaves and flowers.

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