Flavonoid Aglycones from the Bud Exudates of Three Betulaceae

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Bud Exudates of three Betulaceae were analyzed for minor flavonoid aglycones. Three flavonoids not previously reported were found in *Alnus koehnei,* four in *Betula nigra.* A novel flavonol, morin-3,2′,4′-trimethyl ether, is described for *Ostrya carpinifolia.*

Introduction

The presence of flavonoid aglycones in bud excretions has been stressed and they have been studied in great detail by one of us in the 1970s. Exudate flavonoids were reported for members of the genera *Aesculus* (Hippocastanaceae, [1]), for *Alnus, Betula,* and *Ostrya* (Betulaceae, see [2]), for *Populus* (Salicaceae; see *e.g.* [3] and ref. therein), for *Prunus* (Rosaceae, [4]), and for *Rhamnus* (Rhamnaceae, [4]). Some more recent results were reported and concerned rare flavonoid aglycones in *Betula nigra* [5], phenolic triglycerides in *Populus lasiocarpa* [6], cinnamic acid derivatives in *Populus nigra* [7], and a stilbene in *Alnus viridis* [8]. In the course of these studies, several minor products remained unidentified, due to paucity of material and/or lack of markers. After isolation by preparative TLC, some of these have been identified by direct comparisons with authentic samples; one structure was elucidated by NMR analysis.

Materials and Methods

The origin of Betulaceae bud material, recovery of bud exudates, and separation procedures have been reported in previous papers (*cf.* [2, 5, 9]). The products now dealt with were obtained, in minute amounts, from residues by preparative TLC on silica (solvents: A, toluene–methylethyl ketone 9:1 and B, toluene–dioxane–glacial acetic acid 18:5:1). Fractions were monitored and comparisons with authentic flavonoids were performed on polyamide DC-11 (solvents: C, toluene–petrol$_{100-140}$–methylethyl ketone–MeOH 12:6:2:1, D, toluene–dioxane–MeOH 8:1:1, and E, toluene–methylethyl ketone–MeOH 12:5:3) and on silica (solvents A and B). Chromatograms were viewed in UV$_{366}$ before and after spraying with Naturstoffreagenz A (0.5% in MeOH). 6-Hydroxykaempferol-3,6,7- and -3,6,4′-trimethyl ethers and myricetin-3,7,3′,4′-tetramethyl ether were available in E. W.'s laboratory. Myricetin-3,3′,4′,5′-tetramethyl ether, isolated from *Cistus symphytifolius* [10], was obtained from Th. Vogt. Samples of synthetic 6-hydroxykaempferol-6,7,4′-trimethyl ether as well as of quercetagetin-6,7,4′-trimethyl and 3,6,7,3′-tetramethyl ethers [11] were obtained from T. Horie.

Mass spectra were recorded on a Varian MAT 311 at 70 eV by direct inlet. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Nicolet NT-WB 200 FT spectrometer at 200 and at 50 MHz, respectively.

Compound **1,** isolated from the bud exudate of *Ostrya carpinifolia,* has the following spectral data. UV λ^{MeOH} (nm): 336 (305), 261, 252; +NaOH: 352, 301 sh, 267; +AlCl$_3$: 389, 341, 305, 267; unchanged on addition of HCl; +NaOAc: 343, 300, 265; +H$_3$BO$_3$: 336 (305), 261. MS *m/z* (rel. int. %): 344 (M$^+$, 100), 329 (8), 313 (80), 301 (13), 295 (15), 243 (12), 201 (11), 167 (23), 153 (30). ^{1}H NMR δ (ppm): 12.62 (s, 1 H; 5-OH), 7.39 (d, J = 8 Hz, 1 H; H-6′), 6.73 (d, J = 2.5 Hz, 1 H; H-3′), 6.68 (dd, J = 2.5, 8 Hz, 1 H; H-5′), 6.29 (d, J = 2.5 Hz, 1 H; H-8), 6.18 (d, J = 2.5 Hz, 1 H; H-6), 3.85, 3.81, 3.67 (s, 3 H each; 3 × OMe). ^{13}C NMR δ: 156.0 (C-2), 139.0 (C-3), 177.7 (C-4), 161.4 (C-5), 98.6* (C-6), 165.4 (C-7), 93.8 (C-8), 157.1 (C-9), 104.2 (C-10), 111.7 (C-1′), 158.4 (C-2′), 98.9* (C-3′), 162.6 (C-4′), 105.3 (C-5′), 131.4 (C-6′), 60.0 (3-OMe), 55.5$^{\#}$ (4′-OMe), 55.8$^{\#}$ (2′-OMe) (*, $^{\#}$: values may be interchanged).

Results and Discussion

For the bud exudate of *Alnus koehnei,* 3 flavones and 10 flavonols (Table I) were reported earlier [2] to be present as aglycones. (In [2], quercetagetin-

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Table I. Flavonoid profiles in the bud exudates of three Betulaceae.

Plant species	Known flavonoids	New flavonoids
Alnus koehnei	apigenin-4′-Me, scutellarein-6,4′-diMe, scut-6,7,4′-triMe; kaempferol-4′-Me, 6-hydroxykaempferol-3,6-diMe, 6-OH-kae-3,6,4′-triMe, quercetin, qu-3′-Me, qu-3,7-diMe, qu-3,3′-diMe, qu-7,3′-diMe, qu-7,3′,4′-triMe, quercetagetin-3,6,4′-triMe	quercetagetin-6,7,4′-triMe, queg-3,6,7,3′-tetraMe (6-OH-kae-6,7,4′-triMe confirmed)
Betula nigra	apigenin, ap-7,4′-diMe, scutellarein-6,7-diMe, scut-6,7,4′-triMe, luteolin; kaempferol, kae-3-Me, kae-7-Me, kae-3,7-diMe, kae-3,4′-diMe, kae-7,4′-diMe, 6-hydroxykaempferol-6,7,4′-triMe, quercetin-3-Me, qu-3′-Me, qu-3,7-diMe, qu-3,3′-diMe, qu-3,7,3′-triMe, qu-3,3′,4′-triMe, qu-3,7,3′,4′-tetraMe, myricetin-3,7,3′,4′,5′-pentaMe	scutellarein-6-Me, 6-hydroxykaempferol-3,6,7-triMe, 6-OH-kae-3,6,4′-triMe, myricetin-3,7,3′,4′-tetraMe, myr-3,3′,4′,5′-tetraMe
Ostrya carpinifolia	apigenin-4′-Me, kaempferol-3-Me, kae-7-Me, kae-4′-Me, kae-3,4′-diMe, kae-7,4′-diMe	morin-3,2′,4′-triMe

3,6,4′-trimethyl ether had been listed as **A-3**.) The presence of 6-hydroxykaempferol-6,7,4′-trimethyl ether (mikanin), previously cited as dubious, can now be confirmed (TLC, UV and MS identical with a marker). Trace amounts of quercetagetin-6,7,4′-trimethyl ether (eupatin) and quercetagetin-3,6,7,3′-tetramethyl ether (chrysosplenetin) were identified by direct comparisons (TLC, MS) with authentic samples. Chrysosplenetin is now known to be a rather widespread flavonol, while eupatin was found so far only in three genera of Asteraceae (*Brickellia, Eupatoria* and *Heterotheca*).

Twenty flavonoid aglycones, including 5 flavones and 15 flavonols (Table I), have been reported earlier for the bud exudate of *Betula nigra* [2, 5,12]. From residual fractions we now identified in addition: scutellarein-6-methyl ether, the 3,6,7- and the 3,6,4′-trimethyl ethers of 6-hydroxykaempferol, and myricetin-3,7,3′,4′- and 3,3′,4′,5′-tetramethyl ethers. The two latter products are readily distinguishable by polyamide TLC where they show an important R_f difference (solvent C: myr-3,7,3′,4′-tetra-Me R_f 0.75, myr-3,3′,4′,5′-tetra-Me R_f 0.44; important fragments in the MS at m/z 374 (M$^+$), 360, 359, 344, and 331). These two tetramethyl ethers of myricetin are rare flavonols (*cf.* [13]); thus far each has only been reported from four plant genera. They occur together in *Cistus symphytifolius* leaf resin [12].

For *Ostrya carpinifolia* eight bud exudate flavonoids were known earlier, one flavone and seven flavonols (Table I), and the presence of several unknowns had been indicated in [2]. One of these, compound **1,** was isolated from residual fractions in the minimum amount needed to run UV, MS and NMR spectra. On polyamide TLC, it forms a very dark and somewhat reddish/violet spot (UV$_{366}$), that turns greenish-yellow after spraying with "Naturstoffreagenz A". It thus behaves much like kaempferol-3,7-dimethyl ether, but its R_f is slightly higher (solvent C: 0.43 for **1,** 0.50 for kae-3,7-diMe). The mass spectrum (see Materials and Methods section) indicates that it is either a flavone or a flavonol with two hydroxy and three methoxy groups. The presence of an important peak, in the MS, at m/z 313 (M$^+$−31) further indicates 3,2′-methoxy substitution [14]. The UV spectrum also exhibits characteristic features of 3,2′-dioxygenated flavonols, Band I appearing at shorter wavelength than expected for flavonols and about half as intense as Band II [14]. The structure was finally elucidated by studies of the ^{1}H and ^{13}C NMR spectra. In addition to signals of two *meta*-coupled protons (H 6 and H 8), a chelated OH at low field (5-OH) and three OMe groups, the ^{1}H spectrum of **1** showed three aromatic B ring proton signals, two of which were coupled to the third but not to each other. The chemical shift of one of these, an *ortho* doublet, appeared considerably downfield from the other two (a *meta* doublet and an *ortho, meta* double-doublet) unlike spectra of 3′,4′-oxygenated B rings wherein the *ortho* doublet appears substantially *upfield* of the other two B ring proton signals. The foregoing suggests

2′,4′-oxygenation for **1.** The A and C signals of **1** in the ^{13}C spectrum are nearly identical to those of quercetin-3-methyl ether, and indicate 5,7-diOH and 3-OMe substitution. The B ring signals agree well with those of morin [15] if allowances are made for having OMe at C2′ and C4′ rather than OH. Product **1** is thus identified as 5,7-dihydroxy-3,2′,4′-trimethoxy flavone, a trimethyl ether of the long known, though narrowly distributed, flavonol morin. To the best of our knowledge it is not only a novel natural flavonoid, but it is also the first methyl derivative of morin ever found in nature. Its synthesis was reported in 1929 [16], but unfortunately we cannot compare the melting point, the only data available at that time, since the paucity of our sample does not allow to measure the melting point.

In Table I we give a survey on the flavonoid profiles of the three Betulaceae studied, listing the previously known and the newly added products separately.

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