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FLAVONOID GLYCOSIDES FROM *BETULA PUBESCENS* AND *BETULA PENDULA*

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An hplc method for the separation of flavonoid glycosides from *Betula* species was recently developed (1). Using diode-array technology seven flavonoid glycosides were identified based on their characteristic uv spectra. Reference substances were used to identify rutin, hyperin, and quercitrin, which were previously reported in *Betula* species (2,3). By up-scaling of this hplc method to low pressure liquid chromatography (lplc), six flavonoid glycosides could be isolated. In addition to hyperin and quercitrin, myricetin-3-galactoside was obtained, which was known to be present in various *Betula* species (4). Now quercetin-3-glucuronide, quercetin-3-arabinofuranoside, and quercetin-3-arabinopyranoside are reported for the first time as being present in *Betula pubescens* Ehrhart and *Betula pendula* Roth (Betulaceae).

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *B. pubescens* and *B. pendula* were collected in Birmensdorf, 5 km from Zurich, Switzerland. Voucher specimens are deposited in the Swiss Federal Institute of Forestry Research, Birmensdorf, Switzerland.

EXTRACTION AND ISOLATION.—Air-dried, finely powdered leaves (500 g) from both species were separately and exhaustively extracted with 80% MeOH at 40°. The extracts were concentrated in vacuo, and after adding 1 liter of H_2O, the extracts were filtered. Chlorophyll was removed by extraction with light petrol; then the flavonoid glycosides were exhaustively extracted with EtOAc, and extracts of 5.8 g from *B. pubescens* and of 6.2 g from *B. pendula* were obtained. Most of the hyperin was removed by crystallization from both extracts. The remaining residue was chromatographed on a Sephadex-LH-20 column using a step-gradient process with 30-100% MeOH to afford six fractions. These fractions were chromatographed on C-18 reversed phase material with lplc. The mobile phase, optimized with the "PRISMA"-model for hplc (5), was applied to lplc by scaling up.

From fraction 1, quercetin-3-glucuronide was isolated in addition to hyperin with a mobile phase of 15.0% THF, 1.2% acetonitrile, 1.5% MeOH, and 82.3% H_2O. With the same mobile phase, myricetin-3-galactoside was isolated from fraction 2. From fraction 4 quercetin-3-arabinopyranoside and quercitrin were obtained with a mobile phase of 1.4% THF, 16.3% acetonitrile, 2.5% MeOH, and 79.8% H_2O. This latter mobile phase was also used for the isolation of quercetin-3-arabinofuranoside as well as quercitrin and quercetin-3-arabinopyranoside from fraction 5.

IDENTIFICATION.—All flavonoid glycosides were identified by hptlc, hplc, and spectral data (uv, nmr, ms) and are in agreement with those in the literature.

Full details on isolation and identification of the compounds are available on request.

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MINOR ALKALOIDS FROM *NICOTIANA TABACUM*

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Systematic alkaloid investigations of various cultivated strains (Coker-319, Virginia Delcrest, Kallo, Palmonostor) of *Nicotiana tabacum* L. (Solanaceae) were carried out with hplc. The presence of 2,4′-dipyridyl and 4,4′-dipyridyl could be presumed in the Palmonostor strain due to the retention data and uv spectra obtained by diode array detection. The other three dipyridyl isomers have been known for a long time (1,2). In 1980, Saint-Jalm and Moree-Testa (3) identified 2,4′-dipyridyl in tobacco smoke with a gc-ms method. Now 2,4′-dipyridyl and 4,4′-dipyridyl are reported for the first time being present in *N. tabacum*.

EXPERIMENTAL

PLANT MATERIAL.—Leaves of the *N. tabacum* strain Palmonostor were cultivated and collected in Albertirsa, 60 km from Budapest, Hungary. A voucher specimen is deposited in the Institute of Tobacco-Research, Debrecen, Hungary.

EXTRACTION AND ISOLATION.—Air dried, finely powdered leaves (40 kg) were exhaustively extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated in vacuo and mixed with 5% HCl. The acidic extract was alkalinized with NaOH to pH 9 and extracted with $CHCl_3$, this being repeated twice.

The $CHCl_3$ phase was then washed with H_2O and dried, and the solvent was removed to give an alkaloid mixture which was then chromatographed on Si gel. The solvent strength (4) of the mobile phase (containing EtOH, dioxane, hexane, and 0.5% NH_3) was gradually increased from 0.4 to 1.8. The dipyridyl fraction (containing 2,3′-dipyridyl, 2,4′-dipyridyl, 3,3′-dipyridyl, and 4,4′-dipyridyl) was concentrated in vacuo. The mobile phase was optimized with the help of "PRISMA"-model (4, 5) on tlc-plates. The optimized mobile phase was transferred to the sequential centrifugal layer chromatography (sclc) (6) using a diluting factor (7) which was calculated from the tlc pre-assay. The isolation of the 2,4′-dipyridyl and 4,4′-dipyridyl was carried out by sclc with the recycling technique (8), using 1.9% THF, 1.59% dioxane, 1.39% methoxyethanol, and 95.12% hexane as mobile phase.

IDENTIFICATION.—Both alkaloids were identified by standard methods: hptlc, hplc, mp, spectral data (uv, ^1H nmr, eims) and compared with authentic samples (9). Full details on isolation and identification of the compounds are available on request.

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