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A new flavone glycoside from the fern *Pteris cretica*

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Abstract. A new flavone glycoside isolated from the fern *Pteris cretica* has been shown to be luteolin 7-O-robinobioside (**1**) by chemical and spectral methods. In addition luteolin 7-O-rutinoside (**2**) and luteolin 7-O-glucoside (**3**) have been isolated from this plant. Flavonoid **2** is reported for the first time in ferns.
Key words. *Pteris cretica*; ferns; luteolin 7-O-robinobioside; flavone glycosides.

It is well known that flavonoids of ferns are of chemotaxonomic and phylogenetic interest[1,2] but flavonoid data for some fern families are scanty. Previous work on the flavonoids of the genus *Pteris* has led to the isolation[3] of an anthocyanin (apigenidin 5-O-glucoside) from three *Pteris* species (*P. longipinnula*, *P. quadriaurita* and *P. vittata*); in addition a flavonol glycoside (an acylated quercetin 3-O-glucoside) has been reported[4] from *P. grandifolia*, and flavone glycosides (not identified) based on luteolin and apigenin have been found[5] in *P. cretica*. Very recently a new flavonoid (8-C-rhamnosylluteolin 7-O-rhamnoside) has been isolated[6] from *P. cretica*. The present paper deals with the presence of three luteolin glycosides in this fern.

Materials and methods

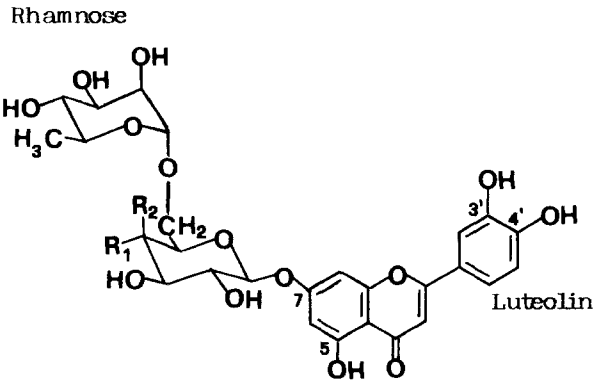
For paper chromatography and TLC the solvent mixtures used, with their abbreviations, were as follows: A, 1-butanol-acetic acid-water (4:1:5, upper phase); B, acetic acid-water (15:85); C, 1-butanol-ethanol-water (4:1:2.2); D, water; E, acetic acid-concentrated HCl-water (30:3:10); F, toluene-chloroform-acetone (8:5:7); G, benzene-pyridine-formic acid (36:9:5); H, 1-butanol-pyridine-water (6:4:3); I, 1-butanol-acetic acid-ethyl ether-water (9:6:3:1); L, chloroform-ethyl acetate (1:1).

Aerial parts (190 g) of *Pteris certica* (collected in the Botanical Garden of the University of Naples) were homogenized and extracted three times with boiling 95% ethanol; the combined extracts were filtered, concentrated to a small volume in vacuo and re-filtered. Flavonoids **1** (circa 15 mg), **2** (circa 18 mg) and **3** (circa 20 mg) were isolated by preparative paper chromatography on Whatman 3MM paper in solvent A. Bands were cut off, eluted with 95% ethanol, concentrated in vacuo and rechromatographed in solvents B and C. Further purification was carried out by Sephadex LH-20 column chromatography, eluting with methanol.

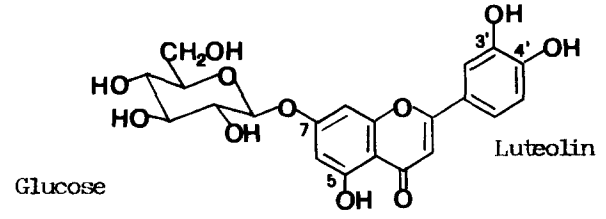
Results and discussion

Colour reactions (brown to yellow in UV + NH_3), chromatographic behaviour (R_f values on Whatman No 1 paper: 0.35 in solvent A; 0.30 in solvent B; 0.46 in solvent C; 0.07 in solvent D) and UV-spectral analysis in the presence of the customary shift reagents[7] (λ_{max}^{MeOH} nm: 253, 265 (sh), 350; +NaOMe 262, 399; +$AlCl_3$ 270, 292 (sh), 337 (sh), 421; +$AlCl_3$/HCl 270, 291 (sh), 350, 386; +NaOAc255, 400; +NaOAc/H_3BO_3 258, 370) were consistent with flavonoid **1** being a flavone glycoside with free hydroxyl groups at positions 5, 3′ and 4′. Total acid hydrolysis with 2 N HCl (1 h at 100 °C) gave luteolin, D-galactose and L-rhamnose; controlled acid hydrolysis with 10% acetic acid (3.5 h under reflux) gave, in addition to the products of total acid hydrolysis, robinobiose (6-O-α-L-rhamnopyranosyl-D-galactose). Luteolin was identified by paper chromatography with an authentic sample (solvents A, C and E), SiO_2 TLC (solvents F and G) and UV-spectral analysis with the customary shift reagents[7]; the sugars were identified by paper co-chromatography (solvents A and H) and SiO_2 TLC (solvent I).

These results suggested that flavonoid **1** might be luteolin 7-O-robinobioside, a new natural product. The structure of this compound was confirmed as follows. Methylation with methyl iodide in dimethylformamide in the presence of silver oxide (18 h in the dark at room temperature) followed by acid hydrolysis with 0.3 N HCl (4 h under reflux) gave 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-galactose, which were identified by paper co-chromatography with authentic samples according to Petek[8], and by SiO_2 TLC (solvent L). The FAB mass spectrum showed $[M + H]^+$ at m/z 594 ($C_{27}H_{29}O_{15}$ requires 593). The ^1H NMR spectrum (DMSO-d_6) showed a doublet at δ 1.11 (J = 6 Hz, rhamnosyl methyl group), a multiplet at δ 3.21–3.91 (rhamnosyl four protons and galactosyl six protons), a doublet at δ 4.58 (J = 4 Hz, rhamnosyl anomer), a doublet at δ 5.10 (J = 8 Hz, galactosyl anomer), a doublet



Flavonoid	R_1	R_2	
1	H	OH	(Galactose)
2	OH	H	(Glucose)



Flavonoid 3

at δ 6.45 (J = 2.1 Hz, H-6), a singlet at δ 6.73 (H-3), a doublet at δ 6.82 (J = 2.1 Hz, H-8), a doublet at δ 6.92 (J = 8 Hz, H-5′) and a doublet at δ 7.44 (J = 8 Hz, H-2′ and H-6′).

Flavonoid **2** was identified as luteolin 7-O-rutinoside by colour reactions, UV-spectral analysis in the presence of usual shift reagents[7], total acid hydrolysis (to give luteolin, D-glucose and L-rhamnose), controlled acid hydrolysis (to give rutinose (6-O-α-L-rhamnopyranosyl-D-glucose) in addition to the products of total acid hydrolysis) and paper co-chromatography with an authentic sample (solvents A, B and C). Identification was confirmed by the FAB mass spectrum which showed $[M + H]^+$ at m/z 594 ($C_{27}H_{29}O_{15}$ requires 593), ^1H NMR spectrum and Khun methylation followed by acid hydrolysis which gave 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-glucose.

Flavonoid **3** was identified as luteolin 7-O-glucoside by colour reactions, UV-spectral analysis in the presence of usual shift reagents[7], total acid hydrolysis, treatment with β-glucosidase (to give luteolin and D-glucose) and paper co-chromatography with an authentic sample (solvents A, B and C). Identification was confirmed by FAB mass spectrum which showed $[M + H]^+$ at m/z 449 ($C_{21}H_{20}O_{11}$ requires 448) and ^1H NMR spectrum. Robinobiose (6-O-α-L-rhamnopyranosyl-D-glucose), the disaccharide component of flavonoid **1**, does not appear to have been reported before in the flavone series; in addition this sugar is here reported for the first time in the flavonoids of ferns. Luteolin 7-O-rutinoside (**2**) is a new constituent of ferns. Luteolin 7-O-glucoside (**3**) is one of the most common flavone glycosides, but has previously been found only in two fern genera[9] (*Dryopteris* and *Arachnoides*). From the biosynthetic point of view, the presence of luteolin 7-O-rutinoside (**2**) and luteolin 7-O-glucoside (**3**) in *Pteris cretica* suggests but does not prove that in the biosynthesis of flavone diglycoside (**2**) the monosaccharides are attached to the appropriate flavone successively rather than as a preformed disaccharide.

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