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# FLAVONOL GLYCOSIDES FROM *PTERIDIUM AQUILINUM*

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**Key Word Index**—*Pteridium aquilinum*; Dennstaedtiaceae; flavonol glycosides; quercetin 3-*O*-β-laminaribioside; isorhamnetin 3-*O*-β-laminaribioside.

**Abstract**—Two new flavonol glycosides from aerial parts of *Pteridium aquilinum* were identified as quercetin 3-*O*-β-laminaribioside and isorhamnetin 3-*o*-β-laminaribioside by chemical and spectroscopic methods.

## INTRODUCTION

Previous work on the flavonoids of *Pteridium aquilinum* (L.) Kuhn has led to the isolation of a number of flavonol *O*-glycosides. These include kaempferol 5-glucoside [1], 3-glucoside [2], 3-*p*-coumaroylglucoside [2] and 3-rhamnosylglucoside [3] and quercetin 7-glucoside [4], 3-glucoside [5] and 3-rutinoside [5]. In addition, the presence of proanthocyanidins in this fern has been reported [6]. The current report concerns the presence of two new flavonol glycosides (**1** and **2**) in aerial parts of *P. aquilinum*.

## RESULTS AND DISCUSSION

Flavonoids **1**–**4** were isolated from an ethanolic extract of aerial parts of *P. aquilinum*. Colour reactions (brown to yellow in UV + $NH_3$), chromatographic behaviour (see Experimental) and UV spectral analysis in the presence of the customary shift reagents [7]: $\lambda_{max}^{MeOH}$ nm: 257, 265 (*sh*), 357; + $AlCl_3$ 273, 303 (*sh*), 431; + $AlCl_3$–HCl 268, 301 (*sh*), 361 (*sh*), 399; + NaOAc 268, 365; + NaOAc–$H_3BO_3$ 262, 377; + NaOMe 270, 324 (*sh*), 404 (increase in intensity) suggested that flavonoid **1** may be a flavonol glycoside with free hydroxyl groups at positions 5, 7, 3′ and 4′. Total acid hydrolysis gave quercetin and D-glucose while controlled acid hydrolysis gave quercetin, D-glucose and laminaribiose (3-*O*-β-D-glucosyl(1 → 3)-D-glucose). These results suggested that **1** is quercetin 3-*O*-laminaribioside, a new natural product. The structure of this compound was confirmed as follows. Kuhn methylation followed by acid hydrolysis gave 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The FAB mass spectrum showed a quasi-molecular ion at *m/z* 627 $[M + H]^+$ ($C_{27}H_{30}O_{17}$ requires 626). The $^1H$ NMR spectrum (DMSO-$d_6$) showed a multiplet in the range δ3.08–3.95 (laminaribiosyl 12 protons), a doublet at δ4.63 ($J$ = 8 Hz, glucosyl anomer), a doublet at δ5.73 ($J$ = 8 Hz, glucosyl anomer), a doublet at δ6.18 ($J$ = 1.9 Hz, H-6), a doublet at δ 6.40 ($J$ = 1.9 Hz, H-8), a doublet at δ 6.88 ($J$ = 8.3 Hz, H-5′), a doublet of doublets at δ 7.20 ($J$ = 1.9 Hz, 8.3 Hz, H-6′) and a doublet at δ 7.32 ($J$ = 1.9 Hz, H-2′).

Colour reactions (brown to yellow in UV + $NH_3$), chromatographic behaviour (see Experimental) and UV spectral analysis in the presence of the usual shift reagents [7]: $\lambda_{max}^{MeOH}$ nm: 256, 265 (*sh*), 354; + $AlCl_3$ 266, 299 (*sh*), 364 (*sh*), 398; + $AlCl_3$/HCl 267, 299 (*sh*), 354, 396; + NaOAc 267, 360, 385 (*sh*); + NaOAc–$H_3BO_3$ 260, 360; + NaOMe 272, 408 (increase in intensity) suggested that compound **2** may be a flavonol glycoside with free hydroxyl groups at positions 5, 7 and 4′. Total acid hydrolysis of **2** gave isorhamnetin and D-glucose; controlled acid hydrolysis gave laminaribiose in addition to the products of total acid hydrolysis. These results suggested that **2** is isorhamnetin 3-*O*-laminaribioside, a new natural product. The structure of this compound was confirmed as follows. Kuhn methylation followed by acid hydrolysis gave 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The FAB mass spectrum showed a quasimolecular ion at *m/z* 641 $[M + H]^+$ ($C_{28}H_{32}O_{17}$ requires 640). The $^1H$ NMR spectrum (DMSO-$d_6$) showed a multiplet in the range δ3.05–3.92 (laminaribiosyl 12 protons), a singlet at δ3.79 (methoxyl three protons), two doublets at δ4.64 and 5.75 (each $J$ = 8 Hz, two glucosyl anomers), a doublet at δ6.19 ($J$ = 2 Hz, H-6), a doublet at δ6.43 ($J$ = 2 Hz, H-8), a doublet at δ 6.93 ($J$ = 8 Hz, H-5′), a doublet of doublets at δ7.53 ($J$ = 2.8 Hz, H-6′) and a doublet at δ7.90 ($J$ = 2 Hz, H-2′).

Flavonoids **3** and **4** were identified as kaempferol 3-*O*-β-glucoside (astragalin) and kaempferol 3-*O*-β-galactoside (trifolin), respectively by total acid hydrolysis, controlled acid hydrolysis, β-glucosidase treatment, UV spectral analysis in the presence of the customary shift reagents [7] and paper chromatography with authentic samples. Identifications were confirmed by positive FAB mass spectra and $^1H$ NMR spectra.

Laminaribiose (the disaccharide of **1** and **2**) has recently been reported [8] for the first time as a sugar moiety of fern flavonoids. Isorhamnetin (the aglycone of **2**) is here reported for the first time as the aglycone of flavonoid glycosides in Dennstaedtiaceae.

## EXPERIMENTAL

*Plant material.* Aerial parts of *Pteridium aquilinum* (L.) Kuhn subspecies *aquilinum* were collected in Potenza (Italy) in the spring of 1992. The fern was identified by Dr R. Nazzaro (Dipartimento di Biologia Vegetale dell'Università Federico II, Naples, Italy). A voucher specimen has been deposited in the Herbarium Neapolitanum (NAP) of the University of Naples.

*Isolation.* Aerial parts of *P. aquilinum* were homogenized and extracted × 3 with hot EtOH. The combined extracts were filtered, concd and re-filtered. Flavonoids **1**–**4** were isolated by prep PC on Whatman 3MM paper in BAW. They were eluted with EtOH, concd and rechromatographed in 15% HOAc and BEW. Further purification was carried out on Sephadex LH-20 CC eluting with MeOH. $R_f$ values for **1** and **2** (on Whatman No 1 paper) are: BAW 0.44, 0.46; 15% HOAc 0.46, 0.51; $H_2O$ 0.18, 0.19.

*Hydrolysis procedures.* Total acid hydrolysis was carried out with 2M HCl (2 hr at 100°) and controlled acid hydrolysis was carried out with 10% HOAc (3.5 hr under reflux). Kaempferol, quercetin and isorhamnetin were identified by UV spectral analysis with the customary shift reagents [7], PC (4 solvents) and polyammide TLC (2 solvents). D-Glucose, D-galactose and laminaribiose were identified by co-PC (4 solvents) and silica gel TLC.

*Methylation of* **1** *and* **2**. Flavonoids were methylated with MeI in $HCONMe_2$ in the presence of $Ag_2O$ (18 hr in the dark at room temp.) and subsequently hydrolysed with 0.3 M HCl (4 hr under reflux). 2,4,6-Tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose were identified by CO-PC (solvent systems: octane–2-propanol–10% $NH_3$, 50:25:2 and isooctane (2,2,4-trimethylpentane)–2-propanol–10% $NH_3$, 65:25:2) [9] and silica gel TLC.

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