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RHAMNETIN 3-*O*-LAMINARIBIOSIDE FROM *PTERIDIUM AQUILINUM*

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Key Word Index—*Pteridium aquilinum*; Dennstaedtiaceae; flavonol glycosides; flavonoid aglycones; rhamnetin 3-*O*-β-laminaribioside.

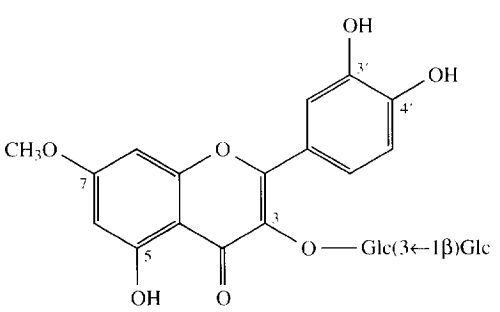
Abstract—A new flavonol glycoside from aerial parts of *Pteridium aquilinum* was characterized as rhamnetin 3-*O*-β-laminaribioside by chemical and spectral methods.

INTRODUCTION

In spite of the fact that polyphenolic analyses of ferns are of taxonomic and phylogenetic interest, flavonoid data available are limited for many fern families [1]. Previous work on the flavonoids of *Pteridium aquilinum* has led to the identification of a number of flavonol glycosides [2–7]; in addition, the presence of proanthocyanidins in this fern has been reported [8]. The present paper deals with the isolation from aerial parts of *Pteridium aquilinum* of rhamnetin 3-*O*-β-laminaribioside (**1**), a new natural product. In addition, kaempferol 3-*O*-(6″-feruloylglucoside) (**2**), kaempferol 3-*O*-(6″-*p*-coumaroylglucoside) (**3**), kaempferol (**4**) and apigenin (**5**) have been found in this plant material.

RESULTS AND DISCUSSION

Five flavonoids (**1**–**5**) have been isolated from an ethanolic extract of aerial parts of *Pteridium aquilinum*. Colour reactions (brown to yellow in UV + NH_3), R_f data (see Experimental) and UV spectral analysis in the presence of the customary shift reagents [9]: λ_{max}^{MeOH} nm: 258 (sh), 265, 355; +$AlCl_3$ 270, 310 (sh), 338, 425; +$AlCl_3$/HCl 270, 298 (sh), 341, 395; +NaOAc 268, 391; +NaOAc/H_3BO_3 261, 370; +NaOMe 270, 404 (increase in intensity), suggested that **1** is a flavonol glycoside with a free hydroxyl group at position 5 (shift with $AlCl_3$/HCl) and a free *o*-dihydroxyl group at positions 3′, 4′ (shift with NaOAc/H_3BO_3 as well as comparison of $AlCl_3$ shift with $AlCl_3$/HCl shift). Total acid hydrolysis gave rhamnetin and D-glucose; controlled acid hydrolysis gave laminaribiose (3-*O*-β-D-glucosyl-D-glucose), in addition to the products of total acid hydrolysis. Kuhn methylation followed by acid hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose and quercetin 5,7,3′,4′-tetramethyl ether. These results show that the isolated flavonoid is rhamnetin 3-*O*-laminaribioside (**1**), a new natural product. The structure of this substance was confirmed as follows. The positive FAB mass spectrum showed a quasimolecular ion $[M + H]^+$ at *m/z* 641 ($C_{28}H_{32}O_{17}$ required 640). The ^1H NMR spectrum (300 MHz; CD_3OD) showed a multiplet at δ 3.02–3.97 (laminaribiosyl 12 protons), a singlet at δ 3.61 (methoxyl three protons), a doublet at δ 4.51 (J = 8 Hz, glucosyl anomer), a doublet at δ 5.70 (J = 8 Hz, glucosyl anomer), a doublet at δ 6.21 (J = 2 Hz, H-6), a doublet at δ 6.41 (J = 2 Hz, H-8), a doublet at δ 6.88 (J = 8.3 Hz, H-5′), a doublet at δ 7.61 (J = 2.1 Hz, H-2′) and a doublet of doublets at δ 7.66 (J = 2.2 Hz, 8.4 Hz, H-6′). Flavonoids **2** and **3** were identified as kaempferol 3-*O*-(6″-feruloylglucoside) and kaempferol 3-*O*-(6″-*p*-coumaroylglucoside) (tribuloside), respectively, by UV spectral analysis with the customary shift reagents [9], total acid hydrolysis, controlled acid hydrolysis, alkaline hydrolysis, ^1H and ^{13}C NMR spectra and positive FAB mass spectrum. Identification of kaempferol 3-*O*-(6″-*p*-coum-



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aroylglucoside) was confirmed by paper co-chromatography with an authentic sample. Kaempferol 3-*O*-(6″-feruloylglucoside) is a new fern constituent and has only recently been reported as a new natural product from *Polylepis incana* (Rosaceae) [10]. The presence of kaempferol 3-*O*-(6″-*p*-coumaroylglucoside) in *P. aquilinum* has previously been reported [2], but the position of the acyl group is reported here for the first time. Flavonoids **4** and **5** were identified as kaempferol and apigenin, respectively, by UV spectral analysis with the customary shift reagents [9], EIMS and paper chromatography. The presence of these free aglycones in *P. aquilinum* is reported here for the first time.

EXPERIMENTAL

Plant material. Aerial parts of *Pteridium aquilinum* (L.) Kuhn subspecies *aquilinum* were collected in Potenza (Italy) in spring 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 **1–5** 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 a Sephadex LH-20 CC eluting with MeOH. R_f values for **1** (on Whatmann No. 1 paper) are: BAW, 0.45; 15% HOAc 0.51.

Hydrolysis procedures. Total acid hydrolysis was carried out with 2 M HCl (2 hr at 100°); controlled acid hydrolysis was carried out with 10% HOAc (3.5 hr under reflux); alkaline hydrolysis was carried out with 2 M NaOH (2 hr at room temp in a sealed tube). Rhamnetin and kaempferol were identified by UV spectral analysis with the customary shift reagents [9], PC (four solvent systems) and EIMS. D-glucose and laminaribiose were identified by co-PC (four solvent systems) and silica gel TLC. Ferulic acid and *p*-coumaric acid were identified by co-PC (four solvent systems), silica-gel TLC, paper electrophoresis and UV spectroscopy. Kaempferol 3-*O*-*β*-glucoside (obtained by alkaline hydrolysis of **2** and **3**) was identified by co-PC (four solvent systems), UV spectral analysis with the customary shift reagents [9], total acid hydrolysis and treatment with *β*-glucosidase.

Methylation. Flavonoid **1** was methylated with MeI in $HCONMe_2$ in the presence of Ag_2O (18 hr in the dark at room temp. under stirring) 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 [11] and silica gel TLC. Quercetin 5,7,3′,4′-tetramethyl ether was identified by UV spectral analysis with the customary shift reagents [9] and EIMS.

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