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KAEMPFEROL 7-*O*-RHAMNOSIDE-4′-*O*-GLUCOSIDE FROM *PTERIDIUM AQUILINUM*

FILIPPO IMPERATO

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Key Word Index—*Pteridium aquilinum*; Dennstaedtiaceae; flavonol glycosides; kaempferol 7-*O*-rhamnoside-4′-*O*-glucoside; quercetin 3-*O*-fructoside.

Abstract—A new flavonol glycoside from aerial parts of *Pteridium aquilinum* was identified as kaempferol 7-*O*-rhamnoside-4′-*O*-glucoside by chemical and spectral methods. In addition a mixture of quercetin 3-*O*-fructoside and isoquercitrin was found in this plant material. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Previous work on the flavonoids of *Pteridium aquilinum* (L.) Kuhn has led to the identification of proanthocyanidins and flavonol glycosides [1], including most recently the 3-laminaribiosides of quercetin and isorhamnetin [2], kaempferol 3-*O*-(5″-feruloylaspioside) [3] and kaempferol 3-*O*-(X″-*p*-coumaroyl-X″-heptaglycylapioside [4]. This paper deals with the identification of a new flavonol diglycoside (**1**) from *P. aquilinum* ssp. *aquilinum* together with a mixture of quercetin 3-*O*-fructroside and isoquercitrin.

RESULTS AND DISCUSSION

Two bands (B1 and B2) were isolated from an ethanolic extract of the aerial parts of *P. aquilinum*. Colour reactions (yellow to bright yellow in UV+NH_3), chromatographic behaviour (see Experimental) and UV spectral analysis in the presence of the customary shift reagents [5]: λ_{max}^{MeOH} nm: 265, 368; +NaOMe 277, 402; +$AlCl_3$ 274 301 (sh), 340, 421; +$AlCl_3$–HCl 269, 304 (sh), 340, 420; +NaOAc 267, 313, 388; +NaOAc–H_3BO_3 265, 370, suggested that band 1 may be a flavonol glycoside with free hydroxyl groups at positions 3 and 5. Both total acid hydrolysis and controlled acid hydrolysis gave kaempferol, D-glucose and L-rhamnose. Treatment with β-glucosidase gave kaempferol 7-*O*-rhamnoside. These results show that band 1 is kaempferol 7-*O*-rhamnoside-4′-*O*-glucoside (**1**), a new natural product. The structure of **1** was confirmed as follows. The negative FAB mass spectrum gave a quasimolecular ion $[M-H]^-$ at *m/z* 593 ($C_{27}H_{30}O_{15}$ requires 594) and significant ions at *m/z* 447 $[(M-H)-146]^-$ (loss of rhamnosyl moiety), *m/z* 431 $[(M-H)-162]^-$ (loss of glucosyl moiety) and *m/z* 285 (aglycone). Kuhn methylation followed by acid hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose and kaempferol 3,5-dimethyl ether. The ^{1}H NMR spectrum (DMSO-d_6) showed a doublet at δ 1.14 (J = 6 Hz, rhamnosyl methyl group), a multiplet at δ 3.18–3.98 (glucosyl six protons and rhamnosyl four protons), a doublet at δ 4.85 (J = 7 Hz, glucosyl H-1), a broad singlet at δ 5.35 (rhamnosyl H-1), a doublet at δ 6.45 (J = 2 Hz, H-6), a doublet at δ 6.78 (J = 2 Hz, H-8), a doublet at 7.16 (J = 9 Hz, H-3′ and H-5′) and a doublet at δ 8.31 (J = 9 Hz, H-2′ and H-6′). Only one flavonol 7, 4′-diglycoside has been reported previously from ferns, namely, quercetin 3-methyl ether 7-*O*-diglucoside 4′-*O*-glucoside from *Ophioglossum vulgatum* [6].

R_f values (see Experimental), colour reactions (brown to yellow in UV+NH_3) and UV spectral analysis in the presence of the customary shift reagents [5]: λ_{max}^{MeOH} nm: 257, 264 (sh), 287 (sh), 360; +NaOMe 272, 324, 404; +$AlCl_3$ 272, 303 (sh), 435; +$AlCl_3$-HCl 269, 301 (sh), 351 (sh), 403; +NaOAc 272, 316, 375; +NaOAc–H_3BO_3 261, 376, suggested that band 2 may be a flavonol glycoside with free hydroxyl groups at positions 5, 7, 3′ and 4′. Both total acid hydrolysis and controlled acid hydrolysis gave quercetin, D-glucose and D-fructose. H_2O_2 Oxidation [7] (which releases the sugars at 3-position) gave D-glucose and D-fructose. The ^{1}H NMR spectrum (DMSO-d_6) showed a multiplet at δ 3.09–3.91 (approximately glycosyl six protons), a doublet at δ 5.43 (J = 8 Hz, glucosyl H-1) and broad signals at δ 6.21 (H-6), δ 6.41 (H-8), δ 6.85 (H-5′) and δ 7.58 (H-2′ and H-6′). The positive FAB mass spectrum gave a quasimolecular ion $[M+H]^+$ at *m/z* 465 ($C_{21}H_{20}O_{12}$ requires 464) and a significant ion at *m/z* 303 (aglycone). These results

Table 1. ^{13}C NMR spectral data (DMSO-d_6) for band B2

Sugar moiety	
Glucose	
1″	101.4
2″	74.3
3″	76.8[a]
4″	70.3
5″	77.7[a]
6″	61.3
Fructose	
1″	62.4
2″	103.1
3″	77.2[a]
4″	75.8
5″	82.1
6″	63.8

[a]Assignments with the same superscripts may be interchanged.

show that band 2 is a mixture of quercetin 3-*O*-glucoside (isoquercitrin) and quercetin 3-*O*-fructoside. This conclusion was confirmed by the ^{12}C NMR data (Table 1) for the fructose and glucose moeties which agreed with those of methyl-β-D-fructofuranoside [8, 9] and isoquercitrin [10], respectively. D-Fructose is not normally found in combination with flavones and flavonols [11] but it has been found [12] as a constituent sugar of a tricin diglycoside from *Hyacinthus orientalis* (Liliaceae) although the complete structure was not determined. In addition quercetin 3-*O*-(6″-*O*-fructofuranosylglucoside) has been reported from tea [13, 14] where it is presumably a product of tea processing because it is absent from fresh tea leaves (*Camellia sinensis*) [13]. However, D-fructose was found in a flavanone glycoside from *Camellia sinensis* [13]. Otherwise, D-fructose has been identified as the carbohydrate component in anthocyanins and leucoanthocyanins of *Mentha piperita* [15] and in an anthocyanin pigment of *Salix purpurea* [15].

EXPERIMENTAL

Plant material. Aerial parts of *P. aquilinum* (L.) Kuhn ssp *aquilinum* were collected in Potenza (Italy) in spring 1992. The ferm was identified by Dr R. Nazzaro (Dipartimento di Biologia Vegetale dell'Università Federico II, Naples, Italy). A voucher specimen has been deposited in 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 refiltered. Bands B1 and B2 were isolated by prep. PC on Whatman 3MM paper in *n*-BuOH–HOAc–H_2O (BAW; 4:1:5, upper phase). They were eluted with EtOH, concd and rechromatographed in 15% HOAc and in *n*-BuOH–EtOH–H_2O (BEW; 4:1:2.2). Further purification was carried out on Sephadex LH-20 CC, eluting with MeOH. R_f values (on Whatmann no. 1 paper) for bands B1 and B2 are: BAW 0.45, 0.57; 15% HOAc 0.24, 0.35.

Hydrolysis procedures. Total acid hyhdrolysis of B1 and B2 was carried out with 2 M HCl (2 hr at 100°) in a sealed tube and controlled acid hydrolysis was carried out with 10% HOAc (3.5 hr under reflux). Treatment with β-glucosidase was carried out in H_2O at 37° for 24 hr. Kaempferol and quercetin, respectively, were identified by UV spectral analysis in the presence of the customary shift reagents [5], PC (4 solvent systems) and polyamide TLC. D-Glucose, L-rhamnose and D-fructose were identified by co-PC (4 solvent systems) and silica gel TLC (*n*-BuOH–HOAc–Et_2O–H_2O, 9:6:3:1). Furthermore, D-fructose gave the expected colours with appropriate sugar reagents [16]. Kaempferol 7-*O*-rhamnoside was identified by UV spectral analysis with the customary shift reagents [5], total acid hydrolysis, controlled acid hydrolysis and co-PC.

Methylation. Band B1 was methylated with MeI in $HCONMe_2$ in the presence of Ag_2O (18 hr in the dark at room temp. with stirring) and subsequently hydrolysed with 0.3 M HCl (4 hr under reflux). 2,3,4,6-Tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose were identified by silica gel TLC (solvent system: $CHCl_3$–EtOAc, 1:1). Kaempferol 3,5-dimethyl ether was identified by UV spectral analysis with the customary shift reagents [5] and EIMS.

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