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KAEMPFEROL 3-*O*-(5″-FERULOYLAPIOSIDE) FROM *PTERIDIUM AQUILINUM*

FILIPPO IMPERATO

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Key Word Index—*Pteridium aquilinum*; Dennstaedtiaceae; acylated flavonol glycoside; kaempferol 3-*O*-(5″-feruloylapioside).

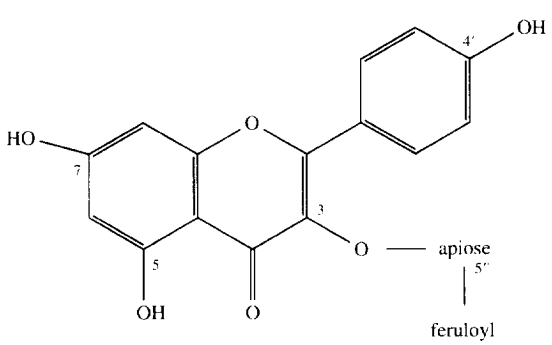
Abstract—A new acylated flavonol glycoside from aerial parts of *Pteridium aquilinum* was characterized as kaempferol 3-*O*-(5″-feruloylapioside) by chemical and spectral methods. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In spite of the fact that polyphenolic analyses of ferns are of phylogenetic and taxonomic 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 *P. aquilinum* of kaempferol 3-*O*-(5″-feruloylapioside) (**1**), a new natural product.

RESULTS AND DISCUSSION

Compound **1** has been isolated from an ethanolic extract of aerial parts of *P. aquilinum*. Colour reactions (brown to yellow in UV + NH_3), R_f data (see Experimental) and UV spectral analysis in the presence of the usual shift reagents [9]: λ_{max}^{MeOH} nm: 265, 300 (sh), 321; +$AlCl_3$ 275, 305, 325 (sh), 401; +$AlCl_3$/HCl 275, 302, 316 (sh), 400; +sodium acetate 273, 312, 378; +sodium acetate/H_3BO_3 265, 301 (sh), 319; +sodium methoxide 272, 379 suggested that **1** may be a flavonoid glycoside acylated with a hydroxycinnamic acid, since the hydroxycinnamic acid spectrum is superimposed on the flavonoid spectrum. In addition, free hydroxyl groups at positions 5 (shift with $AlCl_3$ and $AlCl_3$/HCl), 7 (shift with sodium acetate) and 4′ (shift with sodium methoxide) are present on the flavonoid skeleton. Total acid hydrolysis gave kaempferol and ferulic acid; mild acid hydrolysis gave D-apiose n addition to the products of total acid hydrolysis. Kuhn methylation followed by acid hydrolysis gave 3,4-dimethoxycinnamic acid, 2,3-di-*O*-methyl-D-apiose and kaempferol 5,7,4′-trimethyl ether. These results show that the isolated flavonoid is kaempferol 3-*O*-(5″-feruloylapioside), a new natural product. The structure of this compound was confirmed as follows. The negative FAB mass spectrum showed a



1

Table 1. ^{13}C NMR spectral data (MeOH-d_4) for **1**

Kaempferol		Apiose	
2	158.7[a]	1	112.3
3	134.9	2	79.1
4	178.8	3	78.6
5	162.9	4	76.1
6	100.3	5	68.5
7	165.7	Feruloyl	
8	95.3	1	126.7
9	158.4[a]	2	112.4
10	105.6	3	150.1
1′	122.3	4	148.9
2′	131.7	5	116.9[b]
3′	116.8[b]	6	124.5
4′	161.4	7	146.8
5′	116.8[b]	8	115.7
6′	131.7	C=O	167.9
		OMe	60.2

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

quasimolecular ion $[M-H]^-$ at *m/z* 593 ($C_{30}H_{26}O_{13}$ required 594) and significant ions at m/z 417 $[(M-H)-176]^-$ (loss of feruloyl moiety) and *m/z* 285 (aglycone). The ^1H NMR spectrum for the trimethylsilyl ether (300 MHz; CCl_4) showed a singlet at δ 3.50 (2H, H-4″), a doublet at δ 4.21 (1H, J = 3.5 Hz, H-2″), a singlet at δ 4.62 (2H, H-5″), a doublet at δ 5.57 (1H, J = 3.5 Hz, H-1″), a doublet at δ 6.12 (1H, J = 2.5 Hz, H-6), a doublet at δ 6.42 (1H, J = 2.5 Hz, H-8), a doublet at δ 6.89 (2H, J = 8.5 Hz, H-3′,5′) and a doublet at δ 7.93 (2H, J = 8.5 Hz, H-2′,6′). Feruloyl protons appeared as a singlet at δ 3.85 (3H, methoxyl), a doublet at δ 6.41 (1H, J = 17.1 Hz, H-α), a doublet at δ 6.67 (1H, J = 8.5 Hz, H-5), a doublet of doublets at δ 6.93 (1H, J = 8.5 and 1.8 Hz, H-6), a doublet at δ 7.03 (1H, J = 1.8 Hz, H-2) and a doublet at δ 7.58 (1H, J = 17.1 Hz, H-β). The chemical shift (δ 4.62) of protons at C-5″ of apiose was downfield by 1.1 ppm in comparison with the corresponding protons of non-acylated apiose [10]. Furthermore, in the ^{13}C NMR spectrum of **1** (Table 1) C-5″ showed a 2.5 ppm downfield shift and C-3″ showed a 3 ppm upfield shift in comparison with the corresponding carbons of apiose in the spectrum of quercetagetin 6,7-dimethyl ether 3-*O*-apioside [10]. These findings confirmed 5″-*O*-acylation in **1** [11].

Apiose has been reported previously in the flavonoids of only one fern species, *Polypodium vulgare*, as catechol 7-*O*-apioside [12]. This pentose is of rare occurrence in flavonol monoglycosdies; it has been found in *Lepidagathis cristata* as 6-hydroxyluteolin 7-*O*-apioside [13] and in *Ageratina calophylla* as quercetagetin 6,7-dimethyl ether 3-*O*-apioside [10]. Compound **1** is the first acylated flavonoid apioside to be reported.

EXPERIMENTAL

Plant material. Aerial parts of *P. aquilinum* (L.) Khun 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 refiltered. Compound **1** was isolated by prep. PC on Whatman 3MM paper in *n*-BuOH–HOAc–H_2O (BAW; 4:1:5, upper phase). It was eluted with EtOH, concd and rechromatographed in 15% HOAc and *n*-BuOH–EtOH–H_2O (BEW; 4:1:2.2). Further purification was carried out by Sephadex LH-20 CC, eluting with MeOH. R_f values for **1** (on Whatman No. 1 paper) are: BAW (4:1:5, upper phase), 0.77; 15% HOAc 0.23 and H_2O, 0.05.

Hydrolysis procedures. Total acid hydrolysis was carried out with 2 M HCl (2 hr at 100°); mild 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). Kaempferol was identified by UV spectral analysis with the usual shift reagents [9], PC (4 solvent systems) and EIMS. D-Apiose was identified by co-PC (4 solvent systems) by silica gel TLC. Ferulic acid was identified by UV spectroscopy, PC (4 solvent systems), silica gel TLC and paper electrophoresis. Kaempferol 3-*O*-apioside was identified by UV spectral analysis with the usual shift reagents [9] and mild acid hydrolysis.

Methylation. Compound **1** 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 10% HOAc (4 hr under reflux). 2,3-Di-*O*-methyl-D-apiose was identified by co-PC [14]. Kaempferol 5,7,4′-trimethyl ether was identified by UV spectral analysis in the presence of the customary shift reagents [9] and by EIMS. 3,4-Dimethoxycinnamic acid was identified by co-PC (4 solvent systems) and silica gel TLC.

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