Pergamon

PII: S0031-9422(96)00803-5

Phytochemistry, Vol. 45, No. 1, pp. 199–200, 1997

0031-9422/97 $17.00+0.00

KAEMPFEROL 3-*O*-(6″-CAFFEOYLGLUCOSIDE) FROM *PTERIDIUM AQUILINUM*

FILIPPO IMPERATO* and PINA MINUTIELLO

Dipartimento di Chimica dell'Università della Basilicata, 85100 Potenza, Italy

(*Received* 8 *October* 1996)

Key Word Index—*Pteridium aquilinum*; Dennstaedtiaceae; acylated flavonol glycoside; kaempferol 3-*O*-β-(6″-caffeoylglucoside).

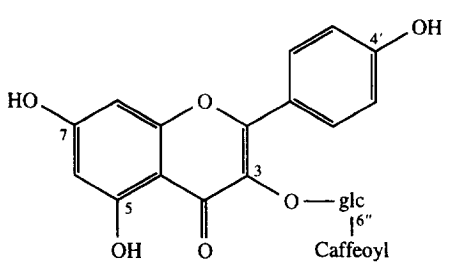
Abstract—A new acylated flavonol glucoside from the aerial parts of *Pteridium aquilinum* was characterized as kaempferol 3-*O*-β-(6″-caffeoylglucoside) by chemical and spectral methods.

INTRODUCTION

Proanthocyanidins and flavonol glycosides have been reported previously from *Pteridium aquilinum* (L.) Kuhn [1], including most recently the 3-laminaribiosides of Kaempferol and quereetin [2]. In the present paper we describe the characterization of a new acylated kaempferol glycoside from the same plant.

RESULTS AND DISCUSSION

Flavonoid **1** was isolated from an ethanolic extract of the 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 [3]—λ_{max}^{MeOH} nm: 265, 300 (sh), 321; +$AlCl_3$ 275, 305, 325 (sh), 401; +$AlCl_3$/HCl 275, 302, 316 (sh), 400; +NaOAc 273, 312, 378; +NaOAc/H_3BO_3 265, 301 (sh), 319; +NaOMe 272, 379, suggested that **1** may be a flavonoid glycoside acylated with an hydroxycinnamic acid. Thus, the hydroxycinnamic acid spectrum could be seen to be superimposed on the flavonoid spetrum. The UV spectral data also indicated free hydroxyl groups at positions 5 (shift with $AlCl_3$ and $AlCl_3$/HCl), 7 (shift with NaOAc) and 4′ (shift with NaOMe) of the flavonoid skeleton. Both total acid hydrolysis and controlled acid hydrolysis gave kaempferol, D-glucose and caffeic acid. Alkaline hydrolysis gave kaempferol 3-*O*-β-glucoside and caffeic acid. Kuhn methylation followed by acid hydrolysis gave 3,4-dimethoxycinnamic acid, 2,3,4-tri-*O*-methyl-D-glucose and kaempferol 5,7,4′-trimethyl ether. These data indicate that it is kaempferol 3-*O*-β-(6″-caffeoylglucoside) (**1**) which is a new natural product. A kaempferol 3-*O*-(caffeoylglucoside) has been found recently [4] in *Eryngium campestre* (Umbelliferae), but the position of the acyl group was not determined. 6″-*O*-Acylation was confirmed in **1** by the ^{13}C NMR spectrum (Table 1), which showed a downfield shift of C-6″ by 2.0 ppm and an upfield shift of 5″ by 2.1 ppm in comparison with the corresponding carbons of glucose in the spectrum of kaempferol 3-*O*-glucoside [5]. Also the negative ion FAB mass spectrum showed a quasimolecular ion [M-H] at *m/z* 609 ($C_{30}H_{26}O_{14}$ required 610) and significant ions at *m/z* 447 [(M-H)-162] (loss of caffeoyl moiety) and *m/z* 285 (aglycone). The ^1H NMR spectrum (300 MHz; DMSO-d_6) showed a multiplet at δ 3.05–3.40 (glucosyl 4 protons), a multiplet at δ 4.12–4.24 (methylene of glucose), a doublet at δ 5.53 (J = 8 Hz, glucosyl anomer), an H-6 doublet at δ 6.25 (J = 2 Hz), an H-8 doublet at δ 6.52 (J = 2 Hz), a doublet at δ 6.94 (J = 8.8 Hz, H-3′, 5′) and a doublet at δ 7.75 (J = 8.8 Hz, H-2′, 6′). Caffeoyl protons appeared as doublet at δ 6.20 (J = 16 Hz) (H-8), a doublet at δ



1

*Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data (DMSO-d_6) of **1**

Kaempferol		Glucose	
2	156.3[a]	1	101.3
3	133.2	2	74.2[b]
4	177.3	3	77.1
5	161.2	4	70.0
6	98.6	5	74.4[b]
7	164.3	6	63.0
8	93.5	Caffeoyl	
9	156.4[a]	1	128.1
10	103.9	2	115.3
1′	120.9	3	146.4
2′	130.0	4	147.2
3′	115.6	5	116.7
4′	159.8	6	127.1
5′	115.6	7	148.4
6′	130.0	8	123.8
		C—O	168.7

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

6.65 (J = 8.2 Hz) (H-5), a doublet at δ 6.75 (J = 8.2 Hz) (H-6), a singlet at δ 6.90 (H-2) and a doublet at δ 7.48 (J = 16 Hz) (H-7).

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. Flavonoid **1** was isolated by PPC on Whatman 3MM paper in BAW. It was 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** (on Whatman no. 1 paper) are: BAW, 0.77; 15% HOAc 0.23; H_2O, 0.05.

Hydrolysis procedures. Total acid hydrolysis with 2 M HCl (2 hr at 100°), controlled acid hydrolysis with 10% HOAc (3.5 hr under reflux) and alkaline hydrolysis with 2 M NaOH (2 hr at room temp. in a sealed tube) were carried out on **1**. Kaempferol was identified by UV spectral analysis with the usual shift reagents [3], PC (four solvent systems) and EIMS. D-Glucose was identified by co-PC (four solvent systems) and silica gel TLC. Caffeic acid was identified by UV spectroscopy, PC (four solvent systems), silica gel TLC and paper electrophoresis. Kaempferol 3-*O*-β-glucoside was identified by co-PC (four solvent systems), UV spectral analysis with the usual shift reagents [3], total acid hydrolysis and treatment with β-glucosidase (at 37° in H_2O).

Methylation. Flavonoid **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 0.3 M HCl (4 hr under reflux). 2,3,4-Tri-*O*-methyl-D-glucose was identified by co-PC [6]. Kaempferol 5,7,4′-trimethyl ether was identified by UV spectral analysis in the presence of the customary shift reagents [3] and EIMS. 3,4-Dimethoxycinnamic acid was identified by co-PC (four solvent systems) and silica gel TLC.

Acknowledgements—The authors thank Consiglio Nazionale delle Ricerche (Rome) and MURST (Rome) for financial support. Mass spectral data were provided by SESMA (Naples).

REFERENCES

1. Markham, K. R., in *The Flavonoids: Advances in Research Since* 1980, ed. J. B. Harborne. Chapman and Hall, London, 1988, p. 427.
2. Imperato, F., *Phytochemistry*, 1995, **40**, 1801.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, Berlin, 1970.
4. Karting, T. and Wolf, J., *Planta Medica*, 1993, **59**, 285.
5. Markham, K. R., in *The Flavonoids: Advances in Research*, eds J. B. Harborne and T. J. Mabry. Chapman and Hall, London, 1982, p. 43.
6. Petek, F., *Bulletin de la Société Chimique de France*, 1965, 263.