Quercetin 3-O-β-D-(6″-caffeoylgalactoside) (**1**). Yellow needles (200 mg), mp 198–200°, $[\alpha]_D^{30} - 19.5°$ (*c*, 0.12, pyridine), UV λ_{max}^{EtOH} nm (ε): 257 (27300), 340 (27900), IR ν_{max}^{KBr} cm^{-1}: 3360, 1650, 1600, 1500, 1353, 1280, 1195, 1160, 1070, FDMS *m/z*: 627 $[M+H]^+$ (100%), 465 (36), ^1H NMR δ (CD_3OD): 5.13 (1H, *d*, *J* = 7.2 Hz, anomeric), 6.00 (1H, *d*, *J* = 16.2 Hz, =CH–Ph), 6.13 (1H, *d*, *J* = 1.8 Hz, H-6), 6.30 (1H, *d*, *J* = 1.8 Hz, H-8), 6.80 (1H, *d*, *J* = 9 Hz, H-5′), 7.32 (1H, *d*, *J* = 16.2 Hz, CO–CH=), 7.55 (1H, *dd*, *J* = 1.8 and 9 Hz, H-6′), 7.80 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.7–7.0 (3H, *m*, aromatic-H of caffeic acid). Found: C, 52.13; H, 4.42. $C_{30}H_{26}O_{15} \cdot 3.5\,H_2O$ requires C, 52.25; H, 4.82%. **1** (46 mg) was hydrolysed with saturated methanolic Na_2CO_3 (10 ml) at room temp. overnight. The reaction mixture was evaporated *in vacuo* and, after addition of H_2O (5 ml), extracted with EtOAc and then *n*-BuOH. The EtOAc extract gave colourless needles (4.3 mg), mp 220–225°, TLC R_f: 0.82 (TEFF), ^1H NMR δ (DMSO-d_6): 6.21 (1H, *d*, *J* = 16.2 Hz), 6.74–7.06 (3H, *m*), 7.46 (1H, *d*, *J* = 16.2 Hz), identical with caffeic acid. The BuOH extract gave yellow needles (11.4 mg), mp 225–230° of quercetin 3-galactoside [mmp, TLC (EAWA) and NMR].

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KAEMPFEROL 3-SULPHATE IN THE FERN *ADIANTUM CAPILLUS-VENERIS*

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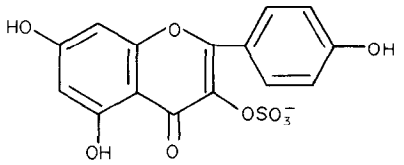
Key Word Index—*Adiantum capillus-veneris*; Adiantaceae; kaempferol 3-sulphate.

Abstract—A new natural product isolated from the fronds of the fern *Adiantum capillus-veneris* has been shown to be kaempferol 3-sulphate by chemical and spectroscopic methods.

Previous work on the phenolic constituents of *Adiantum capillus-veneris* L. has led to the identification of kaempferol 3, 7-diglucoside [1]. Very recently [Imperato, F., unpublished results] four sulphate esters of hydroxycinnamic acid sugar derivatives have been isolated. This paper describes the isolation of kaempferol 3-sulphate from this fern.

This flavonoid (colour reactions: brown to yellow in UV + NH_3) was isolated from an ethanolic extract of fresh fronds of *Adiantum capillus-veneris* by prep. PC. The UV spectral data: λ_{max}^{MeOH} nm: 267, 303 (sh), 347; + NaOAc 275, 303, 378; + NaOAc/H_3BO_3 268, 350; + $AlCl_3$ 278, 304, 349, 396; + $AlCl_3$–HCl 276, 303, 343, 395; + NaOMe 275, 322, 392 (increase in intensity); + $ZrOCl_2$–citric acid 265, 345 are consistent [2] with those of a 3-substituted flavonol with free hydroxyl groups at positions 5, 7 and 4′. The isolated compound was electrophoretically highly mobile (towards anode), strongly suggestive of a sulphate derivative. Total acid hydrolysis, controlled



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acid hydrolysis and alkaline hydrolysis gave kaempferol and sulphate; enzyme hydrolysis with sulphatase (from *Helix pomatia*) gave kaempferol. Kuhn methylation followed by acid hydrolysis gave 5, 7, 4'-tri - *O* - methylkaempferol. The above results show that the isolated flavonoid must be kaempferol 3-sulphate (**1**). This compound has been synthesized [3] by Yamaguchi's procedure but is here reported for the first time in plants.

EXPERIMENTAL

Plant material. Fronds of *A. capillus-veneris* L. were collected in Catania.

Isolation of flavonoid. Fresh fronds (100 g) of *A. capillus-veneris* were homogenized and extracted ×3 with 95% EtOH. The combined extracts were filtered, concd. to small vol. *in vacuo* and refiltered. Kaempferol 3-sulphate (15 mg) was isolated by prep. PC on Whatman 3MM paper in BAW; the pale yellow band was cut off, eluted with 70% EtOH, concd. and rechromatographed in 15% HOAc and BEW. R_f data (on Whatman No. 1 paper) are: BAW, 0.66; BEW, 0.28; 15% HOAc, 0.47; PhOH satd. with H_2O, 0.25.

Hydrolysis procedures. Controlled acid hydrolysis was carried out with 10% aq. HOAc (3.5 hr under reflux); total acid hydrolysis was carried out with 2N HCl (2 hr at 100°); alkaline hydrolysis was carried out with 2N NaOH (0.5 hr at 100° under N_2); enzyme hydrolysis with sulphatase was carried out in citrate–Pi buffer, pH 4.5, at 37° for 12 hr. Kaempferol was identified by UV spectroscopy, PC (4 solvents) and polyamide TLC (2 solvents). Sulphate was identified via $BaCl_2$.

Methylation. The flavonoid was methylated with MeI in $HCONMe_2$ in the presence of AgO and the permethylated product was hydrolysed with 0.3 N HCl (4 hr under reflux). 5, 7, 4' - Tri - *O* - methylkaempferol was identified by UV spectroscopy and PC (2 solvents).

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