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SULPHATE ESTERS OF HYDROXYCINNAMIC ACID–SUGAR DERIVATIVES FROM *ADIANTUM CAPILLUS-VENERIS*

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Key Word Index—*Adiantum capillus-veneris*; Adiantaceae; sulphate esters of 1-*p*-coumarylglucose, 1-caffeylglucose and 1-caffeylgalactose.

Abstract—Two new and two known sulphate esters of hydroxycinnamic acid–sugar derivatives have been isolated from the fronds of the fern *Adiantum capillus-veneris*. The new compounds have been shown to be 1-*p*-coumarylglucose 2-sulphate and 1-caffeylgalactose 6-sulphate by chemical and spectroscopic methods.

Previous work on the chemical constituents of the fern, *Adiantum capillus-veneris* L., has shown the presence of triterpenoids [7, 9(11)-fernadiene, 7-fernene [1], 3α,4α-epoxyfilicane [2], 21β-hydroxy-29-nor-22-hopanone [3] and adiantone [4]], alicyclic acids [5] (quinic acid and shikimic acid) and of a flavonol glycoside (kaempferol 3,7-diglucoside [6]). This paper describes the identification of four sulphate esters of hydroxycinnamic acid–sugar derivatives.

From an ethanolic extract of fresh fronds of *Adiantum capillus-veneris*, two bands (B1 and B2) were isolated by prep. PC and their chromatographic and electrophoretic properties are presented in Table 1. B1 (colour reactions: colourless to blue in UV + ammonia) was electrophoretically highly mobile towards the anode. Both total acid hydrolysis and controlled acid hydrolysis gave *p*-coumaric acid, D-glucose and sulphate. The UV spectral properties (λ_{max}^{MeOH} nm: 230, 315; +$AlCl_3$ 231, 316; +NaOMe 240, 365) showed that the phenolic hydroxyl group of *p*-coumaric acid must be free (large bathochromic shift of the band at 315 nm in the presence of sodium mectoxide [7]). R_f values, colour reactions and UV spectral properties of B1 were in agreement with those of an authentic sample of the sulphate ester of 1-*p*-coumarylglucose from *Adiantum pulverulentum* [8]. Treatment with sulphatase gave 1-*p*-coumarylglucose [7] identified by UV spectroscopy, acid hydrolysis, alkaline hydrolysis, treatment with β-glucosidase and co-PC. Kuhn methylation followed by acid hydrolysis gave *p*-methoxycinnamic acid, 2,3,4-tri-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-glucose. These results show that B1 must be a mixture of 1-*p*-coumarylglucose 6-sulphate (**1**) and 1-*p*-coumarylglucose 2-sulphate (**2**). **1** may already have been found in plants since it was suggested [8] that sulphate is probably attached to the 6-hydroxy group of the glucose in the sulphate ester of 1-*p*-coumarylglucose isolated from *Adiantum pulverulentum* [8]. However, **2** is a new natural product.

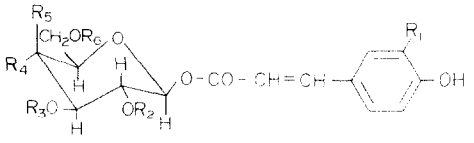
Band B2 (colour reactions: blue to green in UV + ammonia) migrated towards the anode on electrophoretograms. Both total acid hydrolysis and controlled acid hydrolysis gave caffeic acid, D-glucose, D-galactose and sulphate. The UV spectral properties [λ_{max}^{MeOH} nm: 245, 300 (sh), 332; + NaOMe 268, 320 (sh), 372; + $AlCl_3$ 250, 300 (sh), 340; + $AlCl_3$–HCl 250, 318 (sh), 330; NaOAc–H_3BO_3 258, 302 (sh), 344] showed that the *O*-diphenolic group of caffeic acid must be free. Enzymic hydrolysis with sulphatase gave a mixture of 1-caffeylglucose [7] and 1-caffeylgalactose which were identified by UV spectroscopy,

Table 1. R_f and electrophoretic mobility of bands isolated from *Adiantum capillus-veneris*

Band	R_f(×100) in BAW	BEW	15% HOAc	30% HOAc	H_2O	Electrophoretic mobility*
B_1	45	53	72	85	82	12.3
B_2	22	35	68	77	70	9.2

*Relative to *p*-coumaric acid run at pH 4.5 (pyridine formate 0.05 M) at 16 V/cm for 3 hr on Whatman No. 1 paper.

acid hydrolysis, alkaline hydrolysis, treatment with β-glucosidase and co-PC. R_f values, colour reactions and UV spectral properties of B2 were in agreement with those of an authentic sample of the sulphate ester of 1-caffeylglucose [8] from *Pteridium aquilinum*. Kuhn methylation followed by acid hydrolysis gave 3,4-dimethoxycinnamic acid, 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose. These results show that B2 must be a mixture of 1-caffeylglucose 3-sulphate (**3**) and 1-caffeylgalactose 6-sulphate (**4**). **3** has recently been found in the fern *Ceterach officinarum* Lam. et DC. [9]; **4** is a new natural product. This is also the first report of a sulphated cinnamic acid galactoside.



1 $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = OH$; $R_6 = SO_3^-$
2 $R_1 = R_3 = R_5 = R_6 = H$; $R_4 = OH$; $R_2 = SO_3^-$
3 $R_2 = R_5 = R_6 = H$; $R_1 = R_4 = OH$; $R_3 = SO_3^-$
4 $R_2 = R_3 = R_4 = H$; $R_1 = R_5 = OH$; $R_6 = SO_3^-$

Sulphated cinnamic acid glucosides were first discovered [8] in 1975 by Cooper-Driver and Swain who reported the presence of sulphate esters of 1-caffeylglucose and 1-*p*-coumarylglucose in *Pteridium aquilinum* and in 10 *Adiantum* species. Subsequently, 2-*O*-*p*-coumarylglucose 6-sulphate was isolated [9] from *Asplenium fontanum* Bernh var. *obovatum* and the 3- and 2-sulphates of 1-caffeylglucose were found in *Ceterach officinarum* [9]. The occurrence of sulphate esters of hydroxycinnamic acid–sugar derivatives is not restricted to lower plants since sulphate esters of caffeylglucose have been found in higher plants (e.g. in the grass *Paspalum convexum* [10]).

EXPERIMENTAL

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

Isolation. Fresh fronds (100 g) of *A. capillus-veneris* were homogenized and extracted ×3 with hot 95% EtOH. The combined extracts were filtered, concd. to small vol. *in vacuo* and re-filtered. Prep. PC on Whatmann 3MM paper in BAW afforded two bands (B1 and B2) which were eluted with 70% EtOH, concd. and rechromatographed in 15% HOAc; the bands, B1 (30 mg) and B2 (50 mg), were then purified by chromatography in BEW. Another anionic band was isolated but was not present in sufficient amount for analysis; electrophoretic and spectral properties as well as colour reactions suggested that this band may be a sulphate ester of a caffeic acid derivative.

Hydrolysis procedures. Controlled acid hydrolysis was carried out with 10% aq. HOAc (3.5 hr under reflux); total acid hydrolysis was carried out with 2 N HCl (2 hr at 100°); alkaline hydrolysis was carried out with 2 N NaOH at room temp. for 3 hr (under N_2); enzyme hydrolysis with sulphatase (from *Helix pomatia*) was carried out in citrate–Pi buffer, pH 4.5 at 37° for 12 hr. D-Glucose and D-galactose were identified by co-PC (four solvents) and TLC on Si gel (*n*-BuOH–HOAc–Et_2O–H_2O, 9:6:3:1). Sulphate was identified by $BaCl_2$.

Methylation. The bands were methylated with MeI in $HCONMe_2$ in the presence of AgO and the permethylated products were hydrolysed with 0.3 N HCl (4 hr under reflux). 3,4-Dimethoxycinnamic acid and *p*-methoxycinnamic acid were identified by PC [11] and TLC on Si gel; the partially methylated sugars were identified by PC according to Petek [12] and, after reduction ($NaBH_4$) and acetylation (pyridine–Ac_2O), by GC/MS as partially methylated alditol acetates (70 eV; stainless steel column packed with 3% ECNSS-M on Gas-Chrom Q 1.80 m × 1.8 mm, at 180° isothermally and N_2 at 25 ml/min; injector temp. 200°, FID temp. 200°).

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