Two new kaempferol 3,7-diglycosides and kaempferitrin in the fern *Asplenium trichomanes*

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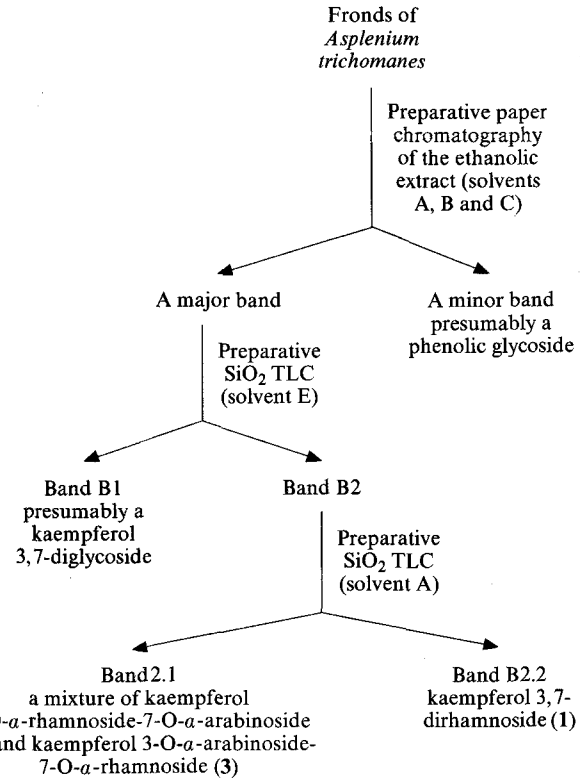
Summary. The fronds of the fern *Asplenium trichomanes* contain kaempferol 3,7-dirhamnoside **(1)** and the new compounds kaempferol 3-O-α-rhamnoside-7-O-α-arabinoside **(2)** and kaempferol 3-O-α-arabinoside-7-O-α-rhamnoside **(3)**. The presence of the above flavonoids has been shown by spectroscopic methods and chemical degradations.

In spite of the fact that *Asplenium* plants provide a classic example of additive inheritance of chemical character, little is known of the chemistry of the substances present. In recent years poliphenolic constituents of some species of ferns belonging to the genus *Asplenium* have been investigated, leading to the isolation of a number of flavonoids[2,3] and xanthones[4]. No detailed study appears to have been made of phenolic constituents of *Asplenium trichomanes;* previous works have led to the identification in this fern of leucoanthocyanidins[5] (very often detected in primitive plants[6]), lignin[7], amino acids (N-acetylornithine[8], 2-amino-4-hydroxypimelic acid and the corresponding lactone[9]), higher alkanes[10] and triterpenoids[11] (22(29)-hopene and cyclolaudenol). The present study deals with the presence of 3 kaempferol 3,7-diglycosides **(1-3)** in the fronds of *Asplenium trichomanes.*

Material and methods. For paper chromatography and TLC, the solvent mixtures used were: A, 1-butanol-acetic acid-water (4:1:5, upper phase); B, 1-butanol-ethanol-water (4:1:2,2); C, acetic acid-water (15:85); D, phenol saturated with water; E, ethyl acetate-butanone-formic acid-water (5:3:1:1); F, 1-butanol-acetic acid-ethyl ether-water (9:6:3:1); G, 1-butanol-pyridine-water (6:4:3); H, acetic acid-conc. HCl-water (30:3:10); I, chloroform-ethyl acetate (1:1); L, chloroform-acetic acid (9:1); M, 1-butanol-2N HCl (1:1, upper phase); N, chloroform-butanone-methanol (70:6:10); O, 1-butanol-ethanol-water (5:1:4).

Plant material was collected on vulcano Etna (Sicily). Fresh fronds (400 g) of *A. trichomanes* were homogenized and extracted 3 times with boiling 95% ethanol; the combined extracts were filtered, concentrated to a small volume in vacuo and re-filtered. Flavonoids were isolated as follows (scheme). Preparative chromatography on Whatmann 3 mm paper in solvent A gave a single band (ca. 80 mg) which was cut off, eluted with 70% ethanol, concentrated and purified by repeated paper chromatography in solvents B and C. Chromatography in solvent C gave a minor band (ca. 2 mg) which was presumably a phenolic glycoside (R_f-values, colour reactions, UV-spectrum) but was in too low concentration for analysis. The major band gave 2 components by separation on SiO_2 TLC (solvent E): B1 (ca. 3 mg; R_f 0.85) and B2 (ca. 60 mg; R_f 0.82). The faster running component (B1) was not isolated in sufficient amount for full structure analysis but enzymic hydrolysis with α-rhamnosidase[12] gave kaempferol identified by paper co-chromatography (solvents A, B, D and H), polyamide TLC (solvent N), SiO_2 TLC (solvent L), UV spectral analysis with shift reagents[13] and MS; colour reactions (dull ochre to fluorescent yellow in UV + NH_3), R_f-values and UV-spectra in the presence of diagnostic reagents[13] suggest that component B1 may be a kaempferol 3,7-diglycoside. The 2nd component (B2) was a mixture since it gave 2 bands by separation on preparative SiO_2 TLC (solvent A, 3 stages): B2.1 (ca. 30 mg; R_f 0.63) and B2.2 (ca. 20 mg; R_f 0.60). Colour reactions (dull ochre to fluorescent yellow in



Scheme. Isolation procedure of flavonoids from *Asplenium trichomanes.*

Spectral properties and R_f-values of band B2.1

Spectral properties Shift reagent	λ^{MeOH}_{max} (nm)	R_f-values Solvent	R_f(× 100)
–	265, 343	A	57[a]
NaOAc	265, 345, 404 (sh)	B	50[a]
NaOMe	266, 358 (sh), 405 (inc)	C	52[a]
$AlCl_3$	265 (sh), 272, 301 (sh), 346, 398	D	69[a]
$AlCl_3$/HCl	262 (sh), 272, 299 (sh), 343, 397	A	63[b]
NaOAc/H_3BO_3	265, 344	E	82[b]
$ZrOCl_2$/citric acid	265, 345	M	71[b]

–, Whithout shift reagent; sh, shoulder; inc, shift accompanied by increase in intensity. [a] On Whatman N1 paper; [b] on SiO_2 TLC.

UV + NH_3), R_f-values and spectral data[13] (table) suggest that band B2.1 may be a 3,7 disubstituted flavonol glycoside with free hydroxyl groups at positions 5 and 4'. α-Rhamnosidase hydrolysis as well as total acid hydrolysis with 2N HCl (2 h at 100 °C) of this band gave kaempferol, L-rhamnose and L-arabinose. The aglycone was identified as above; the sugars were identified by paper co-chromatography (solvents A and G), SiO_2 TLC (solvent F) and GLC of their TMS ethers[14]. Quantitative examination[14] showed that band B2.1 is hydrolyzed by acid to 1 mole each of kaempferol, L-rhamnose and L-arabinose. On controlled acid hydrolysis with 10% acetic acid (3.5 h under reflux) this band gave kaempferol, L-rhamnose, L-arabinose and 2 intermediates (F1 and F2) which were isolated by preparative paper chromatography (solvent A); F1 and F2 were identified as kaempferol 7-0-rhamnoside and kaempferol 7-0-arabinoside respectively in the following way. Acid hydrolysis with 2 N HCl (2 h at 100 °C) of each compound afforded kaempferol and the respective sugar (i.e. L-rhamnose for F1 and L-arabinose for F2); colours (yellow to yellow in UV + NH_3) and UV spectral analysis with shift reagents[13] indicated that only the 7-position in these compounds contains a sugar substituent. On methylation (Me_2SO_4-K_2CO_3-Me_2CO) followed by acid hydrolysis, F1 gave 3,5,4'-tri-O-methylkaempferol and 2,3,4-tri-O-methyl-L-rhamnose; under the same conditions, F2 gave the above partially methylated aglycone and 2,3,4-tri-O-methyl-L-arabinose. Kaempferol, L-rhamnose and L-arabinose were identified as above; 3,5,4'-tri-O-methylkaempferol was identified by UV spectral analysis with shift reagents[13], MS and paper co-chromatography with authentic sample (solvents A and B); 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-L-arabinose were identified by paper co-chromatography (solvent O) and SiO_2 TLC (solvent I). Identification of F1 was confirmed by paper co-chromatography with authentic sample (solvents A, B, C and E). On H_2O_2 oxidation (according to Chandler and Harper [15]), band B2.1 gave 2 sugars which were identified as L-rhamnose and L-arabinose. On methylation (Me_2SO_4-K_2CO_3-Me_2CO) followed by acid hydrolysis, this band gave 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-L-arabinose and 5,4'-di-O-methylkaempferol which were identified as above. Thus band B2.1 must be a mixture of kaempferol 3-O-α-rhamnoside-7-O-α-arabinoside **(2)** and kaempferol 3-O-α-arabinoside-7-O-α-rhamnoside **(3)** which have not been reported previously. Attempts to separate further band B2.1 met with no success. Band B2.2 was identified as kaempferol 3,7-dirhamnoside (kaempferitrin) by UV spectral analysis in the presence of shift reagents[13] and paper co-chromatography with authentic sample (solvent A, B, C and E). The above identification was confirmed as follows. Both acid hydrolysis and α-rhamnosidase treatment gave kaempferol and rhamnose; controlled acid hydrolysis gave kaempferol, L-rhamnose and kaempferol 7-O-rhamnoside; H_2O_2 oxidation[15] gave L-rhamnose. Kaempferol, L-rhamnose and kaempferol 7-O-rhamnoside were identified as above.

Results and discussion. Kaempferol 3,7-dirhamnoside (kaempferitrin) is one of the most common kaempferol glycosides[17] but is reported here for the first time as a constituent of ferns. From the systematic viewpoint, it is of interest that *Asplenium trichomanes* contains 3 flavonol 3,7-diglycosides, since 2 acylated kaempferol 3,7-diglycosides are constituents of *Asplenium rizophyllum*[2], and there is a suggestion that flavonol 3,7-diglycosides are of restricted distribution[16]. The flavonoid pattern of *A. trichomanes* confirms that flavonoids isolated from ferns belonging to the genus *Asplenium* are kaempferol derivatives[2,3] but shows that the sugars (L-rhamnose and L-arabinose) found in the flavonoids of *A. trichomanes* are different from those (D-glucose and sophorose) previously encountered in the flavonoids[2,3] oft this genus.

Fig. 1.

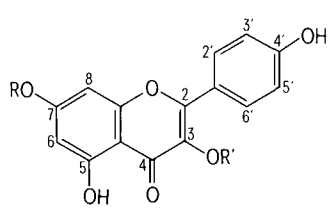


Fig. 2. (**1**) R = R' = α-L-Rhamnopyranosyl.

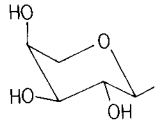


Fig. 3. (**2**) R = α-L-Arabinopyranosyl; R' = α-L-Rhamnopyranosyl. (**3**) R = α-L-Rhamnopyranosyl; R' = α-L-Arabinopyranosyl.

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