## EXPERIMENTAL

*Source of plants.* All vouchers are at OS. *SF* = Stuessy and Funk; *SN* = Stuessy and Nesom; *SRA* = Stuessy, Ricardi and Adamo. *Clibadium glabrescens* S. F. Blake: Colombia, Santander, 15 km NE of Bucaramanga, *SF* 5606. *C. laxum* S. F. Blake: Ecuador, Chimborazo, 2 km NW of Bucay, *SN* 5851; El Oro, 10.5 km W of Piñas, *SN* 5867, 40.6 km ENE of La Avanzda, *SN* 5870. *C. mexiae* S. F. Blake: Ecuador, Pastaza, W edge of Puyo, *SN* 5817, 3.6 km W of Puyo, *SN* 5819. *C. microcephalum* S. F. Blake: Ecuador, Tungarahua, 3.3 km E of Río Topo on rd to Puyo, *SN* 5814, 4.8 km E of Río Topo on rd to Puyo, *SN* 5816, 10.4 km W of Mera, *SN* 5822, 11.9 km W of Mera, *SN* 5823. *C. pedunculatum* Aristeg.: Venezuela, Mérida, La Carbonera, *SRA* 6034, just W of La Carbonera, *SRA* 6038, 5 km W of La Carbonera, *SRA* 6042. *C. pentaneuron* S. F. Blake: Colombia, Antioquía, 3 km SE of Santa Elena, *SF* 5709. *C. sprucei* S. F. Blake: Ecuador, Tungarahua, slopes of Volcán Tangarahua, *SN* 5811. *C. terebinthinaceum* DC.: Colombia, Valle, just S of Cordoba, *SF* 5725, 2 km W of Queremal, *SF* 5737, 6 km W of Queremal, *SF* 5742.

*Isolation of flavonoids.* Flavonoids were isolated by procedures described in Wilkins and Bohm [5] and Gornall and Bohm [6].

*Identification of flavonoids.* Structures were established using standard UV [7], NMR [7] and MS techniques [8]. The unidentified eriodictyol 7-monoglycoside was chromatographically identical to eriodictyol 7-glucoside isolated and identified by these methods in earlier studies. The nature of the sugar in the unknown compound was not determined.

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# FLAVONOL GLYCOSIDES FROM *ASPLENIUM BULBIFERUM*

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**Key Word Index**—*Asplenium bulbiferum*; Aspleniaceae; flavonol glycosides; kaempferol 3,7-diglucoside; kaempferol 3-*O*-rhamnoside-7-*O*-glucoside; kaempferol 3-*O*-β-glucoside-7-*O*-β-galactoside.

**Abstract**—From aerial parts of the fern *Asplenium bulbiferum*, besides kaempferol 3,7-diglucoside and kaempferol 3-*O*-rhamnoside-7-*O*-glucoside, the new glycoside kaempferol 3-*O*-β-glucoside-7-*O*-β-galactoside has been characterized.

Although chemical investigations of some *Asplenium* species have led to interesting results [1, 2] in connection with the identification of hybrid plants, the chemistry of most species of *Asplenium* is not well known. Previous work [3, 4] on the flavonoids of *Asplenium bulbiferum* Forster f. showed the presence of kaempferol 3,7-diglucoside, 3-*O*-rhamnoside-7-*O*-glucoside and 3-*O*-glucoside-7-*O*-rhamnoside. In the present work two flavonoid bands (A and B) were isolated from an ethanolic extract of aerial parts of *A. bulbiferum*.

Colour reactions (dull ochre to yellow in UV + $NH_3$) and UV spectral data: $\lambda_{max}^{MeOH}$ nm 266, 320 (sh), 343; + NaOAc 266, 355, 393 (sh); + NaOAc–$H_3BO_3$ 268, 347; + $AlCl_3$ 273, 298 (sh), 345, 390; + $AlCl_3$–HCl 272, 297 (sh), 340, 389; + NaOMe 270, 300 (sh), 390 (increase in intensity) suggest [5] that band A is a 3,7-disubstituted flavonol glycoside with free hydroxyl groups at the 5 and 4′ positions. Both total acid hydrolysis and treatment with β-glucosidase gave kaempferol, D-glucose and D-galactose; controlled acid hydrolysis gave, in addition to the products of total acid hydrolysis, kaempferol 7-glucoside, kaempferol 7-galactoside and a small amount of kaempferol 3-glucoside. On hydrogen peroxide oxidation [6] band A gave glucose. Methylation followed by acid hydrolysis gave 5,4′-di-*O*-methylkaempferol, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-

galactose. These findings indicate that band A must be a mixture of kaempferol 3-*O*-β-glucoside-7-*O*-β-galactoside (**1**), which is a new natural product, and kaempferol 3,7-diglucoside (**2**). Attempts to further separate this band were unsuccessful. Identification of kaempferol 3,7-diglucoside was confirmed by paper co-chromatography (four solvents) with an authentic marker.

Colour reactions (dull ochre to yellow in UV + $NH_3$) and UV spectral data: $\lambda_{max}^{MeOH}$ nm 263, 338; + NaOAc 263, 393; + NaOAc–$H_3BO_3$ 265, 345; + $AlCl_3$ 277, 295 (sh), 345; $AlCl_3$–HCl 270, 290 (sh), 340, 393; + NaOMe 271, 399 (increase in intensity) suggest [5] that band B may be a 3,7-disubstituted flavonol glycoside with free hydroxyl groups at positions 5 and 4'. Total acid hydrolysis gave kaempferol, D-glucose and L-rhamnose; controlled acid hydrolysis gave, in addition to the products of total acid hydrolysis, kaempferol 7-glucoside and a small amount of kaempferol 3-rhamnoside. Hydrogen peroxide oxidation [6] gave L-rhamnose; methylation followed by acid hydrolysis gave 5,4'-di-*O*-methylkaempferol, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose. These results show that band B must be kaempferol 3-*O*-rhamnoside-7-*O*-glucoside (**3**) which has recently been isolated [7] from *Betula* species.

That the flavonoids found in *Asplenium bulbiferum* are all flavonol 3,7-diglycosides is of some systematic interest since flavonol 3,7-diglycosides have already been found in three other *Asplenium* species (*A. rhizophyllum* [2], *A. trichomanes* [8] and *A. septentrionale* [9]) and it has been suggested that such flavonoids are of restricted distribution [10]. The presence of D-galactose in **1** is also of note since this sugar does not appear to have been reported before in association with flavonoids of the genus *Asplenium*.

## EXPERIMENTAL

*Plant material.* Aerial parts of *Asplenium bulbiferum* were collected in the Botanic Garden of the University of Naples.

*Isolation.* Aerial parts (800 g) of *A. bulbiferum* were homogenized and extracted × 3 with hot 95% EtOH. The combined extracts were filtered, concd to small vol. *in vacuo* and re-filtered. Flavonoid bands A (12 mg) and B (10 mg) were isolated by prep. PC in BAW; the bands were cut out, eluted with 70% EtOH, concd and rechromatographed in 15% HOAc and BEW. $R_f$ data (on Whatman No 1 paper) for bands A and B are: BAW, 0.41, 0.44; 15% HOAc, 0.70, 0.69; BEW, 0.42, 0.50; PhOH satd with $H_2O$, 0.68, 0.70.

*Hydrolysis procedures.* Total acid hydrolysis was carried out with 2 M HCl (2 hr at 100°). Controlled acid hydrolysis was carried out with 10% HOAc (3.5 hr reflux). Enzymic hydrolysis with β-glucosidase was carried out in citrate–phosphate buffer, pH 4.5 at 37° for 20 hr. Kaempferol was identified by co-PC with an authentic sample (four solvents), TLC on polyamide ($C_6H_6$–MeCOEt–MeOH, 3:1:1) and UV spectral analysis with the customary shift reagents [5]. Sugars were identified by co-PC (four solvents) and TLC (*n*-BuOH–HOAc–$Et_2O$–$H_2O$, 9:6:3:1). Kaempferol 7-glucoside, kaempferol 7-galactoside and kaempferol 3-glucoside were identified by UV spectral analysis with the usual shift reagents [5], total acid hydrolysis and co-PC with authentic materials (four solvents).

*Methylation.* Flavonoid bands were methylated with MeI in $HCONMe_2$ in the presence of $Ag_2O$ (18 hr in the dark with stirring at room temp) and subsequently hydrolysed with 0.3 M HCl (4 hr reflux). 2,3,4,6-Tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-L-rhamnose were identified by co-PC [11] and TLC on silica gel. 5,4'-Di-*O*-methylkaempferol was identified by UV spectral analysis with shift reagents [5] and co-PC with authentic material (three solvents).

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