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TWO NOVEL FLAVONOL GLYCOSIDES FROM THE FERN CHEILANTHES FRAGRANS

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Abstract-Two new flavonol glycosides have been found in the fern Cheilanthes fragrans. By chemical trasformations and spectroscopic methods it has been shown that these compounds are quercetin 3-O-glucoside-7-O-neohesperidoside (I) and quercetin 3-O-galactoside-7-O-neohesperidoside (II). The possible phylogenetic interest of these flavonoids is discussed.

In recent years a number of flavonoid aglycones have been reported[1] from farinose exudations (on the lower surface of fronds) of some ferns belonging to the genus Cheilanthes but little is known of the flavonoid glycosides of this genus from which only two such compounds have been reported[2,3] so far.

Now a flavonoid band has been isolated from an ethanolic extract of aerial parts of Cheilanthes fragrans by preparative paper chromatography. Colour reactions (dull ochre to yellow in UV+NH_3), chromatographic behaviour and UV spectrum (Tab.1) suggest that the isolated band may be a flavonol glycoside. The UV spectral analysis in the presence of the customary shift regents[4] gave interesting structural informations. The absence of bathocromic shift of the band at 257 nm (band II) in the presence of NaOAc suggests the absence of a free hydroxyl group at position 7 whereas the 3-hydroxyl group must be substituted because the UV spectrum shows no change with $ZrOCl_2$/citric acid[5]. The bathocromic shifts in the presence of $AlCl_3$, $AlCl_3$/HCl and NaOAc/H_3BO_3 suggest the presence of a free hydroxyl group at position 5 and the presence of a free ortho-dihydroxyl group which presumably is in 3',4' position since the UV spectrum (without shift reagent) shows a shoulder at 265 nm. The presence of a free hydroxyl group in position 4' is confirmed by the bathocromic shifts of the band at 350 nm (band I) in the presence of NaOAc and NaOMe.

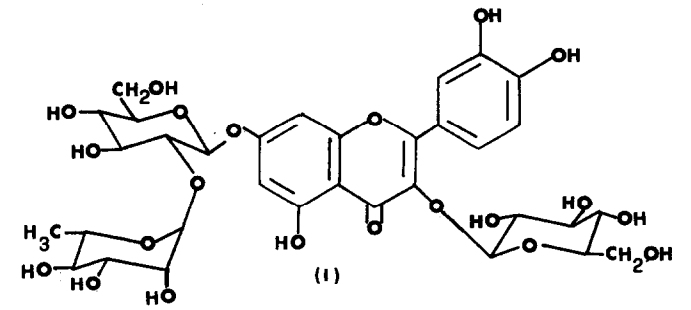
Total acid hydrolysis gave quercetin, D-glucose, D-galactose and L-rhamnose. Controlled acid hydrolysis gave, in addition to the products of total acid hydrolysis, neohesperidose (2-O-α-L-rhamnosyl-D-glucose), quercetin 7-O-glucoside and quercetin 7-O-neohesperidoside. On oxidation with hydrogen peroxide[6] (which releases the sugars at the 3-position of flavonol glycosides), D-glucose and D-galactose were obtained. Treatment with β-glucosidase gave quercetin 7-O-neohes-

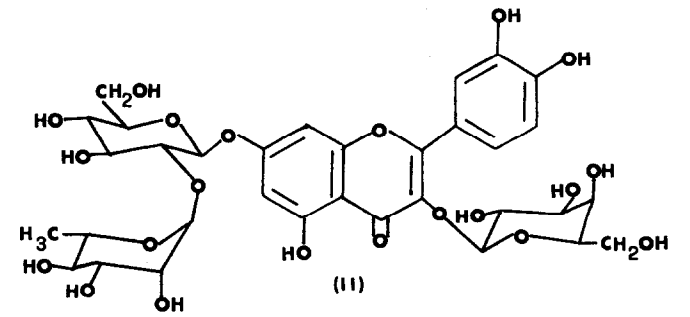
peridoside. The above results show that the isolated band must be a mixture of quercetin 3-O-glucoside-7-O-neohesperidoside (I) and quercetin 3-O-galactoside-7-O-neohesperidoside (II) which are new natural products. Attempts to further separate this band was unsuccessful.

Table 1. UV spectral data and R_f values of the mixture of flavonoids (I) and (II).

UV spectral properties		R_f values (on Whatman N.1 paper)	
Shift reagent	λ^{MeOH}_{max}	Solvent	R_f (×100)
		BAW	31
———	257,265 (sh),350	BEW	18
NaOMe	271,404 (inc.)	15% AcOH	60
NaOAc	257,403	H_2O	25
$NaOAc/H_3BO_3$	260,380		
$AlCl_3$	275,300,(sh),440		
$AlCl_3/HCl$	270,300 (sh),405		
$ZrOCl_2$/citric acid	258,265 (sh),351		

sh=shoulder;inc.=shift accompanied by increase in intensity;BAW=butan-1-ol/acetic acid/water (4:1:5,upper phase);BEW=butan-1-ol/ethanol/water (4:1:2.2).





It is interesting to note that in flavonol 3,7-diglycosides carrying a disaccharide and a monosaccharide attached to their hydroxyl groups,usually[7] the disaccharide is in the 3-position. However three flavonol 3,7-diglycosides (the 3-O-glucoside-7-O-gentiobioside of kaempferol,quercetin and isorhamnetin) in which a disaccharide is the 7-sugar (and a monosaccharide is attached to the 3-hydroxyl group) have been reported[8] from Nerisyrenia linearifolia (Cruciferae). In addition one such compound (quercetin 3-O-glucoside-7-O-rutinoside) has been found[9] in Ba-

ptisia lecontei (Leguminosae). These observations show that the presence of flavonoids (I) and (II) in Cheilanthes fragrans may provide a phylogenetic link between ferns and angiosperms.

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EXPERIMENTAL

Isolation:Aerial parts (108 g) of Cheilanthes fragrans (collected in Catania) were homogenized and extracted three times with hot 95% ethanol. The combined extracts were filtered and evaporated at reduced pressure to afford a residue (35 g) from which the flavonoid band was isolated by preparative paper chromatography in butan-1-ol/acetic acid/water (4:1:5,upper phase);the band was cut out,eluted with 70% ethanol,concentrated at reduced pressure and rechromatographed in 15% acetic acid and in butan-1-ol/ethanol/water (4:1:2.2) to give a mixture of flavonoids (I) and (II) (25 mg).

Total acid hydrolysis: The mixture (4 mg) of flavonoids (I) and (II) was dissolved in 2 N HCl (20 ml) ;the solution was heated on a steam bath for 2 h and then couled and extracted three times with Et_2O;the extract was washed with H_2O,dried and evaporated at reduced pressure. Quercetin was identified by paper chromatography (four solvent systems),polyamide TLC (C_6H_6:MeCOEt:MeOH,3:1:1) and UV spectral analysis[4] with the customary shift reagents.

The aqueous layer was evaporated under reduced pressure and the residue obtained was compared with standard sugars on paper chromatigraphy (four solvent systems) and TLC (silica gel,n-BuOH:AcOH:Et_2O:H_2O,9:6:3:1). L-Rhamnose,D-glucose and D-galactose were identified.

Controlled acid hydrolysis:The mixture (15 mg) of flavonoids (I) and (II) was dissolved in 10% acetic acid (30 ml) and the solution was refluxed for 2 h. The hydrolysate was evaporated to dryness in vacuo and excess acetic acid was removed by adding a small amount of water to the residue and evaporating to dryness in vacuo. By using the above methods,in addition to products of total acid hydrolysis,neohesperidose (2-O-α-L-rhamnosyl-D-glucose) was identified.

The identification of neohesperidose was confirmed in the following way. Authentic samples of 2-O-α-L-rhamnosyl-D-glucose (neohesperidose),6-O-α-L-rhamnosyl-D-glucose (rutinose),3-O-α-L-rhamnosyl-D-glucose and 4-O-α-L-rhamnosyl-D-glucose were hydrolysed with 10% acetic acid (3.5 h under reflux). Sugar analysis of hydrolysates shows that these disaccharides are partially hydrolysed with the single exception of neohesperidose which is completely hydrolysed. When the mixture of flavonoids (I) and (II) is refluxed with 10% acetic acid for 3.5 h,no disaccharide is detected in the hydrolysate;these results confirm that the disaccharide attached to the 7-hydroxyl group of flavonoids (I) and (II) must be neohesperidose.

Authentic samples of the above disaccharides were prepared as follows. Neohesperidose was obtained from naringin (Fluka AG,Buchs) by controlled acid hydrolysis; rutinose was prepared from rutin (Fluka AG,Buchs) by H_2O_2 oxidation[6] ;3-O-α-L-rhamnosyl-D-glucose and 4-O-α-L-rhamnosyl-D-glucose were synthesized by the Koenigs-Knorr reaction[10,11].

From another part of the hydrolysate obtained from the mixture of flavonoids (I) and (II) by controlled acid hydrolysis (10% acetic acid,2 h under reflux),two flavonol 7-glycosides were isolated by preparative paper chromatography in 15% acetic acid. These compounds were identified as quercetin 7-O-glucoside and quercetin 7-O-neohesperidoside by UV spectral analysis in the presence of the customary shift reagents[4],total acid hydrolysis and controlled acid hydrolysis.

Enzymic hydrolysis:The mixture (2 mg) of flavonoids (I) and (II) was dissolved in 4 ml of citrate-phosphate buffer,pH 4.5 and β-glucosidase (about 2 mg) (Fluka AG, Buchs) was added. The mixture was allowed to stand at 37°C for 20 h. After concentration under high vacuum,quercetin 7-O-neohesperidoside was identified by the above methods.

References

1. Wollenweber,E. and Dietz,V.H. (1981) Phytochemistry 20,869.
2. Imperato,F. (1985) Chem. Ind. (London),799.
3. Imperato,F. (1986) Chem. Ind. (London),878.
4. Mabry,T.J.,Markham,K.R. and Thomas,M.B. (1970) The Systematic Identification of Flavonoids,Springer-Verlag,Berlin,Heidelberg,New York.
5. Sosa,F. and Percheron,F. (1970) Phytochemistry 9,441.
6. Chandler,B.V. and Harper,K.A. (1961) Aust. J. Chem. 14,586.
7. Harborne,J.B. and Williams,C.A. (1982) in The Flavonoids:Advances in Research, Harborne,J.B. and Mabry,T.J. (eds), p.261-311,Chapman and Hall,London.
8. Bacon,J.D. and Mabry,T.J. (1976) Phytochemistry 15,1087.
9. Markham,K.R.,Mabry,T.J. and Swift,W.T. (1970) Phytochemistry 9,2359.
10. Imperato,F. (1976) J. Org. Chem. 41,3478.
11. Imperato,F. (1977) Carbohyd. Res. 58,217