A Dihydroflavonol with Taxonomic Significance from the Fern *Notholaena sulphurea*

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A new flavonoid, 2,3-*trans*-5,2′-dihydroxy-7,8-dimethoxy-dihydroflavonol-3-O-acetate, was isolated from the farinose coating on the lower leave surface of the fern, *Notholaena sulphurea.* The 2,3-*cis* diastereoisomer was isolated as a co-constituent. This novel acylated flavonoid is characteristic for the chemotype of *N. sulphurea* exhibiting yellow frond exudate. Its orrurrence underlines the affiliation of the species with the genus *Notholaena.*

Introduction

Notholaena sulphurea (Cav.) J. Sm. is one of the many ferns in the genus *Notholaena* that exhibit a conspicuous farinose (ceraceous) indument on their lower leaf surface. This species is sparingly distributed from northeastern Mexico to Chile (Tryon, 1956). The color of its indument is either white or yellow, and these forms obviously represent different chemotypes. The chemical composition of two populations with white and yellow farina, respectively, has been studied previously (Arriaga-Giner *et al.*, 1987). From the frond exudate of the yellow form we now isolated a further constituent, which has been identified to be a new dihydroflavonol acetate.

Material and Methods

Fronds of the yellow form of *Notholaena sulphurea* were collected in Mexico, Edo. Hidalgo, in May 1983, as reported previously (Arriaga-Giner *et al.*, 1987).

The crystalline deposit from the concentrated acetone rinse of the farinose exudate material, earlier referred to as "crude NS" (Arriaga-Giner *et al.*, 1987), was extracted with boiling toluene to yield a mixture of mostly nonpolar flavonoids. We isolated the product now analyzed using column chromatography over polyamide and subsequently over silica (elution with toluene and increasing proportions of MeCOEt and MeOH).

Thin layer chromatograms were run on polyamide (DC 11, Macherey-Nagel) with the solvents a) petrol$_{100-140}$-toluene-MeCOEt-MeOH 12:6:1:1 v/v/v/v and b) toluene – petrol$_{100-140}$ – MeCOEt – MeOH 12:6:2:1 v/v/v/v, and on silica with c) toluene- MeCOEt 9:1 v/v. Chromatograms were viewed under UV (366 nm) before and after spraying with "Naturstoffreagenz A" (a 1% methanolic solution of diphenyl-boric acid-ethanolamine complex).

Preparative HPLC separation of the two components was achieved on a 10 µm Econosil RP-18 column (250 × 10 mm) using isocratic elution with 34% MeCN in 1% aqueous HCO_2H at 5.6 ml min^{-1}. The UV trace was recorded at 280 nm. The two peak fractions were collected manually, concentrated on a rotavapor and subsequently lyophilized. The separation conditions were optimized using an analytical HPLC column (5 µm RP-18 column, 250 × 4 mm, Phenomenex, Torrance, CA).

Atmospheric pressure chemical ionization (APCI) mass spectra were recorded on a PE Sciex API III Plus triple-quadrupole instrument as described in Stevens *et al.* (2000). HREIMS was performed on a Kratos MS50 mass spectrometer. NMR experiments were run on a Bruker DRX 600 instrument at 600 MHz (^{1}H) and 150 MHz (^{13}C). Samples were dissolved in DMSO-d_6 and

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analyzed at room temperature. DMSO resonances (δ_H 2.50 and δ_C 39.51) were used as internal chemical shift references.

Results and Discussion

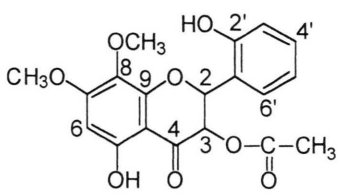
From crystalline material obtained during an earlier examination of the yellow frond exudate of *N. sulphurea* (Arriaga-Giner *et al.*, 1987), a product was isolated that was homogeneous by TLC on silicagel and polyamide. Analytical HPLC revealed the presence of two components, which were separated by preparative HPLC.

Structure elucidation

Both components showed a molecular ion peak at *m/z* 375 in the APCI mass spectrum. The MS-MS spectra of the two components were virtually identical, indicating that the two components were isomers. Mass fragmentation of the *m/z* 375 ion yielded a prominent retro-Diels-Alder fragment ion with *m/z* 197, suggesting the presence of an A-ring bearing one hydroxy and two methoxy substituents. Additional fragments with *m/z* 182 (197–15), 164 (182–18) and 136 (164–28) resulted from loss of a methyl radical, a water molecule, and a CO molecule, respectively. A less abundant fragment ion with *m/z* 315 (375-HOAc) pointed to the presence of an acetate function.

The earlier eluting major component (**1a**) showed a molecular ion peak M^+ at *m/z* 374.10036 in the HREIMS spectrum, consistent with the molecular formula $C_{19}H_{18}O_8$ (calcd. mass 374.10017). The UV spectrum (lmax 292 nm in MeOH; log ε, 4.23) suggested that the compound was a flavanone or a dihydroflavonol. A dihydroflavonol skeleton was apparent from two doublets at δ_H 5.85 (H-2) and δ_H 6.10 (H-3). The magnitude of the coupling constant, $J_{2,3}$ = 12 Hz, indicated that the relative configuration was 2,3-*trans*. Compared with the dihydroflavonol, taxifolin, the large downfield shift of the H-3 doublet indicated that the 3-OH group was substituted. This substituent was identified as an acetyl function, of which the methyl protons resonated at δ_H 1.96. In addition, the ^1H spectrum showed two methoxy substituents (δ_H 3.86 and δ_H 3.58) which were placed at C-7 and C-8 as follows. Prior to examination of the HMBC spectrum, the carbon resonances at δ_C 153.2 and δ_C 158.8 were assigned to C-9 and C-5, respectively, on the basis of comparison with δ_C values reported for 5,2′-dihydroxy-7,8,6′-trimethoxyflavanone ($\delta_{C\text{-}9}$ 154.6, $\delta_{C\text{-}5}$ 159.2) and for 5,2′-dihydroxy-6,7,6′-trimethoxyflavanone ($\delta_{C\text{-}9}$ 154.4, $\delta_{C\text{-}5}$ 159.3) (Tomimori *et al.*, 1985). Carbon-5 could not be identified from HMBC analysis, because the OH-5 proton (d_H 11.39) did not interact with C-5. In the HMBC spectrum, the aromatic proton at δ_H 6.32 showed interactions with all A-ring carbons except for C-9 (δ_C 153.2), indicating that the aromatic proton was located *para* to C-9. This proton was therefore identified as H-6, leaving positions 7 and 8 available for the methoxy groups.

The B-ring protons gave multiplets at d_H 7.44, 7.24, 6.90, and 6.87, the pattern being characteristic of 2′-O-substitution (Markham and Geiger, 1994). The double doublet at δ_H 7.44 (H-6′) showed a cross peak with the multiplet at δ_H 6.87 (H-5′), which, in turn, showed a strong interaction with the multiplet at δ_H 7.24 (H-4′) in the ^1H-^1H COSY spectrum. The latter signal exhibited a strong interaction with the double doublet at δ_H 6.90 (H-3′), thus confirming the presence of an OH (d_H 9.92) group at C-2′. The structure of **1a** was therefore identified as *2,3-trans*-5,2′-dihydroxy-7,8-dimethoxy-dihydroflavonol-3-O-acetate, a novel flavonoid. The assignment of the proton and carbon resonances (Table I) was consistent with ^1H-^{13}C interactions observed in the HMQC and HMBC spectra.



1a (2,3-*trans*)
1b (2,3-*cis*)

The second component (**1b**) showed a molecular ion peak M^+ at *m/z* 374.10021 in the HREIMS spectrum, also consistent with the molecular formula $C_{19}H_{18}O_8$. The UV (l_{max} 294 nm in MeOH; log ε, 4.20) and ^1H data of flavonoid **1b** were very similar to that of compound **1a** (Table I), except for the magnitude of the coupling constant, $J_{2,3}$= 2.4 Hz, indicating that the relative configuration of **1b** is 2,3-*cis*. Compound **1b** was therefore iden-

Table I. NMR data (DMSO-d_6) of compound **1** [δ in ppm, mult. (*J* in Hz)].

Atom no.	**1a** δ_C (2,3-*trans*)	**1a** δ_H (2,3-*trans*)	**1b** δ_H (2,3-*cis*)
2	71.5	6.10 d (11.8)	5.99 d (2.4)
3	75.4	5.85 d (11.8)	5.65 d (2.4)
4	192.5		
5	158.8	11.39 s (OH)	11.52 s (OH)
6	93.7	6.32 s	6.34 s
7	161.6		
8	129.3		
9	153.2		
10	101.3		
1′	121.2		
2′	156.0	9.92 OH	9.98 s (OH)
3′	115.7	6.90 dd (8.1, 0.7)	6.88–6.82 m
4′	130.5	7.24 dt (7.7, 1.5)	7.19 dt (7.7, 1.5)
5′	119.1	6.87 dt (7.7, -)	6.88–6.82 m
6′	128.8	7.44 dd (7.7, 1.5)	7.39 dd (7.7, 1.5)
OCH_3-7	56.5	3.86 s	3.89 s
OCH_3-8	60.4	3.58 s	3.66 s
O=C-**CH_3**	20.1	1.96 s	1.85 s
O=**C**-CH_3	168.8		

tified as *2,3-cis*-5,2′-dihydroxy-7,8-dimethoxy-dihydroflavonol-3-O-acetate.

Flavonoid distribution

Compound **1** is a novel natural flavonoid. Its substitution pattern fits that of earlier identified flavonoid aglycones from the yellow form of *Notholaena sulphurea*: 3,5-diOH-7-OMe-8-OAc-flavonol, 3,5,2′-triOH-7-OMe-8-OAc-flavonol, 5,2′-diOH-7,8-diOMe-flavone (skullcapflavone I) and traces of 3,5,4′-triOH-7-OMe-8-OAc flavonol (+ traces of kaempferol-7-Me, quercetin-7,3′-diMe, and pinocembrin-7-Me) (Arriaga-Giner *et al.*, 1987). It further supports the earlier assumption that the yellow and white "forms" of *N. sulphurea* at least represent two distinct chemotypes, which eventually may be considered as taxonomic varieties. The same type of observation of chemotypes was made earlier for *N. californica* (Wollenweber *et al.*, 1981). In *N. candida*, the two recognized varieties *N. candida* var. *candida* and *N. candida* var. *copelandii* are also characterized by clearly different exudate flavonoid patterns (Wollenweber, 1984), even though the farina is white in both cases.

Products with 8-O-substitution and/or 2′-O-substitution are rather abundant among the methylated exudate flavonoids in *Notholaena*, and natural acetates and butyrates are also abundant in this genus (Scheele and Wollenweber, 1987; Wollenweber *et al.*, 1988). The white form of *C. sulphurea*, where 2′,6′-diOH-4′-OMe-dihydrochalcone, 2′,6′-diOH-4′,4-diOMe-dihydrochalcone and 2′,4′,6′-triOH-4′-OMe-dihydrochalcone (asebogetin) dominate as exudate constituents, also produces the 3-O-acetate, the 3-O-butyrate and the 4′-O-butyrate of aromadendrin-7-Me (Arriga-Giner *et al.*, 1987). One further acetylated dihydroflavonol has been reported from *N. neglecta* (3,5-diOH-7,8-diOMe-2′-OAc dihydroflavonol; Scheele and Wollenweber, 1987). It appears important that flavonoids with 8-O- and/or 2′-O-substitution as well as acylated flavonoid aglycones are completely lacking in *Argyrochosma* (Wollenweber and Schneider, 2000), a genus that has recently been separated from *Notholaena* (*sensu* R. Tryon, 1956) by Windham (1987). This is one of the chemical characters that strongly support the separation of *Argyrochosma* from *Notholaena* (Wollenweber and Schneider, 2000). It should be noted that the latter authors used the generic name *Chrysocosma* (J.Sm.) Kuemmerle for species that are retained in *Notholaena* by most botanists.

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