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NOVEL 2′-SUBSTITUTED FLAVONOLS FROM THE FARINOSE EXUDATE OF *NOTHOLAENA AFFINIS*

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Key Word Index—*Notholaena affinis*; Gymnogrammoideae; Pteridophyta; farinose exudate; novel flavonols; 5,2′,4′-triOH, 3,6,7,8-tetraOMe flavone; 5,4′-diOH, 3,6,7,8,2′-pentaOMe flavone; 5,2′-diOH,3,6,7,8,4′-pentaOMe flavone; 5-OH, 3,6,7,8,2′,4′-hexaOMe flavone.

Abstract—The farinose exudate produced on the undersurface of fronds of *Notholaena affinis* consists of a variety of lipophilic flavonol aglycones. Four of these have been identified as novel interrelated compounds. They all bear methoxyl groups in positions 3,6,7,8 and hydroxyl and/or methoxyl groups at 2′,4′.

INTRODUCTION

As part of a screening program on flavonoid excretion in farinose species of the Gymnogrammoideae [1], the light yellow farina produced by *Notholaena affinis* (Mett.) Moore has been investigated. The major component of the exudate has previously been shown [2] to be the new flavonol aglycone 7,4′-dimethylherbacetin esterified with butyric acid in position 8. We now wish to report the structural elucidation of four additional flavonol aglycones found as minor constituents of this farina.

RESULTS AND DISCUSSION

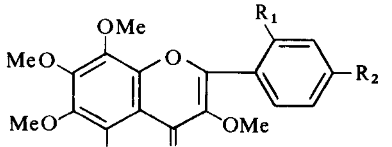
These compounds are lipophilic flavonoids, which on polyamide TLC are best separated by solvents composed of C_6H_6 and petrol. Spots are dark in UV_{365}, turning to different shades of brown after spraying with the aminoethyl ester of boric acid. This behaviour indicates that they are either flavones or 3-methylflavonols, with a free hydroxyl at C-5 and probably a methoxyl at C-6.

Compound **1** is the most polar substance in this series. According to its M^+, it has three hydroxyl and four methoxyl groups. Analysis of the UV spectra following standard procedures [3] confirms a free OH at C-5 ($\Delta\lambda_{max}$ I $AlCl_3$–HCl +12 nm), the localization of a second free OH at C-4′ ($\Delta\lambda_{max}$ I NaOMe +54 nm without decrease of the intensity), and a blocked OH at C-7 ($\Delta\lambda_{max}$ II NaOAc 0 nm). Furthermore it indicates the absence of an *o*-diOH group (reactions with H_3BO_3 and with $AlCl_3$ and $AlCl_3$/HCl). The latter observation, together with MS data (*m/e* 137 showing B-ring with 2 OH; *m/e* 373 showing M-17 fragmentation [4]), indicates that the third OH is located at C-2′. PMR indeed shows the presence of a deshielded proton (δ 7.42 ppm), *o*-coupled with a second H, itself is *m*-coupled with a third H, thus proving the proposed substitution pattern of the B-ring. In consequence, the three remaining OMe groups must be placed at C-3, C-6 and C-8. Compound **1** is thus identified as 5,2′,4′-trihydroxy-3,6,7,8-tetramethoxyflavone.

Compound **2** differs from **1** by an additional OMe group, as shown by M^+ 404; hence it is a dihydroxypentamethoxyflavone. TLC behaviour and UV spectra point to the same structural type as **1**; also PMR shows the same resonance values in the region of the aromatic protons. The position of the supplementary OMe-group is indicated by MS fragmentation (*m/e* 151: B-ring with 1 OH and 1 OMe; *m/e* 387: M-17, typical for 2′-hydroxylation [4]) and UV spectrum ($\Delta\lambda_{max}$ I NaOMe +42 nm with a decrease of intensity indicating a blocked OH at C-4′). Compound **2** is thus 5,2′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone.

Compound **3** is very similar to **2**, especially in the PMR data and MS fragmentations (M^+ 404; important *m/e* 151). However the R_f of **3** is lower than that of **2**; the UV spectrum on addition of NaOH indicates a free OH at C-4′; the peak *m/e* 387 corresponding to the ion M-17 is absent. All these facts indicate the presence of a 4′-OH and a 2′-OMe. Hence the structure is 5,4′-dihydroxy-3,6,7,8,2′-pentamethoxyflavone.

Compound **4** is the least polar product of the series. Its M^+ 418 shows that it is a monohydroxy-hexamethoxyflavone. The region of aromatic protons in the NMR spectrum is similar to that of **2** and **3**; *m/e* 165 in the mass spectrum is characteristic for a B-ring with two OMe groups. Evaluated together with the UV spectrum ($\Delta\lambda_{max}$ I NaOMe +44 nm with decrease of intensity), these observations indicate **4** to be 5-hydroxy-3,6,7,8,2′,4′-hexamethoxyflavone.



1 $R_1 = R_2 = OH$
2 $R_1 = OH$ $R_2 = OMe$
3 $R_1 = OMe$ $R_2 = OH$
4 $R_1 = R_2 = OMe$

Table 1. TLC and spectral data for compounds **1**–**4** from farinose exudate of *Notholaena affinis*

	1	**2**	**3**	**4**
TLC on polyamide				
R_f × 100 solv. B	45	82	74	98
solv. D (2 runs)	19	67	36	91
UV λ_{max} nm				
MeOH	(258), 268, 346	(256), 267, 344	256, 270, 336 (356)	256, 270, 326 (358)
NaOAc	(258), 267, 362	(256), 266, 346	256, 268, 358	256, 270, 328 (358)
NaOAc/H_3BO_3	(258), 268, 348	(258), 267, 346	258, 270, 336	
$AlCl_3$	275, 360, (408)	277, 352, (410)	278, 358, (406)	274, 354, (410)
$AlCl_3$/HCl	275, 358, (410)	276, 350, (412)	282, 358, (406)	280, 348, (410)
NaOMe	270, (307), (358), 400	265, 386	254, 406	272, 370
MS *m/e* (rel. int.)				
M^+	390 (100)	404 (100)	404 (100)	418 (100)
Imp. ions	375 (50), 373 (6), 360 (9), 137 (9)	389 (55), 387 (5), 374 (67), 359 (40), 151 (20)	389 (90), 373 (6), 151 (10)	403 (90), 388 (25), 373 (28), 165 (10)
PMR 100 MHz				
(DMSO/TMS) OMe	3H *s* 3.72; 6H *s* 3.83; 3H *s* 4.03	3H *s* 3.72; 3H *s* 3.79; 6H *s* 3.83; 3H *s* 4.03	3H *s* 3.72; 3H *s* 3.79; 3H *s* 3.82; 3H *s* 3.85; 3H *s* 4.03	3H *s* 3.72; 3H *s* 3.81; 6H *s* 3,84; 3H *s* 3.88; 3H *s* 4.03
H-5′	*dd* 6.39 (*J* 2.5 and 8.5 Hz)	*dd* 6.57 (*J* 2.5 and 8.5 Hz)	*dd* 6.55 (*J* 2.5 and 8.5 Hz)	*dd* 6.72 (*J* 2.5 and 8.5 Hz)
H-3′	*d* 6.46 (*J* 2.5 Hz)	*d* 6.61 (*J* 2.5 Hz)	*d* 6.61 (*J* 2.5 Hz)	*d* 6.78 (*J* 2.5 Hz)
H-6′	*d* 7.42 (*J* 8.5 Hz)	*d* 7.37 (*J* 8.5 Hz)	*d* 7.35 (*J* 8.5 Hz)	*d* 7.48 (*J* 8.5 Hz)

The four compounds **1**–**4** are closely related structurally. Comparison of their chromatographic behaviour and spectral data (Table 1) provides information on the influence of methylation of the 2′- and/or 4′-hydroxyl in the case of flavonoids methoxylated in positions – 3,6,7,8: methylation of OH at C-4′ results in higher R_f on polyamide than methylation of 2′-OH. Methylation of both positions has, as expected, a cumulative effect; in the UV spectra, an OH group at C-2′ causes a slight bathochromic shift of band I in MeOH (compare calycopterin: (223), 278, 340 nm [5]). Methylation of 2′-OH in contrast causes a slight hypsochromic shift. This latter effect is more marked when both positions 2′ and 4′ are methylated; in MS, M^+ occurs as the base peak in all four flavonols. This suggests that 6,7,8-OMe derivatives of 3-methoxyflavones show similar fragmentation as 6-methoxy derivatives [6, 7]. It should be stressed, however, that calycopterin, which is the 2′-deoxy homologue of **1** behaves like an 8-OMe derivative. Hence, it is concluded that substitution of C-2′ can influence the relative intensity of ions M^+ and M-15; in PMR, we observe progressive downfield shifts of signals for H-3′ and H-5′, depending on methylation of the OH groups on the B-ring.

Compounds **1**–**4** have been found for the first time in nature. Farina of the genera *Cheilanthes* and *Notholaena* [1] have already been shown to contain methylated flavonoids, namely derivatives of apigenin, luteolin, kaempferol and quercetin; but these have a lesser degree of methylation in ring A. These present results show, once again, that ferns have a considerable capacity for methylating the flavonoid skeleton [8].

EXPERIMENTAL

Plant material. Fronds of *Notholaena affinis* were collected in Costa Rica (Prov. Gabacaste, Dez. '76). A voucher is kept at the Museo Nacional de Costa Rica at San José (LDG 4725).

Isolation. Exudate material was recovered by rinsing air-dried fronds with Me_2CO and C_6H_6; yield was *ca* 6% dry wt. Most of the major component [2] could be removed by crystallization from concd soln. The remainder was dried onto polyamide and roughly fractionated on a polyamide column, eluted with C_6H_6 and with increasing quantities of MeCOEt and MeOH [9]. The fractions thus obtained were further separated by PLC on Si gel (solvent A: C_6H_6–dioxan–AcOH, 90:12:3) or polyamide (solvents C_6H_6–petrol 100–140°–MeCOEt–MeOH, B: 60:26:7:7:, C: 60:60:7:7, D: 30:60:5:5).

Identification: see Table 1.

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