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3-METHOXYFLAVONES WITH UNUSUAL B-RING SUBSTITUTION FROM TWO SPECIES OF *NOTHOLAENA*

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Notholaena aliena, N. californica*; Pteridaceae; frond exudate; novel 3-methoxyflavonols.

Abstract—The structures of two flavonoid aglycones provisionally described previously from the frond exudate of the fern *Notholaena californica*, need revision. They are now established as 5,2′,5′-trihydroxy-3,7,8-trimethoxyflavone and its 2′-*O*-methyl derivative. The first-mentioned compound is also present in the frond exudate of *N. aliena*, where it is accompanied by its natural 2′-*O*-acetate. A further compound from the latter species is identified as 5,2′,3′-trihydroxy-3,7,8-trimethoxyflavone. These novel natural 3-methoxyflavones are remarkable for their unusual B-ring substitution patterns.

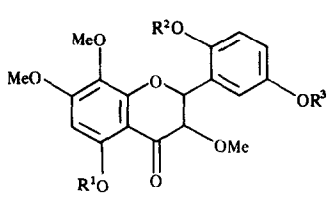
INTRODUCTION

As part of our investigations on exudate flavonoids of Cheilanthoid ferns (Polypodiaceae) [1, 2] we recently reported the occurrence of acylated flavonol aglycones in *Notholaena aliena* Maxon, in *N. aschenborniana* Kl., and in the yellow form of *N. californica* D. C. Eaton [1, 3]. Also flavonoids with rare B-ring substitution were reported from *N. aschenborniana* [4, 5] and from the yellow form of *N. californica* [6]. For two new flavonoids from the latter species the structures had been assigned only tentatively [6]. We have now been able to isolate, from bulk material collected in Arizona and Mexico, some flavonoids in the amounts needed for more detailed spectroscopic analysis. As a result, the previously proposed structures need to be revised. The structural elucidation of these, as well as that of two further novel flavonols, are reported here.

RESULTS AND DISCUSSION

Products **1** and **2**, for which tentative structures have been published previously [6], were re-isolated from the yellow form of *N. californica*. **1** was also found in *N. aliena*, but as a minor constituent only, along with the new flavonoids **3** and **4**. In both species the acylated flavonols **NG-1** (3,5-dihydroxy-7-methoxy-8-butyryloxyflavone) and **NG-2** (3,5-dihydroxy-7-methoxy-8-acetoxyflavone) [3] are the major constituents of the frond exudate.

The data for the new compounds **1**–**4** are compiled in Tables 1 (^{1}H NMR) and 2 (^{13}C NMR). The purple fluorescence of the spots on polyamide plates suggested that these compounds may either be flavones or 3-methylflavonols. They all showed a similar important UV shift on additon of aluminium trichloride, which is unaffected on addition of hydrochloric acid, indicating that they were indeed 5-hydroxyflavones lacking *o*-dihydroxy-substitution. Absence of a significant reaction on addition of sodium acetate as well as a positive NOE in ^{1}H NMR spectroscopy between one H-singlet and a methoxy group further indicated that they were all methylated at C-7. NOE experiments demonstrate the presence of two methoxy groups non-adjacent to protons. Thus the molecules contain a methoxyl at C-3. Also, the EIMS



	R¹	R²	R³
1	H	H	H
2	H	H	Me
3	H	Ac	H
5	Me	Me	Me
6	H	Me	Me

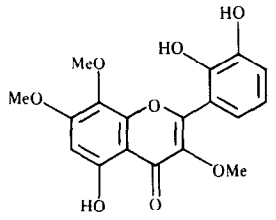


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Table 1. ^{1}H NMR spectra of compounds **1–6** (δ ppm/TMS; *J*: *o* = *ortho*, *m* = *meta*; C_6D_6)

	3	5	6	7	8	2′	3′	4′	5′	6′	OMe	OMe ($CDCl_3$)
1	OMe	OH	6.24	OMe[a]	OMe	OH	6.97	6.57	OH	7.17	3.65–3.44–3.22[a]	3.95–3.88–3.87
J			*	**			*o*	*o*, *m*		*m*		
Mult.			*s*				*d*	*dd*		*d*		
2	OMe	OH	6.24	OMe[b]	OMe	OMe	6.54	7.16	OH	7.4	3.71–3.65–3.15[b]	3.96–3.87–3.84
J			*	**		**	*o	*o*, *m*		*m*		3.81
Mult.			*s*				*d*	*dd*		*d*		
3	OMe	OH	6.23	OMe[c]	OMe	19.1†	6.89	6.59	OH	7.10	3.63–3.55–3.08[c]	
J			*	**			*o*	*o*, *m*		*m*		
Mult.			*s*			s	*d*	*dd*		*d*		
4	OMe	OH	6.23	OMe[e]	OMe	OH	OH	7.09	6.75	7.33	3.60–3.28–3.11[e]	
J			*	**			*o*, *m*	*o*, *o*	*o*, *m*			
Mult.			*s*					*d*	*dd* *d*			
5	OMe	OMe	5.97	OMe	OMe	OMe	6.50	6.82	OMe	7.08	3.87–3.71–3.50 3.31–3.25–3.24	
J							*o*	*o*,*m*		*m*		
Mult.			*s*				*d*	*dd*		*d*		
6	OMe	OH	6.23	OMe	OMe	OMe	6.46	6.81	OMe	7.02	3.75–3.65–3.30 3.22–3.12	3.94–3.84–3.82 3.81–3.80
J							*o*	*o m*		*m*		
Mult.							*d*	*dd*		*d*		

**NOE observed with *
a–e: values assigned by NOE
†Me–CO–.
Mult. = multiplicity.

Table 2. ^{13}C NMR of compounds **1** and **3** (Brukcr 50 MHz, δ ppm/TMS, DMSO-d_6)

C	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′
	C	C	CO	C	CH	C	C	C	C	C	C	CH	CH	C	CH
1	157.2	138.9	178.5	156.5	95.7	158	128.4	148.6	104.9	117.6	148.1	116.0	119.1	149.4	117.2*
3	154.7	139	178.4	156.5	96	158.4	128.4	148.4	104.9	123.6	128.2	124.5	118.6	140.4	116.3†

*OMe at 60.9, 60.0, 56.5 ppm.
†OMe at 60.9, 60.2, 56.5 ppm; *CO-Me* at 168.9 and 20.5 ppm respectively.

fragmentation showed similarities in the structures of these four 3-methylflavonols. Finally the ^{1}H NMR data indicated that in no case was the B-ring symmetrically substituted.

Compound **1**, which exhibits the molecular peak at *m/z* 360, is a hexa-*O*-substituted flavone with three methoxy groups ($C_{18}H_{16}O_8$). As noted above, one hydroxy-group is located at C-5 and one methoxy-group at C-7, so two hydroxy-groups and two methoxy-groups remain to be located. The absence of an NOE with two methoxy-groups in the ^{1}H NMR spectrum shows that the B-ring is substituted at C-2′ and at C-5′ by hydroxy groups. One of the two remaining methoxy groups can be located at C-3 as shown by fluorescence on TLC and by the singlet at 139 ppm in the ^{13}C NMR spectrum. The second methoxy-group is placed on the A-ring, which according to the ^{1}H NMR spectrum bears only one proton (*s*, 6.24 ppm). ^{1}H NMR studies of **5**, obtained by complete methylation of **1**, show an important NOE for two methoxy groups at 3.25 and 3.50 ppm on irradiation of the singlet at 5.97 ppm and inverse. Hence, the single proton on the A-ring can only be located at C-6. *O*-Substitution of C-8 is indicated by the signal at 128.4 ppm in the ^{13}C NMR spectrum and is further confirmed by the ratio of A band I/A band II in the UV spectrum [7]. Compound **1** is thus 5,2′,5′-trihydroxy-3,7,8-trimethoxyflavone.

In the course of these studies we noticed that the structural data relative to 6,7 and 7,8-substitution as given by Goudard *et al.* [8] on the basis of ion fragments observed in the mass spectrum, can be applied to compounds **5** [permethylated product, M^+ *m/z* 360] and **6** (5-desmethoxy derivative of 5; M^+ *m/z* 346; UV $ALCl_3$ BI), but not to compound **1**.

The ^{1}H NMR (Table 1) and the UV data indicate that the substituion patterns of rings A and C in compounds **2**, **3**, and **4** are identical to those of **1**. Compound **2** was assumed, on the basis of its chromatographic behaviour, to be a methyl ether of **1**. This assumption was sustained by the M^+ at *m/z* 384. While the ^{1}H NMR data show a 1′,2′,5′-trisubstituted B-ring, NOE experiments indicate in this case the presence of a methoxy group at C-2′. Moreover we observed that complete methylation of **2** gave **5** (UV, MS, ^{1}H NMR, TLC), obtained by methylation of **1**. Compound **2** is, therefore, 5,5′-dihydroxy-3,7,8,2′-tetramethoxyflavone.

From MS data **3** is an acetyl derivative of **1**: M^+ of **3** is at *m/z* 402, while M^+ of the derivative obtained after saponification is at *m/z* 360, indicating loss of an acetyl group (*m/z* 42). On the basis of ^{13}C NMR data this substituent can be located at C-2′: shielding of the *ipso* C and deshielding of two *ortho* C (C-1′ and C-3′). Thus, **3** is 5,5′-dihydroxy-3,7,8-trimethoxy-2′-acetoxyflavone.

Compound **4** is shown by its MS fragmentation to be an isomer of **1** and ^{1}H NMR data indicate that its B-ring is substituted at C-2′ and at C-3′. The two hydroxy groups of the B-ring are, therefore, located at C-2′ and C-3′. The structure of **4** is thus 5,2′,3′-trihydroxy-3,7,8-trimethoxy flavone. It should be mentioned that **4** is distinguished by a red fluorescence exhibited on polyamide TLC after spraying with Naturstoffreagenz A. This rare colour reaction is similar to that observed, e.g. with eriodictyol-7-methyl ether [9] or with 2′,2-dihydroxychalcone [10].

Compound **1** from *Notholaena californica* was suggested in a preliminary communication [6] to have 5,7-dihydroxy-8-methoxysubstitution at ring A and at ring B either 4′-hydroxy-3′,5′-dimethoxy or 4′-hydroxy-2′,6′-dimethoxy substitution, i.e. flavone structure with a symmetrically substituted B-ring. As stated above, we have now established that it is a 3-*O*-methyl flavonol with an asymmetrically 2′,5′-diOMe-substituted B-ring and **2** is its 2′-methyl derivative. To our knowledge, this type of B-ring substitution has only been found a few times before. First it was ascribed to a flavonol (3,5,2′-trihydroxy-7,5′-dimethoxy), a flavanone (5,7,2′,5′-tetrahydroxy) and two dihydroflavonols (3,5,7,2′-tetrahydroxy-5′-methoxy and 3,5,2′-trihydroxy-7,5′-dimethoxy) from aerial parts of *Inula cappa* (Compositae) [11]. Two further flavones with 2′,5′-diubstitution were found in *Scultellaria* species: 5,2′,5′-trihydoxy-7,8-dimethoxyflavone in *S. rehderiana* (rehderianin, revised structure; synthesis see [12]) and 5,8,2′,5′-tetrahydroxy-6,7,8-trimethoxyflavone in *S. baicalensis* [13].

Compounds **3** and **4** from *N. aliena* are also novel natural flavonols **3** being the 8-*O*-acetate of **1** and **4** having the unusual 2′,3′-*O*-substitution of ring B. This type of B-ring substitution has so far only been reorted for three flavones. One is 5,3′-dihydroxy-7,8,2′-trimethoxyflavone (wightin), from stems and leaves of *Andrographis wightiana* (Acanthaceae) [14]. We should mention here that the monomethyl ether of wightin, erroneously cited in refs [15, 16], was in fact obtained by methylation of wightin and is, therefore, not a natural product. 6,2′,3′-Trimethoxyflavone was reported from aerial parts of *Pimelea decora* (Thymeleaceae) [17] and 5,7,2′,3′-tetrahydroxyflavone was found in roots of *Scutellaria baicalensis* [18].

It is already known that several species of *Notholaena* are characterized by the production of flavonol acetates and flavonol butyrates as major components of their frond exudates: *N. aliena, N. aschenborniana, N. californica, N. galapagensis, N. galeottii, and N. neglecta* [2]. These species are not necessarily closely related within the genus [cf 19]. We recently found that the yellow form of *Notholaena sulphurea* produces a flavonol acetate, too (3,5,2′-trihydroxy-7-methoxy-8-acetoxy flavone) [20]. All these acetates and butyrates are esterified at C-8. Compound **3** is remarkable as the first acylated flavone found to be esterfied at C-2′, although it is *O*-substituted at C-8. We recently detected a 2′-acetoxyflavanone as a minor exudate constitutent in *Notholaena neglecta* (3,5-dihydroxy-7,8-dimethoxy-2′-acetoxyflavanone [21]. The production of **1**, which is a major flavonol of *N. californica* and a minor constituent also of *N. aliena*, along with its 8-acetyl derivative (**3**), points to similar biogenetic capability of these two species. In terms of morphology they are, however, well separated within the genus.

In *Notholaena californica* it has previously been pointed out [6] that two chemical races exist, which can be clearly distinghished by their flavonoid exudate chemistry. The form with yellow farina produces the esterified flavonols **NG-1** and **NG-2** and **1** and **2**, whereas the form with white farina produces a total of 14 methyl derivatives of the common compounds kaempferol, quercetin, apigenin, and luteolin. It has therefore been suggested recently [22] that these two forms must be treated as recognized varieties. This statement is, of course, not affected by the present revision of the provisional structures of **1** and **2**.

EXPERIMENTAL

N. aliena was collected on 17 Dec. 1981 SE of Delicias on Hwy 45 (Christopher Columbus Hwy), Edo Chihuahua, Mexico, where it was growing on volcanic outcrops at an altitude of some 1500 m. Voucher specimens (T, Reeves, L. Reeves and E. Wollenweber 7507) were deposited at Morris, MN and at Darmstadt. 170 g of dry fronds were rinsed with acetone to yield 2.33 g (1.4% dry wt) of crude exudate material. Further rinsing of the fronds with toluene yielded 1.06 g of lipoid material which is awaiting further study. Most of the acetone-dissolved material consisted of the two esterified flavonols, **NG-1** and **NG-2** [3], which could be eliminated by crystallization from ethanol. The residue was further analysed by CC on polyamide.

N. californica was collected on 5 Dec. 1981 south of Bumblebee near interstate 17 in Yavapai Co., AZ and on 6 Dec. 1981 at Salt River Canyon on Hwy 60 in Gila Co., AZ. Voucher specimens of the latter collection (E. Wollenweber and G. Yatskievych 81-489) were deposited at Tucson, AZ and at Darmstadt. From 194.4 g of dry fronds an acetone extract produced 2.28 g (1.2% dry wt) of crude exudate. Further rinsing with toluene yielded 1.12 g of lipoid material. From the concentrated Me_2CO solution part of the esterified flavonols **NG-1** and **NG-2** were crystallized and filtered off. The mother liquor was subjected to CC on polyamide and on silica.

Isolation of exudate constituents was achieved by CC on silica and on polyamide as reported in previous papers [4, 5]. TLC was mainly on polyamide with toluene–petrol$_{100-140}$–MeCOEt–MeOH 12:6:2:1 and on silica with toluene–MeCOEt 9:1. Plates were evaluated under UV_{366} before and after spraying with NA. ^{1}H NMR spectra were recorded at 369 (**3**, **4**) and 350 MHz (**1**, **2**, **5**, **6**), respectively; TMS was used as int. standard. Mp: uncorr. Hydrolysis of **2** was performed by addition of a few drops of HCl to a solution of a sample in boiling HOAc.

5,2′,5′-*Trihydroxy*-3,7,8-*trimethoxyflavone* (**1**). Mp: 230–231° (from AcOH–H_2O); R_f: 0.26 (pol.) 0.44 (Si), dark brown after spraying with; UV λ_{max}^{MeOH} nm (% height peak): 356 (0.3), 305 sh, 263 (0.85); $AlCl_3$: 416, 322, 277; unchanged with HCl; NaOH: 380, 322 sh, 267 (unstable); NaOAc: 356, 306 sh, 263; H_3BO_3: unchanged; EIMS *m/z* (rel. int.): 360 [M^+; 100; $C_{18}H_{16}O_8$), 345 (M − Me; 24), 343 (13), 330 (12), 181 (74; $C_8H_5O_5$), 165 (73; $C_9H_9O_3$), 153 (34; $C_7H_5O_4$), 137 (21), 125 (11), 69 (17); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

5,5′-*Dihydroxy*-3,7,8,2′-*tetramethoxyflavone* (**2**). Mp: 165–166° (from EtOH); R_f 0.53 (pol.), 0.51 (Si); dark before and after spraying with NA; UV λ_{max}^{MeOH} nm (% height peak): 353 (0.29), 306 sh, 262 (0.50); $AlCl_3$: 416, 322, 278; unchanged with HCl; NaOH: 372, 264 (stable); NaOAc: 353, 306 sh, 262; unchanged with H_3BO_3; EIMS *m/z* (rel. int.): 374 (M^+; 55; $C_{19}H_{18}O_8$), 359 (M − Me; 100), 180 (8), 164 (8), 153 (8); ^{1}H NMR: see Table 1.

5,5′-*Dihydroxy*-3,7-8-*trimethoxy*-2′-*acetoxyflavone* (**3**). Mp: 189–191° (from benzene–petrol); R_f: 0.53 (pol.), 0.51 (Si); dark before and after spraying with NA; UV λ_{max}^{MeOH} nm (% height peak): 350 (0.31), 305 sh, 267 (0.60); $AlCl_3$: 415, 325, 275; unchanged with HCl; NaOH: 373, 265; NaOAc: 350, 307 sh, 264; EIMS *m/z* (rel. int.): 402 (M^+; 6), 360 (5), 345 (3), 343 (4), 329 (3), 181 (9), 165 (9), 153 (18), 137 (9), 125 (8), 69 (28), 43 (100); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

5,2′,3′-*Trihydroxy*-3,7,8-*trimethoxyflavone* (**4**). Mp: 203° (from EtOH); R_f: 0.39 (pol.), 0.57 (Si); dark before, fluorescent red after spraying with NA; UV λ_{max}^{MeOH} nm (% height peak): 352, 296 sh, 269; $AlCl_3$: 412, 322sh, 275; unchanged with HCl; NaOH: 371, 295 sh, 268; NaOAc: 351, 296 sh, 269; EIMS *m/z* (rel. int.): 360 (M^+; 100), 345 (53), 343 (12), 329 (7), 271 (15), 181 (5a), 165 (49), 153 (26), 137 (20), 125 (11), 69 (16); ^{1}H NMR: see Table.

3,5,7,8,2′,5′-*Hexamethoxyflavone* (**5**). Prepared by methylation of **1** with diazomethane in MeOH–Ether. Purification of this compound was achieved by circular centrifugal TLC on silica gel with *n*-hexane–$CHCl_3$–*i*PrOH–MeOH: (14:2:1:1); UV λ_{max}^{MeOH} nm: 335, 250; EIMS: *m/z* (rel. int.): 402 (67), 387 (45), 371 (100), 355 (14), 342 (25), 313 (9), 211 (17), 195 (13), 194 (4), 167 (50), 165 (12). ^{1}H NMR: see Table 1.

5-*Hydroxy*-3,7,8,2′,5′-*pentamethoxyflavone* (**6**) was obtained during methylation of **1**. It was separated from **5** by circular centrifugal TLC on silica gel with *n*-hexane–$CHCl_3$–*i*PrOH–MeOH: 96-2-1-1. UV λ_{max}^{MeOH} nm: 345, 305 sh, 258; $AlCl_3$: 405, 320 sh, 275; NMR ^{1}H: see Table 1. EIMS *m/z* (rel. int.): 388 (60), 373 (100), 357 (7), 187 (9).

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