New Flavonol Acetates from the Frond Exudate of the Fern *Notholaena aschenborniana*

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Notholaena aschenborniana, Pteridophyta, Frond Exudate, 5-Hydroxy-3,7,2′,3′,4′-pentamethoxy-8-acetoxyflavone, 5,4′-Dihydroxy-3,7,3′-trimethoxy-8-acetoxyflavone

The farinose frond exudate of the fern *Notholaena aschenborniana* was previously shown to contain a mono-acetate and a mono-butyrate of a 8-hydroxyflavonol as major constituents and the same pair of esters of a second 8-hydroxyflavonol as minor components. Now we have been able to identify the 8-acetates of two further flavonols. The structures of 5-hydroxy-3,7,2′,3′,4′-pentamethoxy-8-acetoxyflavone and 5,4′-dihydroxy-3,7,3′-trimethoxy-8-acetoxyflavone have been elucidated by spectroscopic methods. Both are novel natural products.

In ferns of the genus *Notholaena* the lower surface of the frond lamina is covered either with an indument of hairs or scales or with a ‘ceraceous’ indument [1]. *Notholaena aschenborniana* Kl. is one of the few species which exhibit both characters at the same time. The light yellow farinose exudate on the lower surface of its fronds is completely covered with and thus hidden by brownish scales. This exudate was earlier shown to contain two esterified flavonols as major components, namely the 8-acetate and the 8-butyrate of herbacetin 7-methyl ether (compounds *NAS-1* and *NAS-2* in [2]). The 8-acetate and the 8-butyrate of the 7-methyl ether of 8-hydroxygalangin (compounds *NG-1* and *NG-2* in [2]) may be present in trace amounts in some collections [3]. Another minor component is a flavone with tetra-O-substituted B-ring, 5,4′,6′-trihydroxy-6,7,2′,3′-tetramethoxyflavone, *NAS-3* [4]. We now report the structures of two further trace flavonoids, *NAS-4* and *NAS-5*, occurring in the farinose exudate of this fern.

Materials and Methods

The fern material was collected in Mexico (vouchers D. J. Pinkava & T. Reeves R 4301B and R 4320, kept at ASU). The flavonoid exudate was recovered and fractioned by CC and preparative TLC as described earlier [4]. Thus minute amounts of the compounds under investigation could be isolated in pure state. The solvent used for TLC on polyamide (Polyamid DC-11, Macherey-Nagel, Düren) was benzene/petrol$_{100-140\,°}$/methyl ethyl ketone/methanol 60:60:7:7. Mass spectra were recorded on an AEI MS 902. ^{1}H-NMR spectra were recorded on a Varian XL 100. NOE-experiments were done on a Brucker 360 MHz.

Results and Discussion

Both compounds appear on polyamide as dark spots in UV$_{366}$, *NAS-4* at R_f 0.90 and *NAS-5* at 0.40 in the solvent given in the experimental part. On spraying with “Naturstoffreagenz A” *NAS-4* turns dark yellow, *NAS-5* turns brown. *NAS-4* forms light yellow needles from ethanol, m.p. 183–185 °C. *NAS-5* could not be crystallized, due to lack of material.

The mass spectrum of compound *NAS-4* shows the following important fragments. MS m/z (rel.int.): 446 (15%, M$^+$; $C_{22}H_{22}O_{10}$, found 446.1217, calc. 446.1213), 404 (100%; $C_{20}H_{20}O_9$, found 404.1114, calc. 404.1107), 389 (14%), 373 (30%), 361 (5%). The base peak at m/z 404 represents the flavonoid molecule, derived from the original molecule by loss of an acetyl unit (m^* at 366). According to high-resolution MS this flavonoid has two hydroxy groups and five methoxy groups (see also [5]).

The UV-spectrum of *NAS-4* gives some further information. UV λ^{MeOH} 344, 262, (254) nm; AlCl$_3$ 404, 332, 302, 273 nm; AlCl$_3$ + HCl 402, 326, 300, 272 nm; NaOH 372 (decrease), 268 nm; NaOAc 344, 262 (256) nm; NaOAc + H$_3$BO$_3$ 344, 252 nm. These data show the presence of a hydroxy group at C-5 ($\Delta\lambda B_I$ with AlCl$_3$ + HCl: + 58 nm) and they indicate the presence of methoxy groups at C-7 ($\Delta\lambda B_{II}$ with NaOAc: ±0 nm) and at C-4′ ($\Delta\lambda B_I$ with NaOH: + 28 nm, decrease of intensity).

The ^{1}H-NMR spectrum of *NAS-4* exhibits the following signals. ^{1}H-NMR (100 MHz, DMSO-d$_6$/TMS; δ ppm) 7.18 (1H, d, J = 8.5 Hz; H-6′). 6.92 (1H, d, J = 8.5 Hz; H-5′), 6.68 (1H, s; H-6), 3.88 (6H, s; 2 OCH$_3$), 3.80 (6H, s; 2 OCH$_3$), 3.68 (3H, s; 1

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OCH_3), 2.24 (3H, s; -CO-CH_3). These data confirm the existence of 5 methoxyl groups, of the acetoxy group, and of three aromatic protons. The coupling constants of two of these show them to be in vicinal positions, and they are probably located at the B-ring, at C-5′ and C-6′. The third aromatic proton would then be placed at the A-ring.

So far the flavonoid under investigation is thus assumed to have a tri-O-substituted A-ring and a tri-O-substituted B-ring, and one substituent should be placed at C-3. This agrees with the dark appearance of the spot in UV. Further details on the localization of the seven substituents are attributed by the study of the PMR spectrum at 350 MHz in C_6D_6 and by NOE experiments. In C_6D_6 the 5 methoxyl groups appear separately as singuletts at 3.73, 3.57, 3.53, 3.13 and 2.97 ppm. The aromatic protons appear as two doublets at 6.93 ppm and at 6.13 ppm and as a singulett at 6.14 ppm. Irradiation of the OMe at 3.13 brings about increase of the doublet signal at 6.13 ppm. Irradiation of OMe at 2.97 has the same effect on the singulett at 6.14 ppm. Irradiation of the three further OMe-signals produces no NOE. The UV-spectral data, the PMR-signals in DMSO and the NOE experiments thus show that the two *ortho*-protons at the B-ring must be located at C-5′ and C-6′, only the proton at C-5′ showing a NOE on irradiation of OMe at C-4′. The singulett signal of the third aromatic proton, on the other hand, shows an increase on irradiation of the OMe at 2.97 ppm. This suggests that it is located at the A-ring in *o*-position to OMe-7. Due to the UV-spectral data (*c.f.* [6, 7]) and also on the base of biochemical relations to *NAS-1* and *NAS-2* and to *NG-1* and *NG-2*, this proton can be located at C-6, the esterified OH-group being ascribed to C-8. The flavonol *NAS-4* thus is 5-hydroxy-3,7,2′,3′,4′-pentamethoxy-8-acetoxyflavone.

The mass spectrum of compound *NAS-5* exhibits the molecular peak at *m/z* 402 (20% rel.int.) and the base peak at *m/z* 360 (100%), which shows that this molecule also is a monoacetate (*m** at 322). The non-acetylated flavone or flavonol itself should bear 3 hydroxyl groups and 3 methoxyl groups [5]. Further fragments are observed at *m/z* 345 (11%), 329 (7%), and 317 (17%).

NAS-5 shows the following UV-spectral properties. UVλ^{MeOH} 360, (286), 257 nm; $AlCl_3$ 416, 364, (304), 280, 272 nm; $AlCl_3$ + HCl 416, 362, (304), 280, 274 nm; NaOH 400, 266 nm (increase of B_I); NaOAc 420, (374), 260 nm; NaOA + H_3BO_3 364, (268), 258 nm. The spectrum in MeOH is characteristic of a flavonol with di-substituted B-ring [8]. The absence of a NaOAc-shift indicates that the 7-position is blocked and the negative borate shift indicates the absence of two adjacent OH-groups on the B-ring. Since the reaction with NaOH demonstrates the presence of a free OH-group at C-4′, position 3 obviously bearing a methoxyl (no decrease of B_I), it can be assumed that the B-ring substitution is 3′-OMe,4′-OH. The shape of the spectral curve with $AlCl_3$ points to a 8-substituted flavonol and the negative HCl shift indicates OH at C-5. Thus B_I at 360 nm in MeOH is due to a 8-substituted 3-methoxyflavonol

The PMR-spectrum of *NAS-5* exhibits the following signals (100 MHz, DMSO/TMS; δ ppm): 7.50 (2H, m; H-2′ and H-6′), 6.96 (1H, d, $J = 8.5$ Hz; H-5′), 6.66 (1H, s; H-6), 3.90 (3H, s; OCH_3), 3.82 (6H, s; 2 OCH_3), 2.37 (3H, s; -CO-CH_3). These data confirm the presence of 3 methoxyls and of 4 aromatic protons as well as the presence of an acetyl group. Together the spectral findings establish that compound *NAS-5* is 5,4′-dihydroxy-3,7,3′-trimethoxy-8-acetoxyflavone.

Due to the minute amounts of products isolated we could not afford hydrolyzing these compounds. Saponification of *NAS-5* should yield gossypetin 3,7,3′-trimethyl ether. This flavonol has been found only once before as a natural product (*c.f.* [9]). Sakakibara *et al.* reported it from the leaf resin of *Larrea tridentata* [10]. *NAS-5* fits very well in the series of flavonol esters described earlier from *Notholaena* species (*NA*, *NG*, *NAS* [2]). They are all methylated at C-7 and esterified at C-8. It is most closely related to the trace constituent *NA-4* from *N. affinis* [2], the 8-acetate of gossypetin 7,4′-dimethyl ether. It is the first such compound that is methylated at OH-3, too.

NAS-4 agrees in so far as it is also methylated at OH-7 and at OH-3 and acetylated at OH-8. By its bearing an additional OMe-group at C-2′ it is to a certain extent also related to several flavonoids found in the frond exudate of *Notholaena affinis* [11, 12]. *NAS-4* itself as well as the non-acetylated flavonol are novel natural compounds. As to our knowledge no flavonoid with this pattern of O-substitution has been found as yet (*c.f.* [9]).

Recently E. W. had the opportunity to collect bulk material of *N. aschenborniana* in Arizona/USA. The analysis of the frond exudate of this material may allow the identification of further minor flavonoids produced by this fern species.

Note added in proof: The melting point of compound *NAS-3*, not reported previously, could be determined to be 199 – 200 °.

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