# Unusual Flavanones from a Rare American Fern

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*Notholaena fendleri*, Polypodiaceae, Farinose Exudate, Novel Flavanone

*Notholaena fendleri* is a rare representative of the genus, growing mainly in Colorado/USA. The white farinose exudate on the lower surface of its pinnules is composed partly of waxy material and partly of flavonoid aglycones. The flavonoid moiety consists mainly of methyl derivatives of the flavanones naringenin and eriodictyol. The eriodictyol methyl ethers are rare compounds; eriodictyol-7 methyl ether is a novel natural flavanone. Three flavones and two flavonols are found as trace constituents only. – The farina flavonoids of *N. fendleri* form a very typical pattern that is characteristic for this species.

*Notholaena fendleri* Kze. is one species of the genus that produces a white farina on the lower leaf surface. This species is a rather rare fern which is well-marked by its strongly flexuous rachis and other axes [1]. According to [1] it occurs on dry rocky bluffs and cliffs from south-eastern Wyoming south to New Mexico. Its centre of distribution is in Colorado.

The chemical composition of the frond exudate is unique among all fern farina analyzed as yet (*cf.* [2–4]). The most prominent feature on thin-layer chromatograms is a red spot that develops within a few minutes after spraying with "Naturstoffreagenz A". It is brilliant red in UV and after a few hours in daylight appears violet (on polyamide). From small herbarium fragments analyzed by comparative TLC further constituents of the farina have been identified recently as eriodictyol-7,4′ dimethyl ether and eriodictyol-7,3′,4′ trimethyl ether, naringenin-7 methyl ether and naringenin-4′ methyl ether [5]. The "red spot", however, remained unidentified. It has now been possible to analyze in detail the farina recovered from a handful of freshly collected material.

## Materials and Methods

Fronds of *Notholaena fendleri* were collected by S. Sigstedt (11. Nov. 1980) near Colorado Springs, Colorado/USA and made available by J. L. Carter. This material, 10 g of air-dried fronds, comprised relatively few small leaflets with a white farina on the lower surface. The exudate could be dissolved by rinsing with acetone and petrol ether. The concentrated yellow solution on drying at 60° yielded a brownish mass, covered with a yellow layer of wax. Most of the exudate was soluble in benzene. This solution could be used directly for column chromatography on polyamide SC-6 (Macherey-Nagel, Düren). It yielded some wax and most of the less polar flavonoids. The second portion of the crude exudate was dried onto polyamide and then also subjected to CC. Two flavanones (**1** and **4** ) previously described for the farina of this fern [5] and two new flavanones (**2** and **3**) were recovered from different fractions of the two columns as crystalline products. In addition a series of minor constituents, namely three further flavanones, three flavones and two flavonols could be identified by direct comparison with authentic markers.

Columns were eluated as usual with toluene and increasing quantities of methylethyl ketone and methanol. The solvents used for TLC were A) toluene/petrol$_{100-140°}$/methylethyl ketone/methanol 30:60:5:5; B) dto, 60:30:10:5; C) toluene/dioxane/methanol 80:10:10 for polyamide DC-11 (Macherey-Nagel) and D) toluene/methylethyl ketone 9:1 for silica. After evaluation in $UV_{366}$ and $UV_{254}$ the chromatograms were sprayed with "Naturstoffreagenz A" (β-aminoethyl ester of diphenyl boric acid; C. Roth, Karlsruhe; 0.5% in MeOH). Some of the minor constituents were purified by preparative TLC on silica plates with concentration zone (SIL-GUR 25, Macherey-Nagel). Methylation of compounds **2** and **3** was performed according to [6]. Mass spectra were recorded on a Varian MAT 311 A at the Institut für Organische Chemie der TH Darmstadt. PMR spectra were recorded on a Brucker HFX-90 at the Organisch-Chemisches Institut der Universität Heidelberg.

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## Results

The total yield of exudate material as recovered from the 10 g of fern fronds available was 720 mg. About 2/3 of it were soluble in boiling benzene. This portion yielded some 110 mg of waxy material which was not analyzed further, but which is obviously not a true wax according to the chemical definition (*cf.* [7]). Fractions 5–8, which on TLC in solvent B look alike, were combined and after drying crystalized from EtOH. This led to the formation of slightly yellowish crystals and of pure white crystals. Most of the yellowish material formed clusters, so the two types could be separated and were crystallized once more. Finally portions of 40–50 mg each of the pure compounds **1** and **2** could be obtained.

Some later fractions of the second portion were combined and crystallized from EtOH to yield ca. 60 mg of compound **3**. Also several similar fractions from the first and the second column were combined and yielded some 120 mg of compound **4**.

Compound **1** was readily identified as eriodictyol 7,4′-dimethyl ether by direct TLC comparison with authentic marker and was further confirmed by m. p., UV and MS.

Compounds **1** and **2** form one spot on polyamide with solvent B ($R_f$ 0.75) and on silica with solvent D ($R_f$ 0.39). On polyamide with solvent A the $R_f$ of compound **2** (0.41) is slightly higher than that of compound **1** (0.37). On spraying with "Naturstoffreagenz A" compound **1** turns dull-greenish and brownish in $UV_{366}$; compound **2** turns reddish brown. Both substances are hardly visible on TLC when applied in low concentrations, but the spots become visible when the sprayed chromatogram is exposed to daylight for a while. Comp. **1** then appears brown, whereas comp. **2** is dark brown.

Comp. **2** melts at 148–150 °. UV $\lambda_{max}^{MeOH}$: 288 nm; + $AlCl_3$ 367, 310 nm; + NaOMe 358, 288 nm; + NaOAc 288 nm, unchanged on addition of $H_3BO_3$. This spectrum points to a flavanone with a free OH-group at C-5 and probably a methoxyl at C-7. MS *m/e* (rel.int): 316 (69, $M^+$), 193 (23, "Pic F" according to [8]), 180 (37), 167 (100, "Pic A"), 150 (85, Pic B"), 137 (83), 135 (67), 123 (17), 110 (22), 107 (49), 95 (70). $M^+$ 316 corresponds to the molecular formula $C_{17}H_{16}O_6$ for a flavanone with 2 OH-groups and 2 $OCH_3$-groups [9]. The fragmentation pattern shows that both ring A and ring B bear 1 OH-group each. The PMR-spectrum finally shows the following signals. PMR (90 MHz, DMSO-$d_6$, TMS; $\delta$ ppm) 12.16 (1 H, s, OH-5), 9.2 (1 H, s, OH-4′), 7.1 (d, H-6′), ~ 6.9 (2 H, dd, H-2′ and H-5′), ~ 6.1 (2 H, dd, H-6 and H-8), 5.5 (dd, H-2), 3.78 (6 H, s, 2 $OCH_3$), ~ 3.4 (m, H-3a; $J_{H\text{-}2/H\text{-}3a}$ ~ 12 Hz; $J_{H\text{-}3a/H\text{-}3b}$ ~ 17 Hz), ~ 2.7 (m, H-3 b; $H_{H\text{-}2/H\text{-}3b}$ ~ 3 Hz). In total the spectral properties of comp. **2** lead to the assumption that it is another dimethyl derivative of eriodictyol. As a matter of fact the m. p. as well as MS and PMR data are in agreement with literature data for eriodictyol 7,3′-dimethyl ether [10, 11]. UV-spectral data for this flavanone have not been reported previously.

Comp. **3** forms white crystals, m. p. 221 °. Curiously enough, the UV-spectrum is practically identical with that of comp. **2**. $\lambda_{max}^{MeOH}$ 288 nm; + $AlCl_3$ 367, 308 nm; + NaOMe 358, 287 nm; + NaOAc 291 nm; + $H_3BO_3$ 288 nm. Thus this substance also is a flavanone with free OH at C-5 and $OCH_3$ at C-7, but there is no hint of the B-ring substitution. MS *m/e* (rel. int.): 302 (69 m $M^+$), 193 (30 "Pic F"), 180 (53), 167 (100, "Pic A"), 136 (52, "Pic B"), 95 (32). $M^+$ 302 corresponds to the molecular formula $C_{16}H_{14}O_6$ for a flavanone with 3 OH and 1 $OCH_3$ [9]. The fragmentation indicates 1 OH and 1 $OCH_3$ at ring A and the presence of 2 OH-groups at ring B. PMR (90 MHz, DMSO-$d_6$, TMS; $\delta$ ppm): 12.1 (1 H, s, OH-5), 9.1 (2 H, br. sign., OH-3′ and OH-4′), 6.92 (s, H-6′), 6.77 (2 H, d, H-2′ and H-5′), 6.07 (2 H, dd, H-6 and H-8), 5.44 (dd, H-2), 3.83 (3 H, s, $OCH_3$), 3.35 (m, H-3a; $J_{H\text{-}2/H\text{-}3a}$ ~ 11.5 Hz; $J_{H\text{-}3a/H\text{-}3b}$ ~ 17 Hz), 2.65 (m, H-3b; $J_{H\text{-}2/H\text{-}3b}$ ~ 3.8 Hz). Evaluation of all these data indicates that comp. **3** is eriodictyol 7-methyl ether.

Methylation of compounds **1**, **2**, and **3** leads in every case to the formation of eriodictyol 7,3′,4′-trimethyl ether [5], which proves again that the three of them are derivatives of eriodictyol.

Compound **4** has been reported earlier [5] to be the naringenin 4′-methyl ether, isosakuranetin. This identification can now be corroborated by the physical properties of the crystalline product. Further minor constituents of the farina were identified by direct comparison, partly after purification by PTLC. They are eriodictyol 4′-Me (hesperetin), eriodictyol 7,3′,4′-tri-Me, naringenin 7-Me (sakuranetin), luteolin 7,3′-di-Me (velutin) and luteolin 7,4′-di-Me (pilloin), apigenin 4′-Me (acacetin), quercetin 3,7-di-Me and quercetin 3,7,3′-tri-Me (pachypodol).

## Discussion

It has been stressed earlier that flavanones in fern farina normally occur as minor constituents only [2, 5]. Exceptions reported previously are *Pityrogramma pallida* [12] with C-methylated flavanones as major farina constituents, and *Cheilanthes argentea* [5], which produces a series of flavanones, but has a diterpene as by far dominating constituent. Now it is shown that, apart from some unknown waxy material, the farina of *Notholaena fendleri* consists almost completely of flavanones. The predominant compound is isosakuranetin (**4**) with 17% of the total exudate; then there are the monomethyl ether (**3**) and the two dimethyl ethers (**1** and **2**) of eriodictyol with 5–8% each. Hesperetin, eriodictyol 7,3′,4′-trimethyl ether and sakuranetin are minor constituents. The flavones acacetin, velutin and pilloin as well as the flavonols quercetin 3.7-dimethyl ether and pachypodol occur in trace amounts only.

It may be mentioned that the farina flavonoid pattern formed by the constituents described here is very typical and rather constant in all specimens from which fragments could be analyzed hitherto (derived from US National Herbarium, Washington, D.C.; New York Botanical Garden, Bronx, NY; Field Museum of Natural History, Chicago, Ill.; Gray Herbarium of Harvard University, Cambridge, Mass.; British Museum, Natural History, London).

According to [13] the 7,3′-dimethyl ether of eriodictyol (**2**) has been found in nature only twice before. It was described as an aglycone in the bark of *Melicope sarcococca* Laut. (Rutaceae) and in aerial parts of *Eupatorium odoratum* (Asteraceae). Eriodictyol 7-methyl ether (**3**) is a novel natural flavanone. We have no explanation for its strange reaction with "Naturstoffreagenz A". At any rate the appearance of the red spot on TLC is very characteristic and should allow easy detection of this new flavanone in other materials. We have already identified it as a trace constituent in the bud excretion of a *Prunus* species [14].

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