FLAVONOID ESTERS FROM THE FERN, *NOTHOLAENA NEGLECTA*

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ABSTRACT.—The fern *Notholaena neglecta* produces a lipophilic farinose frond exudate that is composed of flavonoid aglycones. A major constituent is one of the very rare flavonol esters, namely, 7-O-methyl 8-acetoxy- galangin (**1**). Traces of the corresponding butyryl derivative (**2**) are also observable. Another prominent constituent of the farina has now been isolated and identified as a novel natural flavonoid. Its structure is 5-hydroxy 7-methoxy 8-acetoxy flavanone (**3**). The occurrence of these esterified flavonoids in the exudate of *N. neglecta* appears to be typical for this species.

Notholaena neglecta Maxon is a small gymnogrammoid fern of dry limestone hillsides and rocky areas. According to *Tryon* (1) it ranges from southeastern Arizona and southern Texas (U.S.A.) to Chihuahua and eastern Coahuila (Mexico). Its fronds are nearly glabrous above, densely whitish to pale yellow farinose-glandular beneath. Taxonomically, *N. neglecta* is probably most closely related to *N. californica* (1).

As in other species of *Notholaena*, the "ceraceous indument" (the lipophilic farinose exudate on the lower surface of the leaflets) of *N. neglecta* is composed of flavonoids. A substance provisionally called NG–2, namely, the acetic acid ester of 8-hydroxy-galangin-7-O-methyl ether, was identified as a major constituent on herbarium fragments of this species by direct comparison on thin-layer chromatograms (2). Within the scope of our chemotaxonomic studies on gymnogrammoid ferns (3), we next had the opportunity to collect and analyze a small quantity of fronds of this fern. This lead to the isolation and identification of a further prominent constituent of the frond exudate which turned out to be a novel esterified flavanone. We wish to report now on the elucidation of its structure and on the distribution of these flavonoid compounds in several herbarium specimens of which fragments could be analyzed.

EXPERIMENTAL[1]

PLANT MATERIAL.—The fronds of *Notholaena neglecta* Maxon collected for this study originated from a population near Sheepshead Pass in the Dragoon Mountains of Cochise County, Arizona. This previously unpublished locality was first discovered by Dr. Pierre C. Fischer, to whom we are indebted. (Voucher specimen *Fischer, Jenkins* and *Yatskievych* 80–767 in E.W.'s herbarium in Darmstadt). Fronds were carefully clipped from plants in the field and air-dried in a paper sack.

At our locality, the species is locally common on a steep, southwest-facing limestone hillside between 1730 and 1770 m in elevation. The plants grow from crevices in and under rocks and are exposed to periodic drought. Pteridophyte associates include: *N. aschenborniana, N. sinuata, N. cochisensis, N. integerrima, Cheilanthes eatoni, C. lindheimeri,* and *Selaginella rupincola.* The habitat borders a lower elevation Chihuahuan Desert zone and a higher elevation Pinyon-Juniper Woodland zone with granitic substrate. Its dominant angiosperms are: *Rhus choriophylla, Quercus pungens, Garrya wrightii, Agave schottii, Cercocarpus breviflorus,* and *Forsellesia spinescens.*

Fragments of herbarium specimens were available from various herbaria. Six of them were collected in Mexico: *Pringle* 452, *Palmer* 424, *Jones* 520, *Sanchez* 625 (all US), *Johnston* 8775 (GH), *Pinkava* and *Reeves* 4310 (ASU). One is from Texas: *Correll* and *Warnock* 14979 (US); one is of unknown origin: *M. E. Jones s.n.* 19.4.1892 (BM).

ISOLATION PROCEDURE.—The dry fern material (15.3g) was rinsed with acetone to yield some 380 mg of exudate material. From a highly concentrated solution in acetone ca 100 mg

[1]Mass spectra were recorded on a Varian MAT 311 at the Institute of Organic Chemistry of the TH Darmstadt. Pmr spectra were recorded on a Brucker HFX–90 at the Institute of Organic Chemistry of the University of Heidelberg. Adsorbants used for tlc and cc were from Macherey-Nagel/Düren. Naturstoffreagenz-A (β-aminoethyl ester of diphenyl boric acid) was from C. Roth/Karlsruhe.

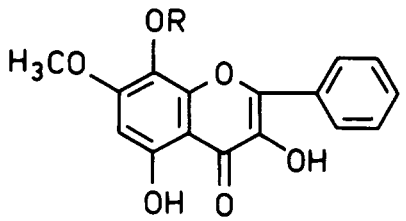
of the major component deposited as light-yellow flakes (comp. **1**), which were filtered off. The wanted unknown product remained in solution, which was subjected to column chromatography. After evaporation of the acetone, the material was dissolved in benzene and applied on top of a colymn with silica gel. Elution was done with toluene and increasing quantities of methylethyl ketone and methanol and 18 fractions (60–80 ml each) were collected. Thin-layer chromatographic control (solvent A) showed the wanted very unpolar substance to be mainly in fraction 6. The fraction was dried and then dissolved in ethanol from which ca 45 mg of **3** crystallized. Recrystallization of fractions 8–10 yielded another 20 mg of compound **1**, together with a small amount of compound **2**. Traces of two trivial flavonols, namely rhamnazin and rhamnocitrin, could be identified in fractions 11–18 by direct comparison with authentic samples (polyamide, solvent B). Efforts to isolate further unknown constituents by preparative tlc were fruitless because of the paucity of material.

Compound **3** was hydrolyzed by the addition of a few drops of concentrated hydrochloric acid to a boiling solution of the compound in glacial acetic acid. This yielded the crystalline compound **4**. Methylation of this product was done according to the method of T. J. Simpson et al. (4) by addition of dimethyl sulfate to a solution of a few mg of **4** in ethanol with sodium hydrogen carbonate. In the same manner a sample of dihydrowogonin (5,7-dihydroxy, 8-methoxy flavanone) was methylated for comparison. Both methylations yielded product **5**. Dihydrowogonin originated from bud excretion of the sweet cherry tree (5).

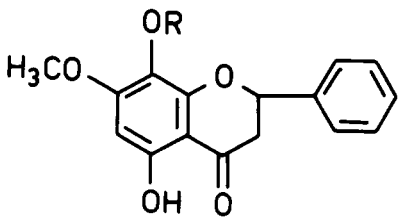
THIN-LAYER CHROMATOGRAPHY.—The solvents used for chromatography of fractions as well as for the comparative study of herbarium fragments were A) toluene-petroleum ether (bp 100–140°)-methylethyl ketone-methanol (30:90:2:1.5), and B) toluene-petroleum ether (bp 100–140°)-methylethyl ketone-methanol (60:30:10:5), both for polyamide. For silica we used solvent C) toluene-methylethyl ketone (9:1). Chromatograms were evaluated in uv_{366} before and after spraying with Naturstoffreagenz-A.

RESULTS

From the farinose frond exudate of *Notholaena neglecta* two flavonoids were isolated in crystalline form by chromatographic procedures. Compound **1** was readily identified by direct comparison of its chromatographic and spectral properties with those of an authentic sample. It is identical with 7-O–methyl 8-acetoxy galangin, isolated previously from the frond exudate of *N. galapagensis* (2). The relevant butyryl ester (2) was present only in a small amount, as identified by thin-layer chromatographic comparison with an authentic sample.



1. R= -CO-CH_3
2. R= -CO-CH_2-CH_2-CH_3



3. R= -CO-CH_3
4. R= -H
5. R= -CH_3

Compound **3** is a colorless substance, mp 146°. The R_f on polyamide in solvent A is 0.73 (comp. **1** occurs at R_f 0.30). Its uv spectrum indicates a flavanone structure: uv λ max (MeOH) 338, 291 nm. The long-wave maximum shows only about ¼ of the intensity of the short-wave maximum. The ms exhibits the molecular peak at *m/z* 328 (10% rel. int.), but fragments, of 100% rel. int., each occur at *m/z* 286 and 182. Hence it can be concluded that compound **3** is a monoacetylated flavanone (loss of an acetyl unit from 328 yields 286) with 1 OH-group and 1 OCH_3-group (cf. 6). The fragmentation 286→182 shows that the B-ring must be unsubstituted (M^+–42–104), and this is stressed also by the presence of a fragment *m/z* 209 (M^+–42–77). According to the uv spectra with classical reagents (7) the OH-group can be placed at C-5 (+$AlCl_3$ λ max: 390, 313 nm) and the OCH_3-group might be at C-7 (no reaction with NaOAc), provided in analogy to the structure of compounds **1** and **2** the acetyl would be situated at C-8. The pmr spectrum corroborates the flavanone structure, the unsubstituted B-ring and the presence of 1 acetyl, 1 methoxyl and 1 H-bonded OH-group by exhibiting the following signals (in ppm/TMS; DMSO-d_6): 12.06 (1H, s; OH-5), 7.42 (5H, m; B-ring), 6.34 (1H, s; H-6), 5.66 (1H, dd; H-2; $J_{H\text{-}2/H\text{-}3a}$=11.8 Hz, $J_{H\text{-}2/H\text{-}3b}$=3.9 Hz), ~3.9–2.96 (2H, m; H-3a/H-3b; $J_{H\text{-}3a/H\text{-}3b}$=16.5 Hz), 3.85 (3H, s; OCH_3), 2.23 (3H, s; CH_3-COO-). The structure deduced for compound **3** from these spectral data thus would be 5-hydroxy, 7-methoxy, 8-acetoxy flavanone.

Hydrolysis of compound **3** yields compound **4** as light yellow crystals, mp 246–247°. Its R_f on polyamide in solvent B is 0.79; on silica in solvent C it is 0.39. After spraying with Naturstoffreagenz A and exposure to daylight the spot exhibits a light greenish color. This reaction has not been observed as yet in any other flavonoid. As expected, the molecular weight of **4** is 286 (88% rel. int.), and diagnostic peaks occur at *m/z* 182 (100%) and 209 (10%). The uv spectrum in comparison with that of compound **3** shows an important shift of Band I: λ max (MeOH) 365, 294 nm, which is indeed in favor of an OH-group at C-8 in this product. We observed an important shift on addition of $AlCl_3$ (λ max: 430, 317 nm) and again no reaction with NaOAc. The pmr spectrum exhibited the following signals: 11.82 (1H, s; OH-5), 8–17 (1H, s; OH-8), ~7.46 (5H, m; B-ring), 6.21 (1H, s; H-6), 5.59 (1H, dd; H-2; $J_{H\text{-}2/H\text{-}3a}$=4 Hz, $J_{H\text{-}2/3b}$=11.2 Hz), ~3.27–2.86 (2H, m; H-3a/H-3b; $J_{H\text{-}3a/H\text{-}3b}$=17.5 Hz), 3.83 (3H, s; OCH_3). These data are in favor of 5,8-dihydroxy, 7-methoxy flavanone. The mp of 192–193° given in literature (8) for the synthetic substance is much lower than that observed here for compound **4**. However, another way was found to confirm the correctness of the structure of comp. **4**, in addition to its spectral properties. Methylation of a sample of 5,7-dihydroxy,8-methoxy flavanone (dihydrowogonin), available from earlier work (5), should yield the same product of methlation as compound **4**. As a matter of fact, partial methylation of both substances with DMS yielded the expected compound **5**, which from both origins was identical in every respect. The R_f on polyamide in solvent A is 0.76, while on silica in solvent C it is 0.63. It formed colorless crystals, mp 101° (Lit. 8: 97°). The uv spectrum was very similar to that of compound **3**: λ max (MeOH) 347, 293 nm; +$AlCl_3$ 402, 316 nm; no shift on addition of NaOAc. The ms showed M^+ at *m/z* 300 (100% rel. int.). Other prominent peaks occurred at *m/z* 285 (11%), 223 (14%; M–77), 196 (75%; M–104), 181 (100%), 153 (42%).

Together, all data presented here establish unambiguously that compound **3** is 5-hydroxy,7-methoxy-8-acetoxy flavanone. This is a novel natural flavanone. The non-acetylated flavanone **4**, isowogonin, is known as yet only as a synthetic product (8).

DISCUSSION

Acetylated flavonoid glycosides occur scattered within angiosperms, gymnosperms, and ferns. However, only two flavanones are known as yet in which esterification takes place directly at the flavonoid molecule. The 3-acetyl deriva-

tive of the flavanone pinobanksin was found in bud excretions of several *Populus* species (9), and the 3-acetate of pinobanksin-7-methyl ether is known from seeds of *Alpinia japonica* (10). Recently Wollenweber *et al.* (2) reported on the identification of esterified flavonols in the farinose frond exudate of several species of the fern genus *Notholaena*. Esters of acetic acid normally were found to occur as twin-pairs together with esters of butyric acid based on the same flavonol. So far, all flavonols encountered are 7-O-methyl flavonols, esterified at C-8 (8-OH-galangin-7-Me, herbacetin-7-Me, herbacetin-7,4'-diMe, gossypetin-7,4'-diMe). The now elucidated compound **3** from *N. neglecta*, identified as 5-hydroxy,7-methoxy,8-acetoxy flavanone, fits into this substitution pattern. The substitution is exactly the same as for compound **1** from this fern's farina.

The scattered distribution of the flavonol esters within the genus has been discussed recently (11). We found them in 7 species out of some 35 which are known to exhibit a "ceraceous indument" (1). Among these seven species, only *N. neglecta* and *N. californica* are closely related taxonomically. The presence or absence of the flavonol esters in *Notholaena californica* allows distinction of two chemical races for this species (12). The same seems to be true for *N. affinis* (13). For *N. neglecta* it is typical that the acetylated flavonol **1** occurs as a major farina constituent. In nine specimens analyzed (see Experimental) only 1 (Johnston 8775) showed more than trace amounts of the butyryl derivative **2**. In this same specimen, the existence of compound **3** remains somewhat dubious, whereas in all others it is present apparently in the same proportion as found for the one case where we could analyze 'bulk' material. This novel flavanone could not be detected in any other farinose species of the genus (only *N. weatherbyana* not studied). Traces of rhamnazin (quercetin-7,3'-diMe) and rhamnocitrin (kaempferol-7-Me) were found in the material analyzed here. Traces of the 8-acetyl- and 8-butyryl ester of 7-hydroxy,7-methyl kaempferol ("NAS-1" and "NAS-2", cf. 2) were detected in the specimen *Pinkava* and *Reeves* 4310. However, compounds **1** and **3** as major components of the farina together with a much smaller amount of compound **2** give rise to a flavonoid pattern typical for the species. In spite of some intraspecific variability, *N. neglecta* is therefore easily discriminated from other species by thin-layer chromatography of the farina flavonoids.

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