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# NEW FLAVONOIDS FROM CHEILANTHOID FERNS

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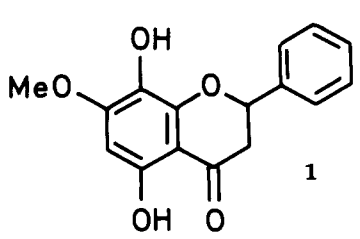
ABSTRACT.—Four species of *Notholaena* and one *Cheilanthes* species were studied for the chemical composition of their frond exudates. *Notholaena neglecta* yielded a new flavanone, two novel dihydroflavonols, and a rare flavone, all substituted at C-8, and the diterpene, lupeone. The frond exudates of *Notholaena greggi*, *Notholaena rigida*, and *Notholaena rosei* consist of methyl derivatives of trivial flavones and flavonols. *Cheilanthes kaulfussii* yields a novel acylated dihydroflavonol, pinobanksin-3-cinnamate.

As part of our continuing studies on the chemical nature of the "ceraceous indument" in Cheilanthoid ferns (1,2), we analyzed the frond exudates of four *Notholaena* species and a *Cheilanthes* species. *Notholaena neglecta* Maxon has been reported earlier to have the new 5-hydroxy-7-methoxy-8-acetoxy-flavanone, along with 7-*O*-methyl-8-acetoxy-galangin as major constituents (3). Reinvestigation of this species with a greater amount of material allowed the isolation and identification of three new flavanones and a rare flavone plus a triterpene. The structure elucidation of these compounds as well as that of a new esterified flavanone from *Cheilanthes kaulfussii* Kunze frond exudate will be discussed in detail. Trivial flavonoids will be reported from this *Cheilanthes* species and from three further Notholaenas, including the rare species *Notholaena rosei* Maxon.

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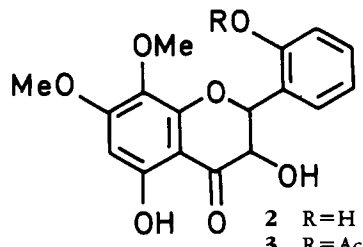


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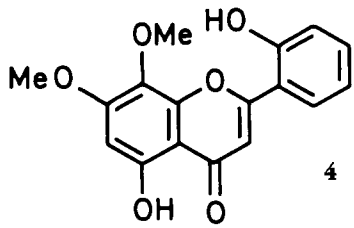
3 R=Ac

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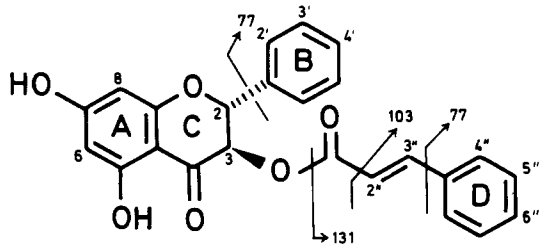


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## EXPERIMENTAL

GENERAL.—Mass spectra were recorded on a Varian MAT 311 at the Institut für Organische Chemie der TH Darmstadt; $^1$H-nmr spectra were recorded on a Bruker WP 200 SY spectrometer at the Departamento de Química Orgánica of the Universidad Autónoma de Madrid. Melting points are uncorrected. Adsorbents used for tlc and cc (Kieselgel N, Polyamid SC-6, Polyamid DC-11) were from Macherey-Nagel/Düren. Naturstoffreagenz-A (NA, β-aminoethyl ester of diphenyl boric acid) was from C. Roth, Karlsruhe, Sephadex LH-20 was from Pharmacia, Uppsala.

PLANT MATERIAL.—Fern fronds were carefully clipped from plants in the field and air-dried in paper sacks. *Notholaena greggi* Maxon was collected in Durango, Mexico (SE end of Sa. del Rosario Canyon of Rio Nazasm, Dec. 20, 1981; voucher T. Reeves, L. Reeves and E. Wollenweber, No. 7513). *N. neglecta* originates from Arizona, USA. (Dragoon Mountains, S of Sheepshead Pass, Dec. 8, 1981; voucher E. Wollenweber, G. Yatskievych 81-492). *Notholaena rigida* Davenport was collected in Tamaulipas, Mexico (along Hwy 101 from Cd. Victoria close to Juamave, May 3, 1983; voucher G. Yatskievych & E. Wollenweber 83-109). *N. rosei* Maxon originates from Oxaca, Mexico (near Hwy 131 from Pto. Escondido to Cd. Oaxaca, Dec. 28, 1983; voucher G. Yatskievych, M.D. Windham & T. Ranker 83-453). *C. kaulfussii* comes from Edo, Mexico (NW of Malinalco, May 5, 1983; voucher G. Yatskievych & E. Wollenweber 83-135). Vouchers are kept in E.W.'s personal herbarium at Darmstadt and, except for *N. greggi*, also in the University of Arizona Herbarium at Tucson.

*Isolation Procedure.*—Dry fern material was rinsed with $Me_2CO$ to dissolve the exudate material, and the solutions were evaporated to solid or semi-solid state. Exudate yields were 16.3 g from 238 g frond material of *N. greggi*, 11.33 g from 368 g of *N. neglecta*, 36 g from 520 g of *N. rigida*, 1.06 g from 15 g of *N. rosei*, and 4.9 g from 46.4 g of *C. kaulfussii*. The *Notholaena* species thus exhibit "farinose" or "waxy" excretions of 3.1% (*N. neglecta*) to 7.1% (*N. rosei*). *C. kaulfussii* produces 10.6% of viscid-resinous exudate. From the concentrated solution from *N. neglecta*, a mixture of the two major flavonoids crystallized on standing. About 4 g of the remainder were subjected to column chromatography (cc) on silica and on polyamide. With *N. greggii* and *N. rigida* only part of the material was chromatographed; while with *N. rosei* and *C. kaulfussii* the total material was used for cc on silica. Elution was done with toluene and increasing quantities of methylethyl ketone and MeOH. Volumes and numbers of fractions were not recorded. Fractions were monitored by tlc on polyamide (for flavonoids) and on silica (for terpenoids). Similar fractions were combined and either separated from terpenoids by passage over Sephadex LH-20, eluted with MeOH, or directly subjected to cc on polyamide, eluted with toluene, methylethyl ketone, and MeOH.

TLC.—The solvents used for chromatography of fractions as well as for comparisons with markers were A) toluene-petroleum ether (bp 100-140°)-methylethyl ketone-MeOH (30:90:2:1.5), B) toluene-petroleum ether (bp 100-140°)-methylethyl ketone-MeOH (12:6:2:1) and C) toluene-MeOH-methylethyl ketone (12:5:3) for tlc on Polyamid DC-11 (4). Chromatograms were viewed at 366 nm before and after spraying with Naturstoffreagenz-A (NA; 0.5% in MeOH). For terpenoids we used precoated silica plates (Polygrams STL G) with solvents D) toluene-methylethyl ketone (9:1) and E) toluene-dioxane-HOAc (18:5:1). Terpenoids were visualized by spraying with $MnCl_2$ reagent (3 g of $MnCl_2$ dissolved in 150 ml $H_2O$, 750 ml MeOH, and 30 ml conc. $H_2SO_4$ added), followed by heating to 130°.

All flavonoid markers were available in E.W.'s lab except for a synthetic sample of scullkapflavone-I obtained from M. Iinuma (5).

## RESULTS

Two major products were obtained directly from the farinose frond exudate of *N. neglecta* by crystallization from the concentrated solution. They were readily identified by direct comparisons to be identical with those described from this species previously (3), namely 7-*O*-methyl-8-acetoxy-galangin (NG-1), accompanied by a small amount of the butyroxy-derivative (NG-2) (6), and 5-hydroxy-7-methoxy-8-acetoxy-flavanone (NNY-6.) From the remainder, after cc on silica and on polyamide, four additional flavonoids [**1-4**] could be obtained in pure form. A terpenoid, TM-KR, was isolated after preparative tlc on silica

Compound **1** forms light yellow crystals, mp 220-225°. The spot on polyamide turns light greenish after spraying with NA and exposure to daylight. This particular reaction indicates **1** is identical with the flavanone obtained on hydrolysis of NNY-6 (3). Its uv, ms, and $^1$H-nmr data confirm this assumption, although the mp is lower than reported previously (246-247°) due probably to different solvent of recrystallization. Thus, **1** is 5,8-dihydroxy-7-methyl-flavanone.

Compound **2** forms almost colorless crystals, mp 212°. Its uv spectrum indicates a flavanone with OMe at C-7 (no shift with NaOAc). According to $M^+$ at *m/z* 332 it has three HO− and two MeO− groups (Table 1). The $^1$H-nmr spectrum shows that ring B is substituted only at C-2′. The remaining HO-group must, therefore, be placed at C-3. The $^1$H-nmr signal at 6.27 ppm (1H, s) further allows the positioning of the second MeO-group at C-8. Thus, the spectral data (Table 2) lead us to establish the structure of **2** as 3,5,2′-trihydroxy-7,8-dimethoxy-flavanone. Mild acetylation of **2** by refluxing with pyridine/$Ac_2O$ (20) yields 3,5,2′-triacetoxy-7,8-dimethoxy-flavone. $^1$H-nmr data δ (ppm) 7.58 (brd, H-6′), 7.36 (brt, H-4′), 7.27 (M, H-3′, H-5′), 6.70 (s, H-6′), 3.97 and 3.86 (s, 2×$OCH_3$), 2.43, 2.26, 2.19 (s, 3×OAc).

TABLE 1. Rf, uv, and ms Data of Compounds **2**, **3**, and **5**

| | Compounds | | |
|---|---|---|---|
| | **2** | **3** | **5** |
| Rf× 100, polyamide, solvent B | 59 | 65 | 53 |
| Spot color/366 nm | dark | dark | dark/gray |
| with NA | reddish brown | dark | brownish/ochre |
| λmax (nm) | | | |
| MeOH | 346, 292 | 347, 295 | 340 s, 296 |
| +$AlCl_3$ | 400, 316, 280 s | 380, 316, 274 | 318, 285 |
| +NaOH | 371, 291 | 375, 289 | 331, 282 |
| +NaOAc | 350, 294 | 343, 292 | 331, 283 |
| +$H_3BO_3$ | 345, 290 | 345, 291 | 333, 284 |
| ms *m/z* (rel. int.) | | | |
| $M^+$ | 332 (56) | 374 (70) | 402 |
| M-$H_2O$ | 314 (9) | 356 (2) | |
| -$CH_3$ | 299 (14) | 341 (2) | |
| -CO | 286 (58) | | |
| -Ac | | 332 (2) | |
| M-Ph-C | 225 (3) | 225 (3) | |
| -O | 209 (8) | 210 (53) | |
| M-Ph-C=C-O | 197 (98) | 197 (100) | |
| M-Ph-C=C-COOH | | | 254 (46) |
| | 196 (33) | 196 (92) | |
| -$CH_3$ | 182 (100) | 182 (22) | |
| | 181 (32) | 181 (51) | |
| | 170 (12) | 170 (16) | |
| -CO | 168 (14) | 168 (29) | |
| -$CH_3$ | 153 (24) | 153 (43) | |
| M-PhC=COO | | | |
| -Ph-C=C | | | 153 (11) |
| | 139 (23) | 139 (9) | |
| Ph-C=C-O | 136 (33) | 136 (20) | |
| | 125 (11) | 125 (22) | |
| | 111 (13 | | |
| Ph-C | 107 (13) | 107 (23) | |
| Ph-C=C | | | 103 (27) |
| | | | 91 (15) |
| Ph | | | 77 (13) |
| $CH_2$=C=O | | 42 (98) | |

Compound **3** was not obtained crystalline but was chromatographically pure. It also is a flavanone. The ms shows the same fragmentation as **2** (Table 1), the B-ring fragments as well as $M^+$ being higher by 42 *amu* than in **2**, suggesting **3** to be a

TABLE 2. $^1$H-nmr Data of Compounds **2**, **5**, and **5** acetate[a]

| Protons | Compounds | | |
|---|---|---|---|
| | **2** (DMSO-$d_6$) | **5** ($CDCl_3$) | **5** acetate ($CDCl_3$) |
| OH-5 | 11.80 s | 11.47 s | |
| OH-2′ | 9.76 | | |
| H-6 | 6.27 s | | 6.60 d |
| | | 6.05 m | |
| H-8 | | | 6.81 d |
| H-2 | 5.50 d | 5.42 d | 5.53 d |
| $J_{H\text{-}2\beta/H\text{-}3\alpha}$ | 9.8 Hz | 11.7 Hz | 11.7 Hz |
| H-3α | 4.81 dd | 5.92 d | 5.90 d |
| OH-3β | 5.92 d | | |
| $J_{H\text{-}3\alpha/OH\text{-}3\beta}$ | 6.2 Hz | | |
| B-ring | | 7.30-7.55 m | 7.30-7.51 m |
| H-3′ | 7.83 d | | |
| H-5′ | 7.20 tr | | |
| H-6′ | 6.88 d | | |
| H-4′ | 6.84 tr | | |
| B-ring +ring D | | 7.30-7.55 m | 7.30-7.51 m |
| H-3″ | | 7.62 d | 7.62 d |
| H-2″ | | 6.35 d | 6.35 d |
| $J_{H\text{-}2''/H\text{-}3}$ | | 16 Hz | 16 Hz |
| OAc | | | 2.37 s |
| $OCH_3$ | 3.84 s | | 2.30 s |
| | 3.55 s | | |

[a]In ppm downfield from TMS.

monoacetate of **2**. Notably, the hydrolysis product of **3** is identical with **2** by direct comparison (tlc, uv, ms). The fragments of ring A with the 3-HO-group are identical in **2** and in **3**; hence, the latter must be the 2′-acetate of the former. Thus, **3** is 3,5-dihydroxy-7,8-dimethoxy-2′-acetoxy-flavanone.

Compound **4** was not crystalline. According to its uv and ms spectra, it is a flavone with two HO− and two MeO− groups ($M^+$ 314). The fragmentation indicates one HO− on the B-ring with the other substituents on the A-ring. One of the MeO− groups that is rather stable in ms is located at C-7, the less stable one is situated at C-8 (M-15>$M^+$) (7). The occurrence of additional maxima in the uv (397 with $AlCl_3$, 395 with $AlCl_3$/HCl) is also in favor of C-8 substitution. Direct comparison (tlc, uv) shows **4** to be identical with the known 5,2′-dihydroxy-7,8-dimethoxy-flavone (scullkapflavone-I).

Compound TM-KR forms colorless crystals, mp 167°. Its color reaction with $MnCl_2$ reagent and its $M^+$ at *m/z* indicate that it is a triterpene. Its mp, ms, and $^1$H-nmr data agree with those reported for lupeone (8).

The farinose frond exudate of *N. greggii* consists partly of terpenoids. Only one of them was isolated in small amount as white flakes, mp 198°. It is assumed to be a triterpene, but has not been studied further. Apigenin 7-methyl ether (genkwanin) obviously is the major flavonoid and was, therefore, obtained crystalline from crude fractions, mp 288°. Direct comparisons with a marker by tlc as well as its uv prove its identity. Further trivial flavonoids present in this material were likewise identified to be apigenin, apigenin 4′-methyl ether (acacetin), apigenin 7,4′-dimethyl ether, and kaempferol 7,4′-dimethyl ether. No further flavonoids could be detected.

In *N. rigida* the exudate again contains a considerable amount of terpenoids. One of

them was obtained as colorless crystals, mp 189°, $M^+$ *m/z* 500, $C_{32}H_{52}O_4$. According to preliminary results, it is assumed to have a cycloartenol skeleton (M. Bokel, Stuttgart, personal communication). Two flavonoids were isolated: the first, mp 188-189°, $M^+$ 328, and the second, mp 255°, $M^+$ 314. Their tlc behavior, uv spectra, and $M^+$ difference of 14 *amu* indicated them to be methyl derivatives of the same flavone. Direct comparisons with authentic markers (tlc, uv, ms) established their identity with scutellarein 6,7,4′-trimethyl ether and with scutellarein 6,7-dimethyl ether (cirsimaritin), respectively. Further flavonoids identified in this material are apigenin, apigenin 4′-methyl ether, and apigenin 7,4′-dimethyl ether. Also, a small amount of a flavonol with unusual properties was obtained, which was later isolated in larger amount from the yellow form of *Notholaena sulphurea*. Its structure elucidation is almost completed and will be reported elsewhere.

The farinose frond exudate of *N. rosei* consists of a series of uncharacterized products which all show the same dark reddish-brown reaction with $MnCl_2$ reagent on silica and are, therefore, assumed to be structurally very closely related terpenoids. They could not be obtained in large enough amounts for spectral studies. The flavonoid aglycones present are apigenin and its 7-methyl, 4′-methyl, and 7,4′-dimethyl derivatives.

*C. kaulfussii* exhibits a resinous frond exudate which is not visible but makes the fronds slightly sticky. From this material we identified the flavone chrysin and the flavonols galangin, galangin 3-methyl ether, galangin 3,7-dimethyl ether, kaempferol 3,7-dimethyl ether, and kaempferol 3,7,4′-trimethyl ether. An unknown phenolic was isolated as a resinous, but chromatographically pure, material [**5**]. Its uv and its chromatographic behavior indicated it to be a flavanone (Table 1). A detailed study of the $^1$H-nmr data of **5** as well as of its acetate and comparisons with literature data and calculated values assigned the $^1$H-nmr signals and ms fragments to the proper structure. The downfield shift observed in the acetate of **5** (5.90 ppm) as compared with alnustinol triacetate (5.75 ppm) is due to the deshielding effect of an aromatic ester. The signal of H-2 β (5.40 ppm) remains unchanged. The signal for H-6 and H-8 in **5** (6.05 ppm) is shifted and occurs as two doublets in **5**-acetate (6.60, 6.81 ppm), due to the deshielding effect of the acetyl groups introduced at C-5 and C-7 (Table 2). The remaining signals for the B-ring and side-chain protons are unchanged. $^1$H-nmr signals and ms peaks that had first been ascribed to a chalcone-component of **5** are now ascribed to a *trans*-cinnamic acid ester moiety. The molecular peak at *m/z* 402 is not observed in the ms. The base peak at 131 is due to the cinnamoyl fragment (Ph-CH=CH-CO) that is split off instantaneously and gives rise to the strong peak at *m/z* 254 which can be explained by the formation of 5,7-dihydroxy-flavone (chrysin). Further important peaks at *m/z* 77 and 103 are explained by the fragments Ph and Ph-CH=CH, respectively (Table 1). Hydrolysis of **5** yields pinobanksin and cinnamic acid, which were identified chromatographically. The structure of **5** is thus established unambiguously at 5,7-dihydroxy-3β-*trans*-cinnamoyloxy-flavanone.

## DISCUSSION

The present analysis of bulk material of farinose exudates ("ceraceous induments") of several cheilanthoid ferns revealed that terpenoids are more important constituents than had been assumed heretofore. Diterpenes have been reported to occur as major farina constituents in *Cheilanthes argentea* (10), in *Notholaena pallens*, and in *Notholaena peninsularis* (11) while in *Notholaena candida* var. *copelandii* a new triterpene was found as the major farina component (12). Now it is evident that terpenoids are also excreted by *N. greggi, N. neglecta, N. rigida,* and *N. rosei*. In *N. greggi, N. rigida*, and particularly in *N. rosei* the terpenoids form remarkable portions of the farinose frond exudate. They should be analyzed in the near future with a view to supporting the chemotaxonomic

studies based so far on flavonoid patterns (1,2). It may be mentioned that the farina in *Notholaena incana* also consists mainly of terpenoids. Three of these were obtained in crystalline form, and their structural analysis is now under way.

The flavonoid pattern of *N. greggi* previously reported to always consist of apigenin and its three methyl derivatives, occasionally accompanied by kaempferol derivatives and traces of luteolin (1), was confirmed by this bulk material analysis. Only small fragments of two herbarium specimens of the rare fern *N. rigida* had been available for an earlier study. They had allowed for the detection of apigenin 4′-methyl ether only, while apigenin 7-methyl ether and kaempferol 4′-methyl ether had been assumed to be minor constituents (13). The present study confirms acacetin as a major flavonoid. In addition, we found apigenin, apigenin 7,4′-dimethyl ether, scutellarein 6,7-dimethyl ether, and scutellarein 6,7,4′-trimethyl ether. We want to stress that this is the first and so far only time (unpublished recent results considered) that 6-substituted flavonoids are encountered in the genus *Notholaena*. A fragment of a *N. rosei* herbarium specimen also had been studied earlier (E.W., unpublished), but this is the first report on the chemical nature of the frond farina of this extremely rare species.

*N. neglecta* is of special interest because of the new compounds analyzed from its frond farina. 5,8-dihydroxy-7-methoxy-flavanone [**1**] was reported previously as a hydrolysis product from its 8-acetate (NNY-6) which is a major constituent in this species (3), but was found here for the first time as a natural flavanone. 3,5,2′-trihydroxy-7,8-dimethoxy flavanone [**2**], and its 2′-acetyl derivative [**3**] are novel dihydroflavonols. 5,2′-Dihydroxy-7,8-dimethoxy-flavone (scullkapflavone-I) [**4**] is a known, but very rare, flavone. It has so far been found in roots of *Scutellaria baicalensis* (14; revised structure, 5) and in callus cultures of *Andrographis paniculata* (15) and later found in aerial parts of *Scutellaria rivularis* (16).

In *C. kaulfussii* the glandular trichomes on its fronds produce a resinous exudate of terpenoid nature that contains methyl derivatives of galangin and of kaempferol as reported earlier, including the rare galangin 3,7-dimethyl ether that had been found in this fern for the first time as a natural product (17). The analysis of bulk material allowed for the isolation of still another novel flavonoid, identified as 5,7-dihydroxy-3β-*trans*-cinnymoyloxy-flavanone (pinobanksin 3-cinnamate) [**5**]. This is the first natural flavanone aglycone found as an ester with an aromatic acid. As yet, one flavone was reported as an aromatic acid ester, namely 7-benzoyl chrysin, isolated from the leaf resin of *Baccharis bigelovii* (18). This confirms the previous assumption that new types of acylated flavonoid aglycones are to be forthcoming (19).

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