Novel Flavonoids from the Fern *Notholaena sulphurea*

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Z. Naturforsch. **42c,** 1063–1069 (1987); received April 21/July 2, 1987

Notholaena sulphurea (Pteridophyta, Pteridaceae), Frond Exudate, 3,5,2′-Trihydroxy-7-methoxy-8-acetoxy Flavone, 5,2′-Dihydroxy-7,8-dimethoxy Flavone, 7-Methyl-aromadendrin 3-*cis*-butyrate

The major constituent of the yellow frond exudate of the fern *Notholaena sulphurea* was identified by spectroscopic methods as 3,5,2′-trihydroxy-7-methoxy-8-acetoxy flavone and its structure was confirmed by synthesis. This novel natural flavonoid was also detected in the frond exudate of five other *Notholaena* species. In the yellow form of *N. sulphurea,* the rare 5,2′-dihydroxy-7,8-dimethoxy flavone was also found, along with some trivial flavonoids. The white form of *N. sulphurea* produces three dihydrochalcones that are accompanied by some kaempferol methyl ethers and apigenin-7-methyl ether. The 3-acetoxy as well as the 3-butyroxy and the 4′-butyroxy derivatives of 7-methyl aromadendrin were also identified in this material. One of them was shown to belong to the extremely rare group of 3-*cis*-dihydroflavonols.

Introduction

In the course of our continuing studies on leaf exudate flavonoids of Cheilanthoid ferns [*cf.* 1, 2] we occasionally observed in *Notholaena* species a constituent with unusual properties. Due to lack of product for analysis it had so far remained unidentified. On a collecting trip in Mexico, bulk material was collected from both forms of *Notholaena sulphurea,* the one with yellow farinose exudate as well as the one with white farina. As it turned out, the yellow material contained the unknown product as a major constituent. Large amounts were obtained from the acetone leaf wash, so its structure could finally be determined. Some further flavonoids, including a very rare flavone with analogous substitution, were identified from the same material. In the white form, the majority of the farinose exudate consists of dihydrochalcones. Among the minor components we identified interesting acyl derivatives of a dihydroflavonol. In the following we want to report the structural elucidation of new as well as rare flavonoids excreted by this fern species.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0900–1063 $ 01.30/0

Materials and Methods

Notholaena sulphurea (Cav.) J. Sm., yellow form, was collected in May 1983 in Mexico, Edo. Hidalgo, on Hwy 85 ca 18 miles SW of Jacala where it was common in a rocky limestone areal at roadside, elev. ca. 1800 m. *N. sulphurea,* white form, was collected in May 1983 in Mexico, Edo. Tamaulipas on Hwy 101 from Cd. Victoria to Juamave, near km post #142 where it grew scattered, but abundand on roadside limestone cliffs; elev. ca. 900 m. Dry leaves were carefully clipped from plants in the field and collected in paper bags. Voucher specimens (yellow: G. Yatskievych and E. Wollenweber 83–182A; white: G. Yatskievych and E. Wollenweber 83–114) are kept at the University of Arizona Herbarium, Tucson, (ARIZ) and in E. W.'s private herbarium.

The dry fern leaves were rinsed with acetone to dissolve the exudate material and the solvent was evaporated. 237 g of the yellow form yielded 7.79 g (3.3% dw) of exudate; ca. 5.35 g of crude "NS" (69%) crystallized from the concentrated solution as fine yellow needles. 406 g of the white form yielded 28.37 g (7% dw) of exudate material. This was fractionated by column chromatography, monitored by TLC, as described earlier [*e.g.* 3]. Thin-layer chromagrograms were evaluated in UV light

(366 nm) before and after spraying with Naturstoff-reagenz A (Nat. A) (C. Roth, 0.5% in MeOH). Mass spectra were run on a Varian MAT 311. ^{1}H NMR spectra were recorded on a Bruker WP200SY at 200 and 50.13 Hz, respectively, and on a JEOL JNM-GX270 at 270 MHz. Melting points are uncorrected.

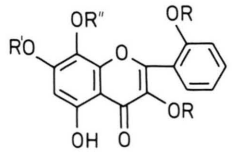
The product NS-II (**1**) was isolated from the crude precipitate "NS" by passage over acetylated polyamide; its m.p. was >300 °C. For UV, MS and ^{1}H NMR data see Tables I and II. ^{13}C NMR (50.3 MHz, DMSO-d_6) 177.6 (C-4), 168.1 ($CH_3\underline{C}O$), 157.8[a] (C-7), 156.8[a] (C-2'), 156.2[a] (C-9), 128.8 (C-6'), 118.8[c] (C-1'), 118.7[c] (C-8), 118.2 (C-5'), 117.6 (C-3'), 103.5 (C-10), 94.6 (C-6), 56.5 ($O\underline{C}H_3$), 19.7 ($\underline{C}H_3CO$) (a, b and c indicate interchangeable assignments).

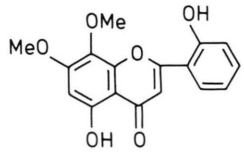
NS-II-ac.: The acetate of NS-II was prepared in the usual manner by reaction of NS-II with acetic anhydride in pyridine overnight. It crystallized from EtOH as colourless needles, m.p. 182–183 °C.

NS-II-sap. (**2**): 10 mg of NS-II (**1**) in 10 ml 6% HCl and 5 ml EtOH was refluxed for 12 h. After cooling, a yellow precipitate (8 mg) was filtered off.

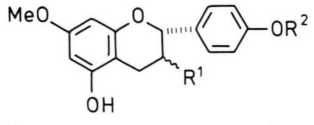
NS-II-sap.-meth. (**4**): Hydrolyzed NS-II (**2**) was treated with excess of CH_2N_2 in DMSO-ether (1:1, 5 ml) at room temp. The reaction mixture was diluted with water, extracted with ether, dried and evaporated to yield the tetramethyl ether (8 mg).

Demethylation of NS-II was achieved by heating 2 mg of NS-II with 20 mg of pyridine-HBr for 5 min [4]. After cooling the reaction mixture was treated





1 NS-II: R=H, R'=CH_3, R''=-CO-CH_3 **5** Skullcapflavone I
2 NS-II-sap.: R=R''=H, R'=CH_3
3 NS-II-dem.: R=R'=R''=H
4 NS-II-sap.-meth.: R=R'=R''=CH_3



6 Aromad.-7-Me-3-Ac.: R^1=O-CO-CH_3, R^2=H
7 Aromad.-7-Me-3-But.: R^1=α-O-CO-CH_2-CH_2-CH_3, R^2=H
8 Aromad.-7-Me-4'-But.: R^1=OH, R^2=-CO-CH_2-CH_2-CH_3

Table I. UV- and Mass Spectra of NS-II and Its Derivatives.

	NS-II, nat. (**1**)	3,5,8,2'-OH-7-OMe flavone (synth.) (**2**)	NS-II-sap. (**2**)	NS-II-ac.	NS-II-dem. (**3**)	NS-II-sap.-meth. (**4**)	*Andrographis* flavone [11]
UV λ_{max}^{MeOH} (nm)	409, 264		398, 275	302	396, 273	345, 264	362, 302 infl. 272
$AlCl_3$	419, 331, 267		442, 350, 307 sh, 281		500, 351, 335, 293	412, 325 sh, 276	362, 278
+ HCl	413, 326, 269		441, 345 307 sh, 280		436, 335 sh, 312, 282	unchanged	unchanged
NaOH	416, 349, 269		356, 295 sh		500, 340, 284, 238 sh	345, 264	360, 272
NaOAc	398, 262		409, 270		500, 345, 285		358, 272
+ H_3BO_3	396, 263		385, 266		500, 344, 281		
MS *m/z* (rel. int.)		316 (M^+, 100%)	316 (100%)	484 (3%)	302 (100%)	358 (M^+, 43%)	358 (90%)
		299 (17)	299 (15)	442 (22)	285 (42)	343 (100)	343 (100)
		287 (9)	287 (6)	400 (50)	273 (6)	327 (11)	328 (9)
		273 (7)	273 (6)	358 (89)	229 (7)	153 (19)	313 (25)
		270 (4)		316 (80)	169 (28)	135 (14)	285 (4)
		242 (2)		287 (21)	168 (20)	91 (17)	181 (20)
		183 (8)	183 (5)	121 (9)	121 (14)	77 (17)	162 (2)
		158 (11)	158 (10)	43 (100)	77 (20)		153 (34)
		139 (8)	139 (8)				147 (5)
		121 (9)	121 (8)				125 (12)

Table II. ^{1}H NMR spectra of NS-II and Its Derivatives.

	nat. NS-II (**1**)	synth. NS-II-sap. (**2**)	NS-II-ac	NS-II-sap.-meth. (**4**)
$-OCOCH_3$	2.26 (3H, s)		2.17, 2.26, 2.33, 2.45 (3H, each, s)	
$-OCH_3$	3.90 (3H, s)	3.89 (3H, s)	3.94 (3H, s)	3.79, 3.84, 3.86, 3.94 (3H each, s)
H-6	6.69 (1H, s)	6.55 (1H, s)	6.75 (1H, s)	6.43 (1H, s)
H-5′	6.92 (1 br. t., *J* 8 Hz)	6.92 (1H, t, *J* 7 Hz)	7.33 (1H, dt, *J* 8 and 1 Hz)	7.18 (2H, m)
H-3′	6.99 (1H, br. d., *J* 8 Hz)	6.95 (1H, d, *J* 7.59 Hz)	7.25 (1H, br. d., *J* 8 Hz)	
H-4′	7.35 (1H, br. t., *J* 8.1 Hz)	6.92 (1H, t, *J* 7 Hz)	7.56 (1H, dt, *J* 8 and 1 Hz)	7.45 (2H, m)
H-6′	7.41 (1H, dd, *J* 8.1 Hz)	7.41 (1H, d, *J* 7.70 Hz)	7.51 (1H, dd, *J* 8 and 1 Hz)	
OH		8.30 (1H, s) 9.64 (1H, s)		
5-OH	12.48 (1H, s)	12.09 (1H, s)		12.51 (1H, s)

with water and acetone and extracted with ethyl acetate to yield NS-II-dem. (**3**).

Synthesis of 3,5,8,2′-tetrahydroxy-7-methoxyflavone (**2**)

Usual condensation [5] of 2-hydroxy-3-isopropyloxy-4,6-dimethoxyacetophenone (350 mg, 1.4 mmol) with 2-isopropyloxybenzaldehyde (226 mg, 1.4 mmol) in the presence of KOH gave 2′-hydroxy-2,3′-diisopropyloxy-4′,6′-dimethoxychalcone (510 mg) as a reddish oil. Crystallized from MeOH it formed yellow rectangles m.p. 76–77 °C. ^{1}H NMR ($CDCl_3$, 60 MHz) δ: 1.30, 1.40 (6H, each, d, $J=6.0$ Hz, $(CH_3)_2$), 3.93 (6H, s, $2\times OCH_3$), 4.38–4.63 (2H, m, $2\times CH<$), 6.01 (1H, s, H-5′), 7.00–7.80 (4H, m, Ph), 7.88, 8.25 (1H, each d, $J=16.0$ Hz, H-α and β). To a methanol soln. (10 ml) containing this chalcone derivative (450 mg) and 15% H_2O_2 (9 ml), 10% aquous soln. of NaOH (10 ml) was added in dropwise. The soln. was stirred for 20 min at room temperature, then acidified with 10% HCl and extracted with AcOEt. Purification of the AcOEt soln. by CC (eluent: AcOEt – C_6H_{14} = 2:1) gave 3-hydroxy-8,2′-diisopropyloxy-5,7-dimethoxyflavone as pale yellow rectangles (210 mg), m.p. 169–170 °C ($C_6H_6-C_6H_{14}$). MS *m/z* (rel. int.): 414 (M^+) (44), 399 (2), 371 (9), 329 (31), 315 (15), 301 (11), 295 (16), 287 (15), 239 (23), 212 (44), 197 (93), 174 (87), 170 (100), 121 (23). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 1.27 (12H, d, $J=6.23$ Hz, $(CH_3)_2$), 3.97, 4.02 (3H, each, s, OCH_3), 4.37, 4.57 (1H, each heptet, $J=6.23$ Hz, $CH<$), 6.43 (1H, s, H-6), 7.03 (1H, dd, $J=8.06$, 1.46 Hz, H-3′), 7.04 (1H, dt, $J=8.06$, 1.46 Hz, H-4′), 7.42 (1H, ddd, $J=8.06$, 7.33, 1.46 Hz, H-5′), 7.55 (1H, dd, $J=7.33$, 1.46 Hz, H-6′). A CH_2Cl_2 soln. containing this flavone derivative (150 mg) was cooled to −60 °C and BCl_3 (0.5 ml) was added. The reaction mixture was left at room temperature for 1 h, and then poured into water. After extraction with AcOEt, recrystallization from $CHCl_3$ gave 3,5,8,2′-tetrahydroxy-7-methoxy flavone (**2**) as a yellow powder, m.p. 249–250 °C. The UV and mass spectra of the synthetic product were identical to those of natural NS-II-sap (Table I). For ^{1}H NMR data of the synthetic product see Table II.

Compound **5** (skullcapflavone I) formed yellow needles, m.p. 255 °C (Lit. 254–255 °C) [6]. $UV_{\lambda max}^{MeOH}$ (nm): 340 sh, 293; + $AlCl_3$ 390, 316, unchanged with HCl; + NaOH 361, 290; + NaOAc 373 sh, 294; + NaOAc + H_3BO_3 335 sh, 291. MS *m/z* (rel. int.): 314 (M^+, 54%), 299 (M-CH_3, 100%). 271 (3), 181 (32), 167 (3), 153 (46), 145 (5), 125 (15).

Compound **7** (aromadendrin-7-Me-3-*cis*-butyrate) formed colourless crystals, m.p. 121 °C. ^{1}H NMR

(ppm, $CDCl_3$): 0.80 (3H, t, $J=7$ Hz; $-CH_3$), 1.52 (2H, sext., J Hz; $-CH_2-$), 2.25 (2H, t, J Hz; $CO-CH_2-$), 3.82 (3H, s; 7-OCH_3), 5.51 (1H, d, $J=3.3$ Hz, H-2), 5.74 (1H, d, $J=3.3$ Hz; H-3), 6.10 (2H, s; H-6/H-8), 6.17 (1H, bs; 4′-OH), 6.80 (2H, d, 8.5 Hz; H-3′/H-5′), 7.28 (2H, d, $J=8.5$ Hz; H-2′/H-6′), 11.61 (1H, s; 5-OH). ^{13}C NMR spectrum: 190.35 (C-4), 171.82 (C-1′), 168.79 (C-7), 164.57 (C-5), 162.11 (C-9), 156.34 (C-4′), 128.37 (C-2′/6′), 126.52 (C-1′), 115.51 (C-3′/5′), 102.46 (C-10), 94.75, 95.56 (C-6/8), 79.96 (C-2′), 70.54 (C-3), 55.71 (7-OCH_3), 35.72 (C-2″), 18.17 (C-3″), 13.32 (C-4″).

Results

Structure elucidation of NS-II

About two thirds of the exudate produced by yellow *Notholaena sulphurea* (Cav.) J. Sm. crystallized from the concentrated acetone leaf wash. This material corresponds to the "unknown product NS" observed earlier in several other *Notholaena* species. It appears on polyamide as a polar spot with strong tailing that is reddish-orange in UV_{366} and turns greenish-yellow on spraying with Naturstoffreagenz A (Nat. A). Chromatographed on silica, the product yields two major spots, one of them strongly tailing from the starting point. The two components cannot be isolated by usual CC on silica or polyamide since the wanted product is irreversibly bound to both adsorbents. They were finally separated by CC on acetylated polyamide and one of them was found to be identical with NG-2 (3,5-diOH-7-OMe-8-acetoxy flavone [7]). The wanted product NS-II after concentration of relevant fractions with a rotation evaporator sticked irreversibly to the glass wall and couldn't be redissolved but with DMSO. It was obtained as a yellow powder, though, when the original fractions were left to evaporate at room temperature.

Acetylation of "NS" yielded a mixture of NG-acetat and NS-II-acetate that can be separated on silica with solvent (9/1). The NS-II-acetate thus isolated by preparative TLC exhibits M^+ at m/z 484 and the base peak at m/z 358. Loss of 4 acetyl units leads to m/z 316 for the underivatized flavonoid, which hence was assumed to be a flavone/flavonol with four OH- and one OMe-group. According to the colour behavior of the corresponding spot in UV before and after spraying with Nat. A, compound NS-II appeared to be a flavonol. Saponification of NS-II yielded a product (**2**) that forms a dark spot on polyamide, turning reddish-brown in daylight after spraying with Nat. A. Demethylation of this latter product with pyridine-HBr yielded a substance (**3**) that forms a dark spot which turns bluish-violet in daylight with Nat. A. Both colour reactions indicated that the flavonol we investigated had on OH-group at C-8 and that in the natural product NS-II the 8-OH-group has to be acetylated. The brownish daylight colour is observed *e.g.* with 8-OH-galangin-7-Me, with herbacetin-7-Me and with herbacetin-7,4′-Me, while the bluish-violet colour is observed with 8-hydroxygalagnin, herbacetin, herbacetin-3-Me, platanetin (3,5,7,8-OH-6-C_5), and also with norwogonin and isocutellarein. It obviously is indicative of 5,7,8-tri-OH-substitution (except for gossypetin that turns reddish). It hence was concluded that our product NS-II exhibits 3,5-OH-7-OMe-8-OAc-substitution. One further OH-group remained to be placed at the B-ring.

The UV-spectrum of NS-II (**2**) is unusual in so far as its exhibits Band I at extremely high wavelength (409 nm). The demethylated product (**3**) shows Band I at 401 nm, and for deacetylated NS-II (NS-II-sap., **2**) it even is found at 415 nm. It should also be mentioned that is was not possible to obtain a mass spectrum with the latter product in standard conditions (EI, DIP, ion source 300 C). The ^1H NMR spectrum of NS-II differed from that of NG-2 (3,5-OH-7-OMe-8-acetoxy flavone) in the shift and multiplicites of the B-ring protons. Four multiplets, corresponding to one proton each, were clearly observed and comparing coupling constants and calculated values [8] a 2′-oxygenated B-ring was assumed for NS-II. The remaining signals were the same as found for NG-2. The ^{13}C NMR spectrum also agreed with the proposed structure of 3,5,2′-trihydroxy-7-methoxy-8-acetoxy flavone [9].

Flavonoids having a 5,7,8,2′-O-substitution pattern are unusual. In the literature we found only 5,2′-diOH-3,7,8-triOMe flavone = dechloroflavonin from *Aspergillus candidus* [10] and 5-OH-3,7,8,2′-tetra-OMe flavone from *Andrographis paniculata* [11]. The structures of the latter compound and of the methylation product of hydrolyzed NS-II should be identical, but direct comparisons of both products showed that neither their R_f-values nor their UV- and ^1H NMR spectra agreed. Both are 3,7,8-trimethoxy-flavones since M^+ is the base peak [12]. A significant peak appeared in the tetramethoxy-

flavone obtained from NS-II ($M^+ - 31$, 11%), which was not important in the *Andrographis* product. This loss of 31 m.u. was suggested by Kingston [13] as being typical for 3,2′-dimethoxy flavones. Further the MS of the methylated fern product showed a good A^+ fragment at 197, while the *Andrographis* product didn't. These observations emphasized that the original fern product NS-II is in fact 3,5,2′-trihydroxy-7-methoxy-8-actoxy flavone, whereas the structure of the *Andrographis* product needs revision.

The structure we deduced for NS-II was finally confirmed by synthesis of the hydrolyzed product, 3,5,8,2′-tetrahydroxy-7-methoxy flavone (**2**). By condensation of 2-hydroxy-3-isopropyloxy-4,6-dimethoxy-acetophenone with 2-isopropyl-oxybenzaldehyde, 2′-hydroxy-2,3′-diisopropyloxy-4′,6′-dimethoxy chalcone was obtained. The chalcone was transformed to 3-hydroxy-8,2′-diisopropyloxy-5,7-dimethoxy flavone by hydroperoxyde oxidation. The flavone was deisopropylated and partially demethylated by treatment with boron trichloride to give the desired flavone: 3,5,8,2′-tetrahydroxy-7-methoxy flavone. This compound is identical with the hydrolyzation product of NS-II (NS-II-sap. **2**).

Further constituents of the yellow form

The flavonoid mixture that remained after crystallization of NS from the concentrated leaf wash was first chromatographed over silica. Individual flavonoids where then purified by CC on polyamide, by preparative TLC on silica and crystallization. They were characterized by UV-spectroscopy and/or co-chromatography with markers.

One compound that was obtained in crystalline form (m.p. 255 °C) appeared on TLC as a dark spot that turned yellow brown with Nat. A. The mass spectrum showed it to be a flavone/flavonol with 2 OH- and 2 OMe-groups. Interpretation of the UV-spectrum led us to assume that it was 5,4′-diOH-7,8-diOMe-flavone. Direct comparisons with authentic markers showed, however, that the fern product is, in fact, identical with the isomeric 5,2′-diOH-7,8-diOMe flavone, skullcapflavone I [15]. This identification was further confirmed by the ^{1}H NMR spectrum of the natural compound (**5**).

With respect to its chromatographic behavior, a less polar component might be the 4′-methyl ether of skullcapflavone I, but this assumption could not be confirmed, due to lack of material and lack of a marker. Further trace constituents were readily identified by direct comparisons with authentic samples as NAS-2 (4,5,4′-triOH-7-OMe-8-OAc flavone, [7]), kaempferol-7-Me, quercetin-7,3′-Me and pinocembrin-7-Me.

Constituents of the white form

A mixture of 2′,6′-diOH-4′-OMe-dihydrochalcone and 2′,6′-diOH-4′,4-diOMe-dihydrochalcone crystallized from the concentrated crude exudate solution of the white form of *N. sulphurea*. A third dihydrochalcone, 2′,6′,4-triOH-4′-OMe-dihydrochalcone (asebogetin), was obtained in good amount after CC of the remainder on silica. These compounds were identified by their UV and mass spectra and their structures were confirmed by comparisons with authentic samples. Also some trivial flavonols, kaempferol-7-Me, kaempferol-7,4′-di-Me as well as apigenin-7-Me were identified by TLC comparisons. A further "constituent" turned out to consist of three components. Two of them were obtained by preparative TLC on silica while the third was crystallized from the relevant fractions after repeated CC on silica. According to their UV- and mass spectra they were assumed to be dihydroflavonols. MS fragmentation indicated that they are acyl derivatives and pointed to monomethyl aromadendrin as the basic molecule. Hydrolysis of a sample of the original mixture furnished indeed aromadendrin-7-Me as the sole reaction product. One of the compounds (**6**) was found to be identical (TLC, UV, MS) with an authentic sample of aromadendrin-7-Me-3-Ac [16]. The two others are butyrates with the butyryl group attached to different positions, in one case at 4′ (**8**) in the other at 3 (**7**). The latter structure (5,4′-diOH-7-OMe-3-butyroxy flavanone) was confirmed by ^{1}H NMR and ^{13}C NMR spectra. Assignment of the ^{13}C NMR signals was made by comparison of the values reported for 3-acetoxy-5,4′-diOH-7-OMe flavanone [16]. In the ^{1}H NMR spectrum, the doublets corresponding to H-2 and H-3 show surprisingly low coupling constants (3,3 Hz) as compared with the corresponding signals (about 12 Hz) in some normal 3-trans-acyloxy-flavanones ("CS-CK-1", [17]; alnustinol acetate). Such a small coupling constant is typical for $J_{2\,ax-3\,eq}$ in flavanones. Assuming that the B-ring exists in the thermodynamically favoured equatorial conformation, the 3-C_4-substituent must be axial. Compound **7** thus is the 3-*cis* butyrate of 7-methyl aromadendrin.

Discussion

With the structure elucidation of NS-II we finally achieved the identification of a compound that first had raised our attention when we analyzed the farina constituents of *Notholaena affinis* years ago [18, 19]. At that time we were unable to isolate the compound that caused that conspicuous tailing spot on polyamide-TLC and which we observed later in several other species. Only the facts that NS is the major farina constituent in the yellow-exudate population of *N. sulphurea* now studied and that we came upon acetylated polyamide to separate NS-II from NG (3,5-diOH-7-OMe-8-acetony flavone), with which it seems to form kind of a complex, allowed us to execute the necessary spectral analyses. The structure now deduced for NS-II, namely 3,5,2′-trihydroxy-7-methoxy-8-acetoxy flavone [1] is beyond doubt as it was confirmed by synthesis. The products reported strange properties (TLD, UV, MS) were now observed in some related compounds and seem to be typical for 5,8-dihydroxy flavones, *i.e.* related to kind of quinoid structure. Studies on this phenomenon are now in progress (M.I., T.T. and M.M.) and will be published elsewhere.

In a screening for further occurrence of NS-II we found it in representatives of the species *Notholaena affinis, N. californica, N. grayi, N. rigida, N. sulphurea* (yellow), and *N. trichomanoides*. It should be stressed, though, that it is not present in all samples of the cited species. For the material of *N. sulphurea* used in the present study it is noteworthy that it also contains skullcapflavone I (5,2′-dihydroxy-7,8-dimethoxy flavone), the flavone with the same substitution pattern as NS-II.

The novel product NS-II was found in several samples of yellow *Notholaena sulphurea,* but in none is it as prominent as in the material here studied. The yellow colour of the exudate is in most cases (12 out of 14 samples) due to the presence of 2′,6′-dihydroxy-4′-methoxy chalcone, which is in general accompanied by the corresponding flavanone (pinocembrin-7-methyl ether), by kaempferol-7-Me, and often also by quercetin-7,3′-diMe. The presence of skullcapflavone and herbacetin-7,4′-diMe can not be stated with certainty in the samples of herbarium fragments as their concentration is too low.

Notholaena sulphurea with white farina, on the other hand, is mostly characterized by rich production of 2′,6′-diOH-4′-OMe dihydrochalcone, 2′,6′-diOH-4′,4-diOMe-dihydrochalcone and 2′,6′,4-triOH-4′-OMe dihydrochalcone (asebogenin) (8 out of 13 samples). The presence of the newly identified acyl-flavonols **6**–**8** seems to be correlated to the presence of these dihydrochalcones. With compound **7** we identified another representative of the so far extremely rare natural *cis*-3-substituted flavanones. The majority of naturally occurring dihydroflavonols exists in the (2R:3R) configuration, but a few compounds are known with (2S:3R) stereochemistry [*cf.* 20]. In addition, this is the first *cis*-3-acyl-dihydroflavonol reported as a natural product. It is possible that compounds **6** and **8** also belong to this series, but the amounts available were too scanty to allow the relevant studies. Further flavonoids occasionally detected in the white exudate are apigenin-7-Me, kaempferol-7-Me, kaempferol-3,7-diMe, kaempferol-7,4′-diMe, and quercetin-7,3′-diMe.

The novel flavonol NS-II (**1**) fits well in the scope of other previously identified *Notholaena* flavonoids (NA, NG, NAS etc.) that were all found to be 8-acetoxy- and 8-butyroxy-compounds, respectively [*cf.* 7, 22], while 7-methyl aromadendrin-3-*cis*-butyrate is the first flavonoid from fern exudates that is esterified at OH-3.

NS-II is not present in any of the white samples. With respect to the major constituents of their leaf exudates, and hence to the fern's biosynthetic capacity, the two colour forms of *Notholaena sulphurea* are clearly distinct and should be treated at least as chemical races, if not as varieties. It can be assumed that a colour effect mentioned by Tryon [21] and observed on a few specimens only, "young fronds being white and the old ones yellow", has nothing to do with the here reported chemical distinctness.

Acknowledgements

The senior author (E.W.) is grateful to Dr. K. R. Markham (Petone, New Zealand) for informations and helpful comments, to Dr. K. L. Dhar, Jammu Tawi, India, for a sample of the *Andrographis* flavone and to Dr. J. D. Connolly, Glasgow, Scotland, for a sample of aromadendrin-7-Me-3-acetate. Thanks are due in particular to George Yatskievych, Bloomington, Ind., who accompanied E.W. on a collection trip in Mexico. Financial support by the DFG to this trip as well as to E.W.'s laboratory work is gratefully acknowledged.

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