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ISOORIENTIN 3′-*O*-SOPHOROSIDE AND 3′-*O*-NEOHESPERIDOSIDE FROM THE MOSS *PLAGIOMNIUM AFFINE*

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Abstract—From *Plagiomnium affine* isoorientin, isoorientin 3′-*O*-sophoroside and isoorientin 3′-*O*-neohesperidoside were isolated. The two di-*O*-glycosides are new natural compounds. The flavonoid pattern of *P. affine* was constant, irrespective of geographical and seasonal sampling.

INTRODUCTION

Liverworts have been chemically investigated more than mosses [1], mainly because they possess oil bodies which contain a large variety of novel terpenoids. Since Kozlowski's early paper on saponarin in *Mnium cuspidatum* [2] a number of further reports on flavonoids in mosses appeared and several new structures were found [1, 3]. Among these papers there are also several reports on flavonoids in species of Mniaceae [4–8].

We wish to report here the results of studies on the flavonoid pattern of *Plagiomnium affine* (Funk) T. Kop. This is the first report on flavonoids from this species. According to Koponen '*Mnium affine*' in the study of Melchert and Alston [4] is "obviously not identical with *Plagiomnium affine* (Funk) T. Kop., but some other species of the section Rosulata" [9]. Koponen [9] in his generic revision of the Mniaceae used a number of characters for the differentiation of the various taxa, including "coloured substances and other chemical characters". This contribution is regarded as a part of a series of papers on the chemical constituents of Mniaceae.

RESULTS AND DISCUSSION

Five flavone glycosides (**1**–**5**) were isolated from gametophytes of *P. affine* and several other minor components were detected by 2D-TLC but not isolated.

Compound **5** was found to be identical with isoorientin (= luteolin 6-*C*-*β*-D-glucopyranoside) by comparison with an authentic sample. Wessely–Moser rearrangement [10] gave a second product which was chromatographically indistinguishable from a standard sample of orientin. Although isoorientin is known from many sources of tracheophytes [11], this is the first isolation from a bryophyte.

Compounds **1**–**4** proved to be *O*-glycosides of isoorientin. The hydrolysable sugars are glucose for **3** and glucose and rhamnose for **4**. Both glycosides were similar in their chromatographic behaviour (Table 1), appearing as deep purple spots under UV, turning yellow-green with ammonia and Benedicts reagent. The UV spectra of both are identical. The sodium methoxide and sodium acetate spectra indicate a free 7-hydroxyl and an isolated 4′-hydroxyl group [12]. Both **3** and **4** therefore must be glycosylated at the 3′-hydroxyl. The molecular ions of the permethylated glycosides are observed at *m/z* 968 (**3**) and 938 (**4**) respectively. This means in **3** that there are two

Table 1. Chromatographic and UV visible absorption data for flavones from *Plagiomnium affine*

	1	**2**	**3**	**4**	**5**
hR_f-values in					
BAW	0	5	13	25	47
BEW	5	7	21	34	60
TBA	0	2	14	19	48
3% HOAc	22–50	24–54	26	38	8
15% HOAc	56–73	57–82	52	64	35
40% HOAc	74	79	65	76	54
80% MeOH	66	32–55	51	66	35
WEMA	44	79	78	43	34
Colour reactions					
UV (254, 350 nm)	p	p	p	p	p
UV/NH_3	g	g	g	g	y
UV/NA	ol	ol	ol	ol	or
UV/Br*	g	g	g	g	p

UV-visible data	**3** (**1**, **2** and **4**)	**5**
MeOH	335, 272	347, 267 sh, 255
NaOMe	405, 335 sh, 277†	412, 335 sh, 279, 270†
$AlCl_3$	385 sh, 350, 296 sh, 278	423, 331, 302 sh, 273
$AlCl_3$–HCl	385 sh, 348, 296 sh, 279	361, 296 sh, 279, 264
NaOAc	395, 312 sh, 278	403, 323, 267
NaOAc–H_3BO_3	340, 272	389, 265

*For Benedicts reagent see refs [23, 24].

†Increasing intensity, stable; g = green; ol = olive; or = orange; p = purple; y = yellow; for other abbreviations see Experimental.

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hydrolysable glucose molecules attached to isoorientin and in the case of **4** one glucose and one rhamnose. In both glycosides mass and ^{13}C NMR spectra prove a 1 → 2 linkage between the hydrolysable sugars, a glycosylation at the *C*-linked glucose being thus excluded. According to Schmid [13] a strong *OS*−methoxyl peak (*OS* = disaccharide fragment) indicates the presence of a 1 → 2 linkage. Such a signal is observed in the MS of **3** at *m/z* 391 (67 %) and in the MS of **4** at *m/z* 361 (88 %), whereas a signal $S+62$ (S = glycoside fragment minus terminal sugar of diglycosides) observed for 1 → 6 linked glycosides is absent in the MS of both compounds. The value for the *S*-fragment of **4** at *m/z* 733 (9 %) and for T_1 (terminal sugar fragment) at 189 (> 100) clearly shows rhamnose as the terminal sugar in this glycoside. These observations are confirmed by the ^{13}C NMR spectra of both glycosides (Experimental). The values for the aglycone and *C*-linked sugar moiety are as expected for isoorientin [14] and 3′-*O*-glycosylation is indicated by the downfield shifts of the adjacent *ortho*-related (C-2′, 4′) and *para*-related (C-6′) signals relative to the corresponding signals of isoorientin. The signals for the two *O*-linked glucose molecules of **3** are almost identical to those observed for sophorosides [14]. Thus the C-2‴ signal is shifted 7.7 ppm downfield compared with the C-2 signal of glucose in luteolin 3′-*O*-glucoside and the C-1‴ signal (terminal glucose) shifts 7.5 ppm downfield compared with C-1 of glucose in D_2O [14]. In contrast the C-6‴ signal is almost at the same position as in luteolin 3′-*O*-glucoside. For compound **4** chemical shift values for the carbons of the *O*-linked sugars are almost identical to those observed for neohesperidosides [rhamnosyl (1–2) glucosides], whereas in rutinosides [rhamnosyl (1–6) glucosides] the signal for C-6 of the glucose is about 6 ppm further downfield [14].

Thus **3** is isoorientin 3′-*O*-sophoroside and **4** isoorientin 3′-*O*-neohesperidoside. To our knowledge these two isoorientin-*O*-diglycosides are new natural compounds. The minor compounds **1** and **2**, from chromatographic and UV absorption data (Table 1), also appear to be isoorientin-*O*-glycosides. For both, acid hydrolysis produces isoorientin and glucose.

Isoorientin-*O*-glycosides in general are not exceptionally rare in the plant kingdom. Especially in the genus *Gentiana* they are found frequently, e.g. isoorientin 3′-*O*-monoglucoside in *G. nivalis* [15] and *G. pedicellata* [16] and isoorientin 4′-*O*-monoglucoside in *G. verna* [17]. An isoorientin 2″-*O*-glucoside was found in *G. lutea* [18], in *G. verna* [17] and in *G. depressa* [19]. Isoorientin *O*-diglycosides however are rare, e.g. isoorientin 2″,4′-di-*O*-*β*-glucoside was isolated from *G. asclepiadea* [20] and isoorientin 3′,6″-*O*-glucoside from *G. pedicellata* [19].

To date flavonoids have been reliably identified from only two species of the Mniaceae *Plagiomnium cuspidatum* (Hedw.) T. Kop. and *P. undulatum* (Hedw.) T. Kop. Both species were shown to produce saponarin (isovitexin 7-*O*-glucoside) and this is the only fully identified flavonoid from *P. cuspidatum* [7]. Österdahl [8] isolated 6 further flavone di-*C*-glycosides from *P. undulatum*: schaftoside, isoschaftoside, neoschaftoside, neosisoschaftoside, vicenin-2- and a chrysoeriol 6-*C*-arabinosyl-8-*C*-hexoside. Flavone *C*-glycosides thus are the predominant flavonoids of the three *Plagiomnium* species investigated. In *P. affine* further *O*-glycosylation of a phenolic hydroxyl is evident although this is also a structural feature of saponarin (found in *P. cuspidatum* and *P. undulatum*). Twenty-four samples of *P. affine* collected in West Germany, France and Switzerland were surveyed to check the variability of the flavonoid pattern. The main flavonoids **1–5** were observed on 2D-TLCs of all samples, from all localities. To check for seasonal variation plants were collected from four different places in West Germany in February, May, August and November. Although some quantitative variation in the main flavonoids was noted, a strict correlation with ecological factors was not observed.

EXPERIMENTAL

Plant material. The plant material for isolation and identification of the flavonoids **1–5** was collected in April and May 1982 near St. Ingbert, Saarland, W. Germany. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken. The plants were identified by Dr. T. Koponen, Helsinki.

Extraction and isolation. After careful cleaning 220 g air dried gametophytes were ground in a Waring blendor and extracted with $CHCl_3$ to remove chlorophyll and lipids, later with 80 % aq. EtOH, 80 % aq. MeOH and finally with 50 % aq. MeOH. Flavonoids were detected in the combined alcoholic fraction. This fraction was evaporated to the H_2O phase and extracted first with $CHCl_3$ to separate the rest of the chlorophyll, and later with EtOAc. Compound **5** was isolated from the EtOAc fraction by repeated PC on Whatman 3 MM in 15 % HOAc, and purified by CC on Sephadex LH-20 with 70 % aq. MeOH. Glycosides **1–4** were isolated from the water fraction by repeated CC on cellulose, (microcrystalline, Merck) with 3 % HOAc, on Sephadex LH-20 with 70 % aq. MeOH and by repeated PC on Whatman 3 MM with 15 % HOAc, BAW and BuOH–HOAc–H_2O (14:1:5, BEW). Final purification of each glycoside was achieved on a Sephadex LH-20 column with 80 % aq. MeOH as solvent. The yields were for **1**: 2 mg, **2**: 1 mg, **3**: 15 mg, **4**: 12 mg and **5**: 1.5 mg.

The extracts for 2D-TLC of 24 samples were prepared as follows: 200 mg gametophytic air-dried plant material were ground with a pestle and mortar in 20 ml 80 % aq. MeOH, stirred 1 hr, filtered, evaporated to a small vol. and finally dissolved in 2 ml aq. MeOH. From these 30 μl were spotted on TLC-plates with cellulose (0.25 mm, microcrystalline, Merck). 2D-TLC-plates were developed in TBA (1) and 15 % HOAc (2); compounds were identified under UV (254, 350 nm) before and after treatment with NH_3 and NA.

Chromatography. TLC solvent systems: (a) glycosides: BAW, BEW, TBA, 3 %, 15 %, 40 % HOAc (cellulose), 80 % MeOH, H_2O–MeCOEt–MeOH–3,5-pentanedione (13:3:3:1, WEMA) (polyamide). TLC on precoated sheets: cellulose F 1440 (Schleicher and Schüll), polyamide 6, Polygram (Macherey and Nagel). (b) sugars: EtOAc–C_5H_5N–HOAc–H_2O (36:36:7:21), cochromatography with standards on cellulose. Spray reagents: Naturstoffreagenz A (NA) [21]; 0.1 % in MeOH (flavones), anilinephthalate, Merck (sugars).

Hydrolysis. Glycoside (0.5–1 mg) was hydrolysed in 6 ml 1N TFA under reflux for 30–120 min (no Wessely–Moser rearrangement under these conditions!). The product was extracted with EtOAc. The flavone part was identified from the EtOAc-fraction and sugars from the H_2O.

Permethylation (PM) was carried out according to ref. [22].

Spectroscopic methods. UV spectra according to ref. [12]. Mass spectra: EI, 70 eV, ion source 150°, probe temp. 160–290°. ^{13}C NMR spectra: measured on a Varian A-60 A; solvent DMSO-d_6; temp. 30°.

Compound **3**. MS PM-derivatives; base peak: peak $m/z > 200$ with highest intensity for Pa 3 and Pa 4 (rel. int. in parentheses):

968 (12), 953 (3), 937 (10), 905 (1), 865 (2), 805 (5), 793 (3), 733 (4), 560 (12), 546 (100), 547 (51), 531 (1), 515 (4), 483 (3), 430 (7), 422 (3), 413 (10), 391 (67), 385 (50), 371 (14), 357 (7), 341 (5), 327 (6), 218 (12). ^{13}C NMR (C-x: aglycone; C-x″: C-linked glucose; C-x‴: O-linked sugar at C3′-OH; C-x⁗: terminal O-linked sugar at C-3′-OH): 181 (C-4), 162.6 (C-2), 162.6 (C-7), 160.6 (C-5), 156.5 (C-9), 153.1 (C-4′), 146 (C-3′), 122 (C-1′), 119.7 (C-6′), 116.7 (C-5′), 114 (C-2′), 108.9 (C-6), 103.7 (C-1⁗), 102.1 (C-10), 101.9 (C-3), 99.9 (C-1‴), 94.4 (C-8), 81.4 (C-5″), 81.2 (C-2‴), 79.1 (C-3″), 77.1 (C-3‴), 76.7 (C-3⁗), 76.1 (C-5‴, 5⁗), 74.1 (C-2⁗), 73.5 (C-1″), 69.9 (C-2″, 4″), 69.4 (C-4‴, 4⁗), 60.7 (C-6″, 6‴, 6⁗).

Compound **4**. MS PM-derivative *m/z* (rel. int.): 938 (10), 923 (8), 907 (23), 875 (2), 835 (3), 775 (7), 763 (7), 733 (9), 560 (12), 546 (100), 547 (72), 531 (1), 515 (6), 483 (4), 430 (20), 393 (18), 412 (28), 361 (88), 385 (92), 371 (24), 357 (14), 341 (8), 189 (> 100). ^{13}C NMR: 182 (C-4), 162.6 (C-2), 162.6 (C-7), 160.7 (C-5), 156.6 (C-9), 152.7 (C-4′), 145.9 (C-3′), 121.5 (C-1′), 120.4 (C-6′), 116.8 (C-5′), 114.3 (C-2′), 109.1 (C-6), 102.3 (C-10), 102 (C-3), 100.3 (C-1⁗), 99.1 (C-1‴), 94.5 (C-8), 81.1 (C-5″), 79.2 (C-3″), 77.4 (C-2‴), 76.8 (C-3‴, 5‴), 73.6 (C-1″), 72.1 (C-4⁗), 70.5 (C-2″, 3⁗), 70.1 (C-4″, 4‴, 2⁗), 68.4 (C-5⁗), 60.9 (C-6″), 60.7 (C-6‴), 17.9 (C-6⁗).

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