Rhizomnium magnifolium and *R. pseudopunctatum*, the First Mosses to Yield Flavone Glucuronides

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Rhizomnium magnifolium and *R. pseudopunctatum* show an identical flavonoid pattern with six flavone glucuronides.

Three of them; namely apometzgerin 7-O-glucuronide, selgin 7,5′-di-O-glucuronide and tricetin 7,3′-di-O-glucuronide are new natural compounds. This is the first report of flavonoid glucuronides in mosses.

Introduction

Koponen and Nilsson [1] reported remarkable differences in the patterns of phenolic compounds of so-called species pairs (according to Lowry [2]) of *Plagiomnium* and *Rhizomnium* detected by means of two dimensional thin-layer chromatography (2 D-TLC). Recently we started the isolation and structure determination of flavonoids in Mniaceae species, including where possible, the local and seasonal variation of these compounds in distinct species (eg. *Plagiomnium affine* [3]). We describe here the identification of flavonoid glycosides from *Rhizomnium magnifolium* and *R. pseudopunctatum*. Koponen and Nilsson [1] have previously detected, but not identified flavonoid compounds in extracts of both species.

Table I. The flavone glucuronides from *Rhizomnium magnifolium* and *R. pseudopunctatum*.

Compound No.	Structure
1	apometzgerin 7-O-glucuronide
2	luteolin 7-O-glucuronide
3	chrysoeriol 7-O-glucuronide
4	luteolin 7,3′-di-O-glucuronide
5	selgin 7,5′-di-O-glucuronide
6	tricetin 7,3′-di-O-glucuronide

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Results and Discussion

Rhizomnium magnifolium and *R. pseudopunctatum* gave identical flavonoid patterns. Six flavonoid glycosides were isolated and at least partly identified (Table I). All glycosides are flavone glucuronides with glucuronic acid as the only detectable sugar moiety. This was identified by cochromatography with a standard sample and clearly distinguished by TLC from similar sugars, *e.g.* galacturonic acid. After strong acid hydrolysis in each case glucuronolactone was additionally observed as expected [4].

Compounds **2** and **3**

Chromatographic and UV-visible data revealed both compounds to be flavone 7-O-glycosides. After acid and enzymic hydrolysis the aglycones luteolin (ex 2) and chrysoeriol (ex 3) were detected and identified by UV- and mass-spectrometry (compound **3**; Table II and Experimental) and by cochromatography with authentic samples. The structural identity of both glycosides was further established by cochromatography with authentic samples [20] in various TLC-systems and by HPLC. Glycosides **2** and **3** are thus identified as luteolin- and chrysoeriol 7-O-β-monoglucuronides, respectively.

Compound **4**

Total acid hydrolysis of this glycoside yielded luteolin as aglycone. The UV-spectra indicated the

Table II. Chromatographic and UV-visible data of flavonoids from *Rhizomnium magnifolium* and *R. pseudopunctatum*.

Compound	**1**	**1**Aglycone	**2**	**3**	**4**	**5**	**5**Aglycone	**6**	**6**Aglyc.
Colour reactions									
UV (350 nm)									
untreated	dark	dark	dark	dark	dark	dark	dark	dark	dark
NH_3[a]	dark	dark	yellow-green	yellow-green	yellow-green	yellow-green	yellow	yellow	yellow
NA[b]	olive	yellow-green	yellow	yellow-green	green	green	yellow	yellow	orange
BR[c]	yellow-green	dark olive	dark	green	green	green	dark	dark	dark
TLC, h R_f values									
Sorbens: Cellulose									
H_2O	65	0	36	38	84	82	0	74	0
15% HOAc	22	7	9	10	14	11	2	7	2
40% HOAc	61	45	34	42	38	36	15	28	16
TBA	46	85	22	28	11	7	61	6	53
BAW[d]	57	92	35	45	24	18	69	15	57
Sorbens: Polyamide									
W E M A[e]	4	–	2	3	3	–	–	–	–
C_6H_6-MeOH-MeCOEt 4:3:3	–	65	–	–	–	–	25	–	–
Sorbens: Si-Gel									
Toluene-Ethylformiate-HCOOH = 5:4:1	–	38	2	–	–	–	46	–	7
$CHCl_3$-Me_2CO-HCOOH 9:2:1	–	48	0	–	–	–	31	–	7
UV-visible data									
MeOH	327, 270, 261sh	328, 296sh 270	347, 267, 254	343, 270, 252sh	339, 269	–	349, 297sh, 267	350, 294sh, 263	–
NaOMe	369, 321sh, 298sh, 267[f]	367, 309sh, 274[f]	406, 268[g]	402, 299sh, 264[g]	400, 342sh, 302sh, 274sh, 263[g]	–	–	400, 301sh, 269[g]	–
$AlCl_3$	379sh, 350, 300, 276	380sh, 350, 300sh, 277	422, 366sh, 321, 298sh, 272	382, 361, 297sh, 272, 262sh	382sh, 355, 299sh, 275	–	–	–	–
$AlCl_3$/HCl	379sh, 336, 299sh, 276	378sh, 338, 300sh, 275	387sh, 360, 296sh, 274sh, 263	384sh, 356, 297sh, 274, 260sh	377sh, 347, 297sh, 275	–	–	–	–
NaOAc	331, 268	350sh, 276	406sh, 360, 265sh, 261	411, 340, 266sh	396, 290sh, 265sh, 256	–	–	–	–
NaOAc/H_3BO_3	330, 269	331, 271	369, 266sh, 259	344, 269	346, 268	–	–	–	–

[a] According to ref. [5].
[b] NA = Naturstoffreagenz A [16, 17, Fa. Roth, Karlsruhe].
[c] BR = Benedikts Reagenz [18, 19].
[d] BAW = *n*-BuOH-HOAc-H_2O (4:1:5; upper phase).
[e] W E M A = H_2O-MeCOEt-MeOH-3,5-pentanedione (13:3:3:1).
[f] decreased intensity; stable.
[g] increased intensity (band I); stable.

presence of free 5- and 4′-and substituted 7-and 3′-OH-groups in the glycoside (Table II) [5]. Mild acid hydrolysis produced a monoglycoside identical with an authentic sample of luteolin 7-O-monoglucuronide. Compound **4** was identified as luteolin 7,3′-di-O-glucuronide by cochromatography with an authentic sample from Marchantia macropora [21].

Compound **1**

The aglycone of this glycoside resulting from acid and enzymic hydrolysis was different from any of the common flavones. On TLC no change in fluorescence was observed with NH_3 and in the UV-spectrum a decrease in band (Bd)I-intensity was observed after addition of NaOMe, both indicating the presence of a substituted 4′-hydroxylgroup [4, 5]. The mass spectrum of the underivatized aglycone showed a molecular ion at $m/z = 330$ and RDA-fragments consistent with two OH-groups on ring A and one OH- and two OMe-groups on ring B. Partial methylation of the glycoside with CH_2N_2 and subsequent acid hydrolysis gave an aglycone identical with tricetin 3′,4′,5′-trimethylether. Thus the structure of the original aglycone is tricetin 3′,4′-dimethylether (= apometzgerin) and compound **1** is its 7-O-glycoside. For structure confirmation ^1H- und ^{13}C-NMR spectra of the glycoside were recorded. In both spectra the signals for the two OMe-groups appear as separated singlets, in the ^1H-spectrum at $\delta = 3.75$ and 3.89 ppm respectively and in the ^{13}C-spectrum at $\delta = 56.3$ (C3′-OMe) and $\delta = 60.2$ ppm (C4′-OMe). The chemical shifts of the OCH_3-groups in both spectra are in accordance with those recorded recently for apometzgerin 6,8-di-C-arabinoside from the liverwort Apometzgeria pubescens (unpublished results) and differ from those observed for tricin-derivatives in which they appear as singlets at 3.88 ppm (^1H) and 56.7 ppm (^{13}C) respectively. The other signals in the ^1H-spectrum of glycoside **1** are in general accordance with those reported earlier for apometzgerin [6].

Thus compound **1** is defined as tricetin 3′,4′-dimethylether-7-O-β-monoglucuronide (-apometzgerin 7-O-monoglucuronide). Apometzgerin was first found as the aglycone moiety in two flavone di-C-glycosides from the liverwort Apometzgeria pubescens [7] and has since been isolated as an aglycone from Poa huecu [6].

The present finding is the first report of an apometzgerin O-glycoside as a natural compound.

Compounds **5** *and* **6**

These glycosides are minor flavonoids in both investigated Rhizomnium species and were isolated only in traces. Acid hydrolysis yielded two aglycones, the chromatographic and UV data for which were similar but still different from luteolin (Table II). Cochromatography with authentic samples proved the identity of aglycone of **5** with selgin (tricetin 3′-methylether) and aglycone of **6** with tricetin. After mild acid hydrolysis of compound **5** a monoglycoside was isolated which still moved in H_2O on TLC and gave glucuronic acid and selgin after acid hydrolysis. Glycoside **5** lacks the B-ring ortho-dihydroxy group (NA and Benedicts reagent fluorescence) which is evident in its aglycone and in the above monoglycoside and since it does possess a free 4′-hydroxyl group (NH_3 and NA-fluorescence) is assigned the structure selgin 7,5′-di-O-glucuronide.

Chromatographic colour tests with glycoside **6** (Table II) indicate the presence of both an *ortho*-dihydroxy group and a free 4′-hydroxyl. The free 4′-hydroxyl is also supported by the NaOMe induced shift of BdI in the UV-spectrum, and the stability of the compound in NaOMe suggests in addition that one of the B-ring hydroxyls is substituted. No band III is evident on addition of NaOMe indicating that the 7-hydroxyl is substituted [8]. As with compound **5**, the mild acid hydrolysis produced a monoglycoside the mobility of which in H_2O (R_f 0.26) is indicative of a monoglucuronide. On these limited data compound 6 is considered to be tricetin 7,3′-di-O-glucuronide.

Both glycosides **5** and **6** are new compounds. This is the first report of the aglycone selgin from a moss species and the second report of this aglycone in any bryophyte, after it being previously first isolated as a di-C-glycoside from the liverwort *Plagiochila asplenioides* [9]. As an aglycone it has also been isolated by Voirin and Jay [10, 11] from *Huperzia selago*. Tricetin has been found in various liverworts mostly as di-C-glycosides [12] or rarely in mono-C-glycosides [13, 14]. This is the first report of an O-glycosylated tricetin from any bryophyte.

The chromatograms of three samples of *R. magnifolium* from Switzerland (2) and Finland (1) were compared and proved to be essentially identical. Thus a rather stable and consistent flavonoid pattern of this species is indicated. Unfortunately so far only one sample of *R. pseudopunctatum* was available and so no comment on the stability of its flavonoid pattern is yet possible. Sporophytes of this sample showed a totally different flavonoid pattern as compared to the corresponding gametophytes. Only two very weak flavonoid − like spots were detected on 2D-TLCs of alcoholic extracts from immature sporophytes. Further investigations of different de-

velopmental stages of sporophytes could be as interesting as those of sporophytes of *Bryum capillare* [15].

A chromatographic analysis of a third *Rhizomnium* species, *R. punctatum*, revealed no flavonoids but a variety of other unidentified phenolics. This finding is in accord with those of Koponen and Nilsson [1] and indicates that even morphologically similar Rhizomnium species may show strikingly different gametophytic phenolic patterns.

Experimental

Plant material

The samples used for isolation of flavonoids were:

Rhizomnium magnifolium (Horik.) T. Kop. – Finland, Uusimaa, Tenhola parish, spring-fen, 6. X. 1982 Koponen 38249.

R. pseudopunctatum (Bruch & Schimp.) T. Kop. – Finland, Varsinais-Suomi, Lohja parish, edge of spring in Picea abies forest, 6. X. 1982 Koponen 38251.

Voucher specimens are deposited in the Herbarium of Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken and in the Botanical Museum, University of Helsinki (H).

Extraction and isolation

Air-dried gametophytes of *R. magnifolium* (19.53 g) and *R. pseudopunctatum* (9.21 g) were repeatedly extracted by 80% aqu.EtOH after preextraction with $CHCl_3$ for removing the chlorophyll and lipids. The plant material was finally extracted with 80% and 50% MeOH. The alcoholic extracts containing mainly the same compounds, were combined, concentrated and preseparated by CC (Sephadex LH-20, Pharmacia, Upsala, with 70% aqu.MeOH and Cellulose, microcrystalline for CC, Merck, Darmstadt, with BEW = BuOH-2 – HOAc – H_2O, 70:5:25). Further separation and purification of compounds was achieved by repeated PC with 15% and 40% HOAc. Final purification of each compound was achieved by CC, Sephadex LH-20, with 80% aqu.MeOH, followed by crystallization from MeOH/H_2O mixtures (in case of compounds **1**–**4**).

Yields: compound **1**: 3,5 mg; **2**: 6.3 mg; **3**: 3.2 mg; **4**: 2.4 mg (all crystallized); **5**: ~1 mg (not crystallized; **6**: ~1 mg (not crystallized).

HPLC: Rp 8, Li-Chrosorb, 10 μ; UV-detector 254 nm; solvents: MeOH-HOAc-H_2O between 50/5/45 to 25/5/70 (v/v).

Hydrolysis

a) Total: 1 N TFA, 4 h under reflux;
b) partial: 1 N TFA, 0.5–1 h under reflux;
c) enzymic: β-glucuronidase (Fluka), 4–6 h at room temperature in dest. H_2O.

The sugar moiety was identified by cochromatography (TLC) with an authentic sample of glucuronic acid on cellulose (Microcrystalline for TLC, Merck), using C_5H_5N–EtOAc–HOAc–H_2O = 36–36–7–21.

Spectroscopic methods

UV-spectroscopy: according to ref- [5].

Mass spectroscopy: Varian MAT 311; 70 eV; ion source temp.: 150 °C; probe temp.: 150–230 °C.

Aglycone 1: $M^+ = 330$ (100); $M^+ - 15 = 315$ (21); $M^+ - 43 = 287$ (16); $A_1 + H = 153$ (17); $A_1 - 28 = 124$ (5); $B_1 = 178$ (2); $B_1 - 15 = 163$ (6).

Aglycone 3: $M^+ = 300$ (100); $M^+ - 43 = 257$ (14); $A_1 + H = 153$ (25); $A_1 - 28 = 124$ (9); $B_1 = 148$ (13).

NMR-spectroscopy: ^1H, Bruker AM 400; 297° Kelvin, DMSO-d_6. ^{13}C, Varian FT-80 A; 303° Kelvin, DMSO-d_6.

Glycoside 1: ^1H-NMR: δ = 3,75; 3,89 (2H, s, OMe-3′4′); 6.46 (1H, d, J = 7,18; 7,21 (2H, d, J = 2 Hz, H-2′,6′); 12.9 (1H, s, OH-5). ^{13}C-NMR: δ = 60.3 (OCH_3-4′); 56 (OCH_3-3′).

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