A New Biflavone and Further Flavonoids from the Moss *Hylocomium splendens*

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Seven flavonoids were isolated from *Hylocomium splendens* (Hedw.). B. S. et G. The structures of two of them, the new biflavone 5′,3‴-dihydroxyrobustaflavone, a 5′,6″-C-linked biluteolin, and apigenin 7-O-(2,4-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside were completely elucidated.

Introduction

Whereas many reports now exist on flavonoids of liverworts only few publications are concerned with the flavonoid pattern of mosses [1–5]. The detection of an apigenin 7-O-rhamnoglucoside from *Hylocomium splendens* was reported [6]. Screening chromatograms of samples of *H. splendens* collected at about 20 different localities showed however a much richer flavonoid pattern as supposed previously [6]. In this paper the isolation of several unknown flavonoids in gametophytic tissue of *H. splendens* is described.

Results and Discussion

Fig. 1 represents a composite 2 D-TLC which includes all flavonoids detected in aqueous alcoholic extracts of gametophytic material of *H. splendens*. The compounds **Hs 2** and **Hs 6** were isolated in crystalline state and their complete structure elucidation is reported here. The structures of the other compounds could only be partly established to date. The chromatographic and spectral data of all isolated compounds are given in Table I.

Compound ***Hs 2***

The chromatographic and UV spectral data indicate an apigenin 7-O-glycoside containing at least two sugar moieties [7, 8]. The total acidic and enzymic hydrolysis yields apigenin, rhamnose and glucose. A partial hydrolysis produces apigenin 7-O-glucoside and two apigenin-O-di-glycosides. **Hs 2** is clearly separated by TLC from standard samples of apigenin 7-O-rutinoside and 7-O-neohesperidoside. The FD-MS molecular ion-peak at m/z 725 is in ac-

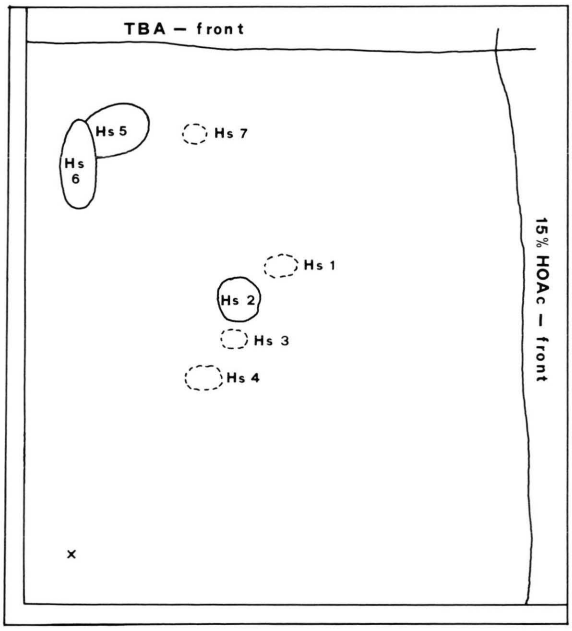

Fig. 1. TLC pattern of the flavonoids obtained from gametophytic material of *Hylocomium splendens*, after extraction with 80% EtOH, dotted lines indicate minor concentrated compounds. Sorbens: Cellulose. Solvent: (1) TBA; (2) HOAc 15%. Detection: UV (350) with and without NA.

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Table I. Chromatographic and UV data of flavonoids **Hs 1–7** from *Hylocomium splendens*.

Compound:	**Hs 1**	**Hs 2**	**Hs 3**	**Hs 4**	**Hs 5**	**Hs 6**	**Hs 7**
Colour reactions UV (350 nm) untreated	dark	dark	dark	dark	dark	dark	dark
NH_3	dark	green	dark	olive	weakly olive	weakly olive	dark
NA[1]	green	green	olive	yellow	yellow	yellow	orange[3]
BR[2]	brownish	green	olive	dark	dark	dark	dark
TLC hRf values (see also Fig. 1) Sorbens: Cellulose 40% HOAc	79	69	68	59	42	29	65
50% HOAc	91	84	83	71	58	34	74
BAW[4]	60	54	44	42	93	92	93
Sorbens: Polyamide EtOAc–MeCOEt–HCOOH–H_2O (5:3:1:1)	72	63	57	40	51	40	63
C_6H_6–MeCOEt–MeOH (4:3:3)	55	27	38	5	6	4	13
C_6H_6–EtOAc–MeOH (60:26:34)	59	26	31	–	–	–	9
Sorbens: Si-Gel EtOAc–MeCOEt–HCOOH–H_2O (5:3:1:1)	34	32	29	28	–	–	–
Toluene–Ethylformiate–HCOOH (5:4:1)	–	–	–	–	20	17	33
UV Data MeOH	269, 323	268, 332	253, 267, 342	256, 266, 342	256, 265 sh, 351	249, 254, 295 sh, 354	225, 287, 332 sh
NaOMe	245 sh, 291, 371[5]	243 sh, 270, 300 sh, 349 sh, 386[6]	268, 296 sh, 377[5]	270, 389[6]	272, 330, 401[6]	233 sh, 269, 401[6]	273 sh, 325, 390
$AlCl_3$	276, 299, 343, 376	230 sh, 275, 299, 348, 379 sh	265, 296 sh, 318 sh, 361, 380 sh	273, 299 sh, 349, 420	273, 363 sh, 415	273, 301 sh, 426	268, 308, 376
$AlCl_3$/HCl	276, 298, 337, 376	275, 298, 341, 377 sh	262, 274 sh, 294 sh, 359, 381 sh	271, 296 sh, 350, 380 sh	256, 270 sh, 357, 385 sh	261, 275 sh, 295 sh, 362, 384 sh	263, 305, 363
NaOAc	268, 325	257 sh, 267, 352 sh, 386	255, 266, 341	269, 331 sh, 403	369, 324 sh, 394	269, 324 sh, 382	273, 293, 322, 388 sh
NaOAc/H_3BO_3	268, 326	267, 338	254, 267, 340	266, 343, 401 sh	263, 376	260, 376	287

[1] NA = Naturstoffreagenz A [14, 15, Fa. Roth, Karlsruhe].

[2] BR = Benedikts Reagenz [16, 17].

[3] Changing to red fluorescence and red colour after 1 h.

[4] BAW = *n*-BuOH–HOAc–H_2O (4:1:5; upper phase).

[5] Decreased intensity; stable.

[6] Increased intensity; stable.

cordance with an apigenin derivative containing one glucose and two rhamnose moieties. The fragment at *m/z* 579 shows the loss of a rhamnose unit [9]. Using ^{13}C NMR spectroscopy the complete structure was elucidated. The aglycone moiety is identical with an apigenin 7-O-glycoside [10]. The sugar signals (see Experimental) show the same values as those published by Österdahl [11]. Thus the structure of *Hs 2* can be assigned as 7-O-(2,4-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside. This is the second report of this glycoside which was found for first time in the moss *Dicranum scoparium* [12].

*Compound **Hs 6***

The spot appearance and UV spectra of **Hs 6** are very similar to those of luteolin. The FD mass spectra of the underivatized compound and its octamethylether showed molecular masses of 570 and 682 respectively. This reveals a C-C linked biluteolin. The site of the interflavonyl linkage was deduced from the ^{13}C NMR spectrum of the underivatized compound, whereby the quaternary carbons were identified by the DEPT-technique. Comparison of the ^{13}C NMR of 5′,8″-biluteolin [13] and **Hs 6** showed differences only for the C-6- and C-8-signals. Due to the interflavonyl linkage the signal for C-8″ of 5′,8″-biluteolin is shifted about 10 ppm downfield relative to the C-8-signal of luteolin. In the case of **Hs 6** a similar downfield shift is observed only for C-6″ (Fig. 2). The degree of this shift is comparable to those typical for flavone 6-C-glycosides [10]. However, in a published spectrum of robustaflavone – the apigenin analogue of **Hs 6** – a signal around 109 ppm was not observed [10], probably due to the long relaxation times of quaternary carbons. Nevertheless, in a recently recorded ^{13}C NMR spectrum of robustaflavone the signal for a quaternary carbon at 109.3 ppm is observed [Markham and Geiger, unpublished]. Thus compound **Hs 6** is assigned the structure 5′,3‴-dihydroxyrobustaflavone.

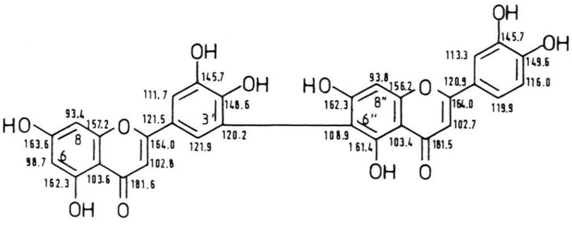

Fig. 2. 5′,3‴-Dihydroxyrobustaflavone (**Hs 6**) and its ^{13}C NMR values.

*Compounds **Hs 1, 3–5** and **7***

Total hydrolysis of **Hs 1, 3** and **4** yielded glucose and rhamnose as sugars and as aglycones acacetin (ex **Hs 1**), diosmetin (ex **Hs 3**) and luteolin (ex **Hs 4**), respectively. The aglycones and sugars were characterized by co-chromatography with authentic samples. UV spectra of the aglycones essentially agree with published spectra [7]. UV spectroscopy of the glycosides reveals only 7-O-glycosylation in each case. The chromatographic behaviour of **Hs 1** and **Hs 3** suggests a similar glycosylation pattern as for compound **Hs 2**. This was confirmed by GC of the hydrolyzed sugars showing a glucose/rhamnose relation of 1:2. **Hs 4** seems to be luteolin 7-O-rhamnoglucoside; the relation of glucose/rhamnose by GC being 1:1.

The spot appearance of **Hs 5** and its UV spectra are also very similar to luteolin; the FD mass spectrum shows a molecular ion at *m/z* 570, as for **Hs 6**. Therefore it must also be a biluteolin type biflavone. In various TLC systems **Hs 5** co-chromatographs with 5′,8″-biluteolin [18], but a final proof of the structure of **Hs 5** is at present not possible. **Hs 7** was obtained in minute amounts only. The molecular ion in the FD-MS spectrum is observed at *m/z* 574, four mass units higher than required for a biluteolin. Some time after spraying with NA this compound shows a bright red colour and fluorescence on TLC. A similar colour reaction has been observed earlier with 2,5,7,3′,4′-pentahydroxyflavanone and its 5-glucoside [19] as well as for 5,7,3′,4′-tetrahydroxyflavanone (= eriodictyol) and its 7-methylether [20]. Taking this colour reaction and the molecular mass into account, one might consider **Hs 7** as a dimer of eriodictyol.

The 2 D-TLCs from fourteen samples of *Hylocomium splendens* investigated, which were collected at different localities, show a fairly identical flavonoid pattern regarding the main compounds (Fig. 1). Beside the flavonoids there are also several other unknown phenolic components.

This is now the second time that a biflavone was detected in a moss [18]. According to chromatographic screening, however, biflavones seem to be widespread in mosses. This phenomenon will be further investigated and is possibly of some chemotaxonomic relevance.

Experimental

The plant material was collected at the Giesbächen Falls near the lake of Brienz in Switzerland. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes.

Extraction and isolation

After pre-extraction with CHCl$_3$ for removing the chlorophyll and lipids, 520 g air-dried gametophytic material was repeatedly extracted by 80% ETOH. The concentrated extract was separated by CC on polyamide 6 by an aqueous acetone gradient rising from 10% up to 80%. Further separation and purification: **Hs 1** and **Hs 2** by CC on Sephadex LH 20 (Me$_2$CO/MeOH/H$_2$O, 2:1:1) followed by MeOH/H$_2$O (3:7). **Hs 2** was crystallized from aqueous MeOH (24 mg); **Hs 1** was isolated in amounts less than 1 mg; **Hs 3** and **4** on Sephadex LH 20 (MeOH/H$_2$O, 3:7) and Polyclar AT (Serva; MeOH/H$_2$O, 1:1), yields: less than 1 mg. **Hs 5–7** on Sephadex LH-20 (Me$_2$CO/MeOH/H$_2$O, 2:1:1 and MeOH/H$_2$O, 8:2 non crystalline, yields: 11 and less than 1 mg respectively). **Hs 6** was crystallized from aqueous acetone (21 mg).

Hydrolysis: a) total: 1 N TFA 1 h under reflux;
b) partial: 40% HOAc 15 min under reflux.

Methylation of **Hs 6** according to [21]

UV spectroscopy according to [7].

Mass spectroscopy: Varian MAT 311a with FD-source

Hs 2: $[M+H]^+$ 725, $[M^+ + H\text{-rhamnose}]$ 579.

Hs 5: M^+ 570.

Hs 6: M^+ 570.

Hs 6 – Octamethylether: M^+ 682.

Hs 7: M^+ 574.

^{13}C NMR spectroscopy: Brucker (AM 400), 400 MHz, 297° Kelvin, DMSO-d$_6$.

Hs 2: 181.4 (C-4); 164.6 (C-2); 162.8 (C-7); 161.7 (C-4′); 161.5 (C-5); 157.3 (C-9); 128.9 (C-2′, C-6′); 116.4 (C-3′, C-5′); 105.8 (C-10); 103.5 (C-3); 101.0, 100.8 (2 × rhm C-1); 99.6 (C-6); 97.9 (glu C-1); 77.4 (glu C-2); 76.3, 76.0 (glu C-3, C-4, C-5); 72.3 (2 × rhm C-4); 71.1, 71.0, 70.8, 70.7 (2 × rhm C-2 and C-3); 69.0, 68.7 (2 × rhm C-5); 60.0 (glu C-6); 18.4, 18.1 (2 × rhm C-6).

Hs 6: see Fig. 2!

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