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THE BIFLAVONOIDS OF *PLAGIOMNIUM UNDULATUM*[*]

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Key Word Index—*Plagiomnium undulatum*; Mniaceae; Musci; 3‴-desoxydicranolomin; 2,3-dihydro-3‴-desoxydicranolomin; 2,3-dihydro-5′, 3‴-dihydroxyrobustaflavone; biflavonoids.

Abstract—The biflavonoid pattern of *Plagiomnium undulatum* has been studied. Besides two known biflavonoids the new 3‴-desoxydicranolomin, 2,3-dihydro-3‴-desoxydicranolomin and 2,3-dihydro-5′,3‴-dihydroxyrobustaflavone have been isolated, and their structures elucidated spectroscopically.

INTRODUCTION

So far only flavone C-, and flavone C-O-glycosides have been isolated from *Plagiomnium undulatum* [1]. However, it has been demonstrated by 2D-TLC that this species also contains several biflavonoids [2]. Because the R_f-values of some of these compounds (see Table 1) differed markedly from those of the known moss-biflavonoids, it was decided to reinvestigate this moss.

RESULTS AND DISCUSSION

Five biflavonoids were isolated from an 80% methanolic extract of *P. undulatum*, as described in the Experimental section. Three of these (**1**, **2** and **3**) were new natural compounds. Their structures were deduced from their ^{1}H NMR spectra by comparison with those of known biflavonoids [3, 4].

The ^{1}H NMR spectrum of **1** is, within the limits of experimental error, composed of the signals H-3 to 6′ of the "left hand" luteolin moiety of dicranolomin (**6**) and of the apigenin part of 5′-hydroxyrobustaflavone (**4**) (H-3″ to 6‴) (see Table 2). A comparison of the ^{13}C NMR spectra of the three compounds shows that the resonances of C-2 to 6′ in the spectrum of **1** are nearly identical with those in **6** and those of C-2″ to 6‴ with those in **4**. Thus, **1** is a 2′ → 6″-linked luteolin-apigenin biflavone, i.e. 3‴-desoxydicranolomin. The M_r 554, as determined by mass spectrometry is also in accordance with this structure.

Compound **2** appeared dark absorbing under UV light, and after spraying with NA it fluoresced first dark olive and after some hours fluoresced bright red (Table 1). This colour reaction is typical of eriodictyol and biflavonoids having an eriodictyol moiety [5] and a M_r of 556 was also in agreement with **2** being an eriodictyol-apigenin biflavonoid. This was verified by a comparison of the ^{1}H NMR spectrum of **2** with the spectra of **1** and **7** (Table 2), which revealed, that **2** is 2,3-dihydro-3‴-desoxydicranolomin. Although the sample of **2** was too small to run a ^{13}C NMR spectrum, the interflavonyl linkage could be defined unequivocally as 2′ → 6″: 2′ follows from the coupling pattern of the eriodictyol B-ring protons, and 6″ from the fact, that the B-ring protons of the apigenin part are not shielded by an aryl substituent at C-8″ (cf. [4]).

The spot appearance of **3** (Table 1) and its M_r (572) pointed to eriodictyol and luteolin as building blocks. The structure of **3** was elucidated from its ^{1}H NMR spectrum (see Table 2) as 2,3-dihydro-5′,3‴-dihydroxyrobustaflavone, because the signals of H-2 to 6′ are almost superimposable with the corresponding signals of **4**, and the signals of H-3″ to 6‴ are, with the exception of the H-8″ signal, nearly identical with those of 5′, 3‴-dihydroxyrobustaflavone (**8**). The deviation of the H-8″ resonance of **3** is 0.24 ppm up-field from its position in the spectrum of **8** as reported *l.c.* [4]. Similar up-field shifts of this signal are, however, sometimes observed in very dilute solutions of **8**. In lit. [6], for example, this signal is reported to occur even 0.35 ppm upfield from the position given *l.c.* [4]. Therefore, it can be assumed that this deviation in the spectrum of **3** is caused by the rather low concentration of the sample.

2,3-Dihydro-5′-hydroxyrobustaflavone (**4**) and 2,3-dihydro-5′-hydroxyamentoflavone (**5**) are known compounds, that were identified by their ^{1}H NMR spectra. The ^{1}H NMR data of **4** are included in Table 2 for comparison, and the ^{13}C NMR data of **4** are presented in Table 3 for the first time.

In conclusion it can be stated, that the biflavonoid pattern of *P. undulatum* differs markedly from that of

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Table 1. hR_f-values, spot appearance and UV spectroscopic data for biflavonoids **1–5**

	TLC											
	hR_f			Appearance under UV*		UV absorption maxima [nm]						
	I	II	III	untreated	NA	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
3‴-Desoxydicranolomin (**1**)	04	55	47	d	y	263, 337	272, 330, 394	274, 300sh, 357	273, 300sh, 348	271, 375	264, 347
2,3-Dihydro-3‴-desoxydicranolomin (**2**)	10	69	56	d	ol → r	271, 330	323, 393	265, 300sh, 353	262, 300sh, 350	271, 323	272, 334
2,3-Dihydro-5′,3‴-dihydroxyrobustaflavone (**3**)	00	49	38	d	y → ro	281, 346	243sh, 284sh, 323, 403	292, 413	294, 365	283, 323, 383	283, 372
2,3-Dihydro-5′-hydroxyrobustaflavone (**4**)	02	64	50	d	ol → r	287, 335	285, 324, 394	307, 367	304, 350	289, 315	290, 335
2,3-Dihydro-5′-hydroxyamentoflavone (**5**)	02	66	46	d	ol → r	288, 335sh	287, 323, 396	287, 304, 349	283, 306, 346	288, 315	281, 335

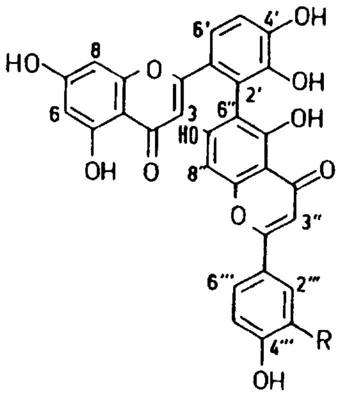
I = Cellulose with 15% HOAc; II = Micropolyamide (S & SNrF-1700) with EtOAc–EtCOMe–HCO_2H–H_2O (5:3:1:1); III = Micropolyamide (S & SNrF-1700) with Me_2CO–HOAc–H_2O (3:1:1); NA = 0.1% diphenylboric acid β-aminoethylester in MeOH; d = dark absorbing, y = yellow; y → ro = yellow becoming reddish orange; ol → r = dark olive becoming red.

* In systems II and III.

Table 2. ^{1}H NMR data of the biflavonoids **1**–**4** from *P. undulatum* and the related biflavonoids **6**–**8**

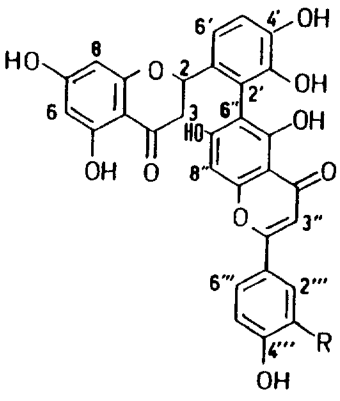
Assignment	Dicranolomin (**6**) [3]	3‴-Desoxy-dicranolomin (**1**)	2,3-Dihydro-3‴-desoxy-dicranolomin (**2**)	2,3-Dihydro-dicranolomin (**7**) [9]	5′,3‴-Dihydroxy-robustaflavone (**8**) [3]	2,3-Dihydro-5′,3‴-dihydroxy-robustaflavone (**3**)	2,3-Dihydro-5′-hydroxy-robustaflavone (**4**)
H-2	—	—	4.95 *dd*	4.95 *dd*	—	5.37 *dd*	5.39 *dd*
ax			3.10 *dd*	3.08 *dd*		3.22 *dd*	3.23 *dd*
H-3	6.04 *s*	5.99 *s*			6.68 *s*		
eq			obscured by DMSO	2.51 *dd*		2.64 *dd*	2.66 *dd*
H-6	6.08 d^m	6.07 d^m	5.79 *s*	5.80 *s*	6.19 d^m	5.86 d^m	5.87 d^m
H-8	5.97 d^m	5.98 d^m	5.79 *s*	5.80 *s*	6.43 d^m	5.88 d^m	5.89 d^m
H-2′	—	—	—	—	7.27 d^m	6.82 *br s*	6.76 d^m
H-5′	6.94 d^o	6.87 d^o	6.85 d^o	6.88 d^o	OH	OH	OH
H-6′	7.17 d^o	7.12 d^o	6.95 d^o	6.95 d^o	7.40 d^m	6.82 *br s*	6.86 d^m
H-3″	6.65 *s*	6.66 *s*	6.58 *s*	6.67 *s*	6.60 *s*	6.58 *s*	6.74 *s*
H-6″	—	—	—	—	—	—	—
H-8″	6.53 *s*	6.46 *s*	6.54 *s*	6.54 *s*	6.61 *s*	6.37 *br s*	6.50 *s*
H-2‴	7.40 d^m	7.91 d^o	7.91 d^o	7.39 d^m	7.43 d^m	7.39 d^m	7.92 d^o
H-3‴	OH	6.92 d^o	6.92 d^o	OH	OH	OH	6.93 d^o
H-5‴	6.90 d^o	6.92 d^o	6.92 d^o	6.86 d^o	6.92 d^o	6.88 d^o	6.93 d^o
H-6‴	7.41 dd^{om}	7.91 d^o	7.91 d^o	7.41 dd^{om}	7.44 dd^{om}	7.40 dd^{om}	7.92 d^o

DMSO-d_6, ambient temperature, 400 MHz, except **6** and **8** which were recorded at 500 MHz.



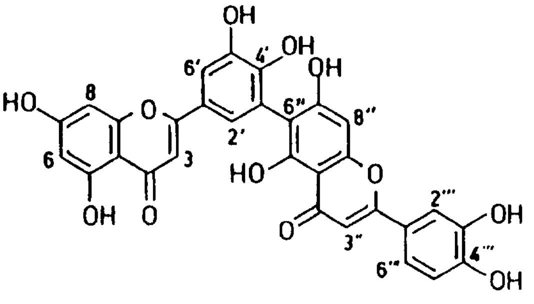
R = H : **1**

R = OH : **6**

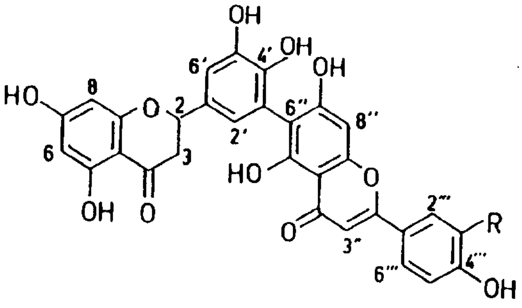


R = H : **2**

R = OH : **7**



8



R = OH : **3**

R = H : **4**

Table 3. ^{13}C NMR data of **1**, **4** and **6**

Assignment	Dicranolomin (**6**)* [10]	3'''-Desoxy-dicranolomin (**1**)	2.3-Dihydro-5'-hydroxy-robustaflavone (**4**)*
C-2	166.3	167.0	78.8 t
C-3	106.2 t	105.6	42.1 s
C-4	181.6	181.2	196.3
C-5	161.2	160.8	163.1
C-6	98.6 t	98.5	95.7 t
C-7	163.6	163.5	166.6
C-8	93.1 t	93.3	94.9 t
C-9	157.3	157.4	163.0
C-10	102.8	102.0	101.8
C-1'	123.7	123.8	127.5
C-2'	119.7	119.6	121.4 t
C-3'	144.2	145.5	120.7
C-4'	148.2	149.0	145.6
C-5'	114.3 t	113.2	145.0
C-6'	121.5 t	121.4	112.2 t
C-2''	163.9	163.8	163.5
C-3''	103.3 t	102.4	102.6 t
C-4''	181.2	181.1	181.6
C-5''	158.7	158.6	159.0
C-6''	107.9	109.0	110.3
C-7''	161.8	162.8	163.0
C-8''	93.3 t	94.5	94.0 t
C-9''	156.2	156.5	156.2
C-10''	103.3	103.3	102.6
C-1'''	120.2	120.7	121.4
C-2'''	113.3 t	128.2	128.3 t
C-3'''	145.7	115.8	115.9 t
C-4'''	149.6	161.2	161.0
C-5'''	116.0 t	115.8	115.9 t
C-6'''	118.9 t	128.2	128.3 t

*s = Secondary C and t = tertiary C, as determined by proton detected C-H correlation.

DMSO-d_6, ambient temperature, 100 MHz.

P. elatum and *P. cuspidatum*, which have been studied previously [7]. The taxonomic relevance of this finding will be discussed elsewhere.

EXPERIMENTAL

Plant material. Gametophytes of *Plagiomnium undulatum* (Hedw.) T. Koponen were collected in spring 1994 at two sites in France and Germany. Voucher specimens are deposited at SAAR (Nos 4885 and 4886). After it had been shown that the two collections were chromatographically identical they were pooled.

Extraction and isolation. 200 g (dry wt) of *P. undulatum* were extracted by a seven-fold maceration overnight with 80% MeOH (8 l each). The combined extracts were freed from lipids and chlorophyll by solidphase extraction with 500 ml (dry vol.) Lichroprep RP-18 (40–63 μm) on a Buchner funnel. The filtrate from this process was reduced *in vacuo* to about 200 ml. This soln was adjusted to 30% MeOH, and then applied to a 7 × 45 cm MPLC column of Lichroprep RP-18 (40–63 μm). Elution was performed as described *l.c.* [8] with a MeOH–H_2O gradient ranging from 30% to 80% methanol (*ca* 2 l per each 10% step). This gave, after a forerun of various glycosides and other hydrophilic substances, three biflavonoid fractions containing **1**, **2** + **3** and **4** + **5**, respectively. Further separation of the two mixed fractions was achieved by CC on Sephadex LH-20 with a gradient ranging from 50% to 80% MeOH. For the final clean-up CC on Sephadex LH-20 with Me_2CO–MeOH–H_2O (2:1:1) was used. Yields: 7 mg **1**, 3 mg **2**, 5 mg **3**, 35 mg **4** and 5 mg **5**.

The M_rs of all compounds were determined by laser induced microprobe mass analysis (LAMMA) using nicotinic acid amide as matrix.

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