New Dihydrobiflavones from the Moss *Plagiomnium cuspidatum*

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In *Plagiomnium cuspidatum* the three new dihydrobiflavones 2,3-dihydro-5′-hydroxyamentoflavone, 2,3-dihydro-5′,3‴-dihydroxyamentoflavone and 2,3-dihydro-5′-hydroxyrobustaflavone were detected.

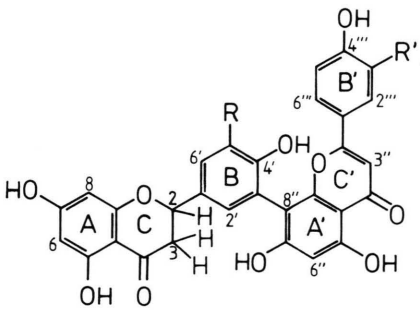
Introduction

For a long time 5′,8″-biluteolin (= 5′,3‴-dihydroxyamentoflavone) from *Dicranum scoparium* [1, 2] was the only known biflavonoid in bryophytes. Meanwhile this compound and other biflavonoids have been isolated from other mosses [3–6].

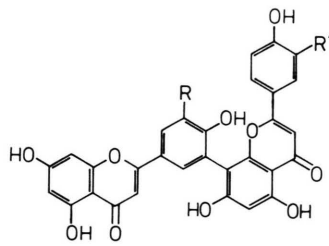
Most recently we described the isolation of a luteolin-apigenin dimer, 5′-hydroxyamentoflavone, from *Plagiomnium elatum* [3]. In continuation of the chemical investigation of the moss family Mniaceae the biflavonoids of *Plagiomnium cuspidatum* were studied.

Results and Discussion

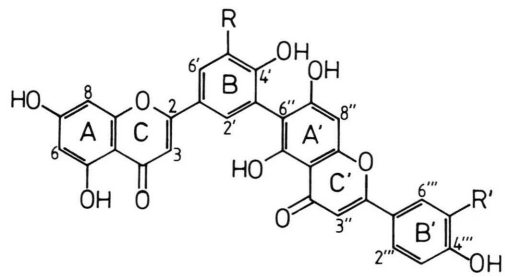
From *Plagiomnium cuspidatum* three biflavonoids **2**, **3**, **7** were isolated. By ^{1}H NMR studies, however, it was obvious that **2** contains additionally a small amount of the further compound **8**.



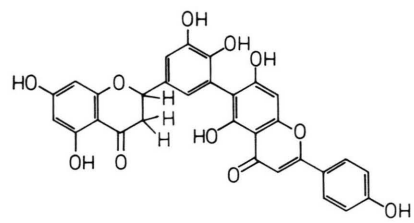
,3-Dihydroamentoflavone (**1**) R = R′ = H
,3-Dihydro-5′-hydroxyamentoflavone (**2**) R = OH, R′ = H
,3-Dihydro-5′,3‴-dihydroxyamentoflavone (**3**) R = R′ = OH



Amentoflavone (**4**) R = R′ = H
5′,3‴-Dihydroxyamentoflavone (**5**) R = R′ = OH
(= 5′,8″-Biluteolin)



Robustaflavone (**6**) R = R′ = H
5′,3‴-Dihydroxyrobustaflavone (**7**) R = R′ = OH
(= 5′,6″-Biluteolin)



2,3-Dihydro-5′-hydroxyrobustaflavone (**8**)

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Compound **7** was identified by its ^{1}H and ^{13}C NMR spectra and cochromatography with an authentic sample as the known 5′,3‴-dihydroxyrobustaflavone [5].

On the TLC plate compound **2** turned after spraying with NA from its original yellowish fluorescence to bright red (Table I). A similar colour reaction is also given by eriodictyol [7]. Together with a molecular mass of 556 this suggested an eriodictyol-apigenin dimer.

Its structure as 2,3-dihydro-5′-hydroxyamentoflavone (**2**) was deduced from its ^{1}H and ^{13}C NMR spectra by comparison with the spectra of some known biflavonoids (Tables II and III). The ^{1}H NMR spectrum of **2** shows the typical ABX-system of the protons at C-2 and C-3 of a flavanone at 2.78, 3.18 and 5.46 ppm as well as two *meta* coupled doubletts at 5.91 and 5.92 ppm which must be attributed to the protons at C-6 and C-8 of the flavanone moiety, which must be linked *via* its B-ring to the second flavonoid. The signals of the protons at the carbon atoms C-3″, 6″, 2‴, 3‴, 5‴ and 6‴ are found at almost the same position and with identical multiplicity in the spectra of 2,3-dihydroamento- (**1**) and amentoflavone (**4**). Therefore, the second flavonoid moiety is apigenin, linked *via* C-8. The remaining two *meta* coupled doubletts at 6.87 and 7.04 ppm must be attributed to the C-2′ and C-6′ protons of the eriodictyol moiety which must therefore be linked as shown by the structural formula of **2**. These arguments are corroborated by the ^{13}C NMR spectrum (Table III) in which again the only significant differences compared with **1** are in the range of the B-ring signals of the eriodictyol moiety, which shows the chemical shift and protonation pattern which one expects. Thus the structure of **2** is proved.

The elucidation of the structure of **3** was carried out analogously. In the proton spectrum all signals except those at C-2‴, C-5‴ and C-6‴ have the same chemical shift and multiplicity as in the spectrum of **2**. The signals for the protons at C-2‴, C-5‴ and C-6‴ are identical with those of **5**. Similarly the ^{13}C signals, with the exception of the signals of C-1‴, C-2‴, C-3‴, C-4‴ and C-5‴, are almost superimposable with those of **2**. The signals of C-1‴ to C-5‴ have the same chemical shift as in **5**. Thus the structure of 2,3-dihydro-5′,3‴-dihydroxyamentoflavone (**3**) is proved.

Table I. Chromatographic and UV data of compounds **2** and **3** from *Plagiomnium cuspidatum*.

Compound	**2**	**3**
Colour reactions		
UV (350 nm)	dark	dark
NH_3	dark	dark
"Naturstoffreagenz A" (NA)	yellowish, turning bright red	yellow, turning brick red
"Benedikt's Reagenz" (BR)	dark	dark
TLC hRf values		
Sorbent: Cellulose (Avicel, Schleicher & Schüll, F 1440)		
15% HOAc	4	4
40% HOAc	55	43
BAW	95	94
Sorbent: Polyamide-6 (Polygram, Macherey & Nagel)		
EtOAc–MeCOEt–HCOOH–H_2O (5:3:1:1)	67	53
Sorbent: Silica (KG $60F_{254}$, Merck)		
$CHCl_3$–Me_2CO–HCOOH (9:2:1)	25	14
Toluene–Ethylformiate–HCOOH (5:4:1)	29	21
UV-data		
MeOH	277 sh/288/326	278 sh/288/339
NaOMe	289/323/397	290/321/411
$AlCl_3$	263/303 sh/319/359	275 sh/292/306/375 sh/426
$AlCl_3$/HCl	286 sh/307/350/371 sh	258 sh/283/306/359/385 sh
NaOAc	289/312 sh/365 sh	290/321/370 sh
NaOAc/H_3BO_3	288/327	265 sh/276/375

Table II. PMR-spectra of **2**, **3**, **7**, **8** and related substances (DMSO-d_6, ambient temperature unless otherwise stated).

	8 (373 K)	**6** [9]	**7**	**5** [3]	**3** (373 K)	**2** (373 K)	**1** (373 K)*	**4** [3]
H-2	~ 5.4+	–	–	–	5.44 dd (3; 12 Hz)	5.46 dd (3; 12 Hz)	5.49 dd (3; 13 Hz)	–
H-3	3.1–3.2+	[6.80 s]	6.65 s	6.68 s	3.16 dd (12; 17 Hz)	3.18 dd (12; 17 Hz)	3.19 dd (13; 17 Hz)	6.74 s
H-3	obscured	–	–	–	2.79 dd (3; 17 Hz)	2.78 dd (3; 17 Hz)	2.74 dd (3; 17 Hz)	–
H-6	~ 5.9	6.23 d (2 Hz)	6.20 d (2 Hz)	6.21 d (2 Hz)	5.89 d (2 Hz)	5.91 d (2 Hz)	5.89 **	6.22 d (2 Hz)
H-8	~ 5.9	6.52 d (2 Hz)	6.45 d (2 Hz)	6.45 d (2 Hz)	5.92 d (2 Hz)	5.92 d (2 Hz)	5.89 **	6.50 d (2 Hz)
H2′	6.73 d (2 Hz)	7.87 d (2 Hz)	7.35 d (2 Hz)	7.51 d (2 Hz)	6.84 d (2 Hz)	6.87 d (2 Hz)	7.37 d (2 Hz)	8.07 d (2 Hz)
H-5′	–	7.09 d (9 Hz)	–	–	–	–	7.02 d (8 Hz)	7.19 d (9 Hz)
H-6′	6.95 d (2 Hz)	7.94 d (2; 9 Hz)	7.40 d (2 Hz)	7.52 d (2 Hz)	7.12 d (2 Hz)	7.04 d (2 Hz)	7.39 dd (2; 8 Hz)	7.99 dd (2; 9 Hz)
H-3″	6.68 s	[6.80 s]	6.72 s	6.72 s	6.53 s	6.65 s	6.65 s	6.81 s
H-6″	–	–	–	6.41 s	6.38 s	6.40 s	6.37 s	6.45 s
H-8″	6.63 s	6.68 s	6.59 s	–	–	–	–	–
H-2‴	7.89 d (9 Hz)	7.97 d (9 Hz)	7.45 d (2 Hz)	7.09 d (2 Hz)	7.02 d (2 Hz)	7.56 d (9 Hz)	7.54 d (9 Hz)	7.57 d (9 Hz)
H-3‴	6.97 d (9 Hz)	7.03 d (9 Hz)	–	–	–	6.81 d (9 Hz)	6.78 d (9 Hz)	6.77 d (9 Hz)
H-5‴	6.97 d (9 Hz)	7.03 d (9 Hz)	6.92 d (8 Hz)	6.70 d (8 Hz)	6.77 d (8 Hz)	6.81 d (9 Hz)	6.78 d (9 Hz)	6.77 d (9 Hz)
H-6‴	7.89 d (9 Hz)	7.97 d (9 Hz)	7.46 dd (2; 8 Hz)	7.07 dd (2; 8 Hz)	7.03 dd (2; 8 Hz)	7.56 d (9 Hz)	7.54 d (9 Hz)	7.57 d (9 Hz)

* Isolated from *Cycas revoluta* [8].

** Due to identical chemical shift, the signals of these two protons appear as a singulett.

\+ Partly obscured.

Table III. ^{13}C NMR spectra of compounds **2**, **3** and related substances (**1**, **4**, **5**). (DMSO-d_6, ambient temperature unless otherwise stated.)

Assignment	**5**	**3** (373 K)	**2** (373 K)	**1** [10]	**4** [10]
C-2	163.9	78.2	78.2	78.8	164.1
C-3	102.9	42.1	42.1	42.5	103.2
C-4	181.9	195.4	195.3	196.5	181.9
C-5	161.4	163.1	163.1	163.6	161.6
C-6	98.7	95.5	95.5	96.0	98.8
C-7	164.0	166.2	166.2	166.8	163.9
C-8	93.8	94.7	94.7	95.2	94.2
C-9	157.3	162.6	162.6	163.2	157.6
C-10	103.6	101.6	101.6	101.9	104.0
C-1′	120.6	128.5	128.6	128.7	120.3
C-2′	122.2	120.8	120.9	127.8	127.9
C-3′	120.0	119.2	119.2	119.2	121.7
C-4′	148.2	145.1	145.1	156.2	159.6
C-5′	145.5	143.9	143.9	115.9	116.4
C-6′	112.1	112.8	112.8	131.6	131.6
C-2″	164.0	163.6	163.4	163.7	164.3
C-3″	102.5	102.1	102.1	102.6	102.8
C-4″	181.5	181.5	181.6	182.2	182.2
C-5″	160.4	160.0	160.5	160.4	160.8
C-6″	98.6	98.4	98.4	98.8	99.1
C-7″	161.7	161.5	161.4	162.2	161.9
C-8″	104.0	104.6	104.5	104.9	104.1
C-9″	154.5	154.2	154.2	154.5	154.7
C-10″	103.6	103.5	103.5	103.7	104.0
C-1‴	121.8	121.8	121.3	121.5	121.4
C-2‴	113.7	113.5	127.7	128.4	128.3
C-3‴	145.8	145.2	115.4	115.9	116.0
C-4‴	149.4	149.1	160.0	161.2	161.1
C-5‴	115.5	115.4	115.4	115.9	116.0
C-6‴	118.6	118.4	127.7	128.4	128.3

Assignments of carbons having almost the same chemical shift may be reserved.

As mentioned above the ^1H NMR-spectrum of **2** revealed the presence of traces of another dihydrobiflavone (see Experimental) in our sample. Its proton signals, as far as they are not obscured by the signals of the main compound, show the chemical shift and multiplicity, which are expected for a 2,3-dihydro-5′-hydroxyrobustaflavone (**8**) (Table II). The signals for the protons at C-3″, C-8″, C-2‴, C-3‴, C-5‴ and C-6‴ occur at almost the same position as the corresponding signals of robustaflavone (**6**). The remaining clearly separated signals – two *meta* coupled doubletts – can be assigned to the protons at C-2′ and C-6′ of **8**. Although the remaining signals are partly concealed there is no evidence against the proposed structure of **8**.

Experimental

Plant Material

Gametophytic material of Plagiomnium cuspidatum (Hedw.) T. Kop., collected from two different localities, was thoroughly cleaned and air-dried:

1. Laukenmühle, Hessen, W. Germany: 69 g.
2. Waldfriedhof Kaiserslautern, Rheinland-Pfalz, W. Germany: 431 g.

Voucher specimens are deposited in the Herbarium of Fachrichtung Botanik, Universität des Saarlandes (Indexed in Index Herbariorum, "SAAR").

Extraction and isolation

Since the flavonoid patterns of samples from both localities were identical, as determined by 2D-TLC, they were combined. The total air-dried material was ground and defatted with $CHCl_3$. The flavonoids were then extracted by repeated treatment with EtOH/H_2O (8:2). The combined extracts were evaporated. The residue was partitioned between EtOAc/H_2O. The EtOAc phases were reduced *in vacuo* and dissolved in EtOAc–MeCOEt–HOAc–H_2O (5:3:1:1) and chromatographed on a polyamide column with the above solvent as eluent. The different portions of the eluate were pooled into three fractions (A, B, C). Fraction A contained the dihydrobiflavones, **2**, **3** and **8**, fraction B compound **7**, and fraction C a small amount of **7** and some minor biflavonoids. By repeated CC with Sephadex LH-20 and Me_2CO/MeOH/H_2O (2:1:1) and polyamide with the above mentioned eluent from fractions A and B were obtained: 85 mg **2** which contained traces of **8**, 35 mg **3** and 20 mg **7**. Prior to spectroscopy the compounds were freed from high molecular material by CC on Sephadex.

TLC see Table I.

UV spectroscopy according to [11].

^1H NMR spectroscopy: Bruker (AM 400), 400 MHz, DMSO-d_6.

^{13}C NMR spectroscopy: Bruker (AM 400), 100 MHz, DMSO-d_6.

Mass spectra were recorded by FAB-technique (negative mode) on a Finnigan MAT 90 in a glycerol matrix with 4–6 keV Xenon atoms.

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