The Biflavonoid Pattern of *Rhytidiadelphus squarrosus* (Hedw.) Warnst.

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Dedicated to Dr. Ella Campbell, Massey University, Palmerston North, New Zealand, on the occasion of her 80th birthday

Mosses, Hylocomiaceae, *Rhytidiadelphus squarrosus,* Biflavonoids, 5′-Hydroxyrobustaflavone

From *Rhytidiadelphus squarrosus* the hitherto unknown biflavone 5′-hydroxyrobustaflavone and the three known biflavonoids 5′-hydroxyamentoflavone,5′,3‴-dihydroxyamentoflavone and 2,3-dihydro-5′-hydroxyamentoflavone have been isolated.

Introduction

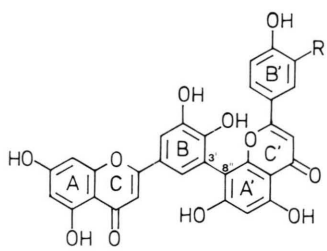
Various biflavonoids have been isolated so far from eleven species of seven different moss families, namely Dicranaceae [1–3], Grimmiaceae [4], Bryaceae [5], Mniaceae [4, 6], Bartramiaceae [7], Leucodontaceae [4] and Hylocomiaceae [4, 8].

The most common moss biflavonoids are dimers of luteolin and their 2,3- or 2″,3″-dihydro-derivatives. Biflavonoids containing monomers other than luteolin or eriodictyol (= 2,3-dihydroluteolin) have been found until now only in species of Bryaceae and Mniaceae [9], both belonging to the suborder Bryineae Fleisch. [10]. Here the isolation of three biflavonoids, containing an apigenin moiety, from *Rhytidiadelphus squarrosus* which belongs to the Hypnineae Fleisch. [10] is reported.

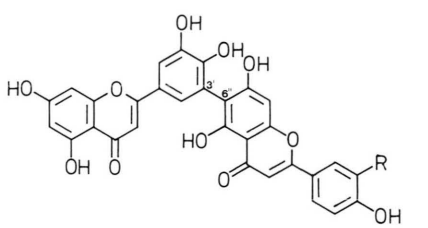
Results and Discussion

From *Rhytidiadelphus squarrosus* four biflavonoids **1, 2, 3** and **4** were isolated. **1, 2** and **4** are known compounds. Their identity was demonstrated by cochromatography with authentic samples, by their FAB-MS, PMR and ^{13}C NMR spectra (only **1** and **2**).

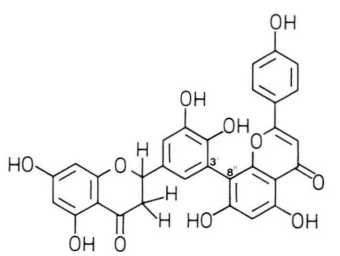
5′-Hydroxyrobustaflavone (**3**) is a new compound. The FAB-MS $[M-H]^-$ signal at 553 mu is in accordance with a heptahydroxybiflavone. The



5′-Hydroxyamentoflavone (**1**) R = H
5′-3‴-Dihydroxyamentoflavone (**2**) R = OH



5′-Hydroxyrobustaflavone (**3**) R = H
5′,3‴-Dihydroxyrobustaflavone (**5**) R = OH



2,3-Dihydro-5′-hydroxyamentoflavone (**4**)

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Table I. Chromatographic data of compounds **1, 2, 3** and **4** and UV data of compound **3.**

Compound	**1**	**2**	**3**	**4**	unknown*
Spot appearance					
UV (350 nm)	dark	dark	dark	dark	dark
NH_3	dark	dark	dark	dark	dark
"Naturstoffreagenz A" (NA)	yellow	yellow	yellow	yellowish, turning to red	orange
"Benedikt's Reagenz" (BR)	dark	dark	dark	dark	dark
TLC hRf values					
Adsorbent: Cellulose (Avicel, Schleicher & Schüll, F 1440)					
15% HoAc	3	4	3	4	5
40% HOAc	50	40	47	54	47
BAW	91	88	91	90	90
Adsorbent: Polygram, Polyamide-6 (Macherey & Nagel) EtOAc–MeCoEt–HCOOH–H_2O (5:3:1:1)	64	53	59	68	50
Adsorbent: Silica (KG 60 F_{254}, Merck) $CHCl_3$–Me_2CO–HCOOH (9:2:1)	20	10	17	23	9
Toluene–Ethylformiate–HCOOH (5:4:1)	20	13	19	27	17

* The crude fractions of **1** and **2** contained traces of this compound, which were decomposed during the further separation.

UV data of compound **3**	
MeOH	256sh–267–288sh–350
NaOMe	274–327–400
$AlCl_3$	273–302–363–408
$AlCl_3$/HCl	264sh–277–300–356–387sh
NaOAc	270–298sh–377
$NaOAc/H_3BO_3$	265–294sh–366

Table II. PMR data of compound **3** and related substances (**1, 2, 5**), DMSO-d_6, ambient temperature.

	2 [4]	**1** [4]	**3**	**5** [6]
H-3	6.68 s	6.70 s	6.64 s	6.65 s
H-6	6.21 d (2 Hz)	6.18 d (2 Hz)	6.19 d (2 Hz)	6.20 d (2 Hz)
H-8	6.45 d (2 Hz)	6.42 d (2 Hz)	6.45 d (2 Hz)	6.45 d (2 Hz)
H-2′	7.51 d (2 Hz)	7.48 d (2 Hz)	7.33 d (2 Hz)	7.35 d (2 Hz)
H-6′	7.52 d (2 Hz)	7.52 d (2 Hz)	7.39 d (2 Hz)	7.40 d (2 Hz)
H-3″	6.72 s	6.78 s	6.81 s	6.72 s
H-6″	6.41 s	6.38 s	–	–
H-8″	–	–	6.63 s	6.59 s
H-2‴	7.09 d (2 Hz)	7.58 d (9 Hz)	7.96 d (9 Hz)	7.45 d (2 Hz)
H-3‴	–	6.70 d (9 Hz)	6.95 d (9 Hz)	–
H-5‴	6.70 d (8 Hz)	6.70 d (9 Hz)	6.95 d (9 Hz)	6.92 d (8 Hz)
H-6‴	7.07 dd (2; 8 Hz)	7.58 d (9 Hz)	7.96 d (9 Hz)	7.46 dd (2; 8 Hz)

structure was deduced from its PMR and ^{13}C NMR spectra by comparison with those of known biflavonoids (see Tables II and III). In the PMR spectrum two ortho coupled doubletts, integrating to two protons each, are revealing a p-hydroxy substituted B'-ring as in robustaflavone [6]. Chemical shifts and multiplicities of all other signals are almost identical with the corresponding signals of 5',3'''-dihydroxyrobustaflavone (**5**) *cf.* Table II.

In the ^{13}C NMR spectrum the signals of the luteolin moiety are almost identical with those of **5** (Table III), whereas those of the apigenin moiety are as of robustaflavone [11]. Thus the proposed structure of **3** is established.

The occurrence of **1** and **3** shows that biflavonoids containing different flavone moieties are not restricted to the suborder Bryineae. Further investigations on other species are in progress.

Table III. ^{13}C NMR data of compound **3** and related substances (**1, 2, 5**), DMSO-d_6, ambient temperature.

Assignment	**2** [4]	**1** [4]	**3**	**5** [8]
C-2	163.9	163.7	163.5	164.0
C-3	102.9	102.9	102.7	102.8
C-4	181.9	182.0	181.7	181.6
C-5	161.4	160.9	161.1	162.3
C-6	98.7	98.8	98.7	98.7
C-7	164.0	164.0	164.0	163.6
C-8	93.8	93.8	93.8	93.4
C-9	157.3	157.3	157.3	157.2
C-10	103.6	103.6	103.4	103.6
C-1'	120.6	120.5	121.0	121.5
C-2'	122.2	122.3	121.9	121.9
C-3'	120.0	120.1	120.0	120.2
C-4'	148.2	148.4	148.8	148.6
C-5'	145.5	145.7	145.7	145.7
C-6'	112.1	112.0	111.6	111.7
C-2''	164.0	163.9	164.0	164.0
C-3''	102.5	102.5	102.7	102.7
C-4''	181.5	181.5	181.5	181.5
C-5''	160.4	160.4	159.1	161.4
C-6''	98.6	98.6	109.0	108.9
C-7''	161.7	161.9	162.5	162.3
C-8''	104.0	104.1	93.6	93.8
C-9''	154.5	154.5	156.3	156.2
C-10''	103.6	103.6	103.6	103.4
C-1'''	121.8	121.4	121.2	120.9
C-2'''	113.7	128.2	128.4	113.3
C-3'''	145.8	115.7	115.9	145.7
C-4'''	149.4	161.4	161.4	149.6
C-5'''	115.5	115.7	115.9	116.0
C-6'''	118.6	128.2	128.4	119.0

Experimental

Plant material

Gametophytic material of *Rhytidiadelphus squarrosus* (Hedw.) Warnst. was collected at St. Ingbert, Saarland, West Germany. Voucher specimens are deposited in the Herbarium of Fachrichtung Botanik, Universität des Saarlandes ("Saar").

Extraction and Isolation

400 g thoroughly cleaned air-dried material was ground and defatted with $CHCl_3$. Afterwards the flavonoids were exhaustively extracted by 80% aq. EtOH. To eliminate chlorophyll the combined extracts were evaporated and the residue subjected to a four step Craig distribution between the upper and lower phases of DMF/H_2O/Et_2O (4:1:8). The combined lower phases were reduced *in vacuo* to a thin syrup (about 50 ml). After addition of 30 ml dry polyamide-6 powder it was diluted with 800 ml water. The resulting suspension was cautiously poured on top of a 1 l polyamide-6-column (wet packed). The column was eluted with 2 l each of Me_2CO:H_2O (1:9, 2:8, 3:7, ..., 8:2) and 4 l 9:1. The compounds were eluted as follows: **4, 1 + 2, 3.** Further purification and separation was done by CC on Sephadex LH 20 with Me_2CO/MeOH/H_2O (2:1:1) and MeOH:H_2O [(65:35) – (85:15)] as solvent.

Yields: 160 mg of **1,** 13 mg of **2,** 25 mg of **3** and 2 mg of **4.**

TLC see Table I.

UV spectroscopy according to [12],

PMR spectroscopy: Bruker AM 400, 400 MHz, DMSO-d_6, ambient temperature.

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

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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