Biflavones from Some Mosses

Hans Geiger

Institut für Chemie der Universität Hohenheim, D-7000 Stuttgart 70, Bundesrepublik Deutschland

Siegbert Anhut, H. Dietmar Zinsmeister

FB 15, Botanik, Universität des Saarlandes, D-6600 Saarbrücken, Bundesrepublik Deutschland

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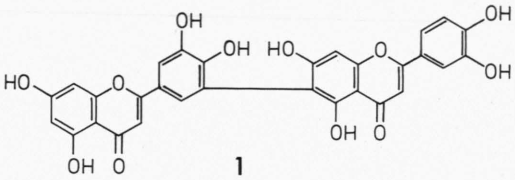
From *Antitrichia curtipendula* and *Racomitrium lanuginosum* 5′,3‴-dihydroxyrobustaflavone and 5′, 8″-biluteolin were isolated. In *Plagiomnium elatum* the new biflavone 5′-hydroxyamentoflavone was detected.

Introduction

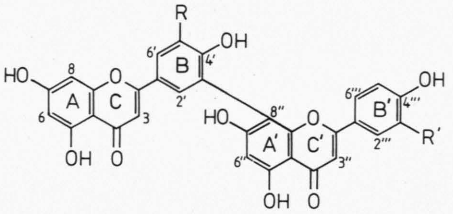
The distribution of biflavonoids among mosses is only incompletely known, mainly because their identification requires the isolation of at least about 20 mg of pure compounds to run PMR and ^{13}C NMR spectra. Biflavonoids have been isolated from the mosses *Dicranum scoparium* [1, 2], *Hylocomium splendens* [3], and *Bryum capillare* [4], all of which belong to different taxa. We now report the isolation of biflavonoids from the mosses *Antitrichia curtipendula, Racomitrium lanuginosum* and *Plagiomnium elatum* which were chosen from three other relatively unrelated taxa, to see how widespread these compounds are in mosses.

Results and Discussion

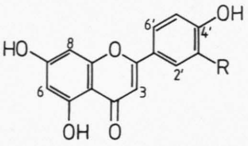
Antitrichia curtipendula and *Racomitrium lanuginosum* both yielded, by the isolation method described earlier [3], 5′,3‴-dihydroxyrobustaflavone (**1**) and 5′,8″-biluteolin (**2**) which were identified by their FD-MS spectra and NMR spectra, as well as by cochromatography with authentic samples. These data were all identical with published results [2, 3]. From both mosses no other flavonoids could be isolated in appreciable amounts. *Plagiomnium elatum* contains besides several flavonoid glycosides, which will be reported in another context, a new biflavone 5′-hydroxyamentoflavone (**3**).



1



2 : R = R′ = OH
3 : R = OH, R′ = H
4 : R = R′ = H



5 : R = H
6 : R = OH

The FD mass spectrum of **3** shows a molecular ion at 554 mu indicating a heptahydroxy-biflavone. Since *Plagiomnium elatum* contains glycosides of luteolin (**6**) and apigenin (**5**) it seemed likely that the biflavone consists of these two flavones. This is corroborated by the UV spectra, with and without added shift reagents (Table I), of **3** and of an equimolar mixture of **5** and **6** which are superimposable. The structure of **3** can be easily deduced from the PMR spectrum by comparison with the spectra of amentoflavone (**4**) and 5′,8″-biluteolin (**2**) (see Table II). The multiplicities and chemical shifts

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Reprint requests to Prof. Dr. H. D. Zinsmeister.

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Table I. Chromatographic and UV data of compound **3** from *Plagiomnium elatum* and related substances.

Compound	5′-OH-Amentoflavone **3**	Luteolin **6**	Apigenin **5**	Luteolin/Apigenin 1:1 equimolar mixture
Colour reactions				
UV (350 nm)	dark	dark	dark	
NH_3	dark	yellow	green	
NA	yellow	yellow	green	
BR	dark	dark	green	
TLC hRf values				
Sorbent: Cellulose (Avicel, Schleicher & Schüll)				
15% HOAc	6	2	3	
40% HOAc	58	19	33	
BAW	96	74	90	
TBA	91	66	83	
Sorbent: Polyamide (Mikropolyamid, F 1700, Schleicher & Schüll)				
EtOAc–MeCOEt–HCOOH–H_2O (5:3:1:1)	40	35	49	
Sorbent: Si-Gel (KG 60 F 254, Merck)				
$CHCl_3$–Me_2CO–HCOOH (9:2:1)	14	28	43	
Toluene−Ethyl Formate−HCOOH (5:4:1)	20	30	35	
UV-data				
MeOH	265−291sh−343	253−265sh−289−348	267−295sh−334	264−292sh−349
NaOMe	272−325sh−388	266−327sh−401	275−324−390	272−325−395
$AlCl_3$	272−306sh−352−410	272−300sh−324sh−422	275−301−349−375sh	273−300−351−406
$AlCl_3$/HCl	255−269sh−304−345	260−268sh−294sh−354−380sh	269−299−339−375sh	268−295−344−377sh
NaOAc	269−313sh−371	267−322sh−384	274−302−383	273−309sh−386
NaOAc/H_3BO_3	263−360	260−371	267−339	265−356

NA, Naturstoffreagenz A.
BR, Benedikt's Reagenz.
BAW, *n*-BuOH−HOAc−H_2O (4:1:5; upper part).
TBA, tertiary BuOH−HOAc−H_2O (3:1:1).

Table II. PMR-spectra of **2**, **3** and **4** (DMSO-d_6, ambient temperature, 400 MHz).

5′,8″-Biluteolin **2**		5′-OH-Amentoflavone **3**		Amentoflavone **4**	
6.68s	H-3	6.70s	H-3	6.74s	H-3
6.72s	H-3″	6.78s	H-3″	6.81s	H-3″
6.21d ($J=2$ Hz)	H-6	6.18d ($J=2$ Hz)	H-6	6.22d ($J=2$ Hz)	H-6
6.41s	H-6″	6.38s	H-6″	6.45s	H-6″
6.45d ($J=2$ Hz)	H-8	6.42d ($J=2$ Hz)	H-8	6.50d ($J=2$ Hz)	H-8
7.51d ($J=2$ Hz)	H-2′	7.48d ($J=2$ Hz)	H-2′		
7.52d ($J=2$ Hz)	H-6′	7.52d ($J=2$ Hz)	H-6′		
7.09d ($J=2$ Hz)	H-2‴				
6.70d ($J=8$ Hz)	H-5‴				
7.07dd ($J=2$; 8 Hz)	H-6‴				
				8.07d ($J=2$ Hz)	H-2′
				7.19d ($J=9$ Hz)	H-5′
				7.99dd ($J=2$; 9 Hz)	H-6′
		6.70d ($J=9$ Hz)	H-3‴ H-5‴	6.77d ($J=9$ Hz)	H-3‴ H-5‴
		7.58d ($J=9$ Hz)	H-2‴ H-6‴	7.57d ($J=9$ Hz)	H-2‴ H-6‴

of the A,A′- and C,C′-ring protons are identical in all three spectra. Two *ortho*-coupled doublets at 6.70 and 7.57 ppm, each integrating to two protons and attributable to the B′-ring protons, are identical in the spectra of **3** and **4**. Two metacoupled doublets at 7.47 and 7.51 ppm each integrating to one proton, which must be assigned to the B-ring protons, are found at the same position in the spectra of **2** and **3**. The comparison of the ^{13}C spectrum of **3** with the spectra of 5′,8″-biluteolin (**2**), apigenin (**5**) and luteolin (**6**) leads also to the structure of **3** (Table III), when the spectrum of **5** is subtracted from the spectrum of **3** one obtains the signals which must be assigned to the "biflavone core" consisting of rings B, A′ and C′. These signals are almost identical with those of the "biflavone core" of **2** obtained by subtraction of the spectrum of **6** from that of **2**.

The 4′,5′-dimethylether of **3** named 5′-methoxybilobetin has previously been isolated from *Ginkgo biloba* [5]. Thus 5′-hydroxyamentoflavone (**3**) is the first example of a biflavone with an identical oxygenation pattern in tracheophytes and bryophytes. Furthermore, the interflavonylic linkage between C-3′ and C-8″ also seems to be common to both plant

Table III. ^{13}C NMR spectra of compounds **2**, **3**, **5** and **6** (DMSO-d_6, ambient temperature, 100 MHz).

Apigenin	A.	5′-OH-Amentoflavone	A.	5′-8″-Biluteolin	A.*	Luteolin	A.
181.8	4	182.0	4^{a}	181.9	4^{a}	181.6	4
		181.5	4″^{a}	181.5	4″^{a}		
164.1	7^{a}	164.0	7^{b}	164.0	7^{b}	164.1	7^{a}
		163.9	2″^{b}	164.0	2″^{b}		
163.8	2^{a}	163.7	2^{b}	163.9	2^{b}	163.9	2^{a}
		161.9	7″^{c}	161.7	7″^{c}		
161.5	4′^{b}	161.4	4‴^{c}				
161.1	5^{b}	160.9	5^{c}	161.4	5^{c}	161.4	5
		160.4	5″^{c}	160.4	5″^{c}		
157.3	9	157.3	9	157.3	9	157.2	9
		154.5	9″	154.5	9″		
				149.4	4‴^{d}	149.6	4′
		148.4	4′	148.2	4′^{d}		
				145.8	3‴^{e}	145.7	3′
		145.7	5′	145.5	5′^{e}		
128.4 t}	2′, 6′	128.2 t}	2‴, 6‴				
128.4 t}		128.2 t}					
		122.3 t	2′	122.2 t	2′		
121.3	1′	121.4	1‴^{d}	121.8	1‴^{d}	121.5	1′
		120.5	1′^{d}	120.6	1′^{d}		
		120.1	3′^{d}	120.0	3′^{d}		
				118.6 t	6‴	118.9 t	6′
116.0 t}	3′, 5′	115.7 t}	3‴, 5‴	115.5 t	5‴	116.0 t	5′
116.0 t}		115.7 t}		113.7 t	2‴	113.3 t	2′
		112.0 t	6′	112.1 t	6′		
		104.1	8″^{e}	104.0	8″^{e}		
103.7	10	103.6	10^{e}	103.6	10^{e}	103.7	10
		103.6	10″^{e}	103.6	10″^{e}		
102.8 t	3	102.9 t	3^{f}	102.9 t	3^{f}	102.8 t	3
		102.5 t	3″^{f}	102.5 t	3″^{f}		
98.8 t	6	98.8 t	6^{g}	98.7 t	6^{g}	98.8 t	6
		98.6 t	6″^{g}	98.6 t	6″^{g}		
94.0 t	8	93.8 t	8	93.8 t	8	93.8 t	8

A., Assignement.

t, tertiary carbon as determined by the DEPT technique.

* The original numbering of this compound [1, 2] has to be changed to bring it in line with the other compounds, it is therefore not consistent with its name!

$^{a, b, c, d, e, f, g}$, assignements bearing the same superscript in any one spectrum may be reversed.

groups (compare [6]). It should be mentioned, in addition, that the occurrence of 5′,8″-biluteolin in *Hylocomium splendens* has definitely been proven (compare [3]). The assumption made earlier, that biflavones might be structures widespread and characteristic for mosses, has been confirmed.

Experimental

Plant material

Airdried gametophytic material of the following species was used:

1. *Antitrichia curtipendula* (Hedw.) Brid. (290 g, near Masevaux, Ht. Rhin, France);
2. *Racomitrium lanuginosum* (Hedw.) Brid. (750 g, Steinbachtal, Saarland, W. Germany);
3. *Plagiomnium elatum* (B.S.G.) Kop. (512 g, Bodental, Carinthia, Austria).

Voucher specimens have been deposited in the Herbarium of Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken.

Extraction and isolation

Antitrichia curtipendula

The airdried material was defatted with 5×2 l CH_2Cl_2 and extracted by percolation with 16 l MeOH followed by 20 l 80% aq. Me_2CO. 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, phase volume 100 ml). The combined lower phases were reduced *in vacuo* to a thin syrup, which, after addition of 70 ml dry polyamide 6, was diluted with 1 l H_2O. The resulting suspension was cautiously poured on a 1 l-polyamide-6 column (wet packed). The column was eluted with 2 l of each 20%, 30%, 40%, 50%, 60%, 70% and 80% aq. Me_2CO. Elution was continued with the latter solvent until all flavonoids had been eluted, **2** was eluted, with only a slight overlap, before **1**. Further purification was effected by CC on Sephadex LH 20 with $Me_2CO/MeOH/H_2O$ (2:1:1) as solvent, whereby some high molecular weight material were eluted before **2** and **1**. The appropriate fractions yielded 19 mg of **2** and 43 mg of **1**.

Racomitrium lanuginosum was treated in the same way as *Antitrichia* and yielded 65 mg of **1** and 430 mg of **2**.

Plagiomnium elatum

The detailed extraction procedure is described elsewhere in connection with its flavonoid glycosides [8]. The aglycone fraction remaining after removal of the glycosides was also treated as mentioned above and yielded 20 mg of **3**.

TLC see Table I.

UV spectroscopy according to [7].

Mass spectroscopy: Varian MAT 311 with FD-source.

PMR spectroscopy: Bruker (AM 400), 400 MHz, 297 K, DMSO-d_6.

^{13}C-NMR spectroscopy: Bruker (AM 400), 100 MHz, 297 K, DMSO-d_6.

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