3′,3‴-Binaringenin, a New Biflavonoid from *Pilotrichella cuspidata* (Meteoriaceae, Musci)*

Tassilo Seeger, Hans Geiger, and Hans Dietmar Zinsmeister
Fachrichtung Botanik, Universität des Saarlandes,
D-W-6600 Saarbrücken, Bundesrepublik Deutschland

Jan-Peter Frahm
Fachbereich Botanik, Universität Duisburg,
D-W-4100 Duisburg 1, Bundesrepublik Deutschland

Ludger Witte
Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30,
D-W-3300 Braunschweig, Bundesrepublik Deutschland

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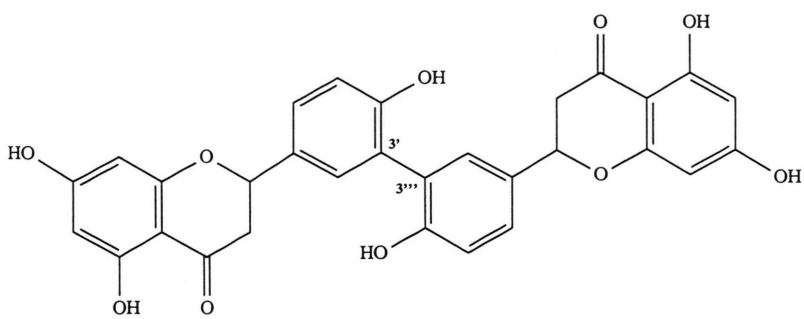
From *Pilotrichella cuspidata* the new biflavonoid 3′,3‴-binaringenin was isolated. Its structure was elucidated spectroscopically.

Recently we screened mosses from various families for biflavonoids [1]. During this study it turned out, that *Pilotrichella cuspidata* contains a biflavonoid, which was chromatographically different from the known moss biflavonoids.

The UV spectra of the unknown compound **1** with and without added shift reagents (see Experimental) are almost identical with those of naringenin. The relative molecular mass of **1** was 542 U, the expected value of a hexahydroxy-biflavanone. The ^{13}C NMR spectrum (Table I) however shows only 15 signals, thus **1** must be a symmetric biflavonoid. A comparison of the ^{13}C NMR spectra of **1** and naringenin reveals, that the chemical shifts of all signals except the one, which must be attributed to the C-3′ are nearly identical. This suggests that **1** is a 3′,3‴-binaringenin. This is corroborated by the ^{1}H NMR spectra (Table I). The signals of the A- and C-ring protons show similar chemical shifts and coupling patterns, whereas in the B-ring part of the spectrum **1** shows a ABX system instead of the A_2X_2 system of naringenin. Thus **1** is 3′,3‴-binaringenin, the first naturally occurring 3′,3‴-linked biflavanone. The only other



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examples of natural 3′,3‴-linked biflavonoids are Hypnogenol A (3′,3‴-biaromadendrin) and Hypnogenol B (3′,3‴-aromadendrin-naringenin) from the moss *Hypnum cupressiforme* [2] and 5′,5‴-bisdihydroquercetin from the conifer *Pseudotsuga menziesii* [3].

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

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Table I. ^{1}H and ^{13}C NMR data of naringenin and **1.** In parentheses the coupling constants in [Hz].

Assignment	Naringenin [4]	**1**	**1***	Naringenin [5]	Assignment
H-2 ax	5.43 dd (2.8/12.7)	5.47 dd (3/13)	78.5 t	78.4	C-2
H-3 ax	3.26 dd (12.8/17.1)	3.31 dd (13/17)	42.1 sec	42.0	C-3
H-3 eq	2.69 dd (2.8/17.1)	2.69 dd (3/17)			
			196.2	196.2	C-4
H-5	OH 12.15 s	OH 12.16 s	163.5	163.6	C-5
H-6	5.90 s	5.89 d (2)	95.8 t	95.9	C-6
H-7	OH	OH	166.6	166.7	C-7
H-8	5.90 s	5.88 d (2)	95.0 t	95.0	C-8
–			162.9	162.9	C-9
–			101.8	101.8	C-10
–			128.8	128.9	C-1′
H-2′	7.32 d (8.5)	7.29 d (2)	127.0 t	128.2	C-2′
H-3′	6.81 d (8.5)	C–C	125.6	115.2	C-3′
H-4′	OH	OH	155.0	157.8	C-4′
H-5′	6.81 d (8.5)	6.93 d (8)	115.7 t	115.2	C-5′
H-6′	7.32 d (8.5)	7.31 dd (2/8)	130.2 t	128.8	C-6′

* sec, t = secondary respectively tertiary carbon atoms as determined by the DEPT technique; all other carbon atoms quarternary.

Experimental

Plant material

Gametophytic material of *Pilotrichella cuspidata* Broth. was collected in Rwanda, Pref. de Gikongoro, Foret de Nyungwe, Rwasenoko, Africa, leg. J.-P. Frahm (No. 6112). Voucher specimens are deposited in the Herbarium of Fachrichtung Botanik, Universität des Saarlandes ("SAAR").

Extraction and isolation

50 g air-dried plant material (freed from foreign matter) was extracted 3 times with 2 l each $Me_2CO:H_2O$ (8:2) and 2 times with 2 l each $MeOH:H_2O$ (8:2). 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 30 ml). After addition of 30 ml dry polyamide-6 powder it was diluted with 300 ml water. The resulting suspension was cautiously poured on top of a 0.5 l polyamide-6 column (wet packed). The column was eluted with 2 l each of $Me_2CO:H_2O$ (0:10; 1:9; 2:8; 3:7; 4:6; 5:5), 41 (11:9), 21 (6:4; 13:7; 7:3; 8:2; 9:1) and 41 of $Me_2CO:HOAc:H_2O$ (18:1:1). Further purification was done by CC on Sephadex LH 20 with $Me_2CO:H_2O:MeOH$ (2:1:1). Yield: 71 mg 3′,3‴-binaringenin.

TLC: hR_f values of **1** and of naringenin (in parentheses): cellulose/TBA: 86 (86); cellulose/15% HOAc: 4 (38); cellulose/40% HOAc: 67 (73); polyamide-6/$Me_2CO-H_2O-HOAc$ (6:2:2): 56 (69); polyamide-6/EtOAc–MeCOEt–HOAc–H_2O (5:3:1:1): 94 (94). Spot appearance under UV dark, after spraying with NA greenish. UV spectroscopy [6]: MeOH: 287, 327 sh; NaOMe: 250 sh, 319; $AlCl_3$: 309, 370; $AlCl_3$–HCl: 307, 370; NaOAc: 297 sh, 321; NaOAc–H_3BO_3: 290, 323 sh. ^{1}H NMR 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 EI-MS and CI-MS (negative mode, with NH_3).

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