

Bi- and Triflavonoids of Representative Moss Species from Six Different Families

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The identity of the bi- and triflavonoids of six moss species from six different families has been proved by NMR. The individual flavonoids are: 3', 3'''-binaringenin, 5', 3'''-dihydroxy-amentoflavone, 5', 3'''-dihydroxyrobustaflavone, campylopusaurone, aulacomniumtriluteolin, philonotisflavone, dicranolomin, and 2, 3-dihydrodicranolomin.

Introduction

Recently more than 200 species belonging to various moss families have been screened by 2 D-TLC for the possible occurrence of bi- and triflavonoids (Seeger, 1992). This survey indicated, that species from 40 out of the 61 families which were studied, may contain some of these compounds. The definitive identification of individual bi- or triflavonoids requires, however, confirmation by NMR spectra. Therefore we have started to isolate these compounds from various mosses in amounts, that are sufficient for NMR studies. In the present communication we describe the results obtained with six different mosses from six different families.

Results and Discussion

The main biflavonoid compound from *Ptilium crista-castrensis* (Hypnaceae) could be identified as 3', 3'''-binaringenin (**1**). This confirms an earlier tentative identification of **1** in *P. crista-castrensis* by HPLC (Sievers, 1992).

In a paper on flavone glycosides of *Hedwigia ciliata* (Hedwigiaceae) it is mentioned, that this species might also contain a biflavone (Österdahl, 1976). Since this author has meanwhile abandoned the field, we have now checked this point and

found, that this moss contains indeed 5', 3'''-dihydroxyamentoflavone (**2**).

During a study of the genus *Campylopus* (Dicranaceae) by 2 D-TLC we observed that the chromatograms of *C. introflexus* showed a spot with the chromatographic characteristics of a triluteolin. Therefore this moss was worked up on a preparative scale. In addition to 5', 3'''-dihydroxyamentoflavone (**2**), 5', 3'''-dihydroxyrobustaflavone (**3**) and campylopusaurone (**4**), which had been obtained before from two other *Campylopus* spp. (Geiger and Markham, 1992), this species contained also aulacomniumtriluteolin. This triluteolin has been found before only in *Aulacomnium palustre* (Hahn et al., 1995).

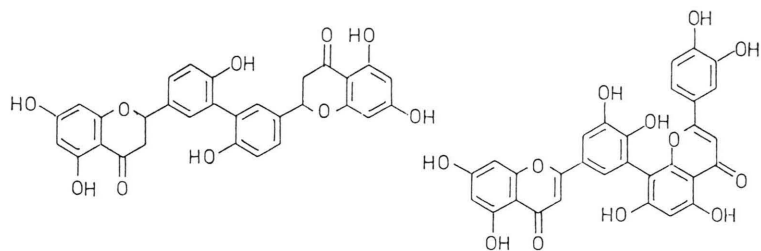
Bryoxiphium norvegicum (Bryoxiphiaceae) and *Dicnemon calycinum* (Dicnemonaceae) are representatives of two small families, that have so far not been studied chemically. They yielded both the two widespread biluteolins **2** and **3**. This is not in contradiction to the usual placement of these families near the Dicranaceae (Allen, 1987, Nyholm, 1986), but it would agree also with various other arrangements.

Pyrrhobryum bifarium (Rhizogoniaceae) was studied, because preliminary tests had already revealed, that this little moss was very rich in biflavonoids. In fact it turned out that the total biflavonoid concentration in this species was 2.5% of the dry weight, that is one to two orders of magnitude more than we find usually in mosses. The individual compounds were identified as philonotisflavone (**6**), dicranolomin (**7**) and 2, 3-dihydrodicranolomin (**8**).

¹ Publ. No 98 des Arbeitskreises Chemie und Biologie der Moose.

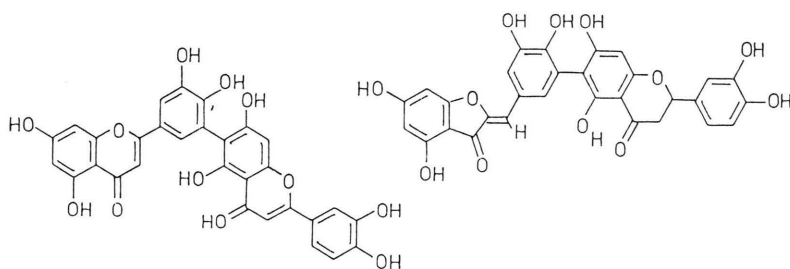
Reprint requests to Prof. Dr. H. D. Zinsmeister.





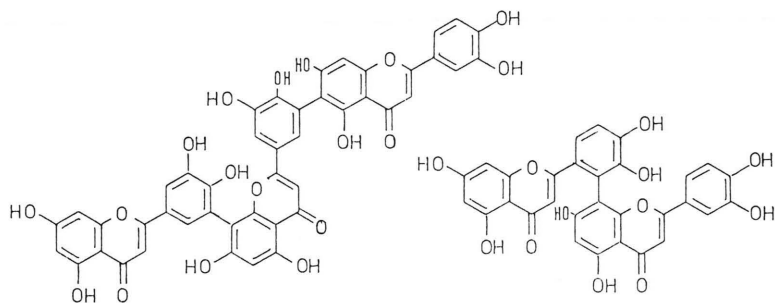
3', 3'''-binaringenin (1)

5', 3'''-dihydroxyamentoflavone (2)



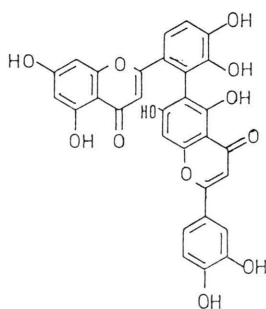
5', 3'''-dihydroxyrobustaflavone (3)

campylobus aurone (4)

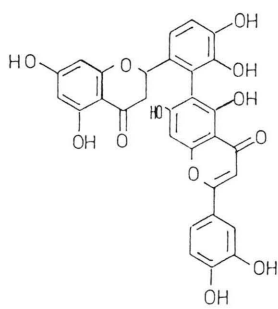


aulacomnium triluteolin (5)

philonotisflavone (6)



dicranolomin (7)



2, 3-dihydrodicranolomin (8)

The taxonomic relevance of the present results will become evident only after many more species will have been studied. The widespread occurrence of some biflavonoids like **1**, **2** and **3**, however, gives already a hint that these constitute beneficial characters, which are not easily lost during evolution, whereas the rarer compounds like **4** – **8** might be taxonomically more important.

Experimental

Plant material, origin and location of vouchers: *Ptilium crista-castrensis* (Hedw.) De Not. and *Hedwigia ciliata* (Hedw.) P. Beauv.: Forest near Umhausener Wasserfall, Ötztal, Austria. *Leg. et det.* R. Mues sept. 3rd, 1993. SAAR 5068, 5069. *Campylopus introflexus* (Hedw.) Brid.: heath near Holz, Saarland, Germany, *leg. et det.* R. Mues *et al.* June 15th, 1992. SAAR 3031. *Bryoxiphium norvegicum* (Brid.) Mitt.: Reykjavik Iceland, *leg. et det.* Á. H. Bjarnason, SAAR 5070. *Dicnemon calycinum* (Hook.) Schwaegr.: On horizontal branches, Waitakere ranges near Auckland, New Zealand, *leg. et det.* H. Geiger february 21st, 1991 no 1791A. Private herbarium of H. G. *Pyrrhobryum bifarium* (Hook.) Manuel: On forest floor near Eastbourne, New Zealand, *leg. et det.* H. Geiger march 24th, 1991 no 1863. Private herbarium of H. G.

The airdried plant material was carefully freed from any foreign matter. *C. introflexus* and *D. calycinum* contained some sporophytes. All other species consisted entirely of gametophytes.

Extraction, isolation and identification

Extraction and column chromatography on polyamide 6 with a H₂O–Me₂CO gradient and on Sephadex LH20 with Me₂CO–MeOH–H₂O (2:1:1) were performed as described earlier (Seeger *et al.*, 1993 a, b). Using these methods the specified amounts of moss material yielded:

160g	<i>P. crista-castrensis</i>	10mg 1 .
130g	<i>H. ciliata</i>	16mg 2 .
170g	<i>C. introflexus</i>	2mg 2 , 23mg 3 , 16mg 4 and 3mg 5 .
6.5g	<i>B. norvegicum</i>	25mg 2 and 3mg 3 .
51g	<i>D. calycinum</i>	5mg 2 and 13mg 3 .
2.7g	<i>P. bifarium</i>	3mg 6 , 17mg 7 and 43mg 8 .

The individual compounds were identified by their ¹H-NMR spectra (DMSO-d₆, 400 MHz, ambient temperature), which were within the limits of experimental error identical with published data (Seeger *et al.*, 1993b; Geiger *et al.*, 1993; Hahn *et al.*, 1995), and by cochromatography with the authentic samples on which the reference spectra had been run.

Acknowledgements

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