# Bryoflavone and Heterobryoflavone, Two New Isoflavone-flavone Dimers from *Bryum capillare*

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*Bryum,* Musci, Biflavonoids: Isoflavone-flavone, Bryoflavone, Heterobryoflavone

From the moss *Bryum capillare* (Bryaceae) two new biflavonoids – bryoflavone and heterobryoflavone – have been isolated. They are the first examples of a new class of biflavonoids formed by oxidative coupling of a flavone and an isoflavone moiety. The structures of both compounds are proved spectroscopically.

## Introduction

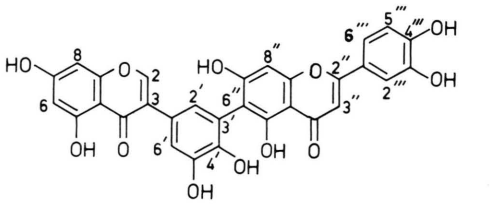
There have been up to now only few reports on the occurrence of biflavonoids in bryophytes. Thus Lindberg *et al.* reported 5′, 8″-biluteolin from *Dicranum scoparium* [1]. Most recently a new biflavone, 5′, 3‴-dihydroxyrobustaflavone was isolated from the moss (Musci) *Hylocomium splendens* [2]. This type of compound has so far not been found in the second important group of bryophytes, the liverworts (Hepaticae), although they have been studied chemically more extensively than mosses. In this paper the isolation of further biflavones from gametophytic tissues of *Bryum capillare* is described.

## Results and Discussion

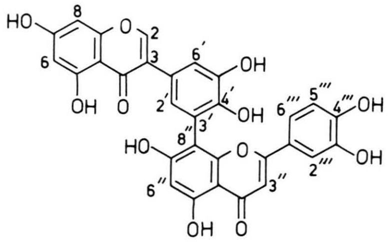
In the course of a study of the flavonoid pattern of *Bryum capillare* Hedw. [3, 4] two new biflavonoids were isolated from gametophytes. These compounds were named bryoflavone (**1**) and heterobryoflavone (**2**). The structures of **1** and **2** were elucidated as follows.

The FD mass spectra of **1** and **2** show a molecular ion at 570 nm and a fragment ion at 552 nm, due to thermal elimination of $H_2O$; this points to octahydroxybiflavones with hydroxyl groups at the ortho positions on either side of the interflavonyl linkage.

If the $^{13}C$ NMR spectra (Table I) of both compounds are compared with those of 5′, 3‴-dihydroxyrobustaflavone (**3**) [2] and 5′, 8″-biluteolin (**4**) [5],



1



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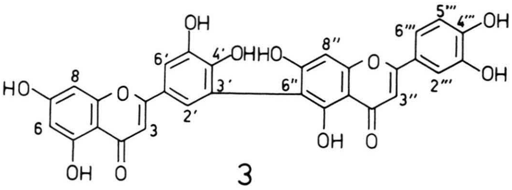

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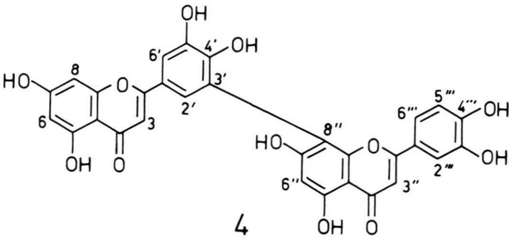

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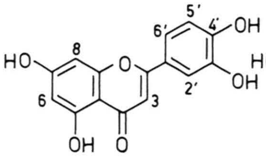
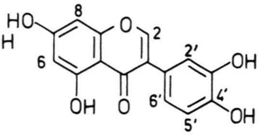


**5**



**6**

the most striking difference is the signal of an oxygenated tertiary carbon at the position of the C-2 signal of an isoflavone (153.8 and 153.5 ppm respectively). On the other hand both spectra show only one signal of a tertiary carbon at 102.7 and 102.2 ppm, respectively, which is attributable to the C-3 of a flavone. Both compounds must therefore contain a flavone and an isoflavone moiety, most likely luteolin (**5**) and orobol (**6**), which have previously been found in *B. capillare* [3, 4]. This is corroborated by the UV-spectra of **1** and **2**, with and without addition of the standard shift reagents [6],

Table I. $^{13}$C NMR spectra of compounds **1–6** (DMSO $d_6$, ambient temperature, 100 MHz).

| Assignment of biflavonoid carbons | 5′,3‴-Dihydroxy-robustaflavone (**3**) | Bryoflavone (**1**) | Heterobryo-flavone (**2**) | 5′,8″-Biluteolin (**4**)* | Luteolin (**5**) | Orobol (**6**) | Assignment of flavonoid carbons |
|---|---|---|---|---|---|---|---|
| 4, 4″ | 181.6 | 181.6 | 181.8 | 181.9 | 181.6 | – | 4 |
| | 181.5 | 180.3 | 180.1 | 181.5 | – | 180.2 | |
| | 164.0 | 164.1 | 164.0 | 164.0 | 164.1 | 165.2 | |
| | 164.0 | – | – | 164.0 | – | – | |
| 2, 2″ | 163.6 | 163.4 | 163.7 | 163.9 | 163.9 | – | 2 (f) |
| 5, 5″ (f) | 162.3 | 162.0 | 161.9 | 161.7 | – | – | 5 |
| 7, 7″ | 161.4 | 162.0 | 161.9 | 161.4 | 161.4 | 163.0 | 7 |
| 9, 9″ | 159.0 | 159.0 | 160.0 | 160.4 | – | – | 9 |
| | 157.2 | 157.2 | 157.4 | 157.3 | 157.2 | 158.5 | |
| | 156.2 | 156.1 | 154.3 | 154.5 | – | – | |
| 2 | – | 153.8 t | 153.5 t | – | – | 154.6 t | 2 (i) |
| | 149.6 | 149.5 | 149.3 | 149.4 | 149.6 | – | |
| 3′, 3‴ (f) | 148.6 | – | – | 148.2 | – | – | 3′, 4′ (f) |
| 4′, 4‴ | 145.7 | 145.7 | 145.3 | 145.8 | 145.7 | – | |
| | 145.7 | – | – | 145.5 | – | – | |
| | – | 144.9 | 145.0 | – | – | 146.4 | 3′, 4′ (i) |
| | – | 144.3 | 144.0 | – | – | 145.7 | |
| | 121.9 t | 122.8 t | 123.0 t | 122.2 t | – | 123.2 | 3 (i) |
| | – | 122.4 | 122.3 | – | – | – | 1′ |
| 3 (i) | 121.5 | 121.6 | 121.8 | 121.8 | 121.5 | 122.5 | |
| 1′, 1‴ | 120.9 | 120.4 | 120.6 | 120.6 | – | – | |
| 2′, 2‴ | 120.2 | 120.3 | 119.2 | 120.0 | – | – | |
| 5′, 5‴ | 118.9 t | 118.9 t | 118.7 t | 118.6 t | 118.9 t | 120.7 t | |
| 6′, 6‴ | 116.0 t | 116.0 t | 115.4 t | 115.5 t | 116.0 t | 117.3 t | 2′, 5′, 6′ |
| | 113.0 t | 114.9 t | 115.3 t | 113.7 t | 113.3 t | 116.1 t | |
| | 111.7 t | 113.2 t | 113.6 t | 112.1 t | – | – | |
| 6″ | 108.9 | 108.9 | – | – | – | – | |
| 8″ | – | – | 104.8 | 104.0 | – | – | |
| 10, 10″ | 103.6 | 104.5 | 104.4 | 103.6 | 103.7 | 105.1 | 10 |
| | 103.4 | 103.2 | 103.4 | 103.6 | – | – | |
| 3, 3″ (f) | 102.8 t | 102.7 t | 102.2 t | 102.9 t | 102.8 t | – | 3 (f) |
| | 102.7 t | – | – | 102.5 t | – | – | |
| 6, 6″ | 98.7 t | 98.9 t | 98.8 t | 98.7 t | 98.3 t | 99.5 t | 6 |
| | – | – | 98.5 t | 98.5 t | – | – | |
| 8, 8″ | 93.8 t | 93.6 t | 93.4 t | 93.8 t | 93.8 t | 94.2 t | 8 |
| | 93.4 t | 93.4 t | – | – | – | – | |

f = Flavone; i = isoflavone; t = tertiary carbon as determined by the DEPT technique.

* The original numbering of this compound [4] had to be changed to bring it in line with the other compounds; it is therefore *not* consistent with its name!

which are almost superimposable with those of an equimolar mixture of **5** and **6**. Table II contains the UV and the chromatographic data. The main differences between the $^{13}$C NMR spectra of **1** and **2** reflect the different interflavonyl linkages (3′ → 6″ in **1** and 3′ → 8″ in **2**). The spectrum of **1** shows two tertiary signals at 93.4 and 93.6 ppm (C-8 and C-8″) and only one at 98.9 (C-6), whereas the quaternary bridgehead C-6″ is shifted to 108.9 ppm [2]. In the spectrum of **2** two tertiary signals at 98.5 and 98.8 ppm (C-6

Table II. Chromatographic and UV-data of the flavonoids **1**, **2**, **5**, **6** from *Bryum capillare* and UV-data of an equimolar mixture of **5** and **6**.

| Compound | Bryoflavone (**1**) | Heterobryoflavone (**2**) | Luteolin (**5**) | Orobol (**6**) | Luteolin/Orobol 1:1 |
|---|---|---|---|---|---|
| Colour reactions | | | | | |
| UV (350 nm) | dark | dark | dark | dark | – |
| $NH_3$ | dark | dark | yellow | dark | – |
| NA[1] | yellow | yellow | yellow | dark | – |
| BR[2] | dark | dark | dark | dark | – |
| TLC | | | | | |
| hRf values | | | | | |
| Sorbens: Cellulose | | | | | |
| 40% HOAc | 39 | 42 | 20 | 58 | – |
| 50% HOAc | 56 | 57 | – | – | – |
| BAW[3] | 91 | 91 | 77 | 87 | – |
| TBA[4] | 86 | 86 | 65 | 75 | – |
| Sorbens: Polyamide | | | | | |
| EtOAc–MeCOEt-HCOOH–$H_2O$ (5:3:1:1) | 37 | 41 | – | – | – |
| $C_6H_6$-MeCOEt-MeOH (4:3:3) | 3 | 5 | – | – | – |
| Sorbens: Si-Gel | | | | | |
| $CHCl_3$–$Me_2CO$–HCOOH (9:2:1) | 10 | 12 | 30 | 30 | – |
| Toluene-Ethyl-Formate-HCOOH (5:4:1) | 20 | 23 | – | – | – |
| UV-data | | | | | |
| MeOH | 262, 288 sh, 344 | 261, 291 sh, 343 | 254, 263 sh, 290, 347 | 261, 286 sh | 260, 288 sh, 346 |
| NaOMe | 267, 329, 405[5] | 269, 325, 403 | 267, 326, 401[5] | 265, 333 (dec.) | 267, 328, 404[5] |
| $AlCl_3$ | 272, 295 sh, 373 sh, 418 | 264, 306 sh, 372, 418 | 272, 299 sh, 324 sh, 363 sh, 423 | 270, 295 sh, 361 | 272, 300 sh, 368, 419 |
| $AlCl_3$/HCl | 274, 297 sh, 363 | 272, 302 sh, 359 | 257, 270 sh, 293 sh, 355 | 271, 361 | 271, 293 sh, 357 |
| NaOAc | 270, 323, 376 | 269, 323 sh, 375 sh | 263, 380 | 264, 319 sh | 268, 320, 387 |
| NaOAc/$H_3BO_3$ | 261, 370 | 261, 304 sh, 373 | 259, 370 | 264, 288 sh | 260, 290 sh, 369 |

[1] NA = Naturstoffreagenz A, see [2].
[2] BR = Benedikt's Reagent, see [2].
[3] BAW = n-BuOH–HOAc–$H_2O$ (4:1:5; upper phase).
[4] TBA = tertiary BuOH–HOAc–$H_2O$ (3:1:1).
[5] Increased intensity; stable
dec. = decomposition.

and C-6″), and only one at 93.4 ppm are shown, and the bridgehead C-8″ is again shifted about 10 ppm downfield [7].

The sites of the other bridgeheads at the 3′-position of **1** and **2** are proved by the multiplicities of the proton resonances (Table III), which are only compatible with interflavonyl linkages at the 3′-position. The question whether the flavone or isoflavone is the "right hand moiety" (*c.f.* formulae!) can be decided by comparing the chemical shifts of the protons at C-5‴ and C-6‴ of the biflavonoids with those at C-5′ and C-6′ of the monomers, respectively. These protons are distinct from all others by their multiplicity [d($J = 8$) and dd($J = 2; 8$)]. Their signals are with **1**, **2**, **3**, **4** and **5** 0.35–0.5 ppm apart from each other whereas they coincide almost with the isoflavone **6**. Thus the structures of bryoflavone and heterobryoflavone are represented by the formulae **1** and **2**. The 0.2–0.4 ppm downfield shift of the 2‴, 5‴ and 6‴ protons with **2** and **4** as compared with **1**, **3**, **5** and **6** cannot be explained at present, but it is consistent with similar shifts in the biapigenin series [8].

## Experimental

The plant material was collected at St. Wendel/Saarland and at Winkel, Rüdesheim/Rheinland-Pfalz FRG. Voucher specimens have been deposited at the herbarium of the Fachrichtung Botanik, Universität des Saarlandes.

### *Extraction and isolation*

The air-dried gametophytic material (153 g) was extracted according to [2]. The concentrated extract was separated by CC on Sephadex LH-20 by MeOH. The fractions containing biflavones were further purified by EtOAc–MeCOEt–HOAc–$H_2O$ (5:3:1:1) on polyamide and finally by MeOH–$Me_2CO$–$H_2O$ (8:1:1) on Sephadex LH-20. Bryoflavone (**1**) and heterobryoflavone (**2**) were crystallized from aqueous acetone. The yields were 15 mg **1** and 30 mg **2**.

UV spectroscopy according to [6].

Mass spectroscopy: Varian MAT 311 A with FD source.

NMR spectroscopy: Bruker AM 400; 297°K, DMSO-$d_6$.

### *Acknowledgements*

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Table III. PMR-spectra of **1**, **2**, **3**, **4**, **5** and **6** (DMSO$d_6$, ambient temperature, 400 MHz).

| Assignment of biflavonoid protons | 5′, 3‴-Dihydroxy-robustaflavone (**3**) | Bryoflavone (**1**) | Heterobryo-flavone (**2**) | 5′, 8″-Biluteolin* (**4**) | Luteolin (**5**) | Orobol (**6**) | Assignment of flavonoid protons |
|---|---|---|---|---|---|---|---|
| H-2 (Isoflavone) | – | 8.28 s | 8.29 s | – | – | 8.25 s | H-2 (Orobol |
| H-3 (Flavone) | 6.67 s | – | – | 6.68 s | 6.69 s | – | H-3(Luteoli |
| H-3″ | 6.75 s | 6.67 s | 6.62 s | 6.72 s | – | – | – |
| H-6 | 6.22 d ($J$ = 2 Hz) | 6.21 d ($J$ = 2 Hz) | 6.22 d ($J$ = 2 Hz) | 6.21 d ($J$ = 2 Hz) | 6.22 d ($J$ = 2 Hz) | 6.20 d ($J$ = 2 Hz) | H-6 |
| H-6″ | – | – | 6.36 s | 6.41 s | – | – | – |
| H-8 | 6.48 d ($J$ = 2 Hz) | 6.37 d ($J$ = 2 Hz) | 6.37 d ($J$ = 2 Hz) | 6.45 d ($J$ = 2 Hz) | 6.47 d ($J$ = 2 Hz) | 6.35 d ($J$ = 2 Hz) | H-8 |
| H-8″ | 6.63 s | 6.54 s | – | – | – | – | – |
| H-2′ | 7.36 d ($J$ = 2 Hz) | 6.76 d ($J$ = 2 Hz) | 6.87 d ($J$ = 2 Hz) | 7.51 d ($J$ = 2 Hz) | – | – | – |
| H-6′ | 7.43 d ($J$ = 2 Hz) | 7.04 d ($J$ = 2 Hz) | 7.12 d ($J$ = 2 Hz) | 7.52 d ($J$ = 2 Hz) | – | – | – |
| H-2‴ | 7.47 d ($J$ = 2 Hz) | 7.41 d ($J$ = 2 Hz) | 7.13 d ($J$ = 2 Hz) | 7.09 d ($J$ = 2 Hz) | 7.43 d ($J$ = 2 Hz) | 7.00 d ($J$ = 2 Hz) | H-2′ |
| H-5‴ | 6.95 d ($J$ = 8 Hz) | 6.90 d ($J$ = 8 Hz) | 6.70 d ($J$ = 8 Hz) | 6.70 d ($J$ = 8 Hz) | 6.92 d ($J$ = 8 Hz) | 6.77 d ($J$ = 9 Hz) | H-5′ |
| H-6‴ | 7.48 dd ($J$ = 2; 8 Hz) | 7.43 dd ($J$ = 2; 8 Hz) | 7.06 dd ($J$ = 2; 8 Hz) | 7.07 dd ($J$ = 2; 8 Hz) | 7.44 dd ($J$ = 2; 8 Hz) | 6.80 dd ($J$ = 2; 9 Hz) | H-6′ |
| OH-5 and 5″ | 13.03 s; 13.27 s | 13.02 s; 13.21 s | 13.01 s; 13.13 s | 13.01 s; 13.14 s | 13.00 s | 12.99 s | OH-5 |

* The original numbering of this compound [4] had to be changed to bring it in line with the other compounds; it is therefore not consistent with its name!

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