New Flavone Glucoside Malonylesters from *Bryum capillare*

Wolfgang Stein, Siegbert Anhut, H. Dietmar Zinsmeister, Rüdiger Mues

FB 16, Botanik, Universität des Saarlandes, D-6600 Saarbrücken

Wolfgang Barz and Johannes Köster

Lehrstuhl für Biochemie der Pflanzen, Westfälische Wilhelms-Universität, D-4400 Münster

Z. Naturforsch. **40c,** 469–473 (1985); received April 15, 1985

Bryum, Bryales, Musci, Apigenin-, Diosmetin-, Luteolin-, 6-OH-Luteolin, 7-O-glucoside-6′′-malonate

From *Bryum capillare* Hedw. a variety of flavone glucosides and their 6′′malonyl esters were isolated. Diosmetin 7-O-β-D-glucoside-6′′-malonylester, luteolin 7-O-β-D-glucoside-6′′malonylester and 6-OH-luteolin 7-O-β-D-glucoside-6′′malonylester are new flavone malonyl esters. This is the first report of flavone glucoside malonylesters in a non vascular plant. The flavonoid pattern of the gametophyte is different from that of the sporophyte. The chemotaxonomic relevance of these results is discussed.

Introduction

Several publications reported on the occurrence of flavonoids in the genus Bryum [1–7].

Thus *Bryum cryophilum* produces luteolinidin 5-monoglucoside and luteolinidin 5-diglucoside [2] and *B. rutilans* and *B. weigelii* contain additionally the aglycone luteolinidin [4]. From *B. weigelii* scutellarein was isolated [6]. Recently we reported on some isoflavones in *B. capillare* [7]. In this report the isolation of further flavonoids from gametophytic and sporophytic tissues of this species is described.

Results and Discussion

Fig. 1 represents a composite 2D-TLC which includes all flavonoids isolated from both the gametophyte and sporophyte of *B. capillare.* A number of these flavonoids are common to both generations, but there are also considerable differences. Thus, compounds 10–15 are typical components found in all (22 samples) investigated gametophytic tissues, whereas the so far extracted sporophytes (6 samples) may contain only traces of a purple spot (254 nm) in the region of compounds 12–15. The

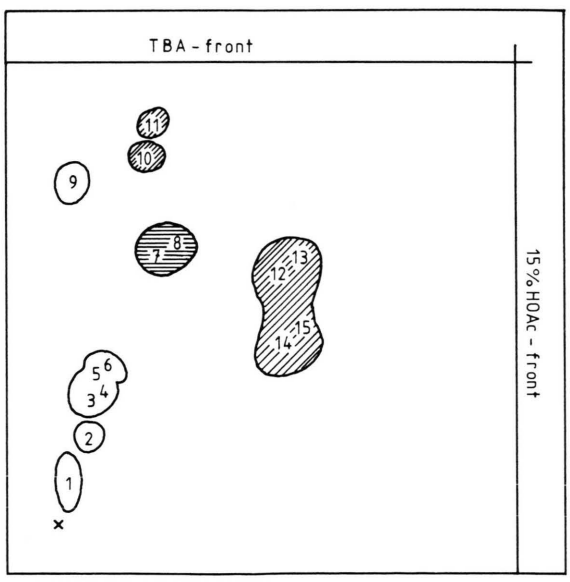

Fig. 1. TLC pattern of the major flavonoids obtained from gametophyte and sporophyte of *Bryum capillare,* after extraction with 80% MeOH. Unhatched spots represent compounds common to both generations. Horizontal hatching indicates compounds unique to the sporophyte, diagonal hatching represents spots unique to the gametophyte.

Sorbens:	Cellulose
Solvent:	(1) TBA (2) HOAc 15%
Detection:	UV (350, 254 nm)

Spot No.	Compound structure
1	6-OH-luteolin 7-O-glucoside
2	6-OH-luteolin 7-O-glucoside-6′′-malonate
3	luteolin 7-O-glucoside
4	luteolin 7-O-glucoside-6′′-malonate
5	diosmetin 7-O-glucoside
6	diosmetin 7-O-glucoside-6′′-malonate
7	apigenin 7-O-glucoside
8	apigenin 7-O-glucoside-6′′-malonate
9	luteolin
10	orobol
11	pratensein
12	pratensein 7-O-glucoside
13	pratensein 7-O-glucoside-6′′-malonate
14	orobol 7-O-glucoside
15	orobol 7-O-glucoside-6′′-malonate

Reprint requests to Prof. Dr. Zinsmeister.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0700–0469 $ 01.30/0

Table I. Chromatographic and UV data of flavonoids **1–9** isolated from *Bryum capillare*.

	1	**2**	**3**	**4**
Colour reactions				
UV (254, 350 nm)				
UV	p	p	p	p
UV/NH_3	p	p	y	y
UV/NA	or	or	y	y
UV/BR	p	p	p	p
TLC				
hRfvalues				
Sorbens: Cellulose				
15% HOAc	2	6	5	9
40% HOAc	21	42	41	54
BAW	24	32	41	43
TBA	15	27	32	41
Sorbens: Polyamide				
C_6H_6-MeOH-MeCOEt (4:3:3)	13	1	21	3
HPLC				
retention time min				
in solvent Nr.				
I	6.3	8.2	–	–
II	–	–	4.3	6.2
III	–	–	–	–
UV data				
MeOH	254 282 344	255 282 346	254 267sh 345	255 265sh 348
NaOMe	260 303 390[1]	261 305 391[1]	265 392	264 388
$AlCl_3$	273 302 426	268 301 412	272 297sh 366sh 425	271 296sh 320sh 366sh 420
$AlCl_3$-HCl	236sh 258sh 295 367	235sh 258sh 294 366	262sh 272 293sh 357 382sh	264 271sh 293sh 359 380sh
NaOAc	265 287 358sh 392	257sh 288 320sh 351 396sh	261 340sh 405	264 329 411
NaOAc-H_3BO_3	262 283 358	262 283 357	258 369	259 372

[1] Decomposition of the spectrum within a few minutes
p = purple, y = yellow, or = orange, ol = olive, gy = greenish yellow, bg = bluish green, yg = yellowish green

occurrence of spots 7/8 seems to be characteristic for the sporophyte.

Compounds 10–15 have been characterized earlier [7]. The chromatographic and UV-spectral data of the hitherto unknown compounds 1–9 are summarized in Table I.

*Compounds **1** and **2***

Both compounds differ in their hRf-values, being almost identical in their colour reactions and their UV-visible data (Table I). From these values they were regarded as 6-OH-luteolin derivatives substituted at C-7 [8]. Acidic and enzymic hydrolysis resulted in 6-OH-luteolin (cochromatography of the underivatized and permethylated (PM) aglycone with the corresponding authentic samples) and glucose (cochromatography with authentic glucose by TLC and GLC). Thus, compound **1** is assigned the structure 6-OH-luteolin 7-O-glucoside. Compound **2** has been shown to be converted to compound **1** upon standing in solution. Compound **1** was esterified by a 7-O-glucoside specific malonyltransferase from *Cicer arietinum* [9] to its 6″-malonylester. The latter product was demonstrated by HPLC analysis to be identical with compound **2** [9].

The FD mass spectrum of 1 shows a M^+ peak at *m/z* 464, while the M^+ peak of 2 appears at *m/z* 550. The difference of 86 mass units corresponds to the esterified malonyl moiety. Therefore compound 2 has been characterized as 6-OH-luteolin 7-O glucoside-6″-malonate.

5	**6**	**7**	**8**	**9**
p	p	p	p	p
p	p	g	g	y
ol	ol	gy	gy	y
yg	yg	bg	bg	p
6	10	9	16	3
52	60	52	60	25
52	57	67	67	85
43	51	63	67	75
63	12	52	7	26
–	–	10.0	12.4	–
–	–	–	–	–
4.2	5.4	–	–	–
252 267 284sh 340	250 266sh 288sh 340	267 332	268 330	255 267 287sh 349
268 297sh 319sh 377	266 300sh 370	268 304sh 349sh 378	266 304sh 346sh 378	266 302sh 330 402
265sh 272 295sh 362 381sh	261 271sh 292sh 361 380sh	273 299 350 376sh	274 299 350 377sh	272 302sh 320sh 368sh 421
262sh 274 292sh 352 280sh	256 273sh 294sh 353 380sh	272 298 343 370sh	275 297 341 375sh	259 274 359 388sh
254 264sh 338	250sh 266sh 341	252sh 265 295sh 388	253sh 265 336sh 388	270 325 399
252 266 343	250sh 267sh 342	266 337	267 337	263 371 432sh

*Compounds **3–6***

Because of the different colour reactions (Table I) and the UV spectra compounds **3** and **4** were assumed to be 7-O-substituted luteolin derivatives, whereas compounds **5** and **6** appeared to be 7-O- and 4′-O-luteolin derivatives. After enzymic (**3** and **5**) and acidic hydrolysis the aglycones were identified by UV analysis and cochromatography with authentic samples as luteolin (**3** and **4**) and diosmetin (**5** and **6**) respectively. In each case the sugar moiety was glucose. Cochromatography with authentic material in at least 9 different solvent systems on cellulose, polyamide and silicagel respectively revealed luteolin 7-O-glucoside for compound **3** and diosmetin 7-O-glucoside for compound **5**. Treatment of substances **3** and **5** with a malonyltransferase as mentioned before [9] and subsequent HPLC analysis resulted in their corresponding malonylesters. Therefore compounds **4** and **6** are luteolin 7-O-glucoside-6″-malonate and diosmetin 7-O-glucoside-6″-malonate, respectively.

*Compounds **7** and **8***

The chromatographic and UV spectral data (Table I) indicated that these compounds are 7-O-substituted flavones with a free 4′-OH function. Enzymic [7] and acidic [7, 8] hydrolysis resulted in the formation of apigenin and glucose. Application of enzymic malonylation as for compounds **3–6** leads to a structure for compound **7** as apigenin 7-O-glucoside and for compound **8** as its corresponding 6″-malonyl ester. FD mass spectra clearly confirmed these structures.

Compound **9** could be identified by its chromatographic and UV-spectral data and comparison with a standard as luteolin (Table I).

All of these compounds were detected on 2 D-TLC plates obtained both with plant extracts prepared with 80% MeOH at room temperature or with iced acetone. This finding indicates [12] that the various aglycones and the flavone -7-O-glucosides are not artefacts formed from the corresponding malonate esters during extraction of the plant material. The occurrence of the malonyl esters of all flavone and isoflavone glucosides in *Bryum capillare* is remarkable, since this is up to now the only representative of bryophytes where those compounds could be detected. In higher plants however malonylated flavonoids seem to be more frequent [10–17].

On the other hand, *Bryum capillare* produces flavone glucoside malonyl esters such as diosmetin 7-O-glucoside-6''-malonate, luteolin 7-O-glucoside-6''-malonate and 6-OH-luteolin-7-O-glucoside-6''-malonate which are new for the plant kingdom. The same is true for the isoflavone glucoside malonyl esters reported earlier [7]. Until now no report on malonyl transferases and malonyl esterases in Bryum has appeared, whereas several authors described such enzymes for higher plants [9, 12, 18–20].

As noted above differences in the flavonoid patterns of gametophytes and sporophytes of *B. capillare* were observed. The pattern of adult gametophytes is almost constant during the year, whereas that of the sporophytes obviously undergoes several changes in course of development. The flavonoids of the sporophyte shown in Fig. 1 are representative for green capsules without calyptra and containing immature spores. During further maturation of the spores, new as yet unknown flavonoids appear and others such as compounds **7** and **8** (Fig. I) disappear. The exclusive O-glucosidation versus C-glucosidation in the investigated species could eventually be useful for taxonomic and evolutionary aspects and this agrees with the findings in other *Bryum* species [3, 4, 6].

Experimental

Plant material. For isolation and structure determination plant material was collected from the three sites described earlier [7] and furthermore from a wall near St. Wendel/Saarland, W. Germany (40 g). From this latter population, the flavonoid patterns of gametophytes and sporophytes were investigated at different developmental stages.

Extraction and isolation

Air-dried gametophytic material was extracted as described in [7]. The sporophytes were dried rapidly (3 hours) at 80 °C in an oven and extracted in the same way as mentioned above. Compounds **1–6** were isolated by repeated 1D-PC on Whatmann 3MM paper in 15% HOAc, *n*-BuOH-HOAc-H_2O (4:1:5, upper layer, BAW), 40% HOAc. The free aglycone (9) was isolated together with the isoflavone aglycones [7]. Compounds **7** and **8** were received after column chromatography on Sephadex LH 20 with MeOH as solvent and following 1D-PC on Whatmann 3MM paper in BAW and 40% HOAc. The flavonoids were eluted from the paper bands with 80% MeOH and finally purified by CC through a Sephadex LH-20 column with MeOH as solvent. All flavone glucosides could be crystallized from aqu. MeOH, whereas the labile malonyl esters were precipitated from EtOAc.

Hydrolysis

Compounds **1–8** were hydrolyzed under the same conditions as described [7].

TLC according to ref. [7].

HPLC: Kontron chromatography: Lichrosorb RP 18 column, 7 μ, 250 × 4 mm; flow of 0,8 ml/min;

gradients: I 20%–60% B in (A + B) within 35 min, II 25% B in (B + C), III 30% B in (B + C) – A = 1.5% H_3PO_4, B = Acetonitrile, C = 3% HOAc.

GC of sugars was carried out with trimethylsilylated derivatives [21] under the following conditions:

Perkin-Elmer Gaschromatography Fraktometer F7 with FID; column: Chromosorb 6 AW-DMCS, 80–100 mesh, packed with 2.5% SE 52, N_2 flow rate of 40 ml/min; temp. 172 °C (isothermal).

The sugar moiety was identified by cochromatography (GC, TLC) with an authentic sample of glucose.

Spectroscopic methods

UV-spectra: according to ref. [22].

FD-Mass spectral data: Nomenclature of the T-fragment according to [23].

Apigenin 7-O-glucoside (7)

MS*: $[M+T+Na]^+$** 617 (12), $[2A+H]^+$ 541 (4), $[M+Na]^+$ 455 (96), $[M+H]^+$ 433 (19), $[M]^+$ 432 (14), $[A+Na]^+$ 293 (14), $[A+H]^+$ 271 (11), $[A]^+$ 271 (8).

Apigenin 7-O-glucoside-6''-malonate (8)

MS*: $[A+2T+Na]^+$** 617 (7), $[A+T+Na]^+$ 455 (95), $[A+T+H]^+$ 433 (23), $[A+T]^+$ 432 (5), $[A+Na]^+$ 293 (16), $[A+H]^+$ 271 (9), $[A]^+$ 270 (9), [malonic acid + Na]$^+$ 127 (29)

6-OH luteolin 7-O-glucoside (1)

MS*: $[M]^+$ 464 (5), $[A+H]^+$ 303 (34), $[A]^+$ 302 (100), $[A]^{++}$ 151 (6), $[T-H_2O+H]^+$ 163 (4), $[T-2H_2O]^+$ 144 (5).

6-OH luteolin 7-O-glucoside-6''-malonate

MS*: $[M+T+Na]^+$** 735 (4), $[M+T-CO_2+Na]^+$ 691 (5), $[A+2T+Na]^+$** 649 (3), [M + malonic acid $-2CO_2+Na]^+$ 571 (21), $[M]^+$ 550 (4), $[M-CO_2+Na]^+$ 529 (88), $[M-CO_2+H]^+$ 507 (9), $[A+T+Na]^+$ 487 (39), $[A+T]^+$ 464 (5), $[A+Na]^+$ 325 (85), $[A+H]^+$ 303 (64), $[A]^+$ 302 (11).

* Relative intensities in parentheses.
** Transfer of a second glucose unit.

Acknowledgements

We are indebted to Dr. Borchers, Kali Chemie AG, Hannover, for running FD-mass spectra.

We thank also Mrs. Stoll, Mrs. Freitag and Mrs. Henn for their assistance.

[1] H. Herzfelder, Beitr. Bot. Zentralblatt **38,** 355 (1921).
[2] G. Bendz and O. Martensson, Acta Chem. Scand. **15,** 1185 (1961).
[3] G. Bendz, O. Martensson, and L. Terenius, Acta Chem. Scand. **16,** 1183 (1962).
[4] G. Bendz and O. Martensson, Acta Chem. Scand. **17,** 266 (1963).
[5] J. W. McClure and H. A. Miller, Nova Hedwigia **14,** 111 (1967).
[6] E. Nilsson, Arkiv kemi **31,** 475 (1969).
[7] S. Anhut, H. D. Zinsmeister, R. Mues, W. Barz, K. Mackenbrock, J. Köster, and K. R. Markham, Phytochemistry **23,** 1073 (1984).
[8] K. R. Markham, Techniques of Flavonoid Identification, Academic Press, London 1982.
[9] J. Köster, R. Bussmann, and W. Barz, Arch. Biochem. Biophys. **234,** 513–521 (1984).
[10] F. Kreuzaler and K. Hahlbrock, Phytochemistry **12,** 1149 (1973).
[11] I. Aguinagalde and M. A. Del Pero Martinez, Phytochemistry **21,** 2875 (1982).
[12] J. Köster, D. Strack, and W. Barz, Planta Med. **48,** 131 (1983).
[13] G. Hrazdina, H. Iredale, and L. R. Mattick, Phytochemistry **16,** 297 (1977).
[14] G. Cornuz, H. Wyler, and J. Lauterwein, Phytochemistry **20,** 1461 (1981).
[15] M. Bloom and T. A. Geissmann, Phytochemistry **12,** 2005 (1973).
[16] J. Krause and D. Strack, Z. Pflanzenphysiol. **95,** 183 (1979).
[17] H. Tamura, T. Kondo, Y. Kato, and T. Goto, Tetrahedron Letters **24,** 5749 (1983).
[18] U. Matern, J. R. M. Potts, and K. Hahlbrock, Arch. Biochem. Biophys. **208,** 233 (1981).
[19] U. Matern, C. Feser, and D. Hammer, Arch. Biochem. Biophys. **226,** 206 (1983).
[20] U. Matern, Arch. Biochem. Biophys. **224,** 261 (1983).
[21] C. C. Sweely, R. Bentley, M. Makita, and W. W. Wells, J. Am. Chem. Soc. **85,** 2497 (1963).
[22] T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, Berlin 1970.
[23] R. D. Schmid, Tetrahedron **28,** 3259 (1972).