New Flavonoids from the Moss *Bryum pseudotriquetrum*

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Dedicated to Professor Robert Hegnauer on the occasion of his 70th birthday

Bryum pseudotriquetrum, Bryaceae, Musci, Flavone-O-glycosides, Flavone-C-glycosides

Fifteen flavonoids have been isolated from the moss *Bryum pseudotriquetrum*. The new natural products kaempferol 3-O-galactoside-4′-O-glucoside, scutellarein 7-O-glucoside-6″-malonyl ester, apigenin 7-O-neohesperidoside-6″-malonyl ester and luteolin 7-O-neohesperidoside-6″-malonyl ester were amongst them. Flavonols were found for the first time in mosses.

Introduction

In the context of an extensive investigation of flavonoids in mosses the occurrence of a variety of flavonoids belonging to different types of compound from *Bryum capillare* was earlier reported. Thus several flavone and isoflavone 7-O-glucosides and their malonyl esters as well as free flavone and isoflavone aglycones and biflavonoids were isolated [1–4]. Many of them were new natural products.

There have been, however, only few reports on the phenolic chemistry of other *Bryum* species. *Bryum cryophilum* [5], *B. rutilans* and *B. weigelii* [6] produce the desoxyanthocyanins luteolinidin 5-O-mono- and diglucoside. From *B. weigelii* scutellarein 7-O-glucoside was isolated in addition [7].

In *B. argenteum,* apigenin, luteolin, their 7-O-glucosides and the 6″-malonyl esters of these were found. Also the 8-hydroxyapigenin and 8-hydroxyluteolin 7-O-glucosides were isolated [8]. The flavonoid pattern of *Bryum pseudotriquetrum* is now reported here.

Results and Discussion

Fig. 1 shows a 2D-TLC of a methanolic extract of *Bryum pseudotriquetrum* containing more than twenty different phenolic compounds. Fifteen of them could be isolated in sufficient amounts for chemical analysis. Compounds **9a** and **9b** were isolated as a mixture. In Table I the chromatographic and UV spectral data, in Table II the ^{1}H NMR data and in Table III the ^{13}C NMR data are given.

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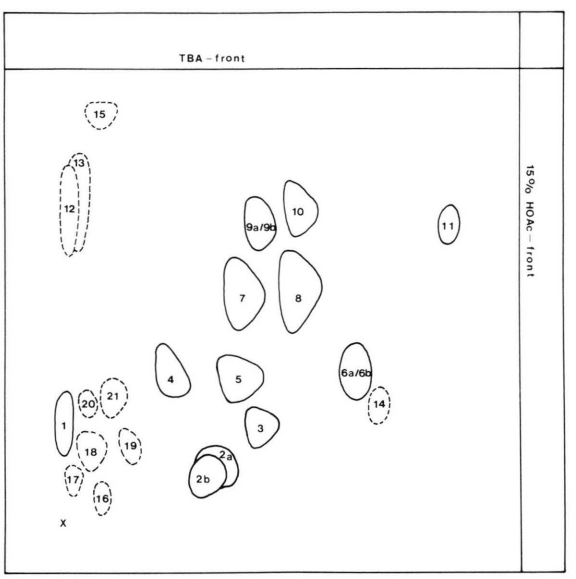

Fig. 1. TLC pattern of the phenolics obtained from dried gametophytic material (2 mg) of *Bryum pseudotriquetrum*. Adsorbent: cellulose; solvents: TBA and HOAc 15%; detection: UV (350 nm) with and without NA, dotted lines indicate minor components.

Spot No.	Compound
2a	Stellarin 2
2b	Lucenin 2
3	Vicenin 2
4	Luteolin 7-O-neohesperidoside
5	Luteolin 7-O-neohesperidoside-6″-malonyl ester
6a	Kaempferol 3,4′-di-O-glucoside
6b	Kaempferol 3-O-galactoside-4′-O-glucoside
7	Apigenin 7-O-neohesperidoside
8	Apigenin 7-O-neohesperidoside-6″-malonyl ester
9a	Kaempferol 3-O-glucoside
9b	Kaempferol 3-O-galactoside
10	Kaempferol 3-O-glucoside-6″-malonyl ester
11	Kaempferol 3-O-rhamnosyl-glucoside
15	3-Methoxy-kaempferol
21	Scutellarein 7-O-glucoside-6″-malonyl ester

Table I. Chromatographic and UV data of the flavonoids isolated from *Bryum pseudotriquetrum*.

	2a	**2b**	**3**	**4**	**5**	**6a**	**6b**	**7**	**8**	**9a/9b** (mixture)	**10**	**11**	**15**	**21**
Colour reactions UV (350 nm)														
UV	p	p	p	p	p	p	p	p	p	p	p	p	p	p
UV/NH_3	g	gy	g	gy	gy	g	g	g	g	g	g	g	p	p
UV/NA[1]	g	y	g	y	y	p	p	gy	gy	g	g	g	gy	p
UV/BR[2]	g	p	g	p	p	p	p	bg	bg	g	g	g	g	p
TLC hR_f values adsorbent: cellulose														
15% HOAc	30	40	28	18	33	58	58	31	44	39	52	73	4	10
40% HOAc	–	64	51	63	76	–	–	69	–	–	–	84	60	48
BAW	–	40	25	51	57	–	–	70	–	82	82	–	–	–
TBA	10	11	20	33	42	39	39	56	57	72	72	69	94	40
PAW adsorbent: polyamide	26	37	–	–	–	48	48	66	–	75	75	67	93	55
WEMA adsorbent: silica gel	63	65	72	35	5	–	–	34	–	13	–	51	–	–
EMAW	–	–	–	35	38	27	24	–	–	66/62	–	40	–	51
UV data						**6a/6b**								
MeOH	251,270, 343	255 sh, 272, 345	272, 330,	254, 267 sh, 348	255, 265 sh, 349	265, 302 sh, 317 sh, 342		267, 322	267, 333	265, 295 sh, 349	265, 296 sh, 350	265, 295 sh, 317 sh, 346	266, 269 sh, 350	285, 332
NaOMe	262, 282, 340 sh, 411	279, 336 sh, 413	282, 330, 398	263, 295 sh, 388	264, 299 sh, 389	275, 293 sh, 366		272, 302, 350 sh, 378	239 sh, 270, 303, 350 sh, 380	276, 324, 398	273, 324, 398	273, 323, 393	274, 324, 397	313, 377 dec.
$AlCl_3$	265 sh, 278, 299, 363, 287 sh	235, 276, 301 sh, 322 sh, 366, 426	234 sh, 278, 305, 356	235, 272, 297 sh, 320, 367 sh, 424	232 sh, 271, 294 sh, 322, 369 sh, 418	275, 297, 341, 386		274, 298, 350, 380	274, 298, 348, 376	232, 272, 302, 353, 390	232, 274, 303, 352, 388	232, 273, 303, 352, 390	232, 274, 303, 353, 396	236, 301 363
$AlCl_3$–HCl	262 sh, 279, 298, 356, 384 sh	231 sh, 261 sh, 276 sh, 298, 357	232, 278, 303, 350	260, 273 sh 294 sh, 359, 380 sh	263, 271 sh, 293 sh, 359, 380 sh	275, 296 sh, 336, 386		275, 298, 340, 378	274, 296, 339, 374	231, 273, 300, 346, 390	232, 273, 301, 348, 387	231, 274, 300, 347, 388	231, 274, 301, 346, 390	235, 299 356
NaOAc	281, 318, 392	238 sh, 274, 323, 395	281, 303 sh, 338 sh, 390	259, 362, 400 sh	264, 412	273, 269 sh, 357		265, 350 sh, 386	267, 338, 387 sh	273, 302, 373	273, 302, 370	270, 298, 358	273, 300, 368	288, 332 386 sh
NaOAc–H_3Bo_3	275, 283 sh, 337	265, 378, 433 sh	276, 322, 345 sh, 403 sh	259, 372	260, 374	266, 302 sh, 319 sh, 341		267, 335	267, 334	265, 297 sh, 350	265, 299 sh, 350	264, 347	266, 300, 350	290, 326

dec. = Decomposition of the compound within a few minutes.
p = purple, y = yellow, g = green, gy = greenish yellow, bg = bluish green.
[1]NA = Naturstoffreagenz A, see [3].
[2]BR = Benedikt's reagent, see [3].

*Compounds **2a, 2b** and **3***

The chromatographic and UV data as well as a positive Wessely-Moser-rearrangement indicate di-C-glycosides of chrysoeriol (**2a**), luteolin (**2b**) and apigenin (**3**). Cochromatography (TLC, HPLC) with authentic samples using various adsorbents/solvent-combinations revealed the occurrence of stellarin 2 (**2a**), lucenin 2 (**2b**) and vicenin 2 (**3**). IR an NMR data confirmed these results.

*Compounds **4** and **5***

The chromatographic and UV spectral data indicate luteolin derivatives substituted in C-7. Acidic hydrolysis of both compounds gave luteolin, d-glucose, l-rhamnose, and for **5**, malonic acid also. Compound **5** proved to be unstable. TLC and HPLC cochromatography with authentic samples, together with NMR studies enabled compound **4** to be assigned the structure of luteolin 7-O-β-neohesperidoside. Compound **5** is the corresponding 6″-malonyl ester which shows a mass of 680 *m/z*, 85 mass units more than compound **4** (595 *m/z*), due to the malonic acid moiety. In ^{13}C NMR the characteristic shifts of 64.3 ppm and 74.8 ppm for C-6″ anc C-5″, respectively, appear, indicating substitution at C-6″. This is confirmed by ^1H NMR showing shifts of 4.50 ppm (d, J = 11 Hz) and 4.02 ppm (multiplet) for H-6″A and H-6″B, respectively.

Table II. ^{1}H NMR data of compounds **4–11, 15** and **21** (DMSO-d_6, ambient temperature, 400 MHz).

Assignment of protons	**4**	**5**	**6a**	**6b**	**7**	**8**
H-3	6.75 s	6.76 s	–	–	6.86 s	6.83 s
CH_3-3	–	–	–	–	–	–
OH-5	13.00 s	13.00 s	12.55 s	12.52 s	12.96 s	12.96 s
H-6	6.38 d (J = 2 Hz)	6.33 d (J = 2 Hz)	6.23 s	6.16 s	6.37 d (J = 2 Hz)	6.36 s
H-8	6.74 d (J = 2 Hz)	6.74 d (J = 2 Hz)	6.46 s	6.39 s	6.79 d (J = 2 Hz)	6.74 s
H-2′	7.40 d (J = 2 Hz)	7.55 d (J = 2 Hz)	8.13 d (J = 9 Hz)	8.14 d (J = 9 Hz)	7.93 d (J = 9 Hz)	7.91 d (J = 8 Hz)
H-6′	7.43 dd (J = 2; 8 Hz)	7.38 dd (J = 2; 8 Hz)				
H-3′	–	–	7.16 d (J = 9 Hz)	7.12 d (J = 9 Hz)	6.94 d (J = 9 Hz)	6.94 d (J = 8 Hz)
H-5′	6.90 d (J = 8 Hz)	6.85 d (J = 8 Hz)				
H-1″	5.25 d (J = 7 Hz)	5.21 d (J = 7 Hz)	5.48 d (J = 7 Hz)	5.38 d (J = 8 Hz)	5.23 d (J = 7 Hz)	5.24 d (J = 7 Hz)
H-6″A*	–	4.50 d (J = 11 Hz)	–	–	–	4.35 d (J = 11 Hz)
H-6″B*	–	4.02 m	–	–	–	4.10 dd (J = 7; 12 Hz)
H-1‴	5.13 s	5.13 s	5.02 d (J = 7 Hz)	5.00 d (J = 7 Hz)	5.13 s	5.13 s
CH_3-6‴	1.21 d (J = 6 Hz)	1.22 d (J = 6 Hz)	–	–	1.20 d (J = 6 Hz)	1.20 d (J = 6 Hz)
Residual sugar protons and CH_2 (malonyl)*	3.1–3.8 m	3.1–3.8 m	3.0–3.8 m	3.0–3.8 m	3.1–3.8 m	3.1–3.8 m

Assignment of protons	**9a/9b** determined as mixture	**10**	**11**	**15**	**21**
H-3	–	–	–	–	6.61 s
CH_3-3	–	–	–	3.77 s	–
OH-5	12.61 s	12.62 s	12.63 s	12.50 s	12.70 s
H-6	6.21 d (J = 2 Hz)	6.21 d (J = 2 Hz)	6.19 d (J = 2 Hz)	6.17 d (J = 2 Hz)	–
H-8	6.43 d (J = 2 Hz)	6.44 s	6.42 s	6.41 d (J = 2 Hz)	6.94 s
H-2′	8.04/8.07 d (J = 9 Hz)	7.97 d (J = 9 Hz)	8.03 d (J = 9 Hz)	7.93 d (J = 9 Hz)	7.94 d (J = 9 Hz)
H-6′					
H-3′	6.86/6.88 d (J = 9 Hz)	6.88 d (J = 9 Hz)	6.88 d (J = 9 Hz)	6.94 d (J = 8 Hz)	6.94 d (J = 9 Hz)
H-5′					
H-1″	5.46 d (J = 7 Hz)/5.40 d (J = 8 Hz)	5.28 d (J = 7 Hz)	5.65 d (J = 7 Hz)	–	5.03 d (J = 7 Hz)
H-6″A*	–	4.11 d (J = 10 Hz)	–	–	4.37 d (J = 12 Hz)
H-6″B*	–	4.03 m	–	–	4.14 m
H-1‴	–	–	5.07 s	–	–
CH_3-6‴	–	–	1.20 d (J = 6 Hz)	–	–
Residual sugar protons and CH_2 (malonyl)*	3.0–3.8 m	3.0–3.8 m	3.0–3.8 m	–	3.0–3.8 m

* For C-6″ malonyl ester.

Compounds 7 and 8

The chromatographical and UV spectral data indicate apigenin 7-O-glycosides. Acidic hydrolysis of **7** and **8** yielded apigenin, d-glucose and l-rhamnose, and for **8,** malonic acid in addition. FD mass spectra of **8** (see Experimental) showed characteristic fragments of its decarboxylation and demalonization products as well as of a transacetylation. According to the results of TLC, HPLC and NMR spectroscopy compound **7** is apigenin 7-O-β-neohesperidoside. The shifts in the ^{1}H and ^{13}C NMR spectrum of **8,** caused by malonylation at C-6″, are analogous to these of compound **5.** The splitting of the signal of H-6″B into a double doublet (4.10 ppm) was observed. This is

Table III. ^{13}C NMR data of compounds **4–10** (DMSO-d_6, ambient temperature 100 MHz).

Assignment of carbons	**4**	**5**	**6a/6b** determined as mixture	**7**	**8**	**9a/9b** determined as mixture	**10**
2	164.9	165.4	155.6[a]	164.3	164.3	156.4/156.3	156.4[a]
3	103.6	103.6	133.8	103.2	103.1	133.2/133.2	133.2
4	182.2	182.2	177.5	182.0	181.8	177.5/177.4	177.3
5	161.6	161.4	161.2	161.4[a]	161.5[a]	161.2	161.1
6	99.7	100.1	98.8	99.3	99.3	98.7	98.8
7	162.9	162.8	164.3	162.5	162.2	164.1	164.5
8	94.8	94.6	93.7	94.5	94.4	93.6	93.7
9	157.4	157.5	156.5[a]	157.0	157.0	156.4	156.7[a]
10	105.8	106.1	104.1	105.4	105.5	103.9	103.7
1′	121.7	121.7	123.7	121.0	120.8	120.9	120.5
2′	113.9	113.8	130.6	128.6	128.5	130.9/130.8	130.7
3′	146.2	146.8	115.8	116.0	116.1	115.0	115.1
4′	150.4	150.5	159.2	161.1[a]	161.1[a]	159.9	160.1
5′	116.4	116.3	115.8	116.0	116.1	115.0	115.1
6′	119.5	119.2	130.6	128.6	128.5	130.9/130.8	130.7
1″	98.3	98.5	100.9/101.7	97.9	97.7	100.9/101.7	101.6
1‴	100.9	100.8	100.0	100.5	100.5	–	–
2″	76.7[a]	76.5[a]	74.2/71.2	76.3[b]	76.2[b]	74.2/71.2	74.0[b]
2‴	70.8[b]	70.8[b]	73.2	70.4[c]	70.3[c]	–	–
3″	77.4[a]	77.3[a]	76.5/73.2	77.0[b]	76.8[b]	76.4/73.1	76.0
3‴	70.9[b]	70.9[b]	76.5	70.5[c]	70.5[c]	–	–
4″	70.1[b]	70.4[b]	69.9/67.4	69.7[c]	69.7[c]	69.9/67.9	69.5
4‴	72.3	72.3	69.7	71.9	71.9	–	–
5″	77.6[a]	74.8	77.5/75.8	77.2[b]	73.8	77.4/75.8	73.9[b]
5‴	68.7	68.8	77.1	68.3	68.3	–	–
6″	60.9	64.3	60.9/60.2	60.5	63.6	60.9/60.2	63.1
6‴	18.5	18.5	60.7	18.0	18.1	–	–
Assignment of malonic acid carbons							
2	–	43.8	–	–	42.9	–	42.4
1/3	–	168.3	–	–	167.7	–	167.9
	–	168.6	–	–	168.1	–	168.0

[a,b,c] Values bearing the same superscript in anyone spectrum may be reversed.

due to coupling with H-6″A (J = 12 Hz) and H-5″ (J = 7 Hz). Thus compound **8** can be assigned the structure of apigenin 7-O-β-neohesperidoside-6″-malonyl ester.

*Compound **21***

The chromatographic and UV spectral data indicate a 7-O-substituted scutellarein derivative. The slow decomposition of **21** to a stable product suggests again a malonyl ester. Acidic hydrolysis resulted in scutellarein, d-glucose and malonic acid. FD mass spectra (see Experimental) show the typical fragmentation pattern for a malonyl ester. The shifting of the H-6″ signals into the lower field in the ^1H NMR spectrum demonstrates the malonylation at C-6″ of the glucose moiety. Thus compound **21** may be assigned the structure of scutellarein 7-O-β-d-glucopyranoside-6″-malonyl ester.

*Compounds **9a, 9b, 10***

As mentioned above **9a** and **9b** were obtained only as a mixture. The chromatographic and UV data indicate 3-O-substituted kaempferol derivatives. Acidic hydrolysis resulted in kaempferol, d-galactose and d-glucose. The TLC and HPLC comparison with authentic substances showed identity with kaempferol 3-O-β-d-glucopyranoside (**9a**) and kaempferol 3-O-β-d-galactopyranoside (**9b**), respectively. The ^1H NMR spectrum confirms the structure of both components of the mix-

ture. All signals of the ^{13}C NMR spectrum can be attributed to the supposed structures and show a good concurrence with values reported earlier [9, 10]. Due to the different substitution of **9a** and **9b** at C-3 the corresponding signal appears twice as do those of C-2, C-4 and C-2′/6′. For the same reason the signals of the B-ring protons in the ^1H NMR spectrum are duplicated. The high-coupling constant of the corresponding anomeric protons (glucose: J = 7.3 Hz, galactose: J = 7.6 Hz) indicates the β-glycosidic linkage for each sugar [11]. The ^{13}C NMR spectrum of **10** shows the signals characteristic for kaempferol 3-O-glucoside and additionally, the typical shifts for malonyl esters (compare compounds **5, 8, 21**). From the FD mass spectrum the typical reactions (transacetylation, decarboxylation) can be deduced. Thus **10** can be assigned the structure of kaempferol 3-O-β-d-glucopyranoside-6″-malonyl ester.

*Compounds **6a, 6b***

Acidic hydrolysis of **6a** resulted in kaempferol and d-glucose, that of **6b** in kaempferol, d-glucose and d-galactose. Partial hydrolysis gave kaempferol 4′-O-glucoside as an intermediate of both compounds (cochromatography, TLC, HPLC, UV data). Enzymatic hydrolysis of **6a** yielded kaempferol 3-O-glucoside, of **6b,** kaempferol 3-O-galactoside as well as d-glucose from both. Combining the hydrolytic with the ^1H and ^{13}C NMR data, compound **6a** can be assigned the structure of kaempferol 3,4-di-O-β-d-glucopyranoside and **6b** that of kaempferol 3-O-β-d-galactopyranoside-4′-O-β-d-glucopyranoside.

*Compound **15***

The chromatographic and UV data indicate again kaempferol derivatives substituted at C-3. The hR_f values reveal, however, an aglycone which could be established by identical TLC and HPLC cochromatography with authentic samples. The EI-MS (see Experimental) shows a molecular ion peak at 300, and the characteristic fragmentation pattern [12] for the supposed structure. The ^1H NMR spectrum (singulet at 3.77 ppm – OCH_3) corroborated the definitive structure of 3-methoxy-kaempferol.

*Compound **11***

Chromatographic, hydrolytic and UV data indicate a kaempferol 3-O-rhamnosylglucoside. Rhamnose is also indicated by a singulett of the anomeric proton at 5.07 ppm and the doublet of $-CH_3$ at 1.22 ppm (J = 6.1 Hz). The β-glycosidic binding of the glucose moiety is demonstrated by the high coupling constant of H-1″ (5.65 ppm, d; J = 7 Hz). Since the amount of substance available was very small the interglycosidic linkage could not be exactly assigned. According to comparative NMR studies with various rhamnoglucosides there is, however, some evidence for a 1,2-linkage. Compound **1** showed a bright blue fluorescence and is extremely unstable, it may not be a flavonoid at all.

The TLC spots (see Fig. 1) 12–14 and 16–20 contain several further phenolic compounds. Since the single components of these spots were available only in traces a structure elucidation is at present not possible. The spot complex 12/13 consists of at least six different compounds. According to their TLC and HPLC behaviour they are assumed to be biflavonoids. Spot 14 shows a reddish colour which changes to blue after alkaline treatment, typical for anthocyanin pigments.

The phenolic pattern described above has so far been found for only one habitat (Bodental, Carinthia, Austria), whereas the TLC patterns of *Bryum pseudotriquetrum* of three other habitats (see Experimental) were somewhat different. The occurrence of flavonols, however, which could be demonstrated for the first time for mosses, is common for plants of all these investigated habitats. Regarding the flavonoid chemistry of *Bryum* species there is a remarkable variety of compounds belonging to different flavonoid classes. Thus, up to now, flavones, flavonols, isoflavones, anthocyanins and biflavonoids have been found; the glycosidation and glycosilation also vary strikingly.

Malonylation seems to be a characteristic feature for the genus *Bryum* as found additionally in *B. capillare* [2], *B. argentum* [8] and *B. schleicheri* [13].

The chemotaxonomic relevance of these results can only be judged after investigation of many further bryophyte species.

Experimental

Plant material

Bryum pseudotriquetrum (Hedw.) Gaertn., Meyer and Scherb. was collected from the following habitats: 1. Bodental, Carinthia, Austria; 2. Wormsa-Tal, Vosges, France; 3. Rimbach, Vosges, France; 4. Peppenkum, Saarland, Federal Republic of Germany. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik (acronym SAAR), Universität des Saarlandes. The species was identified by Prof. Dr. H. Ochi, Tottori, Japan.

Extraction and isolation

After careful cleaning, 100 g air-dried gametophytic material (habitat 1) was ground in a Waring blendor and extracted with $CHCl_3$ to remove chlorophyll and lipids; subsequently the phenolics were extracted with 80% aq. EtOH, 80% aq. MeOH and 50% aq. MeOH. Compound **15** was found in the $CHCl_3$ fraction, whereas all other flavonoids were detected in the combined alcoholic fraction. The compounds were isolated by repeated CC on cellulose (microcrystalline) with aq. HOAc, *n*-BuOH–HOAc–H_2O (4:1:5, upper layer, BAW), BuOH–2-HOAc–H_2O (14:1:5, BEW), *n*-pentanol–HOAc–H_2O (2:1:1, PAW); on polyamide SC 6 with aq. acetone, EtOAc–MeCOEt–HOAc–H_2O (5:3:1:1, EMAW); on silica gel with EMAW; on Sephadex LH 20 with aq. MeOH. Final purification of each flavonoid was achieved on a Sephadex column with 80% aq. MeOH. Most of the compounds could be crystallized from aq. MeOH, whereas the labile malonyl esters were precipitated from EtOAc. The amounts obtained for each compound were between 0.5–96 mg.

Hydrolytic methods

Acidic hydrolysis: 1 N TFA, 1 h under reflux (total); 0.1 N TFA or 40% HOAc, 15 min under reflux (partial). Enzymatic hydrolysis: β-glucosidase (Fluka), 2 h at room temperature in H_2O.

Chromatography

TLC: adsorbents: cellulose microcrystalline (Avicel, Merck); precoated sheets: cellulose F 1440 (Schleicher and Schüll), polyamide-6, Polygram (Macherey and Nagel), silica gel 60 (Merck). Solvent systems: flavonoids: 15% HOAc, 40% HOAc, TBA, BAW, PAW (cellulose); H_2O–MeCoEt–MeOH–3,5-pentanedione (13:3:3:1, WEMA) (polyamide); EMAW (silica gel); sugars: EtOAc–C_5H_5N–HOAc–H_2O (36:36:7:21); detection: aniline phthalate spray reagent (Merck).

HPLC: Waters M·45: Spherisorb ODS II, 5 µ, 250 × 4 mm, flow of 1.0 ml/min: isocratic and/or gradients using H_2O/MeOH mixtures containing 5% HOAc.

Spectroscopic methods

UV spectroscopy: according to [14]. NMR spectroscopy: Bruker AM 400, 297 K, DMSO-d_6.

FD mass spectroscopy: Varian MAT 311 A (relative intensity in parenthesis): Compound **4:** $[M+H]^+$ 595 (100). Compound **5:** M^+ 680 (29), $[M-CO_2+H]^+$ 637 (54), [M–rhamnose–glucose–malonic acid]$^+$ 286 (100). Compound **8:** $[M+HOAc+H-CO_2]^+$ 663 (100), $[M-CO_2]^+$ 620 (68), [M–malonic acid]$^+$ 579 (13). Compound **10:** $[M+HOAc-CO_2]^+$ 532 (46), $[M-CO_2+H]^+$ 491 (100). Compound **21:** $[M+HOAc-CO_2]^+$ 532 (11), $[M-CO_2+Na]^+$ 513 (100).

EI mass spectroscopy: Varian MAT 311, 70 eV, ion source temperature: 150 °C; probe temp.: 200–220 °C; relative intensities in parenthesis: Compound **15:** M^+ 300 (100), $[M-H]^+$ 299 (86), $[M-H_2O]^+$ 282 (25), $[M-CO-H]^+$ 271 (24), $[M-OCH_3]^+$ 269 (19), $[M-CH_3-CO]^+$ 257 (67), $[A_1+H]^+$ 153 (33), $[B_2]^+$ 121 (54), $[B_2-CO]^+$ 93 (29).

Terminology for the fragmentation pattern according to [15].

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