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FLAVONOIDS OF *PLAGIOCHILA ASPLENIOIDES*

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Abstract—Besides an apigenin- and a luteolin-di-*C*-glycoside, 5 previously unknown di-*C*-glycosides of tricetin were identified in the gametophytic and sporophytic tissues of *Plagiochila asplenioides*. Two of them were new 6,8-di-*C*-hexopyranosyltricetins, and two were new 6-*C*-hexopyranosyl-8-C-pentopyranosyltricetins. 6-*C*-Hexopyranosyl-8-C-pentopyranosyltricetin-5'-methyl ether was also found.

INTRODUCTION

In the last 10 years the occurrence of flavonoids in liverworts (Hepaticae) has become well established [1–5]. Whereas the order Marchantiales has been studied in detail, mainly by Markham and coworkers [6–13], the order Jungermaniales has until now been largely ignored. We started some years ago an extensive study of European Jungermaniales; 60 species of 35 genera belonging to 23 families—about 80% of those occurring in Europe—have been so far included. In 18 species of 11 genera and 11 families flavonoids could be detected. They were provisionally identified from their chromatographic data and behaviour on acid hydrolysis to be flavone *O*- and *C*-glycosides [14].

One of the investigated species which has flavonoids is *Plagiochila asplenioides*. Glycoflavones in this species were previously mentioned by Harborne [15] and in a previous communication we reported the occurrence of luteolin-di-*C*-glycosides [16]. The present paper revises this communication and reports additionally the isolation of a variety of tricetin-di-*C*-glycosides and an apigenin-di-*C*-glycoside (vicenin).

RESULTS

From the extract of 1 kg air-dried mixed gametophytic and sporophytic plant material 8 different flavone glycosides (compounds 1–**8**) could be isolated by combined column–PC. The chromatographic and UV-spectral data are given in Table 1; the MS-data of the permethylated (PM) and perdeuteriomethylated (PDM) derivatives according to Brimacombe *et al.* [17] are found in Table 2. The yields were compound 1 (42 mg), 2 (5 mg), 3 (5 mg), 4 (16 mg), 5 (8.5 mg), 6 (tr), 7 (7 mg) and 8 (2 mg).

Compound **8**. This was the only substance clearly separated from the other compounds which appeared on a 2-D chromatogram as a complex streaking spot with different concentration centers after spraying with Naturstoffreagenz A [16] (NA). The deep purple absorbance in UV (254, 350 nm), the fluorescence after spraying with NA and Benedicts reagent and the UV-spectral data indicated an apigenin derivative. After acid treatment no aglycone or free sugar was produced, but after PC an additional spot close to the original one appeared as a consequence of Wessely–Moser-rearrangement [18] of a *C*-glycoside. The MS showed a M^+ at *m/e* 748 and at *m/e* 781 corresponding with the MW of PM- and PDM-di-*C*-hexosylapigenins respectively. The MS-data of the PM-derivative are in agreement with those of Bouillant *et al.* [19, 20] for 6,8-di-*C*-hexosylapigenin (vicenin). However, it did not correspond with vicenin 2 (6,8-di-*C*-*β*-D-glucopyranosylapigenin) after co-chromatography with an authentic sample and from this finding it is concluded that compound 8 is a 6,8-di-*C*-hexosylapigenin with different hexoses at C-6 and C-8. Further structural confirmation of the sugar-moiety was impossible because of the small quantity of the compound isolated.

Compound **5**. The chromatographic behaviour was different from that of compound **8** and its UV-spectrum (Table 1) suggested that it was a flavone glycoside of the luteolin type. Acid treatment indicated *C*-glycosidation and the M^+ at *m/e* 778 and *m/e* 814 corresponded with the MW of PM- and PDM-di-*C*-hexosylluteolins respectively. This is confirmed by the absence of pentose fragment peaks [19, 20]. The periodate –$NaBH_4$ degradation product was glycerol [21]. Compound **5** was thus concluded to be 6-8-di-*C*-hexopyranosylluteolin. After acid isomerization no additional spot was observed, showing that the presence of the same sugar moieties at the *C*-6 and *C*-8 position. Co-chromatography with natural 6-8-di-*C*-*β*-D-glucopyranosylluteolin (lucenin 2) was identical in four different solvents (Table 1).

Compounds **1**–**4** and **7** showed very similar chromatographic behaviour to compound **5** (lucenin). Also the UV-spectra were nearly identical, with one difference that the Band II region [22] of the NaOMe spectrum of these compounds showed only shoulders or very weak peaks. Nevertheless the similarity of the UV-spectra with those of lucenins led in a previous communication [16] to the conclusion that they are also luteolin-6,8-di-*C*-glycosides. However, the correct interpretation of their MS which was first possible by the extensive studies of Chopin's group [19, 20] led us to the conclusion that these compounds are derivatives of 5'-hydroxyluteolin (tricetin).

Table 1. Chromatographic and UV-spectral data of the glycoflavones from *Plagiochila asplenioides*

Compound No.	1	2	3	4	5	6	7	8
spot fluorescence								
UV	deep purple	deep purple	deep purple	deep purple	deep purple	deep purple	deep purple	deep purple
UV/NA*	orange	organe	orange	orange	orange	orange	orange	olive green
UV/BR†	dark	dark	dark	dark	dark	dark	dark	yellow-green
15% HOAc	0.25	0.16	0.26	0.29	0 34	0.23	0.30	0.49
40% HOAc	0.48	0.41	0.50	0 60	0 62	0.64	0.64	0.73
R_f‖ BAW‡	0.11	0.13	0.15	0.17	0 19	0 20	0.22	0.25
BAW 27%§	0.24	0.24	0.28	0.31	0.35	0.32	0 34	0.46
MeOH	262 *sh*, 272, 300 *sh*, 352	262 *sh*, 272, 354	264 *sh*, 272, 300 *sh*, 356	272, 300 *sh*, 350	257 *sh*, 272, 348	262 *sh*, 272, 350	262 *sh*, 272, 300 *sh* 352	272, 304 *sh*, 330
NaOMe	230 *sh*, 284 *sh*, 330 *sh*, 428	224 *sh*, 280 *sh*, 330 *sh*, 428	230 *sh*, 280 *sh*, 330 *sh*, 424	234 *sh*, 268 *sh*, 286, 332 *sh*, 420	234 *sh*, 284, 330, 416	263 *sh*, 276 *sh*, 330 *sh*, 418	268, 332, 426	234 *sh*, 282, 332, 396
λ_{max} $AlCl_3$	234 *sh*, 273, 312 *sh*, 370 *sh*, 420	234 *sh*, 272, 310 *sh*, 370 *sh*, 416	232 *sh*, 274, 310 *sh*, 370 *sh*, 420	236 *sh*, 270, 273 *sh*, 306 *sh*, 370 *sh*, 430	236 *sh*, 278, 302 *sh*, 330, 370 *sh*, 426	240 *sh*, 274, 304 *sh*, 365 *sh*, 428	276, 318 *sh*, 368 *sh*, 432	262 *sh*, 278, 304, 350, 380 *sh*
$AlCl_3$-HCl	236 *sh*, 278, 306, 366, 390 *sh*	272, 306, 362, 390 *sh*	278, 306, 364, 390 *sh*	232 *sh*, 278, 306, 362, 390 *sh*	232 *sh*, 264 *sh*, 278, 298, 360, 386 *sh*	276, 304, 362, 386 *sh*	280, 308, 364, 390 *sh*	278, 304, 350, 380 *sh*
NaOAc	264, 280 *sh*, 330 *sh*, 418	264, 282 *sh*, 328, 412	266, 280 *sh*, 330 *sh*, 414	262, 282 *sh*, 330, 414	272 *sh*, 280, 330 *sh*, 404	264, 280 *sh*, 330 *sh*, 414	264, 280 *sh*, 328 *sh*, 416	282, 304 *sh*, 336, 390
NaOAc-H_3BO_3	272 *sh*, 390 *sh*, 430	272 *sh*, 394 *sh*, 434	264 *sh*, 272 *sh*, 390 *sh*, 432	260 *sh*, 282 *sh*, 390 *sh*, 432	266, 286 *sh*, 380, 426	260 *sh*, 284 *sh*, 385 *sh*, 430	262 *sh*, 284 *sh*, 385 *sh*, 430	282, 304, 342, 400

* Naturstoffreagenz A [16] † Benedicts reagent [16] ‡ *n*-BuOH–HOAc–H_2O 4:1:5, upper phase § *n*-BuOH–HOAc 27% 1:1 ‖ TLC on microcrystalline cellulose

Compounds **1** *and* **4**. After acid isomerization of compound **1** no additional spot was observed, whereas acid isomerization of compound **4** resulted in an additional spot close to the original one on PC. The M^+ of the PM- and PDM-derivatives of both compounds are found at *m/e* 808 (PM) and *m/e* 847 (PDM). Their fragment ions are also the same differing only in relative intensities. Further structure confirmation of the sugar moiety basing on these differences could not be achieved. The M^+ and the number of fragment ions of the PM-derivatives are the same as for compound **5** (lucenin) but 30 a.m.u. higher. This signifies one additional OMe group. The decision whether this group is of natural occurrence or an artifact of methylation was made from the MS of the PDM-derivatives. The difference between the mass of the M^+ ions of the PM- and PDM-derivatives of compounds **1** and **4** is 39 a.m.u. The difference between the mass of the M^+ of the PM- and PDM-derivative of compound **5** is 36 a.m.u. Thus compounds **1** and **4** must have one more free OH-group in the underived molecule than compound **5**. This OH-group must therefore be attached to the aglycone; the UV-spectral data indicate that the 5'-position of the underived compound must be substituted by this OH-group. Thus these compounds are tricetins with *C*-linked hexoses at the *C*-6 and *C*-8 position. A PMR spectrum in DMSO was run for substance 1 and although it was of poor quality, because of the small amount of compound available, it proved the above structure. Signals for A-ring protons were absent, a sharp singlet for the *C*-3 proton (δ = 6,62 ppm) and a broad signal for the *C*-2' and *C*-6' protons (δ = 7,16 ppm) were present; there were no signals for other B-ring protons. The resolution in the sugar proton region was not sufficiently clear to decide the mode of sugar linkage. The periodate –$NaBH_4$ degradation product for both compounds was glycerol. From these data it is concluded, that both compounds are 6,8-di-C-hexopyranosyltricetins. Acid isomerization indicated that compound **1** is substituted by the same hexose, probably glucose [23] and compound **4** by different hexopyranoses, glucose and another hexopyranose.

Compounds **2** *and* **3**. The chromatographic, spectral (UV, MS) and acid isomerization-data showed that these substances were also 6,8-di-*C*-glycosyltricetins. However, in contrast to compounds **1** and **4** the MS of their PM- and PDM-derivatives showed, in addition to the hexose fragments, pentose fragments. In each case the relative intensities of the hexose fragment peaks, $M^+ - 175$ (PM) and $M^+ - 184$ (PDM), were higher than those of the pentose fragment peaks $M^+ - 131$ (PM) and $M^+ - 137$ (PDM). According to the studies of Bouillant *et al.* [19] in both molecules the hexose must be attached to the

Table 2. MS Data for the PM and PDM* derivatives of the glycoflavones from *Plagiochila asplenioides*

Compound No	1	2	3	4	5	7	8
M	808 (847)	764 (800)	764 (800)	808 (847)	778 (814)	764 (797)	748 (781)
Fragments				Relative intensity (%)			
M^+	5 (4)	6 (7)	16 (11)	4 (4)	9 (5)	8 (11)	6 (6)
$M^+ - 14$ (−17)	12 (14)	13 (25)	13 (32)	14 (15)	14 (13)	10 (15)	12 (12)
$M^+ - 15$ (−18)	26 (28)	28 (32)	32 (36)	31 (33)	31 (30)	34 (33)	28 (31)
$M^+ - 29$ (−32)	14 (11)	14 (14)	14 (15)	15 (15)	16 (12)	12 (14)	13 (12)
$M^+ - 30$ (−33)	50 (32)	50 (50)	37 (44)	46 (47)	44 (39)	39 (44)	44 (42)
$M^+ - 31$ (−34)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
$M^+ - 47$ (−52)	10 (11)	14 (13)	11 (13)	12 (12)	13 (9)	11 (10)	13 (8)
$M^+ - 103$ (−109)	8 (23)	22 (22)	13 (23)	18 (24)	19 (17)	— (16)	17 (20)
$M^+ - 131$ (−137)	— (—)	9 (26)	26 (37)	— (—)	— (—)	23 (23)	— (—)
$M^+ - 163$ (−173)	36 (36)	45 (43)	32 (46)	39 (45)	38 (37)	55 (40)	42 (35)
$M^+ - 175$ (−184)	67 (83)	61 (89)	48 (83)	70 (98)	63 (98)	71 (77)	78 (88)
$M^+ - 189$ (−201)	12 (14)	17 (21)	11 (31)	10 (15)	19 (14)	15 (12)	14 (17)
$M^+ - 205$ (−220)	13 (14)	16 (19)	11 (16)	11 (15)	12 (12)	13 (15)	12 (16)

* PDM-values in parentheses.

C-6 position and the pentose to the C-8 position. The periodate $-NaBH_4$ degradation products for both compounds were glycerol and ethyleneglycol derived from an aldohexose in a pyranose form and a pentose in a pyranose form respectively. The occurrence of different sugars at the C-6 and C-8-position is supported by the results of acid isomerization where additional spots were observed. From all these data it is concluded, that compounds 2 and 3 are both 6-C-hexopyranosyl-8-C-pentopyranosyl-tricetins but contain different stereoisomers of the sugar moieties.

Compound **7**. The experimental data (of Tables 1 and 2) led to the conclusion that this compound must also be a 6,8-di-C-glycosyl-tricetin of a similar type to compounds **2** and **3**. The M^+ of the PM-derivative was found to be the same as those of compounds **2** and **3**, but the M^+ of the PDM-derivative was at *m/e* 797, compared with those of the PDM derivatives of compounds **2** and **3** at *m/e* 800; this is due to one free OH group less in the underivatized molecule than in compounds **2** and **3**. Further experiments also indicated that the 5′ (or 3′)-position is methoxylated. The results of acid isomerization and periodate $-NaBH_4$ degradation were similar to those of compound **3**. Compound **7** is therefore a 6-C-hexopyranosyl-8-C-pentopyranosyltricetin-5′ (or 3′)-methylether.

Compound **6** was isolated in such a small quantity that it was not possible to run MS of its derivatives. From the chromatographic, UV-spectral and acid isomerization data it could possibly be another tricetin-di-C-glycoside with different sugars at the C-6 and C-8 positions.

To our knowledge this is the first report of tricetin-di-C-glycosides in plants. Until now only a few reports of the occurrence of O-glycosides or sulphates of tricetin have appeared [24–26]. Our work has also shown that flavone-C-glycosides are more common in liverworts than was previously supposed. This is supported by the fact that most flavonoid containing species of Jungermaniales, investigated in this laboratory, seem to have C-glycosides confirming the earlier results based on acid treatment. Besides the occurrence of the previously reported di-C-glycosides of the apigenin- and luteolin-type in liverworts [3, 4, 8, 12] we have found a third flavone aglycone type with di-C-glycosidation. These tricetin-C-glycosides are very unstable in the presence of oxygen, like luteolin-di-C-glycosides [27]. They decompose or polymerize to products, which are soluble only in water giving a bright yellow colour. After development with standard solvents for flavonoids they cannot be detected on PC in UV or after spraying with the usual reagents for flavonoids.

EXPERIMENTAL

Plant material. Plagiochila asplenioides (L.) *Dum.* was collected in June, 1974 near Klagenfurt, Austria. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken.

Isolation procedure. Air-dried plant material was extracted as described previously [16]. Individual compounds were isolated by column chromatography on cellulose followed by PC (often "overrun" to achieve separation) on Whatman 3 MM paper. Purity of compounds was monitored on TLC (microcrystalline cellulose and polyamide). Solvents: column 5% up to 40% HOAc; PC (see Table 1); TLC-microcrystalline cellulose (see Table 1), polyamide—20 and 50% MeOH. The compounds did not crystallise and were lyophilised. Chromatographic and UV-spectral identification of the compounds was carried out as previously described [16].

Acid isomerization. Compounds (1–2 mg) were treated with 5 ml 2 N HCl under reflux at 100° for 1½–2 hr.

Periodate $-NaBH_4$ degradation was performed according to ref. [21]; detection of the degradation products was done by the method of ref. [28].

PMR. Flavone glycoside (10 mg) dissolved in DMSO d_6 and deuterated TFA, TMS internal standard; 60 MHz.

MS. Preparation of PM- and PDM-derivatives was performed according to ref. [17].

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