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ISOSCUTELLAREIN AND HYPOLAETIN 8-GLUCURONIDES FROM THE LIVERWORT *MARCHANTIA BERTEROANA*

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Key Word Index—*Marchantia berteroana*; Hepaticae; 8-hydroxyapigenin 8-*O*-glucuronide; hypolaetin 8-*O*-glucuronide; hypolaetin 8,4′-di-*O*-glucuronide; apigenin 7-*O*-galacturonide; luteolin 7-*O*-galacturonide; luteolin 3′-*O*-galacturonide; luteolin 7,3′-di-*O*-galacturonide; luteolin 3′,4′-di-*O*-galacturonide.

Abstract—The major flavonoid of *Marchantia berteroana* is hypolaetin 8-*O*-β-D-glucuronide. This is accompanied by apigenin and luteolin, isoscutellarein (8-hydroxyapigenin) 8-*O*-β-D-glucuronide, the 7-*O*-β-D-glucuronide and -galacturonide of apigenin and luteolin, luteolin 3′-*O*-β-D-glucuronide and -galacturonide, luteolin 7,3′-di-*O*-β-D-glucuronide and -galacturonide, luteolin 3′,4′-di-*O*-β-D-glucuronide and -galacturonide, luteolin 7,4′-di-*O*-β-D-glucuronide, and hypolaetin 8,4′-di-*O*-β-D-glucuronide. The isoscutellarein and hypolaetin glucuronides, and the galacturonide flavones are all new natural products.

INTRODUCTION

Our previous studies established the structures of a number of flavone-*O*-glucuronides in *Marchantia foliacea* [1] and *M. polymorpha* [2], the results suggesting that *M. polymorpha* is the less advanced species. The flavonoid chemistry of the Southern Hemisphere [3] liverwort species *Marchantia berteroana* L. & L. is now discussed.

RESULTS AND DISCUSSION

Table 1 lists the chromatographic and spot appearance of the flavonoids isolated from *M.*

Table 1. Flavone glycosiduronic acids of *Marchantia berteroana*

Component number	R_f* TBA	15% HOAc	30% HOAc	Colour UV† Alone	NH_3	Structure
1	0·86	0·11	0·24	d	gy	apigenin
2	0·72	0·03	0·16	d	gy	luteolin
3	0·57	0·26	0·43	d	gy	apigenin 7-glucuronide
4	0·46	0·26	0·43	d	gy	apigenin 7-galacturonide
5	0·48	0·18	0·33	d	gbl	8-hydroxyapigenin 8-glucuronide
6	0·44	0·10	0·26	d	gy	luteolin 3′-glucuronide
7	0·42	0·10	0·26	d	gy	luteolin 3′-galacturonide
8	0·40	0·14	0·31	d	y	luteolin 7-glucuronide
9	0·35	0·10	0·25	d	d	hypolaetin 8-glucuronide
10	0·27	0·12	0·30	d	y	luteolin 7-galacturonide
11	0·12	0·20	0·37	d	d	hypolaetin 8,4′-diglucuronide
12	0·09	0·21	0·37	d	y	luteolin 7,3′-diglucuronide
13	0·06	0·20	0·36	d	y	luteolin 7,3′-digalacturonide
14	0·17	0·30	0·50	d	b	luteolin 7,4′-diglucuronide
15	0·12	0·30		d	b	luteolin 7,4′-digalacturonide
16	0·21	0·40	0·59	d	d	luteolin 3′,4′-diglucuronide
17	0·20	0·39	0·60	d	d	luteolin 3′,4′-digalacturonide

* Quoted R_f values were determined by 1D chromatography of pure material.
† d = dark; g = green; y = yellow; b = brown; bl = blue.

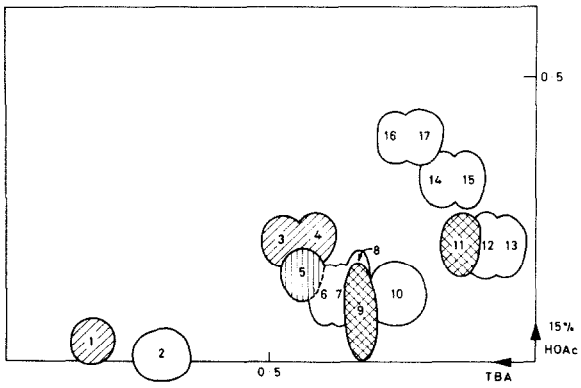


Fig. 1. 2-D PC of the flavonoid constituents of *Marchantia berteroana*. Key: Diagonal hatching—apigenin derivatives; vertical hatching—8-hydroxyapigenin derivatives; unhatched—luteolin derivatives; crossed hatching—hypolaetin derivatives.

berteroana together with their structures. Figure 1 shows the 2-D PC pattern of *M. berteroana* flavonoids. The spot numbering in Fig. 1 corresponds with that of the component numbering in Table 1.

The flavones apigenin and luteolin, components 1 and 2, were the simplest flavonoids present. Their structures were confirmed as described previously [2].

Apigenin and luteolin glycosiduronic acids

Acid and enzyme hydrolyses showed that the glycosides were all derived from either glucuronic or galacturonic acid.* The aglycones were shown to be either apigenin (from components 3 and 4), luteolin (from components 6–8, 10, 12–17) or flavones with lower R_f (TBA) than luteolin (from components 5, 9 and 11).

The apigenin and luteolin glucuronides (components 3, 6, 8, 12, 14 and 16) were thus identical in structure to the flavonoid glycosides previously isolated from *M. polymorpha* [2]. Their structures were confirmed by comparative UV spectroscopy and co-chromatography with the authentic compounds isolated from this source.

Each of the above glucuronides was paired with a less mobile (TBA, see Fig. 1) but UV spectrally identical spot which yielded only galacturonic acid on hydrolysis. Thus was established the structure of the series of apigenin and luteolin galacturonides listed in Table 1.

The structural relationship between the mono- and digalacturonides was confirmed by partial hydrolysis with acid or β-glucosidase.† On enzyme hydrolysis component 17 (luteolin 3′,4′-digalacturonide) yielded luteolin 3′-galacturonide and luteolin 7,3′-digalacturonide yielded luteolin 7-galacturonide. Acid hydrolysis (5% HCl, 100°, 1 hr) of component 13 yielded luteolin plus the very stable [4] luteolin 7-galacturonide, and component 17 yielded luteolin, luteolin 3′-galacturonide, plus a new glycoside, assumed to be luteolin 4′-galacturonide. The latter glycoside had the correct PC relationship with the 3′-galacturonide [a higher mobility in both solvents R_f 0·54 (TBA), 0·16 (15% HOAc)], and had a substituted 4′-hydroxyl group (appearance of spot in UV unchanged in NH_3). The result of acid hydrolysis was thus consistent with that found for luteolin 3′,4′-diglucuronide [5].

The diglycosiduronic acid, component 15, was present at too low a level to establish its structure completely, but by analogy with the firmly established series of flavone glucuronide–galacturonide pairs isolated from *M. berteroana*, its position on a 2D PC suggests that it is almost certainly the 7,4′-digalacturonide of luteolin.

8-Hydroxyapigenin and hypolaetin glucuronides

The remaining flavonoid constituents, 5, 9 and 11, were all glucuronides. Component 9 was the major flavonoid, being present at a level of approximately 0·03% w/w on a dried plant material basis.

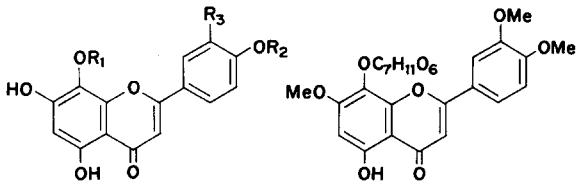
UV data (see Experimental) established that all three compounds possess free 5- and 7-hydroxyl groups and that compounds 5 and 9 possess free 4′-hydroxyl groups. Compound 9 was also shown to have an *ortho*-dihydroxyl group in the B-ring [6]. Partial conversion of compound 11 to 9 was achieved by β-glucosidase hydrolysis (see earlier footnote) thereby establishing compound 11 as a 4′-*O*-glucuronide of 9.

* Comparative runs (0·01 M acetate buffer, 37°, 1 hr) showed that marine mollusc β-glucuronidase hydrolyses β-glucuronides and β-galacturonides with equal facility.

† It was found in the course of this work that sweet almond β-glucosidase has significant β-glycosiduronidase activity. Over a short hydrolysis period (2 hr, 37°, pH 5) it effected preferential cleavage of the more labile β-glycosiduronic acid links, e.g. 4′ > 3′ > 7. This enabled partial hydrolysis of diglycosiduronic acids to the monoglycosiduronic acid.

The presence of additional oxygenation in the A-ring of compounds 5 and 9 was first suspected when it was observed that as chromatographic spots they were virtually unaffected by NH_3 in spite of the possession of free 4′-hydroxyl groups [7,8]. In the case of component 9 this was confirmed by MS of the peracetate which defined 9 as a monosubstituted pentahydroxyflavone. MS data for either 9 or its aglycone included A-ring fragments at *m/e* 168, 140 and 112 which are characteristic of tri-oxygenation [9]. The site of this additional oxygenation must be at C-8 since, although vigorous acid hydrolysis of 9 produced 6-hydroxyluteolin (the thermodynamically more stable isomer [8]), hydrolysis with β-glucuronidase produced a different aglycone. This aglycone had MS, UV and PC properties consistent with those reported [8] for hypolaetin (8-hydroxyluteolin, **1c**), e.g. higher PC mobility and shorter wavelength UV maxima than 6-hydroxyluteolin.

The glucuronic acid moiety in compound 9 was assigned to the 8-hydroxyl group of hypolaetin on the basis of UV and MS data.



Structure (**1**). **1a**: R_1 = Glur; R_2 = H; R_3 = OH. **1b**: R_1 = R_2 = Glur; R_3 = OH. **1c**: R_1 = R_2 = H; R_3 = OH. **1d**: R_1 = Glur; R_2 = R_3 = H. **1e**: R_1 = R_2 = R_3 = H.

* 6- and 8-hydroxyluteolin have closely similar spectra (see Experimental) and it is assumed that the 7-*O*-glucuronide derivatives would also.

The UV spectrum of 9 differs markedly from that reported [10] for 6-hydroxyluteolin 7-*O*-glucuronide (λ_{max} 286, 349) and thus presumably from the spectrum of 8-hydroxyluteolin 7-*O*-glucuronide.* Further, as would be anticipated if the 7-hydroxyl were free, NaOAc causes a band 2 bathochromic shift in the spectrum of 9 whereas the same band in the spectrum of the 7-*O*-glucuronide of 6-hydroxyluteolin is unaffected.

MS of the methyl ester of the trimethyl ether of 9 (**2**) distinguished clearly between the alternative possibilities of glycosylation at the 7- or 8-positions. The MS featured a weak molecular ion at *m/e* 534 [confirming the proposed molecular formulae for 9 (**1a**) and (**2**)] and the base peak was at *m/e* 344 corresponding to the trimethyl ether of the aglycone. Further fragmentation of this trimethyl–aglycone ion (M) gave an intact A-ring fragment and only weak M–Me and M–MeCO ions, in direct contrast to the established [11] behaviour of 6- and 8-methoxyflavones under the same conditions (see Table 2). This is taken to indicate that the methylated derivative of 9 does not possess an 8-methoxyl group and thus that the glucuronic acid moiety is attached to the 8-hydroxyl group. On the basis of the above evidence, component 9 is assigned the structure hypolaetin 8-*O*-β-D-glucuronide (**1a**) and component 11, hypolaetin 8,4′-di-*O*-β-D-glucuronide (**1b**).

The remaining flavonoid, component 5, is assigned the structure 8-hydroxyapigenin 8-*O*-β-D-glucuronide on the basis of similar arguments. The MS confirms that it is a tetrahydroxyflavone with 6- or 8-hydroxylation. Vigorous acid hydrolysis produces scutellarein (6-hydroxyapigenin) whereas hydrolysis with β-glucuronidase produces a different flavone with higher PC

Table 2. Relative intensities of selected ions in the mass spectrum of some methylated 6- or 8-hydroxyluteolins

Compound	M^+	$(M-Me)^+$	$(M-MeCO)^+$	A^+	$(A-Me)^+$	$(A-MeCO)^+$
2	100	17	4		9	21
6-methoxyluteolin	100	72	44		16	20
7-hydroxy-5,8,3′,4′-tetramethoxyflavone	90	100	22		18	12
Sinensetin	84	100	22		26	34

mobility than scutellarein,* paralleling the hydrolytic behaviour of the hypolaetin glucuronide. Moreover, component 5 had a much higher R_f in TBA (0·48) than did scutellarein 7-glucuronide (0·27). The UV spectra are consistent with a flavone containing a free 7-hydroxyl group.

This is the second reported natural occurrence of 8-hydroxyapigenin, the first being from *Pinguicula vulgaris* (Leutibulariaceae) [14].

CONCLUSION

This isolation of flavone glycosiduronic acids from *M. berteroana* fits the now well established pattern of flavonoid types found in liverworts of the order Marchantiales. *M. berteroana* contains all the luteolin mono- and diglucuronides found in *M. polymorpha* [2] and, in addition, a duplication of these glycosides as the galacturonides. This close parallel between the type of luteolin glycosides found in each plant suggests a close affinity between *M. berteroana* and *M. polymorpha* (cf. *M. foliacea* [1]). The presence of galacturonide derivatives extends the known examples of flavone galacturonides occurring in liverworts of the order Marchantiales [15,16]. The only other known example outside this plant group appears to be the methyl ester of apigenin 7-galacturonide isolated from *Centaurea calcitrapa* [17]. The isoscutellarein and hypolaetin glucuronides isolated from *M. berteroana* are the first known flavone 8-*O*-glycosides, although several examples are known in the flavonol series [18].

The occurrence of 8-hydroxyflavones in *M. berteroana* is a feature of particular interest. Extra A-ring hydroxylation is considered to be an advanced chemotaxonomic feature in higher plants [19] and parallels the occurrence of advanced structural features in other liverworts of this order [1,15,16], and also the occurrence of scutellarein in the moss *Bryum weigelii* [13]. It may also indirectly provide some evidence on the systematic position of *Monoclea forsteri* which has been variously placed in the orders Jungermanniales [20], Calobryales [21], Monocleales [22], and most recently, Marchantiales [23]. *M. forsteri*, in common only with *M. berteroana* produces an 8-oxygenated flavone (8-methoxyluteolin) glycosiduronic acid derivative as its major flavonoid [15]. This common biochemical characteristic supports the recently proposed inclusion of *M. forsteri* in the order Marchantiales.

EXPERIMENTAL

A voucher specimen of *Marchantia berteroana* L. & L. has been deposited with Massey University, Palmerston North (MPN 17001). Flavonoid chromatography on paper, sugar analyses, UV and MS data were all carried out as described earlier [1,2].

Isolation procedure. Freshly collected samples of *M. berteroana* gametophyte tissue from both male and female plants were extracted as described for *M. polymorpha* [2]. Larger scale separations were carried out on the equivalent of 80 g of dried plant material. Extract from the bulk sample was applied in bands to 36 sheets of 3MM paper and developed in TBA. Partially separated bands were cut out, eluted and rechromatographed in 15% HOAc. Further separation of the resulting bands in either TBA or 30% HOAc normally resulted in homogeneous material. Flavonoids which had very similar R_f values, e.g. components 8–12, were separated by over-running in 15% HOAc or TBA.

Hypolaetin-8-O-glucuronide (*component* 9, **1a**). Material from PC purification was recrystallized from MeOH to yield 8 mg of chromatographically homogeneous material, mp darkened 195–200°, melted (decomposition) 220–223° (a further 12 mg of **1a**, contaminated with luteolin 7-glucuronide was obtained from the mother liquors). UV data: λ_{max} (MeOH) 259, 270, 290sh, 357; (+NaOMe) 271, 320, 414; (+NaOAc) 279, 326, 396; (+NaOAc–H_3BO_3) 268, 283sh, 379, 427sh; (+$AlCl_3$) 264, 274sh, 301, 357sh, 409; (+$AlCl_3$–HCl) 264, 273sh, 303, 365, 397sh, nm. MS (70eV, 300°) *m/e* 302 (100%), 273(3), 168(46), 140(16), 134(16), 112(16).

The peracetate (C_5H_5N–Ac_2O) was purified by PLC (SiO_2, C_6H_6–Me_2CO, 4:1) and had an MS (70eV, 200°) *m/e* 470, 428(100), 386, 344, 302, 169.

1a was methylated (diazomethane, MeOH/ether, 12 hr) to yield the methyl ester of the trimethyl ether of **1a** (**2**), (50eV, 200°) 534 ($M^{\cdot+}$, 0·2%) 344(100), 329(17), 314(5), 313(5), 312(10), 301(4), 298(7), 183(10), 182(14), 181(8), 167(9), 165(12), 162(12), 153(15), 149(14), 139(21), 136(17).

1a did not hydrolyze with β-glucosidase (37°, pH 5, 2 hr) but was completely hydrolyzed with β-glucuronidase or 5% HCl (100°, 4 hr). Glucuronic acid was produced in either case. Acid hydrolysis produced a flavone which appeared as an intense black spot on paper viewed in UV turning brown in NH_3, R_f 0·27(TBA), 0·50(BAW), 0·01(15% HOAc), 0·09(30% HOAc), 0·18(50% HOAc) which co-chromatographed with authentic 6-hydroxyluteolin (synthesized by demethylation (HI, Ac_2O) of 3′,6-dimethoxyapigenin). The flavone and 6-hydroxyluteolin also possessed identical MS and UV: λ_{max} (MeOH) 248sh, 282, 346; (+NaOMe) decomposed; (+NaOAc) decomposed; (+NaOAc–H_3BO_3) 262, 290, 402; (+$AlCl_3$) 268, 302, 416; (+$AlCl_3$–HCl) 256, 296, 372 nm. Methylation (diazomethane, MeOH–Et_2O, 72 hr) produced

* The 6- and 8-hydroxyflavones have an unusually low R_f in TBA, much lower than those reported [7,8,12,13] in BAW. In contrast the R_f values for the glucuronides are similar in both solvents.

the pentamethyl ether which had an identical MS and co-chromatographed (TLC) with sinensetin (5,6,7,3′,4′-pentamethoxyflavone).

Hydrolysis of **1a** with β-glucuronidase produced the flavone **1c**, which did not co-chromatograph with 6-hydroxyluteolin, R_f 0·39 (TBA), 0·01(15% HOAc), 0·13(30% HOAc), 0·28(50% HOAc), and appeared as a dark spot on paper viewed in UV turning green in NH_3. MS(50eV, 300°) *m/e* 302(100%), 168(73). UV: λ_{max} (MeOH) = 253sh, 280, 340; (+NaOMe) decomposed; (+$AlCl_3$) = 274, 291sh, 338sh, 396, 435sh; (+$AlCl_3$–HCl) = 270sh, 284, 305sh, 362nm.

Hypolaetin 8,4′-di-O-glucuronide (*component* 11, **1b**). PC homogeneous material had UV: λ_{max} (MeOH) 271, 289sh, 337; (+NaOMe) 234, 276, 301sh, 378 (decrease in absorbance cf. 337nm peak in MeOH); (+NaOAc) 270sh, 278, 370; (+NaOAc–H_3BO_3) 273, 322sh, 347sh; (+$AlCl_3$) 257, 279, 342, 378sh; (+$AlCl_3$–HCl) 254, 278, 294sh, 342, 378sh, nm. Hydrolysis with β-glucuronidase produced glucuronic acid plus hypolaetin (**1c**). Hydrolysis with β-glucosidase (37°, pH 5, 2 hr) produced a mixture of **1a** and **1b**.

8-Hydroxyapigenin 8-O-glucuronide (*component* 5, **1d**). PC homogeneous material had UV: λ_{max} (MeOH) 271, 332; (+NaOMe) 279, 329sh, 397; (+NaOAc) 278, 310sh, 389; (+NaOAc–H_3BO_3) 277, 318, 345sh, 412sh; (+$AlCl_3$) 277, 306, 346, 386; (+$AlCl_3$–HCl) 278, 305, 345, 386nm. MS (70eV, 300°) *m/e* 286(100%), 257(6), 168(70), 140(6), 121(15), 118(15), 112(18).

1d was completely hydrolyzed with β-glucuronidase or 5% HCl (100°/4 hr) to yield glucuronic acid plus an aglycone. The product of acid hydrolysis had the appearance on paper and co-chromatographed with scutellarein (6-hydroxyapigenin, obtained by hydrolysis of the 7-glucuronide) R_f 0·47(TBA), 0·03(15% HOAc), 0·10(30% HOAc). Hydrolysis with β-glucuronidase produced 8-hydroxyapigenin (**1e**) which appeared on paper as a purple blue spot turning greenish blue in NH_3, R_f 0·58(TBA), 0·04 (15% HOAc), 0·18(30% HOAc). UV λ_{max} (MeOH) 280, 305, 322sh; (+NaOMe) 272, 333sh, 370 (unstable); (+NaOAc) 310sh, 367 (slowly decomposes); (+NaOAc–H_3BO_3) 302; (+$AlCl_3$) 306, 328, 354 (unstable); (+$AlCl_3$–HCl) 284, 320, 350. It yielded a tetramethyl ether, MS *m/e* = 342 ($M^{\cdot+}$, 93%), 327(100), 299 (33). The sample for UV analysis was isolated by ethyl acetate extraction from the enzyme hydrolysis and used without further purification. 8-Hydroxyapigenin is air sensitive and tends to decompose on PC.

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Note added in proof. The 8-hydroxyapigenin isolated from *M. berteroana* has proved to be chromatographically identical with that isolated by Jay and Gonnet from *Pinguicula vulgaris* [14].

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