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THE MAJOR FLAVONOIDS OF AN ANTARCTIC *BRYUM*

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Key Word Index—*Bryum argenteum*; Bryales; moss; Antarctica, flavonoids; 8-hydroxyapigenin 7-*O*-glucoside; 8-hydroxyluteolin 7-*O*-glucoside; flavone glycoside malonates.

Abstract—The major flavonoid glycosides in two Antarctic *Bryum* samples are apigenin and luteolin glucosides and their 6″-malonyl esters, and the 7-*O*-glucosides of 8-hydroxyapigenin and 8-hydroxyluteolin. 8-Hydroxyflavonoids have not been found previously in mosses and the 7-*O*-glucosides are new to bryophytes. The same major flavonoids are common to *Bryum argenteum* as also are the numerous minor polyglycosides. No biflavonoids of flavone *C*-glycosides were found.

INTRODUCTION

In late 1984, a New Zealand expedition (K053) undertook botanical studies at Terra Nova Bay and Wood Bay, North Victoria Land, Antarctica. Bryophytes and lichens are the dominant macro-plants and at several sites, *Bryum* and *Ceratodon* form extensive patches of ground cover. Fertile plants are rare however and the commonly encountered phenotypic plasticity in this environment often permits only tentative species identification [1, 2]. Studies of comparative flavonoid chemistry have proved to be valuable adjuncts to traditional methods of classification in bryophytes [3, 4] and for this reason, a study of the flavonoids in Antarctic *Bryum* samples was initiated.

Although a number of *Bryum* species have been investigated previously for flavonoids, for only one species has a comprehensive study been reported [5]. In early work, luteolinidin and its 5-mono- and 5-di-glucosides were isolated from several species [6, 7] and a scutellarein glycoside was reported in *B. weigelii* [8]. More recently *B. capillare* has been shown to accumulate a remarkable (for a bryophyte) assemblage of flavonoid types. These include the isoflavone glycosides, orobol and pratensein 7-*O*-glucosides and their 6″-malonyl esters [9], apigenin, luteolin, 6-hydroxyluteolin and diosmetin 7-*O*-glucosides and their 6″-malonyl esters [10] and biflavonoids incorporating both luteolin and orobol [11]. In the present communication, the major flavonoids in *B. argenteum* and an Antarctic *Bryum* are examined for the first time.

RESULTS AND DISCUSSION

Three samples of '*Bryum*' were collected in modest amounts from sites in Wood Bay for flavonoid analysis. 2D-PCs revealed essentially the same flavonoid pattern for two of these samples (Given 13816 and 13828). The third sample (Given 13827) appeared to lack flavonoid glycosides completely and has been redefined subsequently as *Ceratodon purpurea*. The flavonoid pattern of the two *Bryum* samples consisted of two aglycones, six major monoglycosides (**3**–**8**) and up to nine minor di- to tetraglycosides (Fig. 1). Aglycones **1** and **2** were identified as

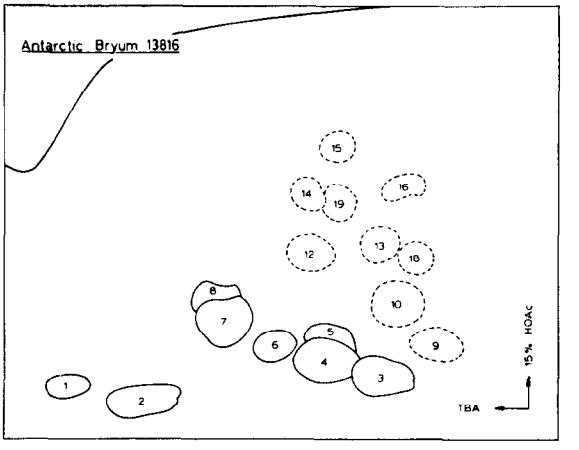

Fig. 1. 2D Paper chromatographic pattern of major (—) and minor (---) flavonoids in Antarctic *Bryum* 13816.

apigenin and luteolin respectively by direct comparison with authentic material.

Compounds **4** and **7** on acid or β-glucosidase hydrolysis, gave **1** and **2** respectively together with glucose. Their absorption spectra (Table 1), high resolution MS (**4**) and chromatographic properties were indistinguishable from those of authentic apigenin and luteolin 7-*O*-β-D-glucopyranosides. Accordingly, **4** and **7** are assigned these structures.

On the 2D-PC, compounds **4** and **7** are accompanied by a related pair of compounds, **5** and **8**, which possess the same R_fs in TBA but which run marginally ahead in 15% HOAc (see Fig. 1). These compounds each gave the same colour reaction, absorption spectra (Table 1), aglycone and sugar as did their respective 2D-PC neighbours. Treatment with 2 N Sodium hydroxide on a microscale [12] converted **5** to **4**, and **8** to **7**, thus suggesting that **5** and **8** are equivalent acylated derivatives of **4** and **7**. TLC analysis of the hydrolysate failed to reveal the acid residue

Table 1. Absorption spectra of *Bryum* flavonoids and reference compounds*

	MeOH	NaOMe	NaOAc	NaOAc–H_3BO_3	$AlCl_3$	$AlCl_3$–HCl
3	257, 273 301 sh, 344	273, 338 sh, 403 dec.	258, 272, 302 sh, 340, 400 sh	264, 302 sh, 375	275, 316, 434	266 sh, 275, 310 sh 358, 410 sh
Hypolaetin 7-*O*-glyc [16]	255 sh, 271 302, 340	266, 398 dec.	262, 298 sh, 340, 398 sh	266, 298 sh, 362	274, 317, 352 sh, 434	263 sh, 275, 317 sh 353, 412 sh
Hypolaetin 8-*O*-glyc [14]	259, 270 290 sh, 357	271, 320 414	279, 326 396	268, 283 sh, 379, 427 sh	264, 274 sh 301, 357 sh, 409	264, 273 sh, 303 365, 397 sh
6	278, 308 327 sh	250 sh, 378 dec	278, 320, 381		278, 322 350, 410 sh	277, 322 347, 410 sh
Isoscutellarein 7-*O*-glyc [17]	277, 308 322 sh	243, 376 dec	—	—	282, 323 343	—
Isoscutellarein 8-*O*-glyc [14]	271, 332	279, 329 sh 397	278, 310 sh 389	277, 318 345 sh, 412 sh	277, 306 346, 386	278, 305 345, 386
4 and **5**	256, 267 sh 350	265, 293 sh 395	256, 267 sh 355, 407 sh	259, 372	272, 295 sh 327, 420	272, 293 sh 355, 385
7 and **8**	269, 333	243 sh, 269 300 sh, 350 sh 383	269, 345 390 sh		275, 298 346, 382	276, 298 342, 381

*Abbreviations: sh = shoulder, dec = decomposed within 5 min.

but FABMS measurements on **8** produced an $(M+H)^+$ ion with *m/z* 519. This is 86 mass units higher than the equivalent ion from **7** and is consistent with the presence of a single malonyl function. ^{13}C NMR studies confirmed this in that the acyl function was represented by signals at 174.5 (CO_2H), 168.0 (CO_2R) and 42.2 (CH_2) ppm. The site of acylation was determined as the glucose 6-hydroxyl, since in the ^1H NMR spectrum the glucose H-6 signals appeared at δ 4.35 and 4.10, well downfield from their normal positions near δ 3.8 and 3.5 [13]. Furthermore, in the ^{13}C NMR spectrum, glucose C-6 acylation was evidenced by the typical [12] 2–3 ppm downfield shift of C-6 to around 63.5 ppm and the concommitant 2–3 ppm upfield shift of C-5 to 73.9 ppm. Compound **8**, and by analogy **5**, are therefore assigned the structures, apigenin and luteolin 7-*O*-β-D-(6″-*O*-malonyl) glucopyranoside respectively. These structures were confirmed by cochromatography (HPLC and TLC) of **5** and **8** with authentic samples from *B. capillare*.

The remaining two major flavonoids, **3** and **6**, appeared as dark UV-absorbing spots on 2D-PC, and were largely unresponsive to NH_3. Compound **6** gave a highly distinctive absorption spectrum in methanol (Table 1) which approximates closely to that of isoscutellarein (8-hydroxyapigenin). Acid hydrolysis gave glucose plus scutellarein (6-hydroxyapigenin). However, β-glucosidase treatment gave glucose plus isoscutellarein, which was identified by direct comparison with authentic material from *Marchantia berteroana* [14]. Wessely–Moser isomerization must therefore account for the production of scutellarein under acid conditions. The absorption spectra require that the 5- and 4′-hydroxyl groups be free in **6**, and therefore that the glucose be sited at the 7- or 8-hydroxyl group. These alternatives are readily distinguished from the absorption spectra. In methanol, **6** possesses the same highly distinctive spectrum as does isoscutellarein. This would not be so if glycosylation is at the 8-hydroxyl however, since 8-*O*-glycosylation causes reversion to a 'normal' apigenin-like spectrum (see Table 1). Furthermore the spectrum of **6** matches closely with that previously reported for an isoscutellarein 7-*O*-glycoside and accordingly, **6** is assigned the structure, isoscutellarein 7-*O*-β-D-glucopyranoside. Compound **3** appears on the 2D-PC in the same position relative to luteolin-7-glucoside (**4**) as does **6** to apigenin-7-glucoside (**7**) (see Fig. 1). Hydrolysis results parallel those for **6**, except that hypolaetin (8-hydroxyluteolin) was produced with β-glucosidase, and again the absorption spectra clearly match those of a 7-*O*-glycoside and not those of an 8-*O*-glycoside (see Table 1). Compound **3** is therefore hypolaetin 7-*O*-β-D-glucopyranoside.

Although 6″-malonyl esters of apigenin and luteolin 7-*O*-glucosides have been reported once before from a *Bryum* species, isoscutellarein and hypolaetin and their 7-*O*-glycosides have not been found previously in any moss. Indeed, the aglycones are rare in nature, the only other known source in bryophytes being in the liverwort, *Marchantia berteroana* where the 8- and 8,4′-diglucuronides are known to occur in the reproductive structures [15]. The present finding of the 7-glucosides is thus the first in a bryophyte.

The low level polyglycosides were not studied in detail. However absorption spectroscopy (MeOH, NaOMe), hydrolyses and aglycone analyses carried out on the nine accessible components, revealed the presence of four equivalent pairs of apigenin (spots 12, 14, 15 and 19) and luteolin (spots 10, 13, 16 and 18) 7-*O*-polyglycosides plus one further luteolin glycoside. No further glycosides of the 8-hydroxyflavones were found, nor were *C*-glycosides or biflavonoids.

The patterns of major and minor flavonoids in the Antarctic *Bryum* samples approximated closely to that of authentic *B. argenteum* collected from mainland New Zealand, although the polyglycosides in the latter were present at lower levels than in the former. Individual components from the New Zealand *B. argenteum* were isolated and compared directly with their equivalents from Given 13816. All major components (**3** to **8**) in both samples were shown to be identical with respect to R_f, colour reactions (NH_3, NA spray), absorption spectra and also aglycones. 2D-PC visible polyglycosides likewise cochromatographed with their equivalents from

Bryum 13816. These results provide independent supportive evidence that Antarctic *Bryum* samples CHR 417359 (Given 13816) and CHR 417371 (Given 13828) are *B. argenteum.** Although apparently the same species these two samples are quite different in habit. Given 13816 is from plants forming flat mats *ca* 4–8 mm thick, but Given 13828 is from convoluted cushions up to 25 mm thick.

EXPERIMENTAL

Plant material. Two samples of *Bryum* were collected in December 1984 from Edmondson Point, Wood Bay, Antarctica and voucher specimens deposited in the Botany Division herbarium: CHR 417359, North Edmondson Point, Wood Bay (74°19′S, 165°06′E), D. R. Given 13816, 12 Dec. 1984; CHR 417371, Edmondson Point (74°20′S, 165°08′E), D. R. Given 13828, 14 Dec. 1984. *Ceratodon purpurea*, D. R. Given 13827, was collected as a *Bryum* at the same time from Edmondson Point, Wood Bay, North Crater. Authentic *B. argenteum* was collected from the DSIR research campus at Lincoln, New Zealand in June 1985 (D. R. Given 14066).

Extraction and work-up. Samples were freed of attached soil, dried at 100°, ground and extracted with EtOH–H_2O (1:1) several times at room temp. Reference 2D-PCs were run on extracts from all three samples at a range of loading levels from 100–500 mg-equiv. of dry plant material; solvents: *t*-BuOH–HOAc–H_2O (3:1:1) (TBA) and 15% HOAc (HOAc). Components were isolated from sample No. 13816 (19 g) by extraction as above, the extract filtered through celite and extracted ×6 and EtOAc. The yellow EtOAc-solubles were chromatographed by ID-PC in 50% HOAc and the resultant bands eluted and rechromatographed by 2D-PC. Individual flavonoids **1**–**8** were isolated from the 2D-PCs in mg quantities and **9**–**19** in trace quantities. Compounds **5** and **8** were separated from **4** and **7** respectively (in a ratio of *ca* 1:1) by further 1D-PC in 50% HOAc, and each was freed from carbohydrate impurities by chromatography on LH-20 in MeOH. Flavonoids from Given 13828 were isolated entirely by 2D-PC. *Bryum argenteum* (30 g) was extracted as above and the extract passed down a cellulose column. Elution with 100% H_2O to H_2O–MeOH (2:3) gave a series of 9 fractions, the later of which contained the major monoglycosides. Further purification of individual fractions was achieved on MCI gel (CHP20P, 75–150μ, Mitsubishi Chemical Industries) eluting with MeOH–H_2O (1:9–3:2). Fractions from this column were PC'd as above to yield pure flavonoids.

Structure elucidation techniques and data. PC (Whatmans 3MM, 46 × 57 cm) and TLC (Schleicher and Schull, F1500 cellulose) cochromatography routinely involved TBA and 15% HOAc, and the use of the spray reagent: 2% tetraphenyldiboroxide ethanolamine complex (K and K) in MeOH ('NA'). For aglycones 15% HOAc was replaced by 50% HOAc. Conditions for acid hydrolyses: 1.5 N HCl, 1 hr, 100°, and for β-glucosidase hydrolyses: β-glucosidase (Koch-Light ex sweet almonds), 10–20 min, 18°. Reference samples of isoscutellarein and hypolaetin were obtained by β-glucuronidase hydrolysis of the 8-glucuronide and 8,4′-diglucuronide respectively, ex *Marchantia berteroana* [14]. Absorption spectroscopy: reagents and conditions etc. as described in ref. [12]. NMR spectra were determined in DMSO-d_6. Compound **8** (^{1}H NMR, δ): 7.96*d*, J = 8.5 Hz (H-2′6′); 6.95*d*, J = 8.5 Hz (H-3′5′); 6.89*s* (H-3); 6.81*d*, J = *ca* 2.5 Hz (H-8); 6.44*d*, J = *ca* 2.5 Hz (H-6); 5.11*d*, J = *ca* 6 Hz (glu H-1); 4.35*d*, 4.10 *m* (glu H-6). Compound **8** (^{13}C NMR, ppm): 182.0 (C-4), *ca* 170(COOH), 168.0 (COOR), 164.3 (C-2), 162.7 (C-7), 161.5/161.1 (C-5,4′), 156.9 (C-9), 128.6 (C-2′,6′), 120.9 (C-1′), 116.0 (C-3′,5′), 105.4 (C-10), 103.1 (C-3), 99.5 (C-6,G-1), 94.8 (C-8), 76.2 (G-3), 73.9 (G-5), 73.0 (G-2), 69.8/69.6 (G-4), 63.9/63.4 (G-6), 42.2 (CH_2). MS (high resolution) were determined on an AEI MS-30. Compound **4** permethylated (NaH MeI–DMF [12]) produced major ions at: m/z 546.224 (PM^+, $C_{28}H_{34}O_{11}$ req: 546.210) 45%, 328.103 (PM^+-PM glucose, $C_{18}H_{16}O_6$ req: 328.095) 100%, 187.112 (PM glucose–OMe, $C_9H_{15}O_4$ req: 187.097) 45%. Compound **5** deacylated during permethylation. FABMS were determined in the positive ion mode on samples in a glycerine–H_2O–DMSO matrix. Spectra: Compound **7**, 433 ($M+H^+$), 271 (Aglyc+H^+); Compound **8**, 519 ($M+H^+$ + malonyl), 433 ($M+H^+$), 271 (Aglyc+H^+). Deacylations were carried out with 2 N NaOH in a microsyringe according to ref. [12]. Compounds **5** and **8** were chromatographically indistinguishable from authentic samples ex *B. capillare* on TLC (cellulose: 40% HOAc and pentan-1-ol–HOAc–H_2O, 2:1:1) and on HPLC (reversed phase; **5**, R_t = 17.75; **8**, R_t = 27.5; **4**, R_t = 10.75; **7**, R_t = 15.5).

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