Phytochemistry, 1974, Vol. 13, pp. 1937 to 1942. Pergamon Press. Printed in England.

FLAVONOIDS OF THE LIVERWORT *MARCHANTIA POLYMORPHA*

K. R. MARKHAM and L. J. PORTER

Chemistry Division, Department of Scientific and Industrial Research, Petone, New Zealand

(*Received* 23 *January* 1974)

Key Word Index—*Marchantia polymorpha*; Hepaticae; liverworts; Marchantiales; apigenin 7,4′-di-*O*-glucuronide; luteolin 3′-*O*-glucuronide; luteolin 7,3′-di-*O*-glucuronide; luteolin 7,4′-di-*O*-glucuronide; luteolin 7,3′,4′-tri-*O*-glucuronide.

Abstract—The major flavonoids of *Marchantia polymorpha* var. *polymorpha* and *aquatica* are the 7-*O*-β-D-glucuronides of apigenin and luteolin, luteolin 3′-*O*-β-D-glucuronide, luteolin 7,3′-di-*O*-β-D-glucuronide, and the 7,4′-di-*O*-β-D-glucuronides of apigenin and luteolin. These are accompanied by minor amounts of apigenin, luteolin, luteolin 3′,4′-di-*O*-β-D-glucuronide and luteolin 7,3′,4′-tri-*O*-β-D-glucuronide. All the luteolin di- and triglucuronides except the 3′,4′-di- substituted compound are new natural products.

INTRODUCTION

EARLIER studies of the flavonoid chemistry of liverworts of the order Marchantiales clearly establish the presence of flavone *O*-glycosiduronic acids in these plants. These include the 7-*O*-rhamnosylgalacturonide of acacetin in *Reboulia hemispherica*,[1] a derivative of 8-methoxyluteolin 7,4′-di-*O*-galacturonide in *Monoclea forsteri**,[2] 7-*O*-glucuronides of apigenin, chrysoeriol and tricin in *Marchantia foliacea*[3] and luteolin 3′,4′-di-*O*-glucuronide in *Lunularia cruciata*.[4]

The present work confirms an earlier brief communication[5] that apigenin and luteolin glycosides occur in *M. polymorpha* and fully defines their structures as a series of glucosiduronic acids.

RESULTS AND DISCUSSION

PC of aqueous acetone extracts from freshly collected gametophyte tissue of *M. polymorpha* var. *polymorpha* and *M. polymorpha* var. *aquatica* revealed an identical pattern of six major flavonoids. The pattern obtained is illustrated in Fig. 1 (which illustrates the position of all flavonoids isolated from var. *polymorpha* on a 2-D PC). The major flavonoids are spots **1b, 1c, 2b, 2c, 2d** and **2e.**

Although initial extractions were carried out using methanol–water or ethanol–water mixtures, 40% aqueous acetone was preferred for the bulk extractions since this avoided the problem of ester formation which was thought to be occurring with the former solvents. It has previously been shown that such esters form under quite mild conditions.[3,6]

---

* *Monoclea forsteri* has been placed in the order Marchantiales according to the classification of GROLLE, R. (1972) *J. Bryology* **7**, 201.

[1] MARKHAM, K. R., MABRY, T. J. and AVERETT, J. E. (1972) *Phytochemistry* **11**, 2875.

[2] MARKHAM, K. R. (1972) *Phytochemistry* **11**, 2047.

[3] MARKHAM, K. R. and PORTER, L. J. (1973) *Phytochemistry* **12**, 2007.

[4] MARKHAM, K. R. and PORTER, L. J. (1974) *Phytochemistry* **13**, 1553.

[5] BREHM, B. G. and COMP, P. C. (1967) *Am. J. Botany* **54**, 660.

[6] ASEN, S., NORRIS, K. H. and STEWARD, R. N. (1972) *Phytochemistry* **11**, 2739.

The simplest flavonoids (spots **1a** and **2a**) were the free aglycones apigenin and luteolin (structures **1a** and **2a**) which were present at relatively low levels. They were concentrated from the crude plant extract by repeated ethyl acetate extraction, purified by PC and their structures established by comparison with authentic material.

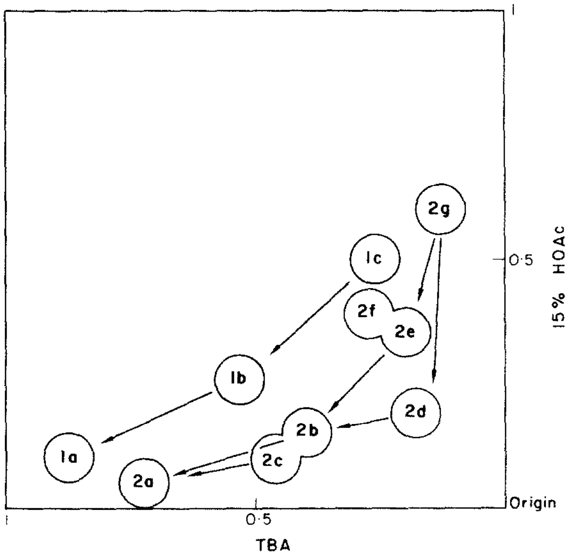
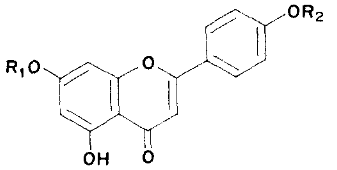
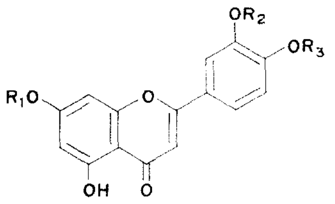


FIG. 1. CHROMATOGRAPHIC RELATIONSHIPS OF LIVERWORT FLAVONOIDS.



(1)



(2)

(**1a**) $R_1 = R_2 = H$
(**1b**) R_1 = glucosiduronic acid; $R_2 = H$
(**1c**) $R_1 = R_2$ = glucosiduronic acid

(**2a**) $R_1 = R_2 = R_3 = H$
(**2b**) R_1 = glucosiduronic acid; $R_2 = R_3 = H$
(**2c**) R_2 = glucosiduronic acid; $R_1 = R_3 = H$
(**2d**) $R_1 = R_2$ = glucosiduronic acid; $R_3 = H$
(**2e**) $R_1 = R_3$ = glucosiduronic acid; $R_2 = H$
(**2f**) $R_2 = R_3$ = glucosiduronic acid; $R_1 = H$
(**2g**) $R_1 = R_2 = R_3$ = glucosiduronic acid

The chromatographic and spectroscopic data for the remaining eight flavonoid constituents are listed in the Table. Their mobilities in TBA and HOAc relative to the aglycones indicate that they are glycosylated and their relative resistance to acid hydrolysis is consistent with their being glycosiduronic acid derivatives. Hydrolyses with β-glucuronidase confirmed this and converted **1b** and **1c** to apigenin (**1a**) and components **2b** to **2g** (inclusive) to luteolin (**2a**). Analysis of the sugars produced, revealed glucuronic acid as the only product and thus established all components as flavone *O*-β-D-glucuronides.

Chromatographic and spectral data (see Table) suggested that spots **1b** and **2b** were the 7-glucuronides of apigenin and luteolin (structures **1b** and **2b**) respectively. These structures were confirmed by the chromatographic and spectral identity of each component with authentic material.

The UV spectral data for spot **2c** (see Table) suggested that it possessed an apigenin-type hydroxylation pattern. However, both acid and β-glucuronidase hydrolyses converted it to luteolin (**2a**), thus liberating an *ortho* dihydroxyl group in the B-ring. Therefore, **2c** must be luteolin 3′*O*-β-D-glucuronide (**2c**) and this was confirmed by its co-chromatography and UV spectral identity with the authentic material earlier isolated from *Lunularia cruciata*.[4]

Mild acid hydrolysis effected partial conversion of **1c** to apigenin 7-glucuronide (**1b**). The UV spectral data for **1c** established that it possessed a free 5-hydroxyl group (complex formation $AlCl_3$/HCl) and lacked a free 4′-hydroxyl group (shift of Band I was accompanied by a decrease in intensity). Interconversion to apigenin 7-glucuronide (**1b**) and apigenin (**1a**) on hydrolysis thus implies that **1c** is apigenin 7,4′-di-*O*-β-D-glucuronide, a compound that has previously been isolated from *Antirrhinum majus* petals[7] Authentic material from this source was spectrally and chromatographically identical with **1c**.

Partial conversion of **2d** and **2e** to luteolin 7-glucuronide (**2b**) on acid hydrolysis and to luteolin (**2a**) on β-glucuronidase hydrolysis establishes their common partial structure. Both **2d** and **2e** possess a free 5-hydroxyl group ($AlCl_3$/HCl shift) but **2d** unlike **2e** has a free 4′-hydroxyl group (the NaOMe shift of Band I is accompanied by an increase in intensity). This data defines the glycosylation pattern of **2d** as 7,4′ and **2e** as 7,3′. The mobility of **2d** and **2e** on paper is in the region expected for luteolin diglycosides[8] and since on hydrolysis no compounds intermediate between **2d** or **2e** and the 7-glucuronide (**2b**) were produced, **2d** and **2e** must be the diglycosides, luteolin 7,3′-di-*O*-β-D-glucuronide and luteolin 7,4′-di-*O*-β-D-diglucuronide respectively. This constitutes the first record of their existence as natural products and **2e** is the first known 7,3′-diglycoside of luteolin. The 7,4′-glycosylation pattern is also rare, the only other known example being luteolin 7-rutinoside-4′-glucoside.[9]

Re-extraction of gametophyte tissue with hot-water provided enriched samples of the diglucuronides and enabled detection of two further minor luteolin glucuronides, **2f** and **2g**. As indicated earlier β-glucuronidase hydrolysis liberated luteolin from both compounds. It was noted that **2f** ran in a similar position on 2-D PC as luteolin 3′4′-di-*O*-β-D-glucuronide earlier isolated from *L. cruciata*,[4] and subsequently **2f** proved to be spectrally and chromatographically indistinguishable from the authentic material.

The remaining flavone glucuronide, **2g**, proved to be largely insoluble in methanol and therefore the spectral data in the Table was obtained in water. This precluded the determination of shifts in $AlCl_3$ or NaOAc. It was hydrolysed to luteolin by β-glucuronidase and its appearance on paper as a dark UV absorbing spot unchanged by NH_3 vapour suggests that is possesses a free 5-hydroxyl group and a substituted 4′-hydroxyl group.[10] Mild acid hydrolysis effected partial conversion of **2g** to a mixture of luteolin 7-glucuronide (**2b**), luteolin 7,3′-diglucuronide (**2d**) and luteolin 7,4′-diglucuronide (**2e**), thus defining the glycosylation pattern as 7,3′,4′. The NaOMe shift of Band II from 269 to 282 nm, accompanied by an increase in intensity is additional evidence that the 5-hydroxyl

---

[7] Harborne, J. B. (1963) *Phytochemistry* **2,** 327.

[8] Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, p. 48, Academic Press, London.

[9] Dranik, L. I. (1968) *Fenolnye Soedin. Ilch, Biol. Funkts., Mater Vses. Simpl.* **1,** 53.

[10] Markham, K. R., Mabry, T. J. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.

TABLE 1. FLAVONE GLUCOSIDURONIC

Structure		**1b**	**1c**	**2b**
Spot colour UV + NH_3		green yellow	Dark	Yellow
R_f	TBA	0·53	0·26	0·40
	15% HOAc	0·26	0·50	0·15
	30% HOAc	0·53	0·33*	0·31
Spectral Maxima (nm)	Alone	268 330	268 314	254 267*sh* 346
	NaOMe	268 288*sh* 386	282 357†	260 400
	NaOAc	266 360*sh* 387	268 300	257 265*sh* 407
	NaOAc/H_3BO_3	268 334	268 314	258 265*sh* 372
(in MeOH)	$AlCl_3$	273 297 344 379	272 292 329 377	272 292*sh* 332 426
	$AlCl_3$/HCl	273 298 344 379	272 292 329 377	269 292*sh* 369*sh* 388

group is the only unsubstituted position in the flavone*. R_m values based on the R_f values of the other glucuronides indicates that the mobility of **2g** is near that expected for a triglucuronide in both solvents. Also on acid hydrolysis no intermediates were formed between **2g** and the diglucuronides. Therefore, **2g** must correspond to luteolin 7,3′,4′-tri-*O*-β-D-glucuronide, a new compound, which contains a unique glycosylation pattern for luteolin glycosides.

The above pattern of flavonoid components detected in *M. polymorpha*, based on apigenin and luteolin glucuronides, is unique to this species amongst the liverworts and constitutes yet another instance of flavone glycosiduronic acids occurring in liverworts of the order Marchantiales. Work on species of this order to date† suggests that flavone glycosiduronic acids are ubiquitous, and may well be characteristic of the order, since they have, as yet, not been found in any species outside the Marchantiales.[11–14]

The flavonoids of *M. polymorpha* differ markedly from those isolated earlier from the endemic New Zealand species *M. foliacea*.[3] The major flavonoids of *M. foliacea* are the 7-glucuronides of tricin, chrysoeriol and apigenin, the latter being the only compound in common with *M. polymorpha*. *M. polymorpha* is seemingly the less advanced biochemically in that it lacks the ability to further oxidise the B-ring of luteolin, to methylate or diglycosylate phenolic hydroxyl groups, and to glycosylate with more than one type of sugar. The remarkable variety of glucuronide derivatives produced by *M. polymorpha*, consisting of every possible glycosylation pattern involving the 7,3′ and 4′ hydroxyl groups, suggests that it also lacks the enzyme specificity exhibited by *M. foliacea* which produces only 7-glycosylated flavones.

---

* For other examples see spectrum **1c** in the Table and spectrum 30 in Ref. 10.

† Species examined, which contain flavone glycosiduronic acids, include: *Monoclea forsteri*; *Marchantia foliacea*; *M. berteroana*; *M. polymorpha*; *Reboulia hemispherica*; *Lunularia cruciata*.

[11] NILSSON, E. (1969) *Acta Chem. Scand.* **23**, 2910.

[12] MARKHAM, K. R., PORTER, L. J. and BREHM, B. G. (1969) *Phytochemistry* **8**, 2193.

[13] TJUKAVKINA, N. A., BENESOVA, V. and HEROUT, V. (1970) *Coll. Czech. Chem. Commun.* **35**, 1306.

[14] NILSSON, E. (1973) *Phytochemistry* **12**, 722.

ACIDS OF *Marchantia polymorpha*

2c	2d	2e	2f	2g
Green -yellow	Yellow	Brown	Dark	Dark
0·44	0·18	0·20	0·27	0·13
0·10	0·19	0·35	0·39	0·60
0·26		0·64		
258 267 285*sh* 337	240*sh* 268 336	264 329*sh*	269 320	269 322‡
265 272*sh* 328 396	257 267*sh* 391	260 378†	277 300*sh* 353†	282 353†‡
268 392	257 264*sh* 292*sh* 393	262 303*sh* 357		
257 267*sh* 344	267 340	260 311*sh* 329*sh*		
272 292*sh* 344 394	275 292*sh* 344 379	260 287*sh* 336 369*sh*		
272 292*sh* 349 379	275 292*sh* 344 379	260 287*sh* 336 369*sh*		

* R_f value in H_2O. † Shift accompanied by a decrease in intensity. ‡ Spectrum in H_2O.

Three varieties of *M. polymorpha* are recognized; var. *polymorpha*, var. *aquatica* Nees and var. *alpestris* Nees.[15] We could detect no difference in the major flavonoid constituents of either var. *polymorpha* or var. *aquatica*. Considering the very marked differences in the flavonoid constituents of all species of the order Marchantiales so far examined*, they appear to be very closely related plants, thus justifying the distinction only at the varietal level.

EXPERIMENTAL

Voucher specimens of *Marchantia polymorpha* L. var. *polymorpha* and *M. polymorpha* L. var *aquatica* Nees have been deposited with Massey University, Palmerston North (MPN 8575 and 8576, respectively). All flavonoid separations were carried out on Whatman 3MM paper, using *t*-BuOH/HOAc/H_2O, 3:1:1 (TBA) and 15% HOAc for 2-D PC. MS were determined by direct insertion on an AEI MS-30 mass spectrometer. UV spectra were measured as described in Ref. 10.

Isolation procedure. Freshly collected samples of *M. polymorpha* gametophyte tissue was pulped with 40% aq. acetone in a Waring-blendor. The resulting suspension was filtered within 30 min of pulping and the filtrate was extracted (4 ×) with petrol. and conc. *in vacuo* at 40°. The extracted plant material was re-extracted with hot H_2O (30 min, steam bath) to yield the di- and triglycoside-rich extract. The individual flavonoid components were isolated by preparative 2-D PC. This gave satisfactory separation for all components except **2b** and **2c**. These were eluted together from the 2-D separations by soxhlet extraction and separated from each other by 1-D PC in 30% HOAc.

Isolation of apigenin (**1a**) *and luteolin* (**2a**). A concentrated aqueous extract of *M. polymorpha* was extracted with EtOAc (3 ×). PC of the resulting extract revealed the presence of luteolin (**2a**), apigenin (**1a**) and its 7-glucuronide (**1b**). The flavones were isolated by preparative 2-D PC, eluted, and purified by passage through an acid-washed microcolumn of SiO_2 (MeOH), to obtain: Apigenin (R_f 0·86 (TBA), 0·12 (15% HOAc); λ_{max} = 266, 332; MS m/e = 270 (M^+), 242 (M-CO), 153 ($C_7H_5O_4$, A-ring fragment) and 121 ($C_5H_5O_2$, B-ring fragment) which co-chromatographed with authentic apigenin on PC and tlc (acid-washed SiO_2, 6% MeOH in $CHCl_3$); and luteolin R_f 0·72 (TBA), 0·06 (15% HOAc); λ_{max} = 256 266*sh*, 344; MS m/e = 286 (M^+), 258 (M-CO), 153 ($C_7H_5O_4$, A-ring fragment) and 137, 134 (B-ring fragments) which co-chromatographed with authentic luteolin on PC, and tlc (acid-washed SiO_2, 6% MeOH in $CHCl_3$).

* Although the flavonoid patterns of *M. foliacea* and *M. berteroana* were reported to be closely similar,[3] subsequent chemical investigation of the latter has revealed distinct differences.

[15] MULLER, K. (1906-11) *Die Lebermoose Deutschlands, Ostereichs,* u.d. Schweiz. In Rabenhorst's *Kryptogamen-Flora von Deutschland usw, Band 6. edn. 2. Leipzig.*

Flavone glucuronides. These were isolated by 2-D PC as described in the isolation procedure. The following experiments were carried out:

(1) *Acid hydrolyses.* Products were analysed by 2-D PC, isolated by elution, and paper chromatographed against relevant standards. Components **1b**, **2b** and **2c** were hydrolysed with 5% HCl, 4 hr (steam-bath), and components **1c**, **2d**, **2e** and **2g** were hydrolysed for 1 hr under the same conditions.

(2) *Enzyme hydrolyses.* These were carried out using β-glucuronidase (ex marine mollusc, Koch-Light) in 0·01 M (pH 5) acetate buffer at room temp. overnight. Products were analysed by 1-D PC (TBA) versus authentic apigenin and luteolin. In the case of components **1b** and **2b** the products of hydrolysis were identified by additional UV spectral, chromatographic and MS data.

(3) *Chromatographic comparisons with authentic materials.* Where possible, components were chromatographed in a range of solvents (TBA, 15% HOAc, H_2O or 30% HOAc) vs authentic glucuronides isolated by preparative PC from plant sources. Thus, **1c** co-chromatographed with apigenin -7,4′-diglucuronide from *Antirrhinum majus* petals.[7] **2b** with luteolin 7-glucuronide from *Digitalis purpurea* leaves,[7] **2c** with luteolin 3′-glucuronide from *Lunularia cruciata*,[4] **2f** with luteolin 3′,4′-diglucuronide also from *L. cruciata*,[4] and **1b** with apigenin 7-glucuronide from *Antirrhinum majus* petals[7] and *M. foliacea* gametophyte tissue.[3]

(4) *Sugar analyses.* Sugars produced by either acid or enzyme hydrolysis were analysed by 1-D PC on Whatman No. 1 paper using *n*-BuOH/Py/H_2O, 2:2:1[16] and compared with authentic D-glucuronic (0·34), D-galacturonic (0·21), D-guluronic (0·25), D-mannuronic (0·37) acids* (the latter two uronic acids were isolated by hydrolysis of alginic acid according to the method of Fischer and Dorfel).[17] In all cases only one sugar could be detected, R_G 0·34. Some determinations were checked using the solvent Py/EtOAc/HOAc/H_2O, 5:5:1:3.[18]

Acknowledgements—We are indebted to Dr. Ella O. Campbell, Massey University, Palmerston North, for the plant material and identifications, and to Mr. G. J. Down for the MS.

---

* Figures in parentheses are R_{glucose} (R_G) values.

[16] SMITH, I. (1960) *Chromatographic and Electrophoretic Techniques*, Vol. 1, Chap. 13. Heinemann Interscience, New York.

[17] FISCHER, F. G. and DORFEL, H. (1955) *Z. Physiol. Chem.* **302**, 186.

[18] FISCHER, F. G. and DORFEL, H. (1955) *Z. Physiol. Chem.* **301**, 224.