Phytochemistry, 1973, Vol 12, pp 2007 to 2010 Pergamon Press Printed in England

FLAVONOIDS OF THE LIVERWORT *MARCHANTIA FOLIACEA*

KENNETH RONALD MARKHAM and LAWRENCE JAMES PORTER
Chemistry Division, D S I R , Petone, New Zealand

(*Received* 13 *February* 1973 *Accepted* 9 *March* 1973)

Key Word Index—*Marchantia*, Marchantiales, liverworts, flavone *O*-glycosides, flavone *C*-glycoside, tricin

Abstract—The major flavonoids of *Marchantia foliacea* are the 7-*O*-β-D-glucuronides of apigenin, chrysoeriol and tricin, and apigenin-6,8-di-*C*-glucoside (vicenin-2) Minor constituents include the rhamnosylglucuronides of the above flavones Apparent isomerization of the glucuronides on hydrolysis (MeOH–HCl) proved to be due to methylation of the sugar carboxyl group

INTRODUCTION

IT IS now well established that flavonoids occur in liverworts [1–5] These flavonoids have been identified as either *C*-glycosyl[1–3,5,6] or *O*-glycosyl[4,5] flavones All species studied to date are clearly distinguished from one another by their flavonoid constituents and it is of phytochemical interest to assess whether chemotaxonomic relationships can be established within genera of the same order To this end we have investigated the flavonoid chemistry of two further species of the order Marchantiales, *Marchantia foliacea* and *M berteroana* Previous work on *Marchantia* appears to be confined to a brief communication,[7] indicating that glycosides of apigenin and luteolin occur in the gametophyte tissue of *M. polymorpha*

RESULTS

Figure 1 shows the 2-D chromatographic pattern of *M foliacea* flavonoids All constituents were present in *M foliacea* (collected from two different locations) and in *M berteroana* Both species are southern hemisphere plants and are clearly distinguished botanically from the ubiquitous *M polymorpha*

Three of the major constituents, A_2, C_2 and T_2, were established as flavones from their UV spectra They proved difficult to hydrolyse with acid and produced, in addition to the aglycones, *A*, *C* and *T*, compounds A_1, C_1 and T_1, which were initially thought to be products of isomerization (see Discussion) The aglycones were isolated by a combination of paper and silica gel chromatography and identified as apigenin (*A*), chrysoeriol (*C*) and tricin (*T*) by direct comparison with authentic samples

The sugar moiety of the glycosides was established as glucuronic acid both by enzymic hydrolysis of A_2, C_2 and T_2 with β-glucuronidase, and by GLC identification of the trimethylsilyl ether derivatives. This, combined with spectral data, establish A_2, C_2 and T_2 as 7-*O*-β-D-glucuronides of *A*, *C* and *T*. A_2, C_2 and T_2 co-chromatographed on paper and polyamide with authentic samples without separation.

[1] NILSSON, E (1969) *Acta Chem Scand* **23**, 2910
[2] MARKHAM, K R , PORTER, L J and BREHM, B G (1969) *Phytochemistry* **8**, 2193
[3] TJUKAVKINA, N A , BENESOVA, V and HEROUT, V (1970) *Coll Czech Chem Commun* **35**, 1306
[4] MARKHAM, K R (1972) *Phytochemistry* **11**, 2047
[5] MARKHAM, K R , MABRY, T J and AVERETT, J E (1972) *Phytochemistry* **11**, 2875
[6] HARBORNE, J B (1967) *Comparative Biochemistry of the Flavonoids*, p 115, Academic Press, London
[7] BREHM, B G and COMP, P C (1967) *Am J Botany (Abstract)* **54**, 660

Mild hydrolysis of the minor constituents, A_3, C_3 and T_3, effected conversion to the major components A_2, C_2 and T_2 Sugar analysis revealed that rhamnose was produced during this conversion and it is therefore concluded that A_3 C_3 and T_3 are the 7-*O*-rhamnosylglucuronides of apigenin, chrysoeriol and tricin respectively

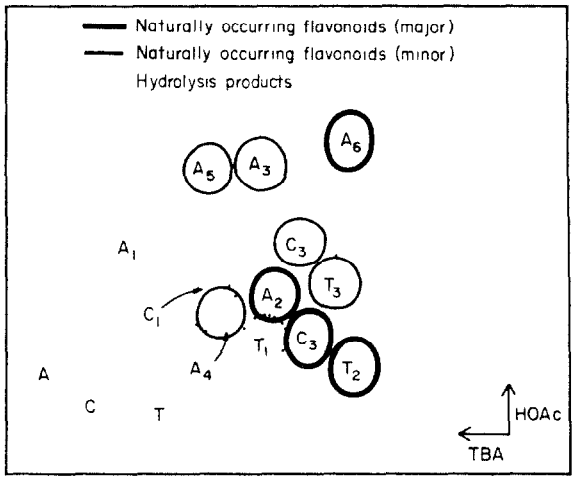

FIG 1 2-D PC OF THE FLAVONOID CONSTITUENTS OF *Marchantia foliacea*

Hydrolysis of A_2, C_2 and T_2 produced compounds A_1, C_1 and T_1 (see earlier discussion) in addition to the aglycones These compounds were shown to be the methyl ester derivatives of A_2, C_2 and T_2 by direct comparison with the esters produced by diazomethane treatment Methylation of glucuronides in the presence of methanolic HCl has also been noted by Asen *et al*,[8] during column chromatography using methanolic HCl as eluent The occurrence of ester formation emphasizes the need for caution in the interpretation of product analysis when flavonoid 7-*O*-glucuronides are hydrolysed with this recommended[9] and widely used reagent

The fourth major constituent of *M foliacea* proved to be completely resistant to acid hydrolysis and no isomerization to other products was observed This information, together with spectral data suggested that A_6 is an apigenin—6,8-di-*C*-glycoside in which the two sugars are identical It proved to be chromatographically indistinguishable from apigenin-6,8-di-*C*-glucoside (vicenin-2) [10]

The only other flavonoids observed in *M foliacea* are compounds A_4 and A_5 which were barely visible on the paper chromatogram The trace quantities isolated were sufficient only for hydrolysis and analysis by UV spectroscopy This data defines A_4 and A_5 as apigenin *O*-glycosides and suggests that they are probably the 7-*O*-glucoside and a 7-*O*-rhamnosyl-glucoside respectively

[8] ASEN, S, NORRIS, K H and STEWARD, R N (1972) *Phytochemistry* **11**, 2739
[9] HARBORNE, J B (1965) *Phytochemistry* **4**, 107
[10] SEIKEL, M K, CHOW, J H S and FELDMAN, L (1966) *Phytochemistry* **5**, 439

DISCUSSION

This work extends further the known examples of methoxylated flavonoids occurring in liverworts To date these have been isolated only from *Reboulia hemispherica*[5] (also Marchantiales) and *Monoclea forsteri*[4] (Monocleales) The species of these two orders also share in common the ability to produce both *O*- and *C*-glycosylated flavones of related structure, and in particular uronide derivatives This apparent biochemical relationship mirrors that previously suggested on botanical grounds Smith[11] and Campbell[12] for example have accepted the placement of *Monoclea* in a monogeneric family, Monocleaceae, within the Marchantiales, although Schuster,[13] while acknowledging a number of marchantioid features in *Monoclea*, prefers its classification in a separate order

The occurrence in *Marchantia* of advanced phytochemical characters such as *O*-glycosylation and methoxylation[14] (especially 8-methoxylation as in *Monoclea forsteri*[4]) seems enigmatic in a group of plants accepted as being exceedingly ancient and apparently unchanged since the mid-Paleozoic [15] However, if the criteria for advanced and primitive characters established for the higher plants[14] are applicable also to the Hepaticae, then the flavonoid chemistry of the group suggests that biochemical evolution parallel to that of the higher plants has occurred in the liverworts Thus the genus *Marchantia* could well comprise the more biochemically advanced liverworts Such a conclusion would be consistent with the observation of Bell and Woodcock[16] that '*Marchantia* seems to represent the highest level of organization achieved by a wholly thalloid gametophyte' Relevant to this is the fact that the flavonoid chemistry of *Marchantia* is not greatly different from that of higher plants such as *Medicago sativa* (which contains compounds A_2, C_2, T_2 and A_4)[17,18] and many grasses (in which *C*-glycosylflavones and *O*-glycosylated methoxylated flavonoids frequently co-occur) [19]

EXPERIMENTAL

Voucher specimens of *Marchantia foliacea*, Mitt and *M berteroana*, L and L have been deposited with Massey University, Palmerston North (MPN 8504) and the Dominion Museum, Wellington (H 396) respectively PCs were run on Whatman 3MM paper using *t*-BuOH–HOAc–H_2O, 3 1 1 (TBA) and 15% acetic acid (HOAc) GLC of sugars as trimethylsilyl ethers was performed on a 90 cm column of 3% SE52 on acid-washed silanized Chromasorb W MS were run on an AEI MS 902 spectrometer using the AEI M S data system DS30

Extraction procedure Air-dried *M foliacea* gametophyte tissue (18 g) was macerated in a Waring-blendor with 20% aqueous MeOH (500 ml) The filtered solution was extracted with light petrol (3 × 100 ml) and then evaporated to yield 1 2 g solids This material was chromatographed on 2-D PC (see Fig 1) and spots were cut out and eluted A similar, but small-scale extraction of *M berteroana* was also carried out for comparison purposes

Tricin 7-O-glucuronide (T_2) The component T_2 appeared on the PC as a dark UV absorbing spot, R_f 0 26 (TBA), 0 13 (HOAc), which turned brilliant yellow in NH_3 It had λ_{max} (MeOH) 249, 269, 348 nm; (NaOMe) 247 sh, 264, 300 sh, 408 nm, (NaOAc) 248 sh, 262 sh, 422 nm, ($AlCl_3$ and $AlCl_3$–HCl) 274, 300 sh, 365, 395 sh, nm Hydrolysis with 5% methanolic HCl for 2 hr produced the aglycone, *T*, together with another product T_1 Hydrolysis of T_2 with β-glucuronidase led solely to the aglycone *T*, R_f 0 66 (TBA) and the sugar (identified by GLC as glucuronic acid) The aglycone was isolated by PC (TBA) and purified on a

[11] Smith, G M (1955) *Cryptogamic Botany*, McGraw-Hill, New York
[12] Campbell, E O (1963) *Tuatara* **11** (Part I) 16, (1954) *Trans Roy Soc N Z* **82**, 237
[13] Schuster, R M (1963) *J Hattori Bot Lab* (26), 185–301
[14] Harborne, J B (1967) Ref 6, p 313
[15] Doyle, W T (1970) *The Biology of the Higher Cryptogams*, p 66, Macmillan, Toronto
[16] Bell, D R and Woodcock, C L (1971) *The Diversity of the Green Plants*, 2nd Edn, p 113, Addison-Wesley, Reading, Massachucetts
[17] Harborne, J B (1967) Ref 6, p 48
[18] Markham, K R and Porter, L J , unpublished work
[19] Harborne, J B (1967) Ref 6, p 246

micro-column (conc HCl washed TLC silica-gel, eluted with H_2O and then MeOH) T had M^+ 330 0732 (100%, $C_{17}H_{14}O_7$ requires 330 0740), fragment $(A + H)^{+}$[23] 153 0190 (15 2%, $C_7H_5O_4$ requires 153 0187), fragment B^{+}[23] 178 0665 (4 5%, $C_{10}H_{10}O_3$ requires 178 0629) It was chromatographically (PC, TLC-SiO_2/MeOH–$CHCl_3$, 2 48) and spectrally identical with tricin (see Ref 20 for data)

Authentic tricin 7-*O*-glucuronide was obtained from powdered *Medicago sativa*[21] leaf material by MeOH–H_2O extraction and preparative PC The material isolated cochromatographed with T_2 on PC (TBA, HOAc) and polyamide (TLC, MeOH–HOAc–H_2O, 18 1 1) The identities of the other major components A_2 and C_2, were established in a manner identical to the above The essential data only, is outlined below,

Chrysoeriol 7-*O-glucuronide* (C_2) R_f 0 35 (TBA), 0 19 (HOAc), dark UV absorbing spot, yellow-green in NH_3 λ_{max} (MeOH) 252, 266, 346 nm, (NaOMe) 250 sh, 262, 302 sh, 395 nm, (NaOAc) 256 sh, 267 sh, 408 nm, (NaOAc–H_3BO_3) 250 sh, 268, 347 nm The aglycone, C, had M^+ 300 0632 (100%, $C_{16}H_{12}O_6$ requires 300 0634), fragment $(A + H)^{+}$[23] 153 0195 (19 4%, $C_7H_5O_4$ requires 153 0187), fragment B^{+}[23] 150 0276 (3 3%, $C_8H_6O_3$ requires 150 0316) Aglycone *C* was chromatographically and spectrally identical with chrysoeriol, and the enzymatically released sugar was glucuronic acid Authentic chrysoeriol 7-*O*-glucuronide, isolated from *Antirrhinum majus* petals,[22] proved to be chromatographically (PC and polyamide) and spectrally identical with C_2

Apigenin 7-*O-glucuronide* (A_2) R_f 0 45 (TBA), 0 27 (HOAc), dark UV absorbing spot, turning yellow-green in NH_3, λ_{max} (MeOH) 268 333 nm, (NaOMe) 270, 300 sh, 384 nm, (NaOAc) 268, 290 sh, 356 sh, 388 nm, (NaOAc-H_3BO_3) 268, 337 nm The aglycone, *A*, had M^+ 270 0521 (100%, $C_{15}H_{10}O_5$ requires 270 0527) Aglycone *A* was chromatographically and spectrally identical with apigenin and the enzymatically released sugar was glucuronic acid Authentic apigenin 7-*O*-glucuronide, isolated from *Antirrhinum majus* petals,[22] proved chromatographically and spectrally identical with A_2

Components A_3, C_3 *and* T_3 The three components, isolated by PC, were resistant to β-glucosidase Brief hydrolysis (reflux with 1 N HCl for 15 min) followed by analysis of the sugars by PC (using ethyl acetate–pyridine–H_2O, 12 5 4) revealed the presence of rhamnose The hydrolysis products co-chromatographed on PC (TBA, HOAc) with A_2, C_2 and T_2

Hydrolysis products A_1, C_1 *and* T_1 Each of the glucuronides A_2, C_2 and T_2 was treated for about 1 min in MeOH with diazomethane The resulting products co-chromatographed on PC (TBA, HOAc) with A_1, C_1 and T_1

Apigenin 6,8-*di-C-glucoside* (*Vicenin-2*) (A_6) R_f 0 28 (TBA), 0 50 (HOAc), dark UV absorbing spot turning green in NH_3 λ_{max} (MeOH) 271 333 nm, (NaOMe) 282, 329, 398 nm, (NaOAc) 280, 301 sh, 387 nm, (NaOAc–H_3BO_3) 273, 280 sh, 320 sh, 340 sh, nm A_6 was isolated by preparative 2-D PC Hydrolysis, 5% HCl, 100°, 6 hr left it paper chromatographically unchanged A_6 co-chromatographed on paper (TBA and HOAc) and polyamide (TLC, MeOH–HOAc–H_2O, 18 1 1), R_f 0 60, with vicenin-2 (ex *Vitex lucens*)[10] but not with violanthin

Minor components A_4 *and* A_5 Compounds A_4, R_f 0 54 (TBA), 0 25 (HOAc) and A_5, R_f 0 57 (TBA), 0 52 (HOAc), both had UV spectra as described above for apigenin 7-*O*-glucuronide (A_2) A_4 was hydrolysed with β-glucosidase to yield apigenin (*A*) and it co-chromatographed on PC (TBA, HOAc) with authentic apigenin 7-*O*-glucoside A_5 did not co-chromatograph with the apigenin 7-*O*-rhamnosylglucoside from *Sophora tetraptera*,[24] but possessed R_fs close to those reported for apigenin 7-*O*-neohesperidoside (see Ref 20)

Acknowledgements—We are indebted to Professor R Hodges, Massey University, Palmerston North for the mass spectra determinations and to Miss S A Dancy of this laboratory for technical assistance.

[20] MARKHAM, K R, MABRY, T J and THOMAS, M B (1970) *The Systematic Identification of Flavonoids*, Springer, New York
[21] HARBORNE, J B (1963) *Phytochemistry* **2**, 327
[22] HARBORNE, J B (1967) Ref 6, p 48
[23] KINGSTON, D G I (1971) *Tetrahedron* **27**, 2691
[24] MARKHAM, K R (1973) *Phytochemistry* **12**, 1091