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FLAVONOID *C*-GLYCOSIDES IN THE HEPATICAE (LIVERWORTS)

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Abstract—Two interconvertible, isomeric, 6,8-di-*C*-glycosides of 5,7,4′-trihydroxyflavone have been isolated from the liverwort *Hymenophytum flabellatum*. This result substantiates earlier indications that flavonoids occur in the liverworts (Hepaticae).

INTRODUCTION

THERE has recently been considerable interest in the range of plants in which flavonoids occur.[1] This has been due in part to their significance as taxonomic and evolutionary markers[2] and also to the fact that the flavonoids are biosynthetically related to lignin.[3] To date flavonoids have been isolated from or identified in a wide range of the lignin-containing angiosperms, gymnosperms and ferns.[1] Of the plants normally considered to be non-ligniferous, only the mosses have so far been proven to contain flavonoids (chiefly flavone *C*-glycosides and anthocyanins).[1,4] However, several existing reports[1,5] indicate that flavonoids might also occur in the liverworts (Hepaticae). In the best substantiated of these[5a] chromatographic information and limited spectral data were presented as evidence for the existence of the flavonols kaempferol and quercetin in *Corsiana*. We discuss in the present paper the isolation and characterization of flavonoid C-glycosides from the liverwort *Hymenophytum flabellatum*.

RESULTS

In the course of studying possible chemical bonding between plant phenolics and the polysaccharide cell wall, we carried out a paper chromatographic survey of a number of different liverworts including *Hymenophytum flabellatum*, *Monoclea forsteri*, *Aneura dentata*,

[1] For a summary see: J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 101, Academic Press, London (1967).

[2] E. C. BATE-SMITH, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 127, Academic Press, London (1963). See also Ref. 1, p. 304.

[3] T. A. GEISSMAN, in *Biogenesis of Natural Compounds* (edited by P. BERNFELD), p. 570, Pergamon Press, Oxford (1963).

[4] Two of us (K. R. M. and L. J. P.) have recently isolated *C*-glycosyl flavonoids from the green alga *Nitella* *Phytochem.* **8**, 1777 (1969).

[5] (a) H. REZNIK and R. WIERMAN, *Naturwiss.* **53**, 1 (1966). (b) H. MOLISCH, *Ber. Deut. Botan. Ges.* **29**, 487 (1911).

Marchantia berteroana, *Symphyogyna frolifer*[6] and a species of *Lunularia*. All but *Symphyogyna* appeared to contain flavonoids as determined by the colour and R_f values of the chromatographic spots and by the u.v. spectra of isolated compounds. *H. flabellatum* was chosen initially for more detailed study because of the relatively good yield of flavonoids obtainable from it.

The paper chromatogram of *H. flabellatum* gametophyte tissue extract (Fig. 1), showed two major u.v. (360 nm) absorbing spots, H-1 and H-2 (as well as a number of minor spots, H-3, 4, 5, 6, 7), which turned yellow-green in the presence of NH_3 vapour. Workable amounts (1 mg) of each of the two major compounds were obtained by polyamide column chromatography of the original extract followed by 2D paper chromatography. Contamination by H-5 (possibly an *O*-glycoside of H-1 or H-2) was avoided by carrying out the initial extraction in the presence of acid.

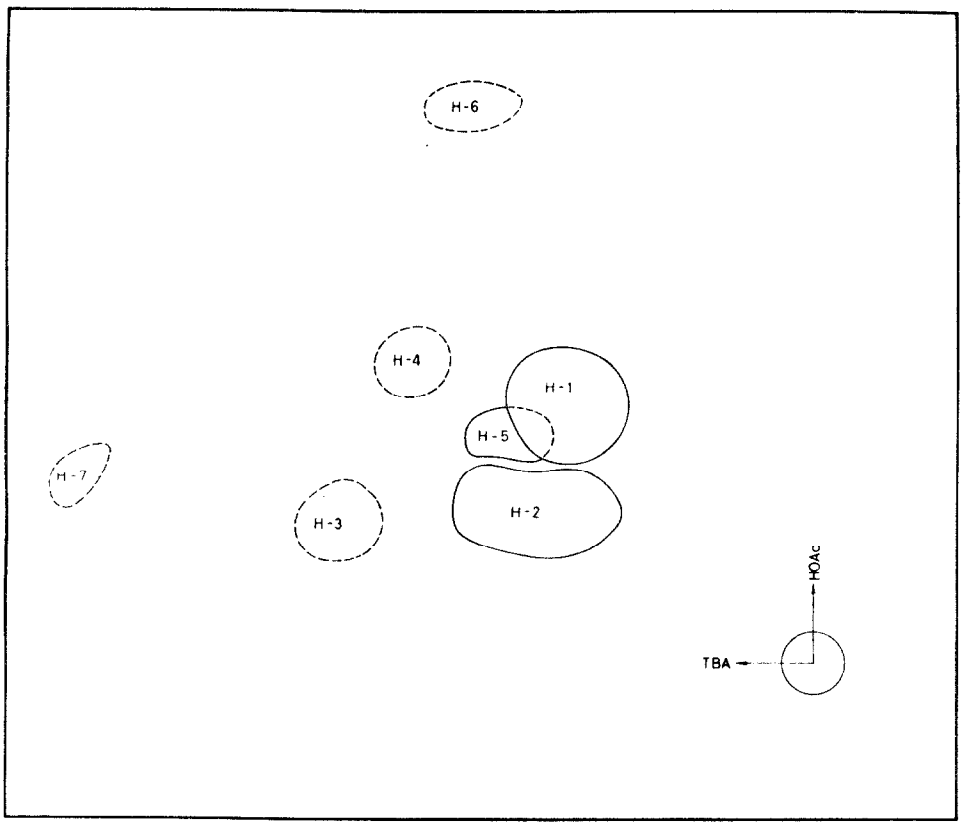


FIG. 1. PAPER CHROMATOGRAM OF *Hymenophytum flabellatum* SHOWING U.V.-ABSORBING SPOTS WHICH TURN YELLOW-GREEN IN NH_3 VAPOUR.

The R_fs and colour reactions of H-1 and H-2 (Table 1) indicate that they are flavonoid glycosides and the u.v. absorption spectra (Table 2) support this. The spectra are almost identical to one another and resemble closely those of certain flavones, e.g. isovitexin. Spectral changes brought about by the diagnostic reagents normally used in flavonoid struc-

[6] Voucher specimen Nos. H 397, H 399, H 398, H 396 and H 395 respectively, Dominion Museum, Wellington, N.Z.

TABLE 1. A COMPARISON OF THE CHROMATOGRAPHIC CHARACTERISTICS OF *H. flabellatum* COMPOUNDS WITH THOSE OF SOME KNOWN FLAVONOIDS

	Spot colour in u.v.	Spot colour in u.v./NH_3	R_f (TBA)*	R_f (HOAc)*
H-1	Purple	Yellow-green	0·33	0·43
H-2	Purple	Yellow-green	0·33	0·30
Apigenin 8-*C*-glucoside (Vitexin)	Purple	Yellow-green	0·48	0·30
Apigenin-6-*C*-glucoside (Isovitexin)	Purple	Yellow-green	0·58	0·54
Apigenin 6,8-di-*C*-glycoside (Vicenin-1)	Purple	Yellow-green	0·30	0·52
Luteolin 6,8-di-*C*-glycoside (Lucenin-1)	Purple	Yellow-green	0·18	0·30

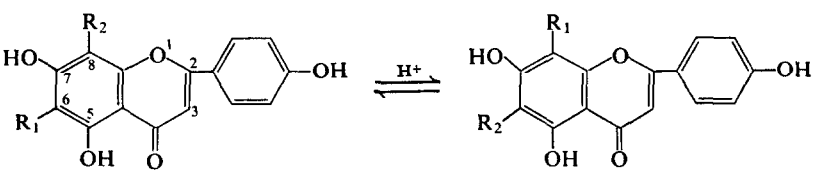
* TBA solvent is *t*-BuOH:HOAc:H_2O (3:1:1) and HOAc solvent is 15% HOAc.

TABLE 2. A COMPARISON OF THE U.V. ABSORPTION SPECTRA (λ_{max}, nm) OF *H. flabellatum* COMPOUNDS WITH THOSE OF A TYPICAL APIGENIN *C*-GLYCOSIDE

Spectrum	H-1	H-2	Isovitexin
MeOH	273, 333	273, 333	269, 335
NaOMe	282, 334, 400	281, 332, 397	277, 332, 395
NaOAc	281, 310sh, 386	282, 308sh, 381	277, 302sh, 385
$NaOAc/H_3BO_3$	276, 323sh, 345sh	276, 326sh, 345sh	271, 346
$AlCl_3$	280, 304, 346, 382	280, 305, 348, 385	277, 302, 350, 383
$AlCl_3$/HCl	280, 304, 346, 382	280, 305, 348, 385	278, 302, 349, 380

ture determinations[7] indicate that both H-1 and H-2 possess an apigenin-type oxygenation pattern (5,7,4′-tri-oxygenated) in which all phenolic hydroxyl groups are unsubstituted.

Although the R_fs of these compounds require that they be glycosides, vigorous acid hydrolyses failed to produce any aglycones, as determined by paper chromatography. Instead, both H-1 and H-2 were partly converted to one another. Such behaviour is typical of flavonoid C-glycosides of type I (in which R_1 and R_2 = H or glycosyl, and $R_1 \neq R_2$) which isomerize by a Wessely-Moser rearrangement to flavonoids of type II or vice versa.[8]



(I) (II)

The NMR spectra of H-1 and H-2 are in complete agreement with the evidence presented above and are consistent with spectra of related flavone *C*-glycosides (Table 3). In the spectra of the trimethylsilyl (TMS) ether derivatives the C-3′,5′ proton doublet is clearly visible at about 6·88 ppm and the C-2′,6′ proton signals appear as a broad doublet at around 7·8 ppm, broadened presumably due to restricted rotation of the B-ring (as has been previously reported for the acetates of 8-*C*-glycosyl flavones[9]). The only other aromatic proton signal

[7] (a) T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer Verlag, New York (1969). (b) L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), pp. 108–154, Pergamon Press, Oxford (1962).

[8] M. K. SEIKEL, J. H. S. CHOW and L. FELDMAN, *Phytochem.* **5**, 439 (1966).

[9] R. A. EADE, W. E. HILLIS, D. H. S. HORNE and J. J. H. SIMES, *Australian J. Chem.* **18**, 715 (1965).

appears as a singlet at about 6·45 ppm and must be due to the C-3 proton rather than a C-6 or C-8 proton since the chemical shift is virtually unchanged after the Wessely-Moser rearrangement. In addition, this signal undergoes a characteristic[7a, 10] 0·15 ppm downfield shift when the TMS group is lost from the 5 position of the completely trimethylsilylated derivatives. Thus it is evident that compounds H-1 and H-2 are 6,8-disubstituted apigenin derivatives. The presence of two *C*-linked glycosyl groups (at C-6 and C-8) is indicated by the signals in the range 3–5 ppm which integrate for 12–14 protons. One of these *C*-glycosyl units could be a rhamnosyl group in view of the presence of a *C*-methyl signal at 1·23 ppm (about 4 protons).[7a, 11]

TABLE 3. CHEMICAL SHIFT VALUES FOR AROMATIC PROTONS IN APIGENIN DERIVATIVES

Flavone	Position of proton (δ, ppm)				
	C-6	C-8	C-2′6′*	C-3′,5′*	C-3†
TMS† derivatives in CCl_4					
Vitexin[7]	6·15	—	7·87	6·92	6·44 (6·32)
Isovitexin[7]	—	6·40	7·72	6·88	(6·39)
Isovitexin-7-*O*-glucoside[7]	—	6·83	7·72	6·88	6·5 (6·38)
Acacetin-8-*C*-glycoside[7]	6·15	—	7·88	6·95	6·48 (6·36)
H-1	—	—	7·82	6·90	6·46 (6·32)
H-2	—	—	7·82	6·88	6·44 (6·30)
Violanthin[11]	—	—	8·15	6·78	6·5
Acetate Derivatives in $CDCl_3$					
Apigenin (Ac_3)[12]	6·86	7·35	7·87	7·26	6·61
Vitexin (Ac_7)[12]	6·83	—	8·15	7·42	6·77
Isovitexin (Ac_6)[12]	—	6·94	7·96	7·42	6·69
H-2	—	—	7·90	7·42‡	6·68

* Doublet (J=9 cps), the C-2′6′ doublet being poorly resolved in many cases.

† Derivatives completely trimethylsilylated except for the 5-hydroxyl group. Bracketed values for the C-3 protons are those observed when the flavonoid is completely trimethylsilylated.

‡ Chemical shift from spectrum in $CDBr_3$.

The NMR assignments above were confirmed by the NMR spectrum of partially acetylated H-2 (in which all hydroxyl groups were acetylated except that at C-5). All aromatic proton signals showed downfield shifts appropriate[12] to their earlier assignments (see Table 3), and the acetyl methyl signals in the region 1·75–2·50 ppm integrated for the 27–30 protons required for a diglycoside.

On the basis of the above evidence, we consider that compounds H-1 and H-2 are interconvertible, isomeric 6,8-di-*C*-glycosides of 5,7,4′-trihydroxyflavone in which the sugar substituent at C-6 differs from that at C-8. Apart from an early publication by Molisch (1911)[5b] this is the first reported occurrence of flavonoid *C*-glycosides in members of the Hepaticae; previously they had only been identified in the angiosperms, ferns and mosses.[13] This finding substantiates earlier indications that flavonoids occur in the liverworts.

[10] M. K. SEIKEL and T. J. MABRY, *Tetrahedron Letters* 1105 (1965).

[11] L. HORHAMMER, H. WAGNER, L. ROSPRIM, T. J. MABRY and H. ROSLER, *Tetrahedron Letters* 1707 (1965).

[12] W. E. HILLIS and D. H. S. HORN, *Australian J. Chem.* **18**, 531 (1965).

[13] R. E. ALSTON, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 305, Appleton-Century-Crofts, New York (1968).

EXPERIMENTAL

NMR spectra were measured on a Varian DA60I spectrometer fitted with a Varian C1024 time averaging computer. Ultra-violet spectra were determined in AR MeOH on a Beckman DK-2A spectrophotometer and diagnostic reagents were made up as directed in Ref. 7a.

Extraction Procedure and Isolation of Flavonoids

Air dried *Hymenophytum flabellatum* gametophyte tissue (12·5 g) was treated with 20% aqueous MeOH (300 ml), to which had been added conc. HCl (20 ml), and then pulverized in a Waring-Blendor. After 5 days' standing the mixture was filtered and the solubles were then evaporated *in vacuo* to dryness. Water (10 ml) was added to the residue and the water solubles were applied to a polyamide (36–100 mesh] column which was subsequently eluted with water containing increasing amounts of MeOH. The column chromatography was monitored by polyamide TLC using MeOH:HOAc:H_2O (90:5:5) as solvent, and fractions containing u.v. absorbing spots were paper chromatographed (in TBA and/or HOAc) for final purification of H-1 and H-2. Approximately 1 mg of each compound was isolated.

Interconversion of H-1 and H-2

Small samples of paper chromatographically pure H-1 and H-2 were dissolved separately in a 1:1 mixture of MeOH:2 N HCl and heated at 100° for 7 hr. Subsequent paper chromatography of the samples revealed that a mixture of H-1 and H-2 had been formed in each case.

Partial Acetylation of H-2

Compound H-2 (1 mg) in AR pyridine (0·25 ml) was treated with Ac_2O (1 ml) and HOAc (0·15 ml). The mixture was kept at 20° for 2 days and was then poured onto crushed ice (10 g). Insoluble material was removed by filtration, dissolved in acetone and applied as a band to a silica thin-layer plate. Benzene:ethyl acetate (1:3) was used as eluant and the acetate derivative was isolated from the single dark band visible in u.v. light. The u.v. spectrum of this derivative changed from λ_{max} (MeOH) 276, 330 nm to λ_{max} 289, 348, 375sh nm on the addition of $AlCl_3$.

Acknowledgement—We thank Mrs. E. A. Hodgson for assistance with the classification of the various liverworts used.

Note added in proof

The R_f values for vitexin and isovitexin quoted in Table 1 of our earlier paper [*Phytochem.* 8, 1777 (1969)] are incorrect. For corrected values see Table 1 in the present paper.