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PHYTOCHEMICAL SUPPORT FOR THE EXISTENCE OF TWO SPECIES IN THE GENUS *HYMENOPHYTON*

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Abstract—Evidence based on flavonoid constituents is cited in support of the existence of two species in the genus *Hymenophyton*, *H. flabellatum* and *H. leptopodum*. A number of apigenin 6,8-di-C-pentosides and pentoside-hexosides are common to both species but the proposed additional species, *H. leptopodum*, is distinguished from *H. flabellatum* by the presence of kaempferol di- and triglycosides. This is the first detailed study of the flavonoid chemistry of any member of the order Metzgeriales and the significance of the findings is discussed.

INTRODUCTION

Stephani's early division of the genus *Hymenophyton* into two species, *H. flabellatum* and *H. leptopodum* [1], has not been accepted by subsequent workers who have preferred to follow Evans's classification [2] in which *H. leptopodum* is reduced to synonomy under *H. flabellatum*. One of the two most recent publications on this topic [3] however, has suggested the reinstatement of *H. leptopodum* as a distinct species and this has found further support in our own morphological [4] and chemical studies. The chemical investigation is detailed in the present communication.

H. flabellatum was the subject of a phytochemical paper in 1969 [5] which fully substantiated the occurrence of flavonoids in liverworts for the first time. The flavonoids isolated were a pair of Wessely–Moser rearrangement related apigenin 6,8-di-C-glycosides and other minor flavonoid constituents were also observed. The present work extends these chemical studies of *H. flabellatum* indirectly through a detailed comparative study of the flavonoids of *H. leptopodum*.

RESULTS

A sample of *Hymenophyton* which appeared to be morphologically distinct from *H. flabellatum* but which was collected in the same location, proved to possess a distinctly different flavonoid pattern (see Fig. 1). This liverwort has since been identified [4] as that referred to by Stephan [1] as *H. leptopodum*. A PC of combined extracts from both species revealed that the major flavonoid components of *H. flabellatum* are also present in *H. leptopodum* (see Fig. 1). However, the group of highly mobile flavonoids, of which HL-4 (i.e. *H. leptopodum*, compound **4**) is dominant, are new to *Hymenophyton* and their presence or absence provides a reliable and readily recognizable character for the distinction of the two species.

Flavone C-glycosides

Compounds HL-**1**, **2**, **3**, **5**, **6** and **11** all have UV absorption spectra consistent with their being apigenin derivatives in which all three OH functions are unsubstituted. As expected for C-glycosyl derivatives these compounds failed to yield aglycones even after prolonged acid treatment.

As with *H. flabellatum*, compounds HL-**1** and **2** are by far the dominant constituents of *H. leptopodum*. They are chromatographically and spectrally identical with the equivalent compounds from *H. flabellatum* which have been shown to be a pair of acid interconvertible apigenin 6,8-di-C-glycosides in which the sugar at C-6 differs from that at C-8 [5]. Chromatographically, HL-**1** behaves as would be expected of a di-C-pentoside [6] in that it is more mobile than vicenin-**1** or **3** on TLC (Si gel) and less mobile on PC (15% HOAc).

MS studies confirm the apigenin di-C-pentoside structures for HL-**1** and **2**. Underivatized HL-**2** exhibited its base ion at *m/e* 295 as expected for an apigenin di-C-glycoside [7]. Although the M^+ was lacking, as is common with di-C-glycosides [6], sequential losses of 2, 3, and 4 molecules of H_2O from the putative M^+ at *m/e* 534 were observed. The observable loss of 4 but not 6 H_2O supports the di-C-pentosyl structure in that C-hexosides are known to lose 3 H_2O per sugar unit whereas C-pentosides lose only 2 [6, 8]. Ions attributable to apigenin (*m/e* 270) and its retro-Diels–Alder fragment (*m/e* 121) were observed, as also were intense ions at *m/e* 397 and 379, thought to be due to the loss of one pentose residue (less $-CH_2$) from the M^+-H_2O and M^+-2 H_2O ions respectively.

MS studies of derivatized HL-2 gave further structural information. In accord with a di-C-pentosylapigenin structure the TMSi-derivative exhibited a weak M^+ at *m/e* 1182 while the permethylated (PM) derivative gave a clearly observable M^+ at *m/e* 660 and a base peak at *m/e* 629. The spectrum of PM HL-2 also suggests the assignment of arabinose as the C-6 linked sugar.

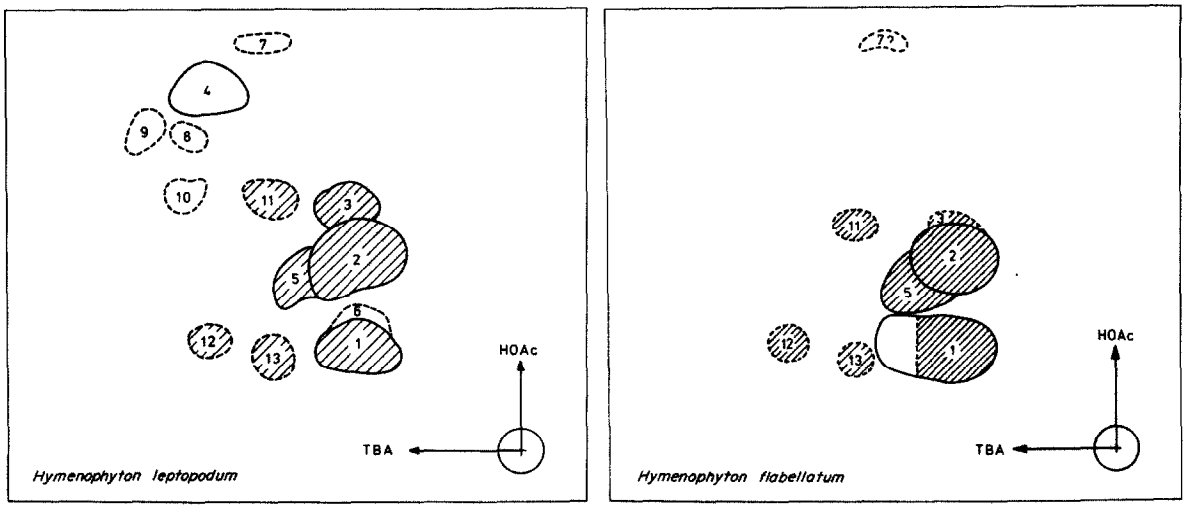

Fig. 1. Two dimensional paper chromatograms of the flavonoids in *H. leptopodum* and *H. flabellatum*. Shaded spots are those common to both species, and weak spots are represented by dotted outlines. Structures assigned are as follows: **1.** Apigenin 6-C-pentoside-8-C-arabinopyranoside; **2.** Apigenin 6-C-arabinopyranoside-8-C-pentoside; **3.** Apigenin 6-C-glucoside-8-C-arabinoside (schaftoside); (Apigenin 6-C-arabinoside-8-C-glucoside, isoschaftoside is hidden by spot 2.); **4.** Kaempferol 3-*O*-rhamnoglucoside-7-*O*-rhamnoside; **5.** Apigenin 6-C-arabinofuranoside-8-C-pentoside; **6.** Apigenin 6-C-arabinoside-8-C-glucoside (isomer of isoschaftoside); **7.** 4,2′,4′,6′-Tetrahydroxydihydrochalcone-3′,5′-di-C-glycoside; **8.** Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside; **9.** Kaempferol 3,7-di-*O*-rhamnoside (kaempferitin); **10.** Kaempferol 3-*O*-rhamnoglucoside; **11.** Neoschaftoside (isomer of schaftoside, HL-3); **12.** Acacetin 7-*O*-glucuronide; **13.** Apigenin 7-*O*-glucuronide.

Studies of model PM flavone 6- and 8-C-xylosides and C-arabinosides have revealed that when the sugar is at C-6, arabinopyranosides are distinguishable from xylopyranosides by the relative intensities of certain fragment ions (in PM 6-C-arabinopyranosides M^+-131 > M^+-119 > M^+-145 instead of M-119 > M^+-131 > M^+-145 in PM 6-C-xylopyranosides) and by the formation of 6-C-formylflavones as PM byproducts from 6-C-arabinopyranosides [9]. HL-2 satisfies both of these criteria and we can therefore reasonably assume the presence of a 6-C-arabinopyranosyl substituent.

The main PM product from HL-1 similarly gave an MS consistent with a di-C-pentosylapigenin. This product however was chromatographically distinct from PM HL-2.

The apigenin di-C-glycoside, HL-5, was separated only with difficulty from HL-2 and was never obtained completely free from it. On acid treatment, it is converted to HL-2 with traces of HL-1 also being formed. After PM, HL-5 gave two products, one of which was chromatographically identical with PM HL-2 and showed the same MS. The second product, PM HL-5, gave a MS which exhibited a dominant M^+ at *m/e* 660 (confirmed by the presence of the usual [11] intense M^+-31 ion at *m/e* 629). HL-5 is thus an apigenin di-C-pentoside and the M^+-119, M^+-131 and M^+-145 ion intensities suggest that the pentose at C-6 is arabinose. However, the effect on this ratio of an additional pentose at C-8 is as yet undetermined.

On the basis of the above data, HL-1, 2 and 5 are all defined as apigenin di-C-pentosides. HL-2 appears to be a 6-C-arabinopyranosyl-8-C-pentosyl-apigenin, and HL-1, because of its ready interconvertibility with HL-2 is probably the Wessely–Moser rearrangement isomer. Since HL-5 yields HL-1 and 2 irreversibly on acid treatment, furanose to pyranose conversion of one or both C-linked sugars is a possible interpretation of this transformation.

Compound HL-3 is an apigenin C-glycoside which undergoes Wessely–Moser rearrangement in acid to produce an isomer which co-chromatographs with HL-2 on 2-D PC. The PC properties of HL-3 suggest [6] that it is a di-C-glycoside and it was found to be chromatographically indistinguishable from schaftoside (apigenin 6-C-glucoside 8-C-arabinoside [10] by TLC and PC in a range of solvents. The MS of PM HL-3 exhibited a M^+ at *m/e* 704 thus confirming the apigenin C-pentoside-C-hexoside structure. The intensity of the M^+-175 (M^+-hexose derived fragment) ion is greater than that of the M^+-131 (M^+-pentose derived fragment) ion, indicating that HL-3 has the hexose linked to the C-6 position and the pentose to C-8 [9, 11]. The PM Wessely–Moser isomer of HL-3, containing the pentose at C-6, was isolated by TLC from a PM sample of HL-2 (which always contains it as an impurity). It was chromatographically identical with PM isoschaftoside [10] and its MS exhibited the ion intensities M^+-131 > M^+-119 > M^+-145 characteristic of 6-C-arabinosides. Further, the formation of 6-C-formyl-8-C-hexosylapigenin as a byproduct of the PM reaction supports the 6-C-arabinosyl structure [9]. The evidence therefore defines the structure of HL-3 as apigenin-6-C-glucoside-8-C-arabinoside (schaftoside) and that of its isomer as apigenin-6-C-arabinoside-8-C-glucoside (isoschaftoside).

Compound HL-11 which is present in only small amounts also undergoes rearrangement in acid. The isomer so formed runs in the vicinity of HL-3 on 2-D PC. The MS of PM HL-11 shows a M^+ at *m/e* 704 and an ion at M^+-175 which is more intense than that at M^+-131, suggesting an apigenin 6-C-hexoside-8-C-pentoside structure for HL-11. Chromatographic comparison of free and PM HL-11 with free and PM neo-

schaftoside (from *Catananche caerulea* [12] confirmed the identity.

Compound HL-6 was separated in small amounts from HL-1 by 'over-run' PC. The MS of the PM derivative exhibited a M^+ at *m/e* 704, an M^+-131 ion of greater intensity than that at M^+-175 and M^+-131 > M^+-119 > M^+-145. This in conjunction with the UV data mentioned above, defines HL-6 as a 6-C-arabinosyl-8-C-hexosylapigenin. The hexose is likely to be glucose since on prolonged acid treatment (2 N HCl, 5 hr) HL-6 was in part isomerized to a mixture of all the C-glucosyl-C-arabinosylapigenin derivatives mentioned above (i.e. schaftoside, isoschaftoside and neoschaftoside). Similar isomerizations have been observed previously with this group of compounds [10, 12] and it is likely that HL-6 is equivalent to the low R_f (in 15% HOAc) isomer produced by acid treatment of isoschaftoside [10]. The subtle structural differences between many of these isomers, however, must await a more detailed investigation.

Flavonol glycosides

The group of highly mobile compounds which distinguish *H. leptopodum* and *H. flabellatum*, HL-4, 8, 9 and 10 were isolated by 2-D PC of early fractions from a cellulose column. On acid hydrolysis all 4 yielded kaempferol. The dominant member of the group, HL-4 displayed UV spectra [13] which defined it as a 3,7-*O*-substituted kaempferol derivative and this was also the case with HL-8 and 9. HL-10 possessed the spectral characteristics of a 3-*O*-substituted kaempferol derivative [13].

Enzyme hydrolyses defined the structure of HL-8 and 9. α-Rhamnosidase* converted HL-9 to kaempferol thus suggesting the structure kaempferol 3,7-di-*O*-rhamnoside (kaempferitin). HL-9 was subsequently shown to be chromatographically identical with authentic kaempferitin. This same enzyme hydrolysed HL-8 to kaempferol 3-*O*-glucoside (identified by PC, β-glucosidase hydrolysis and UV spectroscopy). β-Glucosidase converted it to kaempferol 7-*O*-rhamnoside, the structure of which was established by PC and spectral comparison with authentic material. These data define HL-8 as kaempferol 3-*O*-glucoside-7-*O*-rhamnoside.

Compound HL-4 was unaffected by β-glucosidase but was hydrolysed by α-rhamnosidase to HL-10. The loss of rhamnose from the 7 OH group in this conversion was confirmed by PC analysis of the enzyme hydrolysate. Mild acid hydrolysis of HL-4 also gave HL-10 together with kaempferol-7-*O*-rhamnoside. These conversions suggest that HL-4 is the 7-*O*-rhamnoside of HL-10. Compound HL-10 appears to be a kaempferol 3-*O*-rhamnoglucoside since GLC analysis of the sugars liberated from it on complete acid hydrolysis, established the presence of rhamnose and glucose in the ratio of 1:1. Both rutinoside and glucorhamnoside structures seem to be excluded as possibilities however, since HL-10 is not hydrolysed by α-rhamnosidase or β-glucosidase and is slightly more mobile than authentic kaempferol 3-*O*-rutinoside on PC (TBA and HOAc). This indicates that HL-10 is most likely kaempferol 3-*O*-neohesperidoside (which would not be hydrolysed by the α-rhamnosidase) or kaempferol 3-*O*-rungioside. On the basis of these data, HL-4 is considered to be kaempferol 3-*O*-rhamnoglucoside-7-*O*-rhamnoside and the sugar ratio of 2 rhamnose to 1 glucose was further confirmed by quantitative GLC analysis. Two compounds with this formula have been isolated previously. Kaempferol-3-*O*-rutinoside-7-*O*-rhamnoside was recently reported as a constituent of *Equisetum silvaticum* [14] and is chromatographically similar to HL-4, but the kaempferol-3-*O*-rhamnoglucoside-7-*O*-rhamnoside from *Hesperis matronalis* [15] is reported to have R_f values (relative to the 3-*O*-glucoside-7-*O*-rhamnoside, i.e. HL-8) which preclude its identity with HL-4.

Other flavonoids

Only trace amounts of HL-12 were present in *H. leptopodum* and HL-13 was only observed in heavily loaded chromatograms. Both appeared to undergo isomerization under normal MeOH–HCl hydrolysis conditions, but their complete hydrolysis to aglycone material by β-glucuronidase defines them as glucuronides and their 'isomerization' products as Me esters (see [16]). The aglycone from HL-12 was identified as acacetin by TLC comparison and by MS (M^+ *m/e* 284), and that from HL-13, as apigenin by TLC alone. In both HL-12 and 13, glycosylation at the 7-OH was required by the UV spectra thus defining HL-12 as acacetin 7-*O*-glucuronide and HL-13 as apigenin 7-*O*-glucuronide. The latter structure was confirmed by the co-chromatography (TBA, HOAc, H_2O) of HL-13 with authentic apigenin 7-*O*-glucuronide from both *Ricciocarpus natans* [17] and *Marchantia berteroana* [18], and by the MS of its PM which exhibited a M^+ at *m/e* 530 and M^+-(tetramethylglucuronic acid) at *m/e* 298.

Compound HL-7 is not visibly dominant on the 2-D PC but was isolated in workable quantities from the larger scale column chromatography. It is defined as a dihydroflavonoid type possessing unsubstituted 5- and 7-hydroxyl groups by its UV spectra [13], although it fails to give positive flavanone or dihydroflavonol colour reactions. The R_f values of HL-7 suggest that it is a glycoside, and since it is unaffected by acid hydrolysis (1.5N HCl, 30 min) a C-glycoside structure is probable. This is supported by the PMR spectrum (DMSO-d_6) which exhibits sugar proton signals in the 3–4 ppm region, (the H-1″ appearing at 4.6 ppm) and only two doublets (centred at 7.0 and 6.7 ppm, *J ca* 8 Hz) in the aromatic proton region. The absence of signals attributable to A-ring protons indicates that this 5,7-dihydroxyflavonoid is di-C-substituted in the A-ring. Support for this hypothesis also follows from a PC comparison of HL-7 with the only known [6] flavanone C-glycoside, hemiphloin (naringenin 6-C-glucopyranoside). Although the mobilities are similar in 15% HOAc (HL-7 0.73; hemiphloin, 0.70), HL-7 is significantly less mobile in TBA (0.46; 0.70), as would be expected of a di-C-glycoside [13].

The PMR spectrum is not consistent with a dihydroflavone or dihydroflavonol formulation for HL-7 (a) because it lacks signals in the region of 5.4 ppm which could be attributed [19] to H-2 and (b) because the chemical shifts of H-3′,5′ (6.7 ppm) and H-2′,6′ (7.0 ppm) differ significantly from those reported [20] for naringenin (6.83 and 7.35 ppm) and dihydrokaempferol (6.80 and 7.32 ppm). In fact the only 4′-hydroxyflavonoid type

* Present in Koch-Light pectinase. Removes rhamnose from 3 and 7 positions, from rutinosides but not from neohesperidosides. For full discussion see; Markham, K. R., Porter, L. J., Brehm, B. G., Zinsmeister, H. D. and Mues, R. (1976) *Phytochemistry* **15**, 147.

listed [20] which exhibits B-ring proton signals of the HL-7 type is the dihydrochalcone, phloretin (6.72 and 7.08 ppm). Furthermore, the presence of a broad signal at 2.85 ppm and the lack of an (oxygenated C-2) proton signal at 5.4 ppm supports [21] the presence of the dihydrochalcone $-CH_2-CH_2-$ system.

On the basis of the above evidence, HL-7 is provisionally assigned the structure, 4,2',4',6'-tetrahydroxydihydrochalcone (phloretin-3',5'-di-C-glycoside).

DISCUSSION

The present study of *Hymenophyton* flavonoids has provided an entirely new set of taxonomic characters which may be applied to the now historic problem of whether this genus contains one or more species. The recent re-examination of morphological features [4] which was prompted by the discovery of phytochemical differences within the genus does suggest a distinction of *H. leptopodum* from *H. flabellatum*. The more detailed phytochemistry discussed here also supports this distinction. Whereas *H. flabellatum* is characterized by the presence of apigenin 6,8-di-C-glycosides, *H. leptopodum* exhibits in addition, a high level of kaempferol-*O*-glycosides all of which are absent in *H. flabellatum*. Although the biosynthesis of both flavonoid types begins at the chalcone stage, the biosynthetic pathways from that point are quite distinct. The flavone C-glycosides are produced via chalcone C-glycosides [22] while the biosynthesis of the flavonol-*O*-glycosides involves the important 3-hydroxylation step to yield a dihydroflavonol intermediate [23] which is subsequently oxidised and *O*-glycosylated. A significant biosynthetic difference such as this is certainly consistent with the existence of two distinct species of liverworts. For example, in the liverwort genus Marchantia, the three undisputed separate species, *M. foliacea*, *M. berteroana* and *M. polymorpha* have distinct flavonoid chemistry [18] by differences no greater and generally less than those exhibited by the two *Hymenophyton* specimens.

The present isolation of flavonol glycosides from *H. leptopodum* (order *Metzgeriales*) is the first fully confirmed report of the existence of such compounds in liverworts or mosses. Indeed, until now is was thought that bryophytes were incapable of synthesizing 3-hydroxyflavonoids [24]. An earlier suggestion that they might occur in *Corsinia coriandrina* [25] (order *Marchantiales*) is at variance with all subsequent work on liverworts from this order [18] and needs more detailed investigation. The existence of any flavonoids at all in members of the order *Metzgeriales* is of interest since many other species investigated by us have apparently lacked these compounds. This finding could well be interpreted as providing support for the belief of many bryologists [26] that the family Hymenophytaceae is one of the most highly evolved in the order *Metzgeriales*.

The detection of variable but very low levels of flavone *O*-glucuronides in *Hymenophyton* deserves comment. Such glycosides have previously been detected, in the liverworts, only in members of the order Marchantiales. In this order they are the dominant flavonoids and are apparently ubiquitous [17]. While the present finding does not seriously conflict with previous observations that dominance of flavone-*O*-glycuronides characterizes the flavonoid chemistry of members of the order *Marchantiales*, it does raise the question of a possible phylogenetic relationship between the more advanced *Metzgeriales* and the *Marchantiales*. However, to our knowledge no such suggestion has previously been made on biological grounds, and so further comment must await a more extensive phytochemical survey of the *Metzgeriales*.

EXPERIMENTAL

Samples of *H. leptopodum* and *H. flabellatum* were collected from the same site (Keith George Memorial Park, Silverstream, N.Z.) in Feb. 1968, Dec. 1972, Dec. 1974 and Sept. 1975 and voucher specimens deposited in the Massey University herbarium: *H. flabellatum* (MPN 17029) and *H. leptopodum* (MPN 8546 and 17030). 2-D PC was carried out on Whatman 3 MM paper using *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and 15% HOAc (HOAc). For TLC, Schleicher and Schull plastic backed Si gel (F 1500) sheets were used with the solvent mixtures EtOAc–Pyr–H_2O–MeOH (16:4:2:1) and (80:12:10:5) for C-glycosides and $CHCl_3$–MeOH (47:3) for aglycones, and S. and S. cellulose (F 1440) with the solvents TBA, HOAc and H_2O for glycosides. UV spectra were recorded using shift reagents as described in ref. [13].

Isolation of flavonoids. Dried plant material (10 g) was macerated in a Waring-blendor with MeOH–H_2O (1:1) (400 ml) and left 18 hr to extract. Extraction was carried out several times and the extracts combined and evaporated. Initial investigations were conducted on compounds isolated from 2-D PCs but bulk supplies were isolated by cellulose column chromatography. Extract (3 g) was applied in H_2O to a cellulose (Whatman CF-11) column (6.4 × 40 cm) and elution was carried out with dist H_2O (3.7 l.) followed by MeOH (2 l.). Fractions (15 ml) were collected starting from when the leading band reached half way. 2-D PC analysis of selected fractions revealed the following flavonoid distribution: fractions 10–27 (HL-7), 27–46 (HL-7 and **4**); 47–63 (HL-**4**, **8**, **9**, **10** and HL-3, **11**, **12** and **13**); 64–79 (HL-3, **11** and **2**); 80–125 (HL-**2**); 126–180 (HL-**5** and **2**); 181–246 (HL-**5** and **1**); 247–280, MeOH (HL-**1** and **6**); 281- (HL-**1**). In another isolation, the extract was applied to a Sephadex LH-20 column and elution carried out with MeOH–H_2O (19:1). The separations achieved were comparable to but no better than those described above. Pure samples of each flavonoid were obtained by repeated PC of each group of fractions in HOAc and/or TBA. 'Over-running' was frequently required to achieve separation e.g. for the separation of HL-3 and **2** (HOAc), HL-**2** and **5** (TBA) and HL-**1** and **6** (HOAc).

Comparison of H. leptopodum *and* H. flabellatum. Flavonoid patterns for both species were determined by 2-D PC (see Fig. 1), and flavonoids common to both were identified by chromatographing a mixture of the two extracts.

Hydrolysis conditions. (a) To check for isomerization of the C-glycosides the compound was heated at 100° for 2–3 hr with MeOH–3N HCl (1:1). (b) For complete hydrolysis of the flavonol glycosides, heating with the above acid for 10–15 min was sufficient. (c) For partial hydrolysis of HL-**4** MeOH–0.5% HCl (1:1) was used. Reflux was continued for 15 min, the acid neutralized with NH_4OH and the hydrolysate evaporated to dryness. The products were extracted from the residue with hot MeOH and analysed (and isolated) by 2-D PC. Spot intensity and R_f of products (TBA, HOAc): HL-**4**, strong (0.57, 0.80); HL-**10**, medium (0.68, 0.65); K-3-rha, medium (0.68, 0.15); kaempferol, weak (0.75, 0.02). (d) Enzyme hydrolyses were carried out in H_2O at 20°. Enzymes: β-glucuronidase (ex. Marine Mollusc, Koch-Light); pectinase (ex. *A. niger*, Koch-Light). (e) For the isomerization of HL-**6**, HL-**6** was treated with 2N HCl at 100° for 4.5 hr. Products were analysed and compared with authentic compounds by 2-D PC and TLC (cellulose, HOAc). R_f values on TLC: schaftoside 0.48, isoschaftoside 0.37, HL-**6** 0.14 (cf. ref. [10]: 0.48, 0.35 and 0.13).

MS of flavone glycosides. PM ethers were prepared in DMF with MeI and NaH and purified by TLC (Si gel) in $CHCl_3$–

Me_2CO, 8:2. The MS of the PM derivatives contained the following major ions.

HL-**1** (m/e). 660 (M^+, 29%), M^+-15 (23%), M^+-31 (100%), M^+-45 (10%), M^+-46 (9%), M^+-47 (11%), M^+-59 (11%), M^+-61 (16%), M^+-119 (23%), M^+-131 (61%), M^+-145 (25%), M^+-158 (23%), M^+-163 (13%), M^+-177 (13%).

HL-**2** (m/e). 660 (M^+, 23%), M^+-15 (29%), M^+-31 (100%), M^+-45 (5%), M^+-46 (9%), M^+-47 (10%), M^+-61 (16%), M^+-119 (31%), M^+-131 (47%), M^+-145 (22%), M^+-163 (10%), M^+-175 (3%).

HL-**3** (m/e). 704 (M^+, 20%), M^+-15 (35%), M^+-31 (100%), M^+-47 (8%), M^+-103 (15%), M^+-119 (7%), M^+-131 (15%), M^+-145 (8%), M^+-163 (35%), M^+-175 (41%).

HL-**3** *isomer* ex. *HL*-**2** (m/e). 704 (M^+, 28%), M^+-15 (25%), M^+-31 (100%), M^+-45 (10%), M^+-47 (10%), M^+-63 (13%), M^+-61 (20%), M^+-119 (28%), M^+-131 (33%), M^+-145 (18%), M^+-163 (15%), M^+-175 (18%).

HL-**5** (m/e). 660 (M^+, 34%), M^+-15 (23%), M^+-31 (100%), M^+-45 (8%), M^+-46 (11%), M^+-47 (23%), M^+-119 (27%), M^+-131 (30%), M^+-145 (13%).

HL-**6** (m/e). 704 (M^+, 33%), M^+-15 (16%), M^+-31 (100%), M^+-45 (5%), M^+-46 (7%), M^+-47 (10%), M^+-61 (11%), M^+-119 (22%), M^+-131 (37%), M^+-145 (14%), M^+-163 (8%), M^+-175 (11%).

HL-**11** (m/e). 704 (M^+, 23%) M^+-15 (25%), M^+-31 (100%), M^+-45 (8%), M^+-47 (10%), M^+-78 (14%), M^+-131 (14%), M^+-163 (23%), M^+-175 (31%).

HL-**13** (m/e). 530 (M^+, 100%), M^+-14 (35%), M^+-32 (10%), M^+-232 (dimethyl apigenin, 65%), M^+-246 (29%), M^+-261 (21%). MS of underivatized HL-**2** included the following ions (*m/e*): 534 (M^+, 0%), M^+-$2H_2O$ (4%), M^+-$3H_2O$ (30%), M^+-$4H_2O$ (20%), 397 (38%), 379 (79%), 295 (100%), 270 (14%), 121 (42%). Byproducts isolated by TLC from PM of HL-**2** and HL-**3** isomer ex HL-**2** gave MS containing M^+ = (8-C-pentosylapigenin + 28) and (8-C-hexosylapigenin + 28) respectively.

Qualitative sugar analyses. These were carried out on hydrolysates by PC with the solvent, *n*-BuOH–Pyr–H_2O (2:2:1) using *p*-anisidine HCl for spot detection.

*Quantitative sugar analyses of HL-***4** *and HL-***10**. Samples of HL-**4** and HL-**10** were hydrolysed (as in (b)) but for 30 min and the amount of kaempferol produced was determined spectrophotometrically (367 nm). The hydrolysis products were silylated (TMCS–HMDS–Pyr) and standard solns in hexane prepared. Aliquots of these were analysed by GLC (2 m column, 3% OV-1 on chromosorb WHP, column temp 180°, FID) and compared directly with standard solns of TMSi-glucose and TMSi-rhamnose (both of which had been acid treated prior to derivatization). Response factors for both sugars were determined from parallel sugar analysis on authentic quercetin 3-*O*-rhamnoglucoside. HL-**4** yielded rhamnose, 0.123 mg, and glucose, 0.067 mg, and an equivalent amount (UV) of HL-**10** yielded rhamnose, 0.053 mg, and glucose, 0.068 mg.

Spectrophotometry of H. leptopodum *flavonoids.* The various groups of flavonoids gave relevant UV/visible absorption spectra as follows.

HL-**4**, **8** *and* **9**. λ_{max}(MeOH) 228*sh*, 264, 320*sh*, 345 nm: (NaOMe) 245, 275, 300*sh*, 346, 380 nm; (NaOAc) 262, 292*sh*, 320*sh*, 350 nm.

HL-**10**. λ_{max} (MeOH) 265, 296*sh*, 324*sh*, 345 nm; (NaOMe) 273, 325, 390 nm.

HL-**1**, **2**, **3**, **5**, **6** *and* **11**. λ_{max} (MeOH) 271, 334 nm; (NaOMe) 281, 327, 396 nm; (NaOAc) 279, 300*sh*, 380 nm.

HL-**12**. λ_{max} (MeOH) 267, 320 nm; (NaOMe) 280, 353 nm.

HL-**13**. λ_{max} (MeOH) 270, 332 nm; (NaOMe) 275, 300*sh*, 392 nm; (NaOAc) 269, 350*sh*, 385 nm.

HL-**7**. λ_{max} (MeOH) 285, 329*sh* nm; (NaOMe) 245*sh*, 321 nm.

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