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FLAVONOIDS OF THE PRIMITIVE LIVERWORT *TAKAKIA* AND THEIR TAXONOMIC AND PHYLOGENETIC SIGNIFICANCE

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Key Word Index—*Takakia lepidozioides*; *T. ceratophylla*; Hepaticae; flavonoid glycosides; 8-hydroxyacacetin (takakin) 8-*O*-glucuronide and 8-*O*-xylosylglucoside; luteolin 6-*C*-arabinopyranoside-8-*C*-pentoside; tricetin *C*-glycosides; chemotaxonomy; phylogeny.

Abstract—The flavonoid chemistry of *Takakia* is described for the first time. *T. lepidozioides*, thought to be amongst the most primitive of extant liverworts, contains a high level and wide variety of flavone *C*- and *O*-glycosides, many of which are unique. New flavonoids include the 8-*O*-glucuronide and 8-*O*-xylosylglucoside of takakin (8-hydroxyacacetin), luteolin 6-*C*-arabinoside-8-*C*-pentoside, kaempferol 3-*O*-glucoside-7-*O*-xyloside and a number of tricetin *C*-glycosides. The only other known *Takakia* species, *T. ceratophylla*, contains the same 4 major constituents but significantly lacks flavonols. The often suggested relationship of *Takakia* with the order Calobryales is not supported by the available flavonoid data. Biochemical affinities of *Takakia* with all major liverwort orders are noted and the flavonoid data are interpreted as supporting the concept of *Takakia* as an isolated branch among the ancestors of modern bryophytes.

INTRODUCTION

The genus *Takakia* consists of two species, *T. lepidozioides* and *T. ceratophylla* [1]. They are found only in a few highly disjunct populations in remote areas of Asia and North America and as a result, the first *Takakia* species was not described until 1958 [2]. The genus is generally considered to be the most primitive group of extant liverworts [3–5]. Primitive and unusual features include a chromosome number of 4 for *T. lepidozioides* (the lowest number in any land plant), highly disjunct distribution, isophylly, and a radially symmetrical growth habit [3, 5]. Also, for neither species have sporophytes or male plants yet been found.

Takakia has been placed in a number of different taxonomic ranks. When discovered it was assigned a separate order, Takakiales, near the order Calobryales. However, Proskauer in 1962 [6] included it in the Calobryales and Schuster [7] subsequently gave it suborder rating (Takakiinae) within this order. Grolle [8] preferred a family rating (Takakiaceae). The resemblance of *Takakia* to certain mosses led Mizutani in 1972 (quoted in ref. [3]) to create for it a class, Takakiopsida, within the Musci. On balance, however, a relationship with the Calobryales, appears to be favoured.

Extensive chemical studies have been carried out on many liverwort groups [9], but the only published work relating to the Calobryales (or *Takakia*) describes the flavonoids of *Haplomitrium gibbsiae* (Calobryales) [10]. This plant contains acylated glucosides of apigenin and isoscutellarein whose structures ar unique to the bryophytes and show a degree of biochemical advancement unexpected in a primitive order. The present communication describes the flavonoid chemistry of *Takakia* and provides a preliminary interpretation of the taxonomic and phylogenetic implications of the flavonoid data.

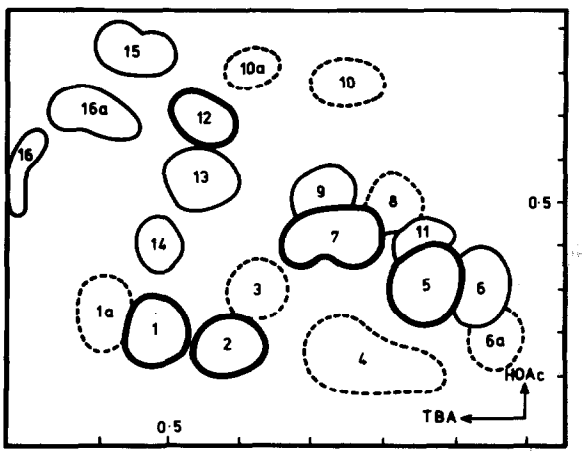

Fig. 1. 2D-PC display of flavonoid glycosides in *Takakia lepidozioides*. Weak spots are represented by dotted outlines and strong spots by thickened outlines. Structures assigned are as follows: **1**, 8-Hydroxyacacetin 8-*O*-glucuronide (takakin 8-*O*-glucuronide). + apigenin 7-*O*-glucuronide; **1a**, apigenin 7-*O*-glucoside; **2**, 8-hydroxyapigenin 8-*O*-glucuronide (isoscutellarein 8-*O*- glucuronide); **3**, tricetin 6-mono-*C*-pentoside? **4**, apigenin 6-*C*-pentoside-8-*C*-arabinopyranoside + luteolin 6-*C*-pentoside-8-*C*-arabinopyranoside + tricetin 8-mono-*C*-pentoside? **5**, luteolin 6-*C*-arabinopyranoside-8-*C*-pentoside; **6**, tricetin 6-*C*-glucoside-8-*C*-arabinoside (3′, 5′-dihydroxyschaftoside; **6a**, tricetin 6-*C*-arabinopyranoside-8-*C*-pentoside; **7**, apigenin 6-*C*-arabinopuranoside-8-*C*-pentoside, **8**, apigenin 6,8-di-*C*-pentoside derivative? **9**, apigenin 6-*C*-glucoside-8-*C*-arabinoside (schaftoside) + apigenin 6,8-di-*C*-glucoside (vicenin-2); **10**, *O*-glycoside of **5**; **10a**, *O*-glycoside of **7**; **11**, luteolin 6-*C*-glucoside-8-*C*-arabinoside (3′-hydroxyschaftoside) + luteolin 6,8-di-*C*-glucoside (lucenin-2); **12**, kaempferol 3-*O*-glucoside-7-*O*-xyloside **13**, 8-hydroxyacacetin 8-*O*-xylosylglucoside (takakin 8-*O*-xylosylglucoside); **14**, quercetin 3-*O*-glucoside, **15–16a**, non-flavonoid dark puple spots (UV).

RESULTS

A 2D-PC of the acetone-H_2O extract of *T. lepidozioides* (Fig. 1) indicated the presence of a high level of flavonoids in this species. All but three of the indicated UV-absorbing spots in Fig. 1 proved to represent flavonoids, comprising flavone *O*-glycosides, flavone *C*-glycosides and flavonol *O*-glycosides.

Flavone O-*glycosides*

Four of the components (**1a**, **1**, **2** and **13**) proved to be flavone *O*-glycosides. Two of these, **1a** and **2** (apigenin 7-*O*-glucoside and isoscutellarein 8-*O*-glucuronide) and a minor component (apigenin 7-*O*-glucuronide) of **1**, have been encountered previously in the Hepaticae [11] and were identified by direct comparison (PC in TBA, HOAc, H_2O; UV and MS) of both glycosides and enzymically produced aglycones with authentic material. The remaining two flavone glycosides (**1** and **13**) both gave an absorption spectrum in MeOH similar to that of component **2** above. On acid hydrolysis they gave the same aglycone which had absorption spectra similar to scutellarein. However, this proved to be an isomer produced by Wessely–Moser rearrangement of the actual aglycone, which was subsequently obtained by enzyme hydrolysis. The enzymically produced aglycone gave an absorption spectrum similar to that of isoscutellarein [11] except that the 4′-hydroxyl group was substituted (decrease in band I intensity on NaOMe addition). Mass spectromety indicated a MW of 300 and RDA fragment ions at *m/e* 168 (A-ring) and 132 (B-ring) establish the aglycone as 5,7,8-trihydroxy-4′-methoxyflavone. This is the first report of its occurrence as a natural product and we propose the name takakin for this aglycone.

The glycoside, **1**, is mobile in H_2O (PC) and is hydrolysed by β-glucuronidase to produce glucuronic acid. In this process the absorption spectrum is changed drastically indicating glycosylation at the 8- rather than the 7-hydroxyl [10]. It is therefore assigned the structure, takakin 8-*O*-glucuronide. Glycoside **13** has the same absorption characteristics as **2** but is relatively immobile in H_2O and is unaffected by β-glucuronidase. Mild acid hydrolysis gave, in addition to the two aglycone isomers, two sugars which were identified by GLC as xylose and glucose. Since the glycoside was unaffected by β-glucusodase its structure is considered to be takakin 8-*O*-xyloglucoside.

Flavonol O-*glycosides*

Flavonol aglycones were isolated from two components **12** and **14**, on mild acid hydrolysis, **14** had the absorption characteristics of a quercetin 3-*O*-glycoside, gave quercetin and glucose on hydrolysis and co-chromatographed with quercetin 3-*O*-glucoside. **12** gave absorption spectra which indicated that it was a 3,7-disubstituted kaempferol derivative and on acid hydrolysis yielded kaempferol which was identified by MS and by chromatographic comparison. The sugars liberated were glucose and xylose (GLC). Hydrolysis of **12** with a crude pectinase preparation gave two products, one of which on PC (TBA, HOAc) appeared as a dark spot in UV and co-chromatographed with kaempferol 3-*O*-glucoside. The other appeared as a yellow fluorescent spot in UV and possessed higher R_f values than authentic kaempferol 7-*O*-glucoside. The structure of **12** is thus defined as kaempferol 3-*O*-glucoside 7-*O*-xyloside, a new natural product.

Flavone C-*glycosides*

A large number of flavone glycosides were present (components **3**, **4**, **5**, **6**, **6a**, **7**, **8**, **9**, and **11**, Fig. 1) which did not hydrolyse in acid (3N HCl, 2 hr, 100°) and therefore are defined as flavone *C*-glycosides.

(1) *Apigenin di-C-glycosides.* A major flavonoid component of *T. lepidozioides* is **7**, which has a UV spectrum consistent with an apigenin *C*-glycoside. **7** undergoes a Wessely–Moser rearrangement when heated in 3N HCl to produce an isomer with the same R_f in TBA and lower R_f in HOAc. This behaviour is characteristic of an unsymmetrical apigenin di-*C*-pentoside, such as those isolated from *Hymenophyton leptopodum* [12]. The molecular constitution of **7** was confirmed by MS of its permethyl ether which displayed the anticipated molecular ion, M^+ 660, and corresponding fragment ions. The fragment ions also suggested the presence of a 6-*C*-arabinopyranoside moiety as judged by the relative intensity of the ions, M-131 > M-119 > M-143 [13]. Moreover, **7** cochromatographed with the *H. leptopodum* 6-*C*-arabinopyranosyl-8-*C*-pentosylapigenin [13, and see Note Added in Proof]. The second pentose moiety is likely to be xylose as this sugar is present in both flavone and flavonol *O*-glycosides in this plant. Compound **10a** is considered to be a readily hydrolysable *O*-glycoside of **7**, since on work-up it converted to **7**.

Two further apigenin di-*C*-glycosides constitute component **9** and these were separated by over-run PC in TBA. Acid hydrolysis of the major component produced an isomer which co-chromatographs with **7** and two minor products, behaviour characteristic of schaftoside (apigenin 6-*C*-glucoside-8-*C*-arabinoside) [12]. This constitution was confirmed by co-chromatography with authentic schaftoside from *Hymenophyton leptopodum* [12] on silica and cellulose TLC (four solvents) and MS of its permethyl ether (M^+ 704 and corresponding fragments). The minor component of **9** was not isomerized by acid, gave a MS (permethyl ether) exhibiting M^+ at 748 *m/e* plus corresponding fragment ions [13], and co-chromatographed with authentic vicenin-2. Vicenin-2 is a constituent frequently found in liverworts [9, 14].

(2) *Luteolin di-C-glycosides.* The other major flavone *C*-glycoside component, **5**, is the luteolin analogue of **7**. This is evidenced (a) by its similar behaviour (to **7**) on acid treatment to produce a less mobile (in HOAc) isomer, and (b) by the MS of its permethyl ether which revealed the anticipated molecular ion, M^+ 690, for a luteolin di-*C*-pentoside (together with a virtually identical fragmentation pattern to **7**). It is therefore proposed that **5** is luteolin 6-*C*-arabinoside-8-*C*-pentoside, by analogy with the apigenin compound. This is the first report of its occurrence as a natural product. Compound **10** which converted to **5** on work-up is considered to be a readily hydrolysable *O*-glycoside of **5**.

Similarly the luteolin di-*C*-glycoside corresponding to schaftoside was also detected. Compound **11** isomerises to give a product which co-chromatographs with **5**, and this isomer was detected in the MS of the permethyl ether of **5**, (M^+ 734 and corresponding fragments). Therefore **11** is luteolin 6-*C*-glucoside-8-*C*-arabinoside, a compound which was reported to be present in *Catananche caerulea* [15]. The properties reported for this latter compound are equivalent to those of **11**. It is probable that lucenin-2 is also present, as ions corresponding to a luteolin di-*C*-hexoside (M^+ 778 and corresponding fragments) are visible in the MS of **5**.

(3) *Tricetin di-C-glycosides.* It was recognised that two of the spots, **6** and the minor component **6a**, which had a very low mobility in TBA and HOAc, had a distinctive appearance when fumed with ammonia. Rather than the dull green-yellow observed with the luteolin di-*C*-glycosides, **6** and **6a** turned a bright orange-yellow, characteristic of tricetin *C*-glycosides. Our structural work on these compounds however was limited to UV absorption and comparative R_f data, since rapid deterioration of both, on storage, made later MS studies meaningless. Tricetin *C*-glycosides have been isolated only once previously (from the leafy liverwort *Plagiochila asplenioides* [16], and they were described in this report as being "very unstable in the presence of oxygen".

The UV spectra of the major component, **6**, and its colouration on a PC when fumed with NH_3, were identical to those of an authentic sample of tricetin 6,8-di-*C*-glucoside from *Plagiochila asplenioides*. The main difference in the UV spectra of lucenin-2 and tricetin di-*C*-glycosides is the presence of a 330 nm peak in the $AlCl_3$/MeOH spectrum of the former. Other evidence is provided by comparative R_f values. The $R_{\text{lucenin-2}}$ values for **6** (0.75 (HOAc) and 0.80 (BAW)) are in exact agreement with those calculated from Mues and Zinsmeister's data [16] for tricetin 6-*C*-hexoside-8-*C*-pentoside (as also are the $R_{\text{tricetin 6,8-di-C-hexoside}}$ values). Therefore **6** is considered to be a tricetin 6-*C*-hexoside-8-*C*-pentoside and correspondingly **6a** is considered to be a tricetin di-*C*-pentoside (from relative R_f values).

From the above data it appears that three homologous series of flavone di-*C*-glycosides exist in *T. lepidozioides*. These are the flavone di-*C*-pentosides, **6a**, **5** and **7**, the flavone 6-*C*-hexoside-8-*C*-pentosides, **6**, **11** and **9**, and the flavone di-*C*-hexosides. The consistent chromatographic relationship of these series will be apparent by inspection of Fig. 1. The chromatographic complexity of the *C*-glycoside region, and the scarcity and small size of the plant, precluded a more precise study of this region.

(4) *Miscellaneous flavone C-glycosides.* Three further minor flavone *C*-glycosides, **3**, **4**, and **8** remain to be discussed. Component **3** gave a UV spectrum similar to that of a luteolin *C*-glycoside [17] and chromatographed near iso-orientin on cellulose TLC (TBA, HOAc) and SiO_2 (APWM). However, it was noted that **3** differed from iso-orientin in two ways. The appearance of the spot when fumed with ammonia was similar to **6** and **6a** and the 330 nm peak was absent in the $AlCl_3$/MeOH spectrum. This compound, like **6** and **6a**, had decomposed by the time MS studies were performed, and is thought to be a tricetin 6-mono-*C*-pentoside.

Component **4** was diffuse and consisted of a mixture of flavone *C*-glycosides. Its components were all immobile in H_2O (cellulose TLC) and unaffected by β-glucuronidase. Acid hydrolysis of **4** produced **5** and **7** as rearrangement products. Therefore **4** must consist of the Wessely–Moser rearrangement isomers of **5** and **7** together with a third component which is possibly the rearrangement product of **3**.

Component **8** was isomerised on acid treatment to an isomer running immediately below it in the HOAc dimension of a 2D-PC and thus behaved like a flavone di-*C*-pentoside. Its absorption characteristics (MeOH and MeOH/NaOMe) resemble those of a 7,4′-disubstituted apigenin derivative.

Only a very small amount of herbarium material was available for the study of *Takakia ceratophylla*. This species as judged by 2D-PC, possesses a markedly lower concentration and simpler pattern of flavonoids than *T. lepidozioides*. Of the 12 UV-absorbing spots visible on the 2D-PC, only 5 proved to be flavonoid. Hydrolyses, and chromatographic and UV absorption studies defined four of these as being identical to compounds **1**, **2**, **5** and **7** of *T. lepidozioides*, and the fifth, as a new (to *Takakia*) 4′-substituted flavone glucuronide. Flavonol glycosides were not found.

DISCUSSION

Two samples of *T. lepidozioides* were studied in the present work, one from Japan and the other from British Columbia and both possessed essentially the same flavonoid constituents as judged by 2D-PC. Thus, although *T. lepidozioides* occurs only in a few highly disjunct populations it would appear to be a chemically discernible species. The diversity and high level of flavonoids found in this species were unexpected and contrast markedly with the lack of diversity and generally low levels found in other morphologically 'simple' liverworts, such as *Riella* and *Sphaerocarpos* [18] and some species of *Riccia* [19]. The range of flavonoid types is in fact the most extensive yet found in any bryophyte. Although flavonoids with phylogenetically 'primitive' characters (such as *C*-glycosylation, and flavonols [20]) are produced by *T. lepidozioides*, the additional presence of flavonoids exhibiting 'advanced' biochemical characteristics (such as tri-oxygenation in the A-ring, *O*-methylation, and mixed and complex *O*-glycosylation) appears enigmatic in a 'primitive' plant.

The only other species in this genus, *T. ceratophylla*, appears to produce lower levels of flavonoids. However, in common with *T. lepidozioides* it contains as its four major flavonoids, the 8-*O*-glucuronides of 8-hydroxyapigenin and 8-hydroxyacacetin and the 6,8-di-*C*-pentosides of apigenin and luteolin. The wide range of flavonoids found in *T. lepidozioides* does not appear in *T. ceratophylla* and, significantly, flavonol glycosides are absent. In a morphologically and chemically conservative genus like *Takakia*, such a difference in flavonoid patterns may well indicate a taxonomic gulf greater than at the species level. Hattori *et al.* [21] have previously proposed that the two species may represent separate subgenera.

The relationship between *Takakia* and the order Calobryales has been discussed in some detail by successive bryologists, and it is of interest to comment upon the contribution provided by the flavonoid data currently available. The comparative flavonoid biochemistry of *Takakia* and *Haplomitrium gibbsiae* (Calobryales) does not support the close relationship between the Calobryales and *Takakia* that is suggested by some bryologists [1, 8, 22]. The *H. gibbsiae* flavonoids are all flavone-*O*-glucosides whereas flavone glucosides are found only as minor constituents in *T. lepidozioides*. In contrast, *Takakia* produces flavone *O*-glucuronides, flavone *C*-glycosides and flavonol *O*-glycosides (in one species) in abundance. Acylation, which is predominant in *H. gibbsiae*, is entirely absent from *Takakia* and, conversely, B-ring di- and tri-oxygenation, methylation and glucosylation with sugars other than glucose, are absent from *H. gibbsiae*. Clearly other *Haplomitrium* species need to be chemically investigated before the evidence is con-

clusive, but on balance the flavonoid chemistry does not support a close relationship between the two groups.

The flavonoids of *Takakia* perhaps relate most closely to those of *Hymenophyton* [12] of the order Metzgeriales. *Hymenophyton* produces most of the main flavonoid types found in *Takakia*, although flavone *O*-glycosides occur at very low levels. The flavonoid features not found in *Hymenophyton* however, are significant characteristics of other liverworts. Thus flavone *O*-glucuronides, *O*-methylation and 'complex' *O*-glycosylation are frequently encountered in the order Marchantiales [9] and certain of the luteolin, and more notably tricetin, di-*C*-glycosides, have been found only in *Plagiochila asplenioides* (order Jungermanniales) [16]. *Takakia* thus shows biochemical links with all three major orders of liverworts, an observation which is interpreted as support for the statement by Hattori *et al.* [3] that *Takakia* represents "an isolated branch among the ancestors of modern bryophytes".

A more extensive appraisal of the taxonomic implications of these data will be published elsewhere.

EXPERIMENTAL

Plant material. Takakia lepidozioides Hatt. et Inoue was collected in May 1977 near a waterfall, Lausmann Creek, Jervis Inlet, British Columbia and a voucher specimen, herbarium No. W. B. Schofield 63888 deposited at the Botany Department, University of B. C., Vancouver. The Japanese sample of *Takakia lepidozioides* Hatt. et Inoue was collected in May 1971 from Mt. Daisetu, Hokkaido by W. B. Schofield and I. Yoshimura. A herbarium specimen is held at the Hattori Botanical Laboratory. *Takakia ceratophylla* (Mitt) Grolle. was collected in June 1972 in Eastern Nepal between Ghopte (Tal Polhari) and Gosa (near Kobche) at an altitude of 3500–4100 m, by Z. Iwatsuki. A voucher specimen is held in the Hattori Botanical Laboratory.

Isolation procedure. T. lepidozioides was carefully separated from other bryophytes under a lens, washed with H_2O and oven-dried to give 3.3 g of dry plant material. This was ground up and extracted with Me_2CO–H_2O (1:1). The total extract was applied to 30 PCs (Whatman 3MM) and chromatographed in 2D, (i) $1\frac{1}{2}$ times 'over-run' in *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and (ii) 15% HOAc (HOAc), to give the pattern of components in Fig. 1. Compounds **9**, **8** and **11** were further purified by 'over-run' 1D-PC. *T. ceratophylla* (0.4 g, d.w.) was treated in the same way and the extract applied to 3 PCs.

Chromatographic comparisons and product analysis. These were routinely carried out on Whatman 3MM paper or Schleicher and Schull F1440 TLC cellulose plates in TBA, HOAc and H_2O (glycosides) and TBA and Bz–HOAc–H_2O, 125:72:3 (BHH) (aglycones). In addition, *C*-glycosides in particular were compared on Schleicher and Schull F-1500 SiO_2 plates in EtOAc–Py–H_2O–MeOH, 80:20:10:5 (APWM). The spray reagent, tetraphenyl diboroxide ethanolamine complex (K&K) was routinely used.

Hydrolysis conditions. Enzyme and acid hydrolyses, and Wessely–Moser rearrangement conditions have been detailed in previous publications [12] as also have details of the GLC analysis of sugars produced.

Absorption spectra. Spectra were measured according to procedures outlined in ref. [17].

Mass spectra. Permethylated derivatives (PM) were prepared of glycosides in DMF with MeI–NaH (overnight) and purified by TLC (SiO_2) in $CHCl_3$–Me_2CO (4:1). Aglycones were underivatized. A probe temperature of 200–300° was generally used for spectra determination.

T. ceratophylla compounds. All 12 UV-absorbing spots were extracted from the 2D-PCs. The absorption spectra were measured and each compound subjected to mild and 'normal' acid treatment (2 N HCl, 100°, 15 min; 3 N HCl, 100°, 1 hr). Products of the acid treatment were analysed by 2D-TLC (TBA, HOAc). Four of the flavonoid components were subsequently co-chromatographed with flavonoids from *T. lepidozioides* (TBA, HOAc, H_2O). The fifth flavonoid, R_f 0.02 (TBA), 0.50 (HOAc), 0.9 (H_2O), had λ_{max} (MeOH) 274, 327; (NaOMe) 270 br, 363 (dec. intensity) and on acid hydrolysis (30 min) gave an unidentified flavone 'aglycone' with R_f 0.25 (TBA). 0.02 (HOAc). It was partly hydrolysed by β-glucuronidase.

Takakin 8-O-*glucuronide* (**1**) *and* 8-O-*xylosylglucoside* (**13**), **1** appeared as a purple spot changing to olive in NH_3 with R_fs 0.53 (TBA), 0.20 (HOAc) and 0.60 (H_2O), and had λ_{max} (MeOH) 273, 323, 349sh nm; (NaOMe) 282, 303sh, 313sh, 377 (decrease) nm; ($AlCl_3$) 277, 306, 342, 380 nm, unchanged on addition of HCl. Acid hydrolysis of **1** gave an aglycone R_f 0.65 (TBA), 0.05 (HOAc) on PC and hydrolysis with β-glucuronidase gave an aglycone R_f 0.78 (TBA), 0.05 (HOAc) on PC and 0.79 (BHH) on SiO_2. The latter had λ_{max} (MeOH) 281, 302, 328sh nm; (NaOMe) 272, 318sh, 380 (decrease) nm; ($AlCl_3$) 271, 278, 295, 323, 350sh nm; ($AlCl_3$/HCl) 272, 277, 313, 341, 388sh nm; and in the MS, ions at *m/e* 300 (100%), 168 (56%), 132 (15%). The presence of glucuronic acid in the hydrolysate was detected by PC (see ref. [12]). **13** appeared as a purple-olive (NH_3) spot on PC with R_fs of 0.46 (TBA), 0.55 (HOAc), 0.15 (H_2O). It had the same spectra as **2** and was unaffected by β-glucosidase, β-glucuronidase and pectinase. Acid hydrolysis (3 N HCl, 1 hr, 100°) gave the scutellarein-like aglycone obtained from **2** but mild acid treatment (2 N HCl, 10 min, 100°) gave a mixture of both aglycones obtained from **2**. GC analysis of the hydrolysate (see ref. [12]) identified xylose and glucose.

Kaempferol 3-O-*glucoside*-7-O-*xyloside* (**12**). Compound **12**, R_f 0.46 (TBA), 0.68 (HOAc), λ_{max} (MeOH) 266, 287sh, 353 nm; (NaOMe) 243sh, 272, 304, 355sh, 395 nm; (NaOAc) 265, 357, 405sh, nm; ($AlCl_3$) 274, 303sh, 352, 395sh nm, unchanged on addition of HCl. Mild acid hydrolysis (1.5 N, 10 min, 100°) gave the aglycone (M^+, 286) which was chromatographically identical to kaempferol (TBA, BHH), together with xylose and glucose. A β-glucosidase preparation (ex sweet almonds, Koch-Light) which removed the glucose specifically from the 7-hydroxyl in kaempferol 3,7-diglucoside in 15 min did not affect **12**, but pectinase gave two products (see Results).

Tricetin 6-C-*glucoside*-8-C-*arabinoside* (**6**). Compound **6** appeared as a dark purple spot R_f 0.07 (TBA), 0.30 (HOAc) on a PC which turned yellow-orange in NH_3. It had λ_{max} (MeOH) 262, 271, 295sh, 350 nm; (NaOMe) 269sh, 280sh, 415 (broad) nm; ($AlCl_3$) 239, 271, 305sh, 422 nm; ($AlCl_3$/HCl) 271, 302sh, 358, 388sh nm. Acid treatment (2 N, HCl, 1.5 hr, 100°) caused partial conversion to an isomer R_f 0.04 (TBA), 0.18 (HOAc) and some degradation. **6** was compared chromatographically on cellulose with lucenin-2 and tricetin di-*C*-hexoside in TBA, BAW and HOAc. The *R* (lucenin-2) values for **6** were 0.75 (HOAc), 0.80 (BAW) and 0.5 (TBA); lit. values [7] for tricetin 6-*C*-hexoside-8-*C*-pentoside, 0.76 (HOAc), 0.79 (BAW). For comparison, the *R* (lucenin-2) values for authentic tricetin 6,8-di-*C*-glucoside were 0.73 (HOAc) and 0.58 (BAW); lit. values, 0.74 (HOAc) and 0.58 (BAW). Compound **6** degraded completely over a period of 4 months as evidenced by TLC analysis, thus MS studies towards the end of this project were of no value.

Luteolin 6-*C-arabinoside*-8-C-*pentoside* (**5**). Compound **5** appeared as a dark purple spot R_f 0.14 (TBA), 0.30 (HOAc) which turned dull yellow in NH_3 on a PC. It had λ_{max} (MeOH) 259, 272, 349 nm; (NaOMe) 268, 282sh, 335sh, 410 nm; ($AlCl_3$)

275, 302sh, 333, 427 nm; ($AlCl_3$/HCl) 274, 297sh, 358, 387sh nm. Acid treatment caused partial conversion to an isomer, R_f 0.14 (TBA), 0.10 (HOAc). *R*(vicenin-2) values for **5** (and isomer) are 0.48, 0.16 (HOAc); 0.47, 0.47 (TBA); 0.23, 0.23 (H_2O). MS of the PM ether of **5** gave major ions at *m/e* 690 (M^+, 7%), M-15 (25), M-31 (100), M-47 (14), M-61 (23) M-77 (12), M-89 (12), M-119 (37), M-131 (63), M-145 (35), M-163 (19).

MS data for PM ethers of other di-C-glycosides

7: 660 (M^+, 3.4%), M-15 (11), M-31 (100) M-47 (5.7) M-61 (20), M-77 (5.7), M-89 (8.0), M-119 (36), M-131 (67), M-145 (34), M-163 (18), M-177 (14).

9: 704 (M^+, 9.0%), M-15 (25), M-31 (100), M-47 (14), M-103 (12), M-119 (52), M-131 (80), M-145 (23), M-163 (43), M-175 (58). The relative intensities of the M-131 and M-175 peaks are not in agreement with formulation, but they can be explained by the coincidence of the M-131 peak with the M-175 peak of the accompanying PM vicenin-2.

Apigenin di-C-hexoside ex MS of PM **9**: 748 (M^+), M-15, M-31, M-47, M-63, M-103, M-133, M-163, M-175, M-189, M-205, M-207, M-221.

11: 734 (M^+), M-15, M-31, M-47, M-61

Luteolin di-C-glycoside ex MS of PM **5**: 778 (M^+), M-15, M-31, M-61.

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NOTE ADDED IN PROOF

Unpublished synthetic work by Prof. J. Chopin, Univ. of Lyon, France, has now shown the *H. leptopodum* compound to be 6,8-di-*C*-α-L-arabinopyranosyl apigenin. Compounds **5**, **7** and **6a** from *Takakia* may therefore also be di-*C*-arabinosides. If this were the case, α–β or pyranose–furanose isomerization of one or both of the sugars would account for the chromatographically observed 'Wessely–Moser rearrangements'.