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UNIQUE FLAVONOID GLYCOSIDES FROM THE NEW ZEALAND WHITE PINE, *DACRYCARPUS DACRYDIOIDES*

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Key Word Index—*Dacrycarpus dacrydioides; Podocarpus*; Podocarpaceae; flavone *C*-glycosides; flavone *O*-glycosides; 3-methoxyflavone *O*-glycosides; flavonol *O*-glycosides; xylosides; ^{13}C NMR; chemotaxonomy.

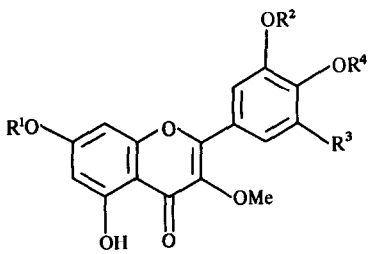
Abstract—A number of new flavonoid glycosides have been isolated from foliage of the New Zealand white pine, *Dacrycarpus dacrydioides*. These include tricetin 3′,5′-di-*O*-β-glucopyranoside; the 3′-*O*-β-xylopyranoside, 7-*O*-α-rhamnopyranoside and 7-*O*-α-rhamnopyranoside-3′-*O*-β-xylopyranoside of 3-*O*-methylmyricetin; the 3′-*O*-β-xylopyranoside, 7-*O*-α-rhamnopyranoside and 7-*O*-α-rhamnopyranoside-3′-*O*-β-xylopyranoside of 3-*O*-methylquercetin, and the 3′-*O*-β-xylopyranoside and 7-*O*-α-rhamnopyranoside-3′-*O*-β-xylopyranoside of 3,4′-di-*O*-methylmyricetin. The accumulation of 3-methoxyflavones and B-ring trioxygenated flavonoids appears to distinguish *D. dacrydioides* from all other New Zealand members of the classical genus *Podocarpus*. Support for De Laubenfels' proposed separation of *Dacrycarpus* from this genus is seen in the present work.

INTRODUCTION

The most authoritative text on the taxonomy of New Zealand plants [1] lists the New Zealand 'white pine' as *Podocarpus dacrydioides* A. Rich. Subsequent to the publication of this volume, De Laubenfels [2] proposed subdivision of *Podocarpus* s.l. into five genera. In this proposal the New Zealand 'white pine' has been separated from the other New Zealand *Podocarpus* species into the genus *Dacrycarpus*, and in more recent popular publications the use of *Dacrycarpus* has found favour over *Podocarpus*. In collaboration with local botanists we have initiated a chemotaxonomic survey of the New Zealand members of *Podocarpus* s.l. in an attempt to substantiate the proposed subdivision. The first member to be studied is *Dacrycarpus dacrydioides* (A. Rich.) de Laub. The study involves comparative flavonoid glycoside chemistry of the group, the analyses being confined to the foliage. No previous survey of these natural products has been made although a survey of biflavonoid aglycones was reported in 1967 [3]. No taxonomic conclusions resulted from this survey. To our knowledge, the only flavonoid glycosides previously isolated from *Podocarpus* species have been flavone *C*-glycosides, e.g. vitexin [4, 5], and documentation is minimal.

RESULTS

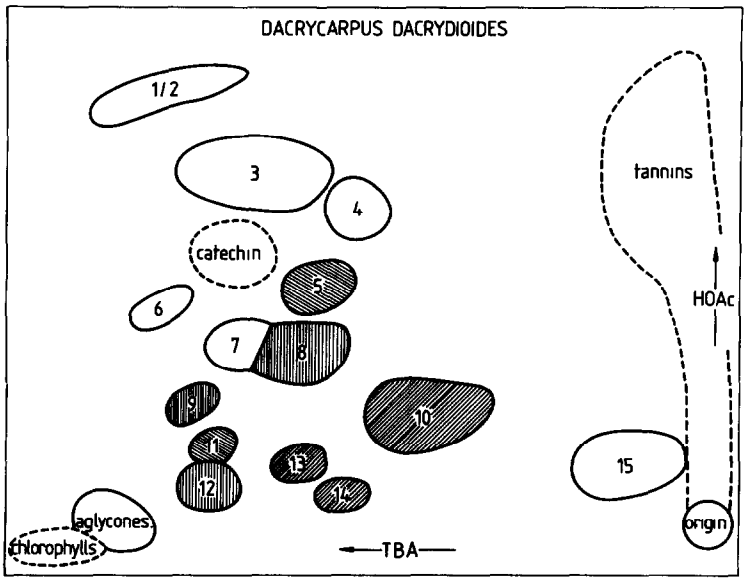
Two-dimensional PC analysis of the ethanol–water extract of *D. dacrydioides* from a number of sources revealed a consistent and spectacular array of flavonoid glycosides (see Fig. 1). Dominant amongst these and typical of the species were glycosides **3**, **8**, **10** and **15** (represented by spots 3, 8, 10 and 15, respectively). These and others were isolated in quantity by column chromatography. Acid treatment of all the observable flavonoid glycosides indicated that all but **1**, **2**, **3** and **4** were flavonoid *O*-glycosides.



	R^1	R^2	R^3	R^4
5	Rha	Xyl	OH	Me
8	Rha	Xyl	H	H
9	Rha	H	H	H
10	Rha	Xyl	OH	H
11	H	Xyl	OH	Me
12	H	Xyl	H	H
13	Rha	H	OH	H
14	H	Xyl	OH	H

Flavone C-glycosides **1–4**

On acid hydrolysis, compounds **3** and **4** yielded vitexin and orientin respectively with the liberation of rhamnose as the only sugar. Since the absorption spectra of each were unchanged by the hydrolysis, the rhamnose must be attached to the *C*-glucosyl residue in both compounds. ^{13}C NMR spectroscopy of **3** confirmed the identification and ratio of the sugars and defined the position of attachment as the C-2 hydroxyl of the C-linked glucose. The spectrum was identical to that previously published for this compound [6]. Since an insufficient sample of **4** was available for ^{13}C NMR study, the attachment site for rhamnose was assigned by analogy with **3**. Compounds **3**



Spot No.		R_f values TBA	HOAc	Colour UV	UV/NH_3
1/2	2″-*O*-Rhamnopyranosylisovitexin/isoorientin	0.8	0.8	dark	olive
3	2″-*O*-Rhamnopyranosylvitexin	0.60	0.67	dark	olive
4	2″-*O*-Rhamnopyranosylorientin	0.49	0.60	dark	olive
5	3,4′-Di-*O*-methylmyricetin 7-*O*-rhamnopyranoside-3′-*O*-xylopyranoside	0.51	0.48	dark	dark
6	Quercetin 3-*O*-rhamnopyranoside	0 66	0.43	dark	olive
7	Quercetin 3-*O*-glucopyranoside	0.57	0.37	dark	olive
8	3-*O*-Methylquercetin 7-*O*-rhamnopyranoside-3′-*O*-xylopyranoside	0.50	0.36	dark	green fluorescent
9	3-*O*-Methylquercetin 7-*O*-rhamnopyranoside	0.61	0.26	dark	yellow
10	3-*O*-Methylmyricetin 7-*O*-rhamnopyranoside-3′-*O*-xylopyranoside	0.39	0.23	dark	yellow
11	3,4′-*O*-Methylmyricetin 3′-*O*-xylopyranoside	0.60	0.20	dark	dark
12	3-*O*-Methylquercetin 3′-*O*-xylopyranoside	0.60	0.15	dark	green
13	3-*O*-Methylmyricetin 7-*O*-rhamnopyranoside	0.51	0.17	dark	yellow
14	3-*O*-Methylmyricetin 3′-*O*-xylopyranoside	0 45	0.08	dark	yellow-green
15	Tricetin 3′,5′-di-*O*-glucopyranoside	0.14	0.11	dark	green fluorescent

Fig. 1. Two-dimensional PC display of the flavonoid glycosides in a crude extract of *Dacrycarpus dacrydioides* foliage. Spots with like hatching represent glycosides of the same aglycone. R_f values measured from a two-dimensional paper chromatogram. UV = 366 nm.

and **4** were therefore assigned the structures 2″-*O*-α-L-rhamnopyranosylvitexin and 2″-*O*-α-L-rhamnopyranosylorientin, respectively.

On acid hydrolysis, compounds **1** and **2** yielded vitexin and a trace of orientin with the liberation of rhamnose. Attempts to isolate **1** and **2** pure from two-dimensional paper chromatograms invariably resulted in the isolation of mixtures of **1**, **2**, **3** and **4**. This ready isomerization of **1** and **2** to **3** and **4** and the matching absorption spectra allowed tentative assignment of the structures 2″-*O*-rhamnopyranosylisovitexin and 2″-*O*-rhamnopyranosylisoorientin to **1** and **2**, respectively.

Flavone O-glycoside **15**

Compound **15** was exceedingly difficult to remove from paper or from a cellulose column. On acid hydrolysis it yielded glucose and tricetin. The absorption spectra of **15** indicated that the 5- and 4′-hydroxyl groups were free but did not clearly define the presence or absence of a free 7-hydroxyl group (see Table 1). In the ^{1}H NMR spectrum of the TMSi-ether, coincidence of the H-2′ and H-6′ signals (δ7.19) was suggestive of a symmetrically substituted B-ring, and integration revealed the presence of two glucose units per flavonoid molecule. The ^{13}C NMR spectrum

Table 1. Absorption spectra for new glycosides from *Dacrycarpus dacrydioides*

Compound	Absorption maxima (nm) MeOH	NaOMe	NaOAc ($NaOAc–H_3BO_3$)*	$AlCl_3$ ($AlCl_3$–HCl)*
5	270, 350	280, 300 sh, 384 ↓	268, 348	278, 304 sh, 348, 394 sh
8	250, 267, 350	248, 265, 287 sh, 393 ↑	261, 403 (250, 265, 352)	275, 302 sh, 352, 400
9	256, 266 sh, 355	270, 387 ↑	265, 370, 400 sh (N.A.)	272, 300 sh, 360, 415 (270, 300 sh, 355, 385 sh)
10	253, 268 sh, 355	245, 266, 400 ↑	261, 410 (260, 270 sh, 372)	275, 303 sh, 350 sh, 435 (275, 302 sh, 353, 400)
11	269, 348	N.A.	274, 360 (269, 350)	N.A.
12	270, 352	273, 328, 402 ↑	276, 398 (270, 352)	262 sh, 278, 303 sh, 356, 400
13	256, 268 sh, 360	rapid dec.	256, 268 sh, 370 (258, 270 sh, 384)	N.A.
14	252, 268, 356	270 br, 328, 406 ↑	268, 390 (264, 298 sh, 370)	275, 300 sh, 350 sh, 430 (276, 300 sh, 352, 390 sh)
15	240, 270, 300 sh, 350	262, 277 sh, 335 sh, 402 ↑	260, 401 (270, 305 sh, 350, 400 sh, 450 sh)	251, 279, 302, 357, 385

*Detailed only where different from NaOAc or $AlCl_3$.
↑↓ Increase or decrease in intensity, N.A. = not available.

confirmed that the sugar units were β-glucopyranosyl and that they were each in a very similar environment. As indicated by the proton spectrum, substitution in the B-ring appeared to be symmetrical, C-2′ and C-6′ being coincident at 113.6 ppm and C-3′ and C-5′ being coincident at 148.3 ppm. The weight of evidence thus favoured a 3′,5′-diglucoside structure rather than a 7,3′-diglucoside. To confirm this, a slow hydrolysis was carried out, the progress of the hydrolysis being closely monitored by TLC. Only one product intermediate between **15** and its aglycone was formed and this was chromatographically identical to tricetin 3′-*O*-glucoside and not to the less labile tricetin 7-*O*-glucoside. Compound **15** was therefore assigned the structure tricetin 3′, 5′-di-*O*-β-D-glucopyranoside. This is a new tricetin glycoside.

Flavonol O-glycosides **6** *and* **7**

Compound **6** gave predominantly quercetin and rhamnose on hydrolysis, had the absorption spectra of a 3-*O*-glycosylated quercetin, and cochromatographed with authentic quercetin 3-*O*-α-L-rhamnopyranoside. Traces of kaempferol detected in the hydrolysate were thought to indicate the presence also of small amounts of kaempferol 3-*O*-glucoside. Compound **7** was separated from **8** with difficulty and was shown to be quercetin 3-*O*-β-D-glucopyranoside by hydrolysis, absorption spectroscopy and cochromatography.

3-*Methoxyflavone*-O-*glycosides* **10**, **13** *and* **14**

Glycoside **10** appeared on paper as a dark spot which turned distinctively yellow in ammonia. It gave absorption spectra akin to those of luteolin 7-*O*-glucoside [7] but with band I a little higher at 355 nm, suggestive of additional B-ring oxygenation. On acid hydrolysis it gave two sugars, rhamnose and xylose, together with an aglycone. This aglycone appeared as a dark spot on a paper chromatogram (i.e. 5-OH free but not 3-OH [8]) and was purified by HPLC. Its ^{1}H NMR spectrum (DMSO-d_6) revealed two doublets (J = 2 Hz) at δ6.26 and 6.05 representing H-8 and H-6, respectively, and a two-proton singlet at δ7.09 which was assigned to H-2′ and H-6′. No H-3 signal was evident but a methoxyl signal was present at δ3.75. The aglycone thus appeared to be 3-*O*-methylmyricetin, a structure which was confirmed by the tricetin-like absorption spectra, the mass spectrum ($[M]^+$ 332 and fragments) and the conversion to myricetin on demethylation.

Glycoside **10** is therefore a xylorhamnoside of 3-*O*-methylmyricetin (annulatin [9]). The ^{13}C NMR spectrum confirmed the presence of these two sugars in the α-(rhamnose) and β- (xylose) linked pyranosyl forms (cf. ref. [8]), and furthermore required that they be linked independently to the aglycone since the pattern of signals was equivalent to a simple superimposition of the signals associated with each *O*-linked sugar [8]. The absorption spectra of **10** indicated that the 7-hydroxyl was substituted, the 4′-hydroxyl was free and that an *ortho*-dihydroxyl system was present in the B-ring. The sugars therefore must be linked to the 7- and 3′-hydroxyl groups. Partial hydrolysis of **10** produced two monoglycosidic products in addition to the aglycone. These monoglycosides were identical to compounds **13** and **14** in all respects and gave distinctly different absorption spectra (see Table 1). Compound **14** possesses a free 7-hydroxyl group and is stable in sodium methoxide, while compound **13** does not possess a free 7-hydroxyl group and is unstable in sodium methoxide. Further, compound **14** gave 3-*O*-methylmyricetin plus xylose on hydrolysis whereas compound **13** gave 3-*O*-methyl-myricetin plus rhamnose on hydrolysis. These results defined the

structures of **13**, **14** and **10** as 3-*O*-methylmyricetin 7-*O*-α-rhamnopyranoside, 3-*O*-methylmyricetin 3′-*O*-β-xylopyranoside and 3-*O*-methylmyricetin 7-*O*-α-rhamnopyranoside-3′-*O*-β-xylopyranoside, respectively. All three are new natural products [9].

3-*Methoxyflavone* O-*glycosides* **8**, **9** *and* **12**

Compound **8** was separated from **7** by either HPLC or by PC in BBPW. Its structure was proved in much the same manner as that of **10** above. As for **10**, hydrolysis produced xylose, rhamnose and an aglycone. The aglycone had the absorption spectra (Table 1) and PC appearance (Fig. 1) of a flavone with free 5-, 7-, 3′- and 4′-hydroxyl groups. It gave quercetin on demethylation and was spectrally and chromatographically identical to 3-*O*-methylquercetin [10]. In the ^{1}H NMR spectrum of the TMSi-ether of **8**, a methoxyl signal was visible at δ3.88 as also were the H-1 signals for α-rhamnose (δ5.26 [7]) and β-xylose (δ4.90). The downfield positions of the H-6, H-8 and H-2′ signals relative to those in rutin [7] were indicative of glycosylation at the 7- and 3′-hydroxyl groups. The pattern of sugar carbon signals in the ^{13}C NMR spectrum was identical to that of **10** requiring again that the sugars be separately attached to the aglycone. Partial hydrolysis of **8** produced **9** and **12**. Compound **9** possesses an *ortho*-dihydroxyl system, a substituted 7-hydroxyl and gave 3-*O*-methylquercetin and rhamnose on hydrolysis, while **12** lacks an *ortho*-dihydroxyl system, possesses free 7- and 4′-hydroxyl groups and gave 3-*O*-methylquercetin and xylose on hydrolysis. The structures of **9**, **12** and **8** were thus defined as 3-*O*-methylquercetin 7-*O*-α-rhamnopyranoside, 3-*O*-methylquercetin 3′-*O*-β-xylopyranoside and 3-*O*-methylquercetin 7-*O*-α-rhamnopyranoside-3′-*O*-β-xylopyranoside, respectively. All are new natural products [9].

3-*Methoxyflavone* O-*glycosides* **5** *and* **11**

These glycosides were present in *D. dacrydioides* at only low levels. As did **8** and **10** above, compound **5** produced xylose and rhamnose (in the ratio of 1:1) together with the aglycone on hydrolysis. Both **5** and its aglycone appeared on paper as dark spots unaffected by ammonia vapour indicating the presence of substituted 4′-hydroxyl groups in both compounds [8]. The absorption spectra of **5** (Table 1) revealed the presence of a free 5-hydroxyl group together with the absence of free 7- and 4′-hydroxyls and of an *ortho*-dihydroxyl grouping. The band I position in methanol at 350 nm was suggestive of a di- or trioxygenated B-ring and the mass spectrum of the aglycone confirmed that the B-ring was trioxygenated. The molecular ion at *m/z* 346 was consistent with a di-*O*-methylmyricetin structure and fragment ions at *m/z* 153 ($[A_1+H]^+$) and 167 ($[B_2]^+$) confirmed the presence of two hydroxyl groups in the A-ring and two hydroxyls and one methoxyl group in the B-ring [8]. The fragment of modest intensity at *m/z* 303 ($[M-COMe]^+$) was consistent with the presence of a 3-methoxyl group [8]. On the basis of this evidence, the aglycone of **5** was assigned the structure 3,4′-di-*O*-methylmyricetin.

Glycoside **5** is thus a rhamnoxyloside of this, and one sugar at least must be attached to the 7-hydroxyl group (absorption data). Partial hydrolysis, as for **8** and **10**, produced two monoglycoside products with different absorption spectra and R_fs, thereby proving that the sugars are attached at different sites on the flavonoid. One of these monoglycosides was identical to **11**, which possesses a free 7-hydroxyl group. The other, higher R_f (TBA, HOAc), monoglycoside had its 7-hydroxyl substituted, and yielded rhamnose on hydrolysis. Its structure was thereby defined as 3,4′-di-*O*-methylmyricetin 7-*O*-rhamnoside and it followed that **11** must be 3,4′-di-*O*-methylmyricetin 3′-*O*-xyloside. On the basis of this evidence, compound **5** was assigned the structure 3,4′-di-*O*-methylmyricetin 7-*O*-rhamnoside-3′-*O*-xyloside. Compounds **5** and **11** are both new natural products.

DISCUSSION

The range of flavonoid glycosides isolated from *D. dacrydioides* is extensive and includes a flavone *O*-glycoside, *O*-glycosylated flavone *C*-glycosides, flavonol 3-*O*-glycosides and a number of 3-methoxyflavone *O*-glycosides. The glycosylation patterns of the *O*-glycosides are most unusual as also are rhamnose/xylose sugar combinations [9]. Most of the glycosides are reported here for the first time. These include tricetin 3′,5′-di-*O*-glucoside (**15**); the 3-*O*-methylmyricetin glycosides, 7-*O*-rhamnoside-3′-*O*-xyloside (**10**), 7-*O*-rhamnoside (**13**) and 3′-*O*-xyloside (**14**); the 3-*O*-methylquercetin glycosides, 7-*O*-rhamnoside-3′-*O*-xyloside (**8**), 7-*O*-rhamnoside (**9**) and 3′-*O*-xyloside (**12**); and the 3,4′-di-*O*-methylmyricetin glycosides, 7-*O*-rhamnoside-3′-*O*-xyloside (**5**) and 3′-*O*-xyloside (**11**). Of the aglycones, tricetin is uncommon, 3-*O*-methylmyricetin (annulatin) has been found only twice before (in *Aegialitis annulata* and as its 3′-*O*-glucoside in *Oenothera speciosa* [11]), and 3,4′-di-*O*-methylmyricetin has been reported once in a thesis [12] but never in a paper [13].

A preliminary chromatographic survey of the other New Zealand members of the classical genus *Podocarpus* indicates that *D. dacrydioides* alone accumulates 3-methoxyflavones and flavonoids with trioxygenated B-rings. In this sense *D. dacrydioides* is distinct from the rest, and support for De Laubenfels' proposed separation of *Dacrycarpus* is seen in the present work. A fuller investigation of the other New Zealand *Podocarpus* species is currently underway.

EXPERIMENTAL

Plant sources. Dacrycarpus dacrydioides bulk supply. Riccarton Bush, Canterbury, New Zealand (voucher CHR 391441); other samples: Wright's farm, near Whangarei, New Zealand and Botanical Gardens, Palmerston North, New Zealand.

Extraction method. Batches of 100 g of dry, ground foliage were extracted several times with 50% aq. EtOH (1 l.). The EtOH was removed on a rotary evaporator and the aq. extract was washed twice with $CHCl_3$ and the $CHCl_3$-solubles were discarded. The extract was then evapd to *ca* 30–40 ml vol. 2D-PCs were typically run on the extract from 100 mg dry plant material (see Fig. 1) using *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and 15% HOAc (HOAc).

Column chromatography and compound purification. Microcrystalline cellulose (Merck) presoaked overnight in 2% HOAc was used to make up a 20 × 10 cm column for chromatography. After further washing with 2% HOAc, the extract in 2% HOAc (30–40 ml) was applied to the top and

elution begun with 2% HOAc. Fractions were monitored by TLC and when compounds **8** and **9** had been eluted the concn of HOAc was gradually increased to *ca* 10%. Finally, compounds **15** and **16** were eluted using EtOH–H_2O. 1D-PC (TBA or HOAc) purification of most compounds was necessary. Compounds **7** and **8** were separated in BBPW (*n*-BuOH–C_6H_6–pyridine–H_2O, 5:1:3:3), and compounds **9–14** were best separated using combinations of TBA and 30% HOAc. Final clean-up of all compounds was carried out on Sephadex LH-20 in MeOH. Additionally, compounds **8** and **10** and the aglycone of **10** were cleaned up by HPLC on a Waters C-18 column in MeOH–1% HOAc (1·1) using a UV detector set at 350 nm. Yields were low by this method, which was therefore not used more extensively.

Absorption data. These are presented in Table 1 and were measured according to the methods of Mabry *et al.* [7]. Spectra not present in the table were essentially as previously reported [7, 8].

Partial hydrolysis conditions. The glycoside in 0.1 N TFA was heated at 100° for 15 min. The evapd soln was applied to 2D-PCs for work-up.

Sugar analyses (PC and GC), trimethylsilylation and demethylation (pyridine–HCl). These were standard techniques as described by Markham [8].

Physical data for glycosides and derived compounds. For absorption data, see Table 1; for R_f values, see Fig. 1.

Glycosides **3** *and* **4**. Absorption data—as previously published [7]. ^{13}C NMR of **3** (ppm in DMSO-d_6): 182.0 (C-4), 163.9 (C-2), 162.2 (C-7), 161.1 (C-4'), 160.6 (C-5), 155.7 (C-9), 128.9 (C-2', 6'), 121.5 (C-1'), 115.8 (C-3', 5'), 104.1/104.4 (C-8, 10), 102.4 (C-3), 100.2 (R-1), 98.2 (C-6), 81.7 (G-5), 79.8 (G-3), 75.0 (G-2), 71.6/71.4 (R-3, G-1), 70.6/70.4/70.2 (R-2, 4, G-4), 68.1 (R-5), 61.1 (G-6), 17.6 (R-6). Hydrolysis products, vitexin and orientin, were identified by co-TLC (cellulose; TBA, HOAc. SiO_2; EtOAc–pyridine–H_2O–MeOH, 16.4:2:1) and absorption spectroscopy. Sugar analyses by PC.

Glycosides **5** *and* **11**. Complete hydrolysis of **5** (2 N HCl, 15 min, 100°) gave rhamnose and xylose, 1:1 (PC analysis) and an aglycone with MS *m/z* (rel. int.): 346 $[M]^+$ (100), 345 (63), 331 $[M-Me]^+$ (16), 303 $(M-COMe]^+$ (16), 167 $[B_2]^+$ (5), 153 $[A_1+H]^+$, (13). Partial hydrolysis of **5** gave **11** plus another monoglycoside; λ (MeOH) 260, 293 sh, 342; (NaOAc) 258, 292 sh, 344; (NaOAc–H_3BO_3) 258, 290 sh, 344 nm. Acid hydrolysis of this glycoside gave rhamnose (PC).

Glycosides **6** *and* **7**. Data as previously published [7, 8].

Glycoside **8** (*and* **9** *and* **12**). ^1H NMR, δ (TMSi-ether in CCl_4): 3.2–3.75 (9H, *m*, sugar protons), 3.88 (3H, *s*, OMe), 4.90 (1H, *d*, J = 7 Hz, xylose H-1), 5.26 (1H, *br s*, rhamnose, H-1), 6.3 (1H, *d*, J = 2.5 Hz, H-6), 6.52 (1H, *d*, J = 2.5 Hz, H-8), 6.83 (1H, *d*, J = 9 Hz, H-5'), 7.6 (1H, *br d*, J = 9 Hz, H-6'), 7.75 (1H, *d*, J = 2 Hz, H-2'). ^{13}C NMR (DMSO-d_6), sugar carbons: 98.0 (X-1, R-1), 76.2 (X-3), 73.2 (X-2), 72.0 (R-4), 70.0/70.5/69.4 (R-2, 3, 5/X-4), 65.9 (X-5), 18.3 (R-6) ppm. Acid hydrolysis of **8** gave xylose and rhamnose (PC) and an aglycone [λ_{max} (MeOH) 260, 270 sh, 356; (NaOMe) 272, 328, 404; (NaOAc) 276, 324, 386; (NaOAc–H_3BO_3) 262, 380; ($AlCl_3$) 278, 305 sh, 335 sh, 438; ($AlCl_3$–HCl) 275, 305 sh, 358, 404 nm; MS *m/z* (rel. int.): 316 $[M]^+$ (100), 273 $[M-COMe]^+$ (20), 153 $[A+H]^+$ (13), 137 $[B_2]^+$ (10)] which cochromatographed with authentic 3-*O*-methylquercetin (from *Ophioglossum vulgatum*) on TLC (cellulose; TBA, 50% HOAc; SiO_2, C_6H_6–dioxan–H_2O, 90:25:4) and on demethylation (pyridine–HCl) gave quercetin (co-TLC; cellulose, TBA, 50% HOAc, C_6H_6–HOAc–H_2O, 125:72:5). Partial hydrolysis gave a mixture of **8**, **9**, **12** and the aglycone. On acid hydrolysis, both **9** and **12** gave 3-*O*-methylquercetin (MS and co-TLC); **9** also gave rhamnose (PC) and **12** gave xylose (GC).

Glycoside **10** (*and* **13** *and* **14**). ^1H NMR, δ (TMSi-ether in CCl_4): 3.25–3.75 (9H, sugar protons), 3.90 (3H, *s*, OMe), 4.85 (1H, *d*, J = 7 Hz, xylose H-1), 5.22 (1H, *br s*, rhamnose H-1), 6.32 (1H, *d*, J = 2.5 Hz, H-6), 6.50 (1H, *d*, J = 2.5 Hz, H-8), 7.25/7.45 (2H, *m*, H-2', 6'). ^{13}C NMR (DMSO-d_6) sugar carbons: 98.5 (R-1, X-1), 76.2 (X-3), 73.1 (X-2), 71.8 (R-4), 70.3/70.0/69.5 (R-2, 3, 4/X-4), 66.0 (X-5), 18.0 (R-6) ppm. Acid hydrolysis of **10** gave xylose and rhamnose (PC) and an aglycone (purified by HPLC); MS *m/z* (rel. int.): 332 $[M]^+$ (100), 331 (78), 289 $[M-COMe]^+$, 153 $[A+H]^+$ and $[B_2]^+$; λ_{max} (MeOH) 253, 270 sh, 300 sh, 358; (NaOMe) decomp.; (NaOAc) 267, 372; (NaOAc–H_3BO_3) 260, 381; ($AlCl_3$) 271, 310 sh, 430; ($AlCl_3$–HCl) 270, 305 sh, 365, 400 nm; ^1H NMR, δ (DMSO-d_6): 7.09 (2H, *s*, H-2', 6'), 6.26 (1H, *d*, J = 2 Hz, H-8), 6.05 (1H, *d*, J = 2 Hz, H-6), 3 75 (3H, *s*, OMe). On demethylation (pyridine–HCl) this aglycone gave myricetin (co-TLC, solvents, etc. as for **8** above). Partial hydrolysis gave a mixture of **10**, **13** and **14** and the aglycone (co-TLC, cellulose, TBA, 50% HOAc). Both **13** and **14** produced 3-*O*-methylmyricetin on hydrolysis and rhamnose and xylose, respectively (PC). Absorption data—see Table 1.

Glycoside **15**. ^1H NMR, δ (TMSi-ether in CCl_4): 7.19 (2H, *s*, H-2', 6'), 6.45 (1H, *d*, J = 2.3 Hz, H-8), 6.28 (1H, *s*, H-3), 6.13 (1H, *d*, J = 2.3 Hz, H-6), 4.95 (2H, *d*, J = 6.5 Hz, glucose H-1), 3–3.75 (12H, glucose protons). ^{13}C NMR (DMSO-d_6), protonated carbons: 113.6 (C-2', 6'), 104.3 (C-3), 100.8 (G-1), 99.2 (C-6), 94.2 (C-8), 77.6/76.8 (G-3/5), 73.7 (G-2), 70.1 (G-4), 61.1 (G-6) ppm, non-protonated carbons C-3' and C-5' also evident at 148.3 ppm. Complete acid hydrolysis gave glucose (GC, PC) and an aglycone [λ_{max} (MeOH) 250 sh, 268, 300 sh, 355; (NaOMe) rapid decomp.; ($AlCl_3$) 271, 310, 416; ($AlCl_3$–HCl) 276, 304, 360, 386 nm; MS *m/z* (rel. int.): 302 $[M]^+$ (100), 274 $[M-CO]^+$ (10), 245 (5), 153 $[A_1+H]^+$ (40), 150 $[B_1]^+$ (12%)] which cochromatographed with tricetin (co-TLC cellulose, TBA, 50% HOAc). When **15** was hydrolysed in 1 N TFA at 100° and the progress of the hydrolysis monitored by TLC (TBA, 50% HOAc) over a period of 70 min, only one product intermediate between **15** and tricetin was observed. This product was subjected to co-TLC against tricetin 3'-*O*-glucoside and tricetin 7-*O*-glucoside standards (cellulose, TBA, 50% HOAc and C_6H_6–HOAc–H_2O, 125:72·5), and the plates were sprayed with Naturstoffreagenz-A. The product was identical only to tricetin 3'-*O*-glucoside.

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