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Phytochemistry 68 (2007) 1407–1416
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Flavonol tetraglycosides and other constituents from leaves of *Styphnolobium japonicum* (Leguminosae) and related taxa

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Received 4 January 2007; accepted 5 March 2007

Abstract

Two flavonol tetraglycosides comprising a trisaccharide at C-3 and a monosaccharide at C-7 were isolated from the leaves of *Styphnolobium japonicum* (L.) Schott and characterised as the 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside-7-*O*-α-rhamnopyranosides of quercetin and kaempferol. The 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside-7-*O*-α-rhamnopyranoside of kaempferol, the 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranosides of kaempferol and quercetin and the 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside of kaempferol were also obtained from this species for the first time. Some or all of these flavonol tetra- and triglycosides were detected in 17 of 18 specimens of *S. japonicum* examined from living and herbarium material, although the most abundant flavonoid in the leaves was generally quercetin 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranoside (rutin). The triglycosides, but not the tetraglycosides, were detected in herbarium specimens of *Styphnolobium burseroides* M. Sousa, Rudd & Medrano and *Styphnolobium monteviridis* M. Sousa & Rudd, but specimens of *Styphnolobium affine* (Torrey & A. Gray) Walp. contained a different profile of flavonol glycosides. The flavonol tetra- and triglycosides of *S. japonicum* were also present in leaves of *Cladrastis kentukea* (Dum. Cours.) Rudd, a representative of a genus placed close to *Styphnolobium* in current molecular phylogenies. An additional constituent obtained from leaves of *Styphnolobium japonicum* was identified as the maltol derivative, 3-hydroxy-2-methyl-4*H*-pyran-4-one 3-*O*-(4′-*O*-*p*-coumaroyl-6′-*O*-(3-hydroxy-3-methylglutaroyl))-β-glucopyranoside.

Keywords: *Styphnolobium japonicum*; *Sophora japonica*; Leguminosae; Fabaceae; Flavonoids; Flavonol tetraglycosides; MS/MS analysis

1. Introduction

Styphnolobium japonicum (L.) Schott (pagoda tree, Chinese scholar tree) is a commonly grown ornamental tree belonging to subfamily Papilionoideae of family Leguminosae. The species is native to eastern Asia, mainly China, where the flowers and fruits are used as a haemostatic agent in traditional medicine (Chen and Chen, 2004; Kuang and Zhang, 2005). Both in horticulture and traditional Chinese medicine, the species is more widely known by its synonym, *Sophora japonica* L.

The genus *Styphnolobium* was re-segregated from *Sophora* on the basis of morphology and chromosome number (Sousa and Rudd, 1993). Subsequent cladistic analyses of DNA sequence data have suggested that *Styphnolobium* is more closely related to *Cladrastis* than *Sophora* sens. strict. (Pennington et al., 2001; Lavin et al., 2005). Indeed, *Sophora* s.s. is now considered to belong to the genistoid group of papilionoid legumes that are characterised phytochemically by the presence of quinolizidine alkaloids; *Styphnolobium* and *Cladrastis* lack quinolizidine alkaloids (Kite and Pennington, 2003) and are placed among the more basally-branching lineages of papilionoid legumes in molecular cladistic analyses.

Most studies on the flavonoids of *Styphnolobium japonicum* have concentrated on the flower buds, flowers, pods

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0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.03.004

and seeds – the parts used medicinally. These organs, and particularly the buds, contain high levels of rutin (Balbaa et al., 1974; Paniwnyk et al., 2001) and are a commercial source of the compound (Tran Cong Khanh, 1999). Other major flavonoids in the flowers include kaempferol 3-*O*-sophoroside (sophoraflavonoloside), kaempferol 3-*O*-gentiobioside and quercetin (Tulaganov and Gaibnazarava, 2001; Wagner et al., 2004), while isoflavonoid glycosides, such as the genistein glycosides sophoricoside and sophorabioside, occur in the fruits and seeds (Tang et al., 2001; Wang et al., 2003). A flavonol tetraglycoside characterised as kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranosyl(1 → 2)-β-glucopyranoside-7-*O*-α-rhamnopyranoside, has been reported from seeds of *Styphnolobium japonicum* (Wang et al., 2003).

As part of further investigations on the phytochemistry of *Styphnolobium* and *Cladrastis* (Kite and Pennington, 2003), we have carried out a detailed analysis of the leaf flavonoids of *S. japonicum*. Two new flavonol tetraglycosides were discovered together with a further flavonol tetraglycoside and three triglycosides that were unreported in this species. A new maltol derivative was also obtained. This paper describes the isolation and characterisation of these compounds and assesses their validity as chemical characters in *Styphnolobium*.

2. Results and discussion

2.1. Characterisation of flavonol glycosides from Styphnolobium japonicum

Following LC-PDA-MS/MS analysis of 80% aqueous methanol extracts of leaves from four species (30 samples) of *Styphnolobium*, 22 of the chromatographic peaks, observed as a significant component of at least one analysis, were identified as showing the UV and mass spectral characteristics of flavonol glycosides or aglycones; Table 1 lists their HPLC retention times and relative molecular masses (indicated by $[M+H]^+$). Eleven of these flavonoids were evident in the analysis of leaves of *S. japonicum* (1897-62202/BI-14276) collected in bulk for the isolation of flavonol glycosides (Fig. 1).

Compounds **3** ($[M+H]^+$, *m/z* 903), **9** ($[M+H]^+$, *m/z* 757) and **15** ($[M+H]^+$, *m/z* 611) were *O*-glycosides of quercetin according to MS/MS analysis of $[M+H]^+$. This analysis also suggested that **15** was a diglycoside comprising hexose and deoxyhexose sugars and **9** and **3** were tri- and tetraglycosides containing one or two additional deoxyhexoses, respectively. The quercetin *O*-diglycoside, **15**, had the same retention time, UV spectrum and product ion spectra (of $[M+H]^+$ and $[M-H]^-$) as an authentic standard of rutin (quercetin 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranoside). MS/MS analysis of $[M+Na]^+$ revealed the neutral loss of quercetin from the molecular species of **9** and **15**, but in **3**, the loss of quercetin occurred only in combination with the loss of a deoxyhexosyl moiety, suggesting that the aglycone of **3** was glycosylated at two positions rather than one (as in **9** and **15**). UV spectra were indicative of *O*-glycosylation at C-3 in **9** and **15** (shoulder at ca. 300 nm), and both C-3 and C-7 in **3** (no shoulder at ca. 300 nm) (Mabry et al., 1970).

Column chromatography and semi-preparative HPLC of aqueous MeOH extracts of *S. japonicum* leaves yielded **3**, **9** and **15** as yellow solids. The structures of **9** and **15** were confirmed as quercetin 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside (manghaslin) and quercetin 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranoside (rutin), respectively, by ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum of **3** was similar to that of **9**, but contained a set of additional resonances corresponding to a fourth sugar moiety, including an anomeric proton resonance at δ 5.55 (1H, *d*, $J = 1.8$ Hz) and a methyl resonance at δ 1.27 (3H, *d*, $J = 6.1$ Hz), both typical of an α-rhamnopyranosyl residue. The identity of the sugar was confirmed from the full set of ^1H and ^{13}C NMR spectral assignments obtained using 2D methods (Table 2), and the multiplicities and coupling constants of the proton resonances of H-1 to CH_3-6 (Duus et al., 2000). The site of substitution of the additional α-Rha sugar in **3** was shown to be C-7 from the downfield shifts of H-6 and H-8 in the ^1H NMR spectrum (compared to those of **9**), the ROE connectivities detected between the anomeric proton at δ 5.55 and both H-6 and H-8, and a long-range connectivity from δ 5.55 to C-7 (δ_C 163.5) in the HMBC spectrum. Thus, **3** was determined to be quercetin 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside, a new flavonol tetraglycoside.

Three isomeric pairs of flavonoid glycosides, **6** and **7** ($[M+H]^+$, *m/z* 887), **12** and **13** ($[M+H]^+$, *m/z* 741), and **17** and **19** ($[M+H]^+$, *m/z* 595) each displayed similar positive ion mass spectra. These appeared to be tetra- (**6** and **7**), tri- (**12** and **13**) and diglycosides (**17** and **19**) of kaempferol, and the neutral losses of sugar residues observed from $[M+H]^+$ were the same as those observed in the corresponding spectra of the quercetin glycosides **3**, **9** and **15**, respectively. MS/MS analysis of $[M+Na]^+$ of **6** and **7** indicated that the kaempferol aglycone was glycosylated at two positions. Compounds **17** and **19** were identified as kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-galactopyranoside (kaempferol 3-*O*-robinobioside) and kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranoside (kaempferol 3-*O*-rutinoside), respectively, from comparison of their HPLC retention times and UV and mass spectra with authentic standards.

Column chromatography and semi-preparative HPLC of aqueous MeOH extracts of *S. japonicum* leaves yielded **6**, a 3:5 mixture of **6** and **7**, and a 1:1 mixture of **12** and **13**, all as yellow solids. Extensive 1D and 2D NMR spectral analysis of the mixture of **12** and **13** confirmed that this comprised the known flavonol glycosides, kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside and kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside,

Table 1
HPLC retention times (t_R), relative molecular masses (M_r), aglycone identity (Agly.)[a] and *O*-linked sugars at C-3 and C-7 of the aglycones of foliar flavonoids in *Styphnolobium*

No.	t_R (min)	M_r	Agly.	C-3[c]	C-7	Det.
1	10.0	918	Q	Hex(1 → 2)[α-Rha(1 → 6)]-β-Glc-	α-Rha-	MS
2	10.4	772	Q	Hex(1 → 2)-β-Glc-	α-Rha-	MS
3	11.4	902	Q	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Glc-	α-Rha-	NMR
4	12.3	902	K	Hex(1 → 2)[α-Rha(1 → 6)]-β-Glc-	α-Rha-	MS
5	12.6	756	K	Hex(1 → 2)-β-Glc-	α-Rha-	MS
6	13.2	886	K	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Gal-	α-Rha-	NMR
7	13.4	886	K	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Glc-	α-Rha-	NMR
8	13.5	772	Q	Hex(1 → 2)[α-Rha(1 → 6)]-β-Glc-	–	MS
9	15.9	756	Q	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Glc-	–	NMR
10	16.2	756	K	Hex(1 → 2)[α-Rha(1 → 6)]-β-Glc-	–	MS
11	17.2	756	Q	α-Rha(1 → 6)-β-Glc-	α-Rha-	MS
12	18.3	740	K	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Gal-	–	NMR
13	18.8	740	K	α-Rha(1 → 2)[α-Rha(1 → 6)]-β-Glc-	–	NMR
14	20.2	594	K	α-Rha(1 → 2)-β-Glc-	–	MS
15	20.6	610	Q	α-Rha(1 → 6)-β-Glc-	–	NMR
16	20.9	740	K	α-Rha(1 → 6)-β-Glc-	α-Rha-	MS
17	23.5	594	K	α-Rha(1 → 6)-β-Gal-	–	Std
18	23.6	610	Q	–	α-Rha(1 → 6)-β-Glc-	MS
19	24.9	594	K	α-Rha(1 → 6)-β-Glc-	–	Std
20	25.8	624	I	α-Rha(1 → 6)-β-Glc-	–	Std
21	30.5	302	Q	–	–	MS
22	34.7	286	K	–	–	MS

Structural assignments (Det.) supported by data from NMR, MS[b] or comparison with an authentic standard (Std).
[a] Aglycones: I, isorhamnetin; K, kaempferol; Q, quercetin.
[b] For assignments from MS data, anomeric configurations of sugars are assumed to be those found in naturally occuring flavonol glycosides (β-Gal, β-Glc and α-Rha).
[c] The primary sugar (*O*-linked at C-3) is the last listed in each entry. Hex, hexosyl.

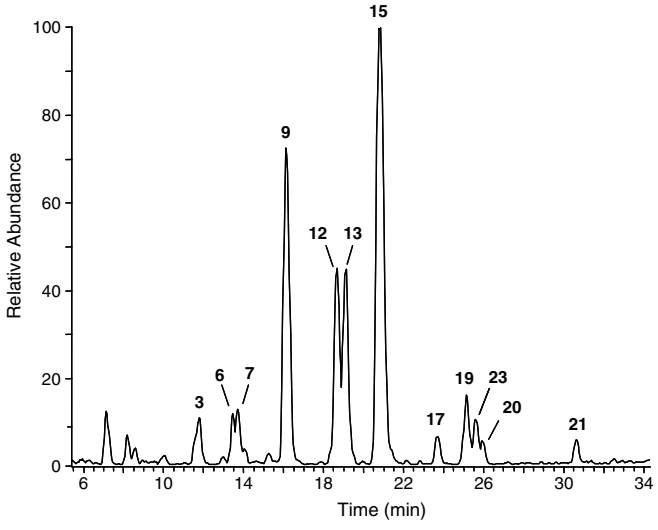


Fig. 1. Base ion chromatogram from a positive ion LC-ESI-MS analysis of an 80% aq. MeOH extract of leaves of *Styphnolobium japonicum* (1897-62202/BI-14276).

respectively. Assignment of the ^1H and ^{13}C NMR spectra of **6** indicated that this was similar to kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside (**12**), but had an additional α-Rha residue (Table 2). A long-range connectivity from the anomeric proton of the latter (δ 5.56) to C-7 (δ_C 163.5) in the HMBC spectrum and the downfield shifted resonances of H-6 and H-8 confirmed the site of substitution as C-7. Thus, **6** was determined to be kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside-7-*O*-α-rhamnopyranoside, a compound first reported (Yahara et al., 2000) from aerial parts of *Astragalus shikokianus* (a species name unrecorded in the Leguminosae literature). The assignments for **6** in CD_3OD are in good agreement with the earlier dataset obtained in DMSO-d_6, taking into account the small solvent effect. This kaempferol glycoside has also been obtained from leaves of *Zollernia ilicifolia* Vog. (Leguminosae) (Coelho et al., 2003), but this is the first report in *Styphnolobium japonicum*. Analysis of the NMR spectra of the 3:5 mixture of **6** and **7** revealed that the latter was the β-glucopyranosyl analogue of **6**, as the assignments in Table 2 confirm. Thus, **7** is kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside, a second new flavonol tetraglycoside.

The presence of kaempferol 3-*O*-robinobioside (**17**) and kaempferol 3-*O*-rutinoside (**19**) in the same extract allowed for detailed examination of the product ion spectra of these isomers. While the spectra of $[M+H]^+$ and $[M+Na]^+$ were essentially the same for both **17** and **19**, in the spectra generated from $[M-H]^-$ the abundance ratios of the deprotonated aglycone ion $[A-H]^-$ to the radical ion $[A-2H]^{\bullet-}$ were different, being approximately 10:8 for **17** but 10:1 for **19**. In the MS/MS spectra of $[M-H]^-$ of the isomeric triglycosides **12** and **13**, the ratio of these product

Table 2
^{1}H and ^{13}C NMR spectral data for flavonol tetraglycosides **3**, **6** and **7** (400 MHz, CD_3OD, 30 °C)[a]

	Atom	**3**		**6**		**7**	
		δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)
Aglycone	6	6.46 *d* (2.2)	100.5	6.47 *d* (2.2)	100.5	6.47 *d* (2.2)	100.5
	8	6.72 *d* (2.2)	95.7	6.73 *d* (2.2)	95.7	6.73 *d* (2.2)	95.7
	2′	7.62 *d* (2.2)	117.5	8.09 *d* (9.0)	132.4	8.05 *d* (8.9)	132.3
	3′			6.91 *d* (9.0)	116.3	6.91 *d* (8.9)	116.3
	5′	6.88 *d* (8.2)	116.2	6.91 *d* (9.0)	116.3	6.91 *d* (8.9)	116.3
	6′	7.64 *dd* (8.3, 2.2)	123.7	8.09 *d* (9.0)	132.4	8.05 *d* (8.9)	132.3
3-*O*-Gal/Glc[b]	1	5.61 *d* (7.6)	100.5	5.63 *d* (7.8)	100.9	5.63 *d* (7.8)	100.6
	2	3.66 *dd* (9.2, 7.6)	80.2	3.93 *dd* (9.3, 7.8)	77.7	3.61 *m*	80.0
	3	3.54 *m*	79.0	3.70 *m*	75.8	3.54 *m*	79.1
	4	3.28 *m*	71.9	3.77 *m*	70.8	3.27 *m*	71.9
	5	3.33 *m*	77.2	3.63 *m*	75.5	3.33 *m*	77.2
	6	3.82 *m*, 3.41 *m*	68.3	3.72 *m*, 3.47 *m*	67.3	3.82 *m*, 3.38 *m*	68.3
2″-*O*-Rha	1	5.23 *d* (1.7)	102.7	5.22 *d* (1.7)	102.7	5.23 *d* (1.7)	102.7
	2	4.00 *dd* (3.4, 1.7)	72.4	4.00 *dd* (3.5, 1.8)	72.5	4.00 *dd* (3.5, 1.8)	72.4
	3	3.79 *dd* (9.6, 3.4)	72.4	3.80 *m*	72.4	3.79 *m*	72.4
	4	3.35 ‘*t*’ (9.5)	74.2	3.34 *m*	74.2	3.34 *m*	74.2
	5	4.07 *dd* (9.7, 6.3)	70.0	4.05 *dd* (9.6, 6.2)	69.8	4.04 *dd* (9.6, 6.2)	69.9
	6	1.01 *d* (6.2)	17.6	0.97 *d* (6.1)	17.6	0.98 *d* (6.1)	17.6
6″-*O*-Rha	1	4.50 *d* (1.7)	101.9	4.52 *d* (1.8)	101.9	4.49 *d* (1.7)	102.3
	2	3.53 *dd* (3.4, 1.7)	72.2	3.53 *dd* (3.4, 1.7)	72.2	3.55 *dd* (3.5, 1.8)	72.1
	3	3.46 *dd* (9.5, 3.4)	72.3	3.48 *m*	72.3	3.46 *m*	72.4
	4	3.22 ‘*t*’ (9.4)	74.0	3.26 ‘*t*’ (9.5)	74.0	3.22 *m*	73.9
	5	3.41 *m*	69.7	3.51 *m*	69.7	3.40 *m*	69.8
	6	1.07 *d* (6.2)	17.9	1.17 *d* (6.2)	18.0	1.07 *d* (6.2)	17.9
7-*O*-Rha	1	5.55 *d* (1.8)	100.0	5.56 *d* (1.8)	100.0	5.56 *d* (1.8)	100.0
	2	4.02 *dd* (3.4, 1.8)	71.9	4.02 *dd* (3.5, 1.8)	71.8	4.02 *dd* (3.5, 1.8)	71.8
	3	3.82 *dd* (9.5, 3.4)	72.2	3.82 *m*	72.2	3.82 *m*	72.2
	4	3.48 ‘*t*’ (9.6)	73.7	3.48 *m*	73.7	3.48 *m*	73.7
	5	3.62 *dd* (9.6, 6.1)	71.4	3.61 *m*	71.4	3.61 *m*	71.3
	6	1.27 *d* (6.1)	18.2	1.26 *d* (6.2)	18.2	1.28 *d* (6.2)	18.2

[a] The ^{13}C NMR spectral assignments were obtained from the inversely-detected dimension of HSQC experiments.
[b] Gal in **6**, Glc in **3** and **7**.

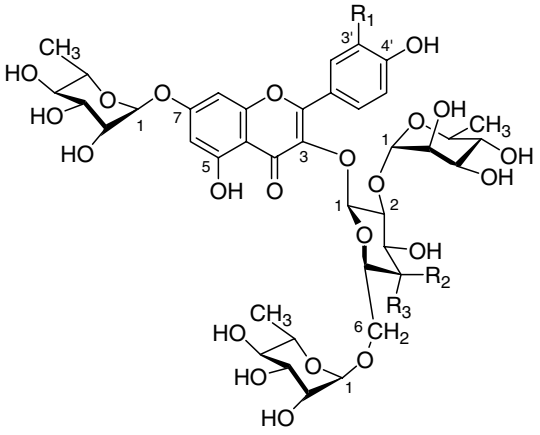
ions did not differ as dramatically; however, it was noted that the MS3 (m/z 739 → 575) spectra showed differences in the relative intensity of a product ion at m/z 393, being 20–40% for **12** and 70–100% for **13**.

A further flavonol diglycoside **20** ($[M+H]^+$, m/z 625) was identified as isorhamnetin 3-*O*-rutinoside by comparison of analytical data with a standard. The free flavonol aglycone **21** was identified as quercetin from MS and UV spectra, and kaempferol (**22**) also became evident during fractionation and was observed in the LC–MS analysis of the crude extract in a single ion chromatogram of m/z 287, $[M+H]^+$.

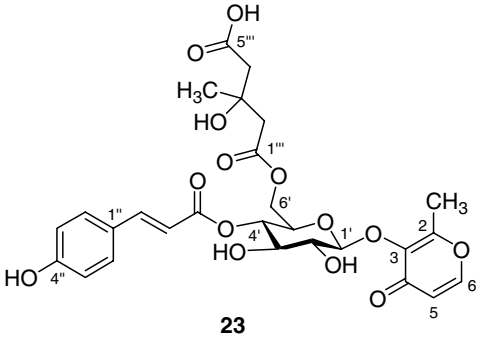
2.2. Characterisation of a maltol derivative

During fractionation of flavonoid glycosides from *S. japonicum*, one non-flavonoid component (**23**) was isolated. The UV spectrum of this compound suggested the presence of a coumaroyl group ($\lambda_{max} = 312$ nm), and it gave $[M+H]^+$ at m/z 579 which generated an ion at m/z 309 following MS/MS, in accordance with [coumaroylhexosyl + H]$^+$. The ^{1}H NMR spectrum of **23** also contained several distinct groups of resonances that suggested the presence of an acylated glycoside of maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one) (Li et al., 2000). The acylating groups were readily identified as coumaric acid and 3-hydroxy-3-methylglutaric acid (Section 3.13). The coupling constants of the ^{1}H resonances of the sugar moiety and the chemical shift values of its ^{1}H and ^{13}C resonances assigned from COSY, HSQC and HMBC data indicated that it was a β-glucopyranoside (Duus et al., 2000). Both H-4′ (δ 4.948) and CH_2-6′ (δ 4.11 and 4.18) of the latter were downfield shifted in the ^{1}H NMR spectrum. Long-range correlations in the HMBC spectrum from H-4′ of β-Glc to the carbonyl of the coumaroyl group at δ_C 168.4, and from CH_2-6′ to δ_C 172.2 of the 3-hydroxy-3-methylglutaroyl group established the substitution sites of these acyl groups. A similar correlation from the anomeric proton of β-Glc at δ 4.952 (1H, *d*, $J = 7.9$ Hz) to C-3 of the maltol group at δ_C 143.3 confirmed the position of the latter. Thus, **23** was identified as 3-hydroxy-2-methyl-4*H*-pyran-4-one 3-*O*-(4′-*O*-*p*-coumaroyl-6′-*O*-(3-hydroxy-3-methylglutaroyl))-β-glucopyranoside, a new maltol derivative. The related compound, maltol 3-*O*-(6′-*O*-(3-hydroxy-3-

methylglutaroyl))-β-glucopyranoside (licoagroside B) has been reported from hairy root cultures of the legume *Glycyrrhiza glabra* L. (Li et al., 2000).



3 $R_1 = R_3 = OH, R_2 = H$
6 $R_1 = R_3 = H, R_2 = OH$
7 $R_1 = R_2 = H, R_3 = OH$



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2.3. Survey of flavonoids in Styphnolobium accessions

Among eleven living specimens of *Styphnolobium japonicum* sampled (10 from RBG Kew and one from a private garden), some variation existed in the range of flavonol glycosides produced (Table 3). Nevertheless, the majority of the profiles were dominated by two series of compounds, the first based on quercetin 3-*O*-rutinoside (**15**) and the second based on kaempferol 3-*O*-robinobioside (**17**) and 3-*O*-rutinoside (**19**). In each series, co-occurring tri- and tetraglycosides were characterised by an additional rhamnosyl group at 2-OH of the primary C-3 sugar (**9**, **12**, and **13**) and, for the tetraglycosides, an additional rhamnosyl group at 7-OH of the flavonol aglycone (**3**, **6**, and **7**). These observations suggest that rhamnosyl transferases specific to the 2-OH group of a hexose (β-Gal or β-Glc) *O*-linked at C-3, and to 7-OH of the flavonol aglycone, are operative. Furthermore, both quercetin and kaempferol glycosides are substrates. The tetraglycosides **3**, **6** and **7** were detected in nine plants, albeit at relatively low levels in some, while in one of the remaining plants only **3** was detected. In the majority of accessions, quercetin 3-*O*-rutinoside was the most abundant flavonol glycoside (assuming similar ionisation responses between flavonoids), but in specimen 1931–18102 (plant 9), 1994-3561 (plant 10) and the plant from a private garden (11), the kaempferol tetraglycosides **6** and **7** were more abundant. Conversely, accession 1982-6282 (plant 7) produced mainly quercetin 3-*O*-rutinoside, and the only higher flavonol glycoside detected was **9**. In three plants (1–3) from which collections were made in different months and years, the flavonoids profiles were reasonably stable for each individual. In herbarium specimens made from trees growing wild in China (plants 12–17 of Table 3), the range of flavonol glycosides detected was similar to the cultivated specimens; the kaempferol tetraglycosides **6** and **7** were detected in five of the six specimens analysed while the quercetin tetraglycoside **3** was only detected in one. Again, there was some variation in the relative abundance of flavonol glycosides, with the kaempferol tetraglycosides predominating in one specimen (plant 15) while the kaempferol triglycosides **12** and/or **13** predominated in another (plant 12); in a specimen from a tree cultivated in Bolivia (plant 18), quercetin 3-*O*-rutinoside was the main flavonol glycoside, and no higher glycosides were detected.

In surveying the cultivated accessions of *Styphnolobium japonicum*, several additional flavonol glycosides were noted. A tetraglycoside **1** ($[M+H]^+$, *m/z* 919) was detected in samples from four trees and consisted of quercetin *O*-glycosylated with two hexose and two deoxyhexose sugars. MS/MS analysis of $[M+Na]^+$ and $[M-H]^-$ showed one major neutral loss of a deoxyhexosyl unit (as with **3**, **6** and **7**) suggesting **1** was similarly substituted with rhamnose at C-7. Glycosylation at both C-3 and C-7 was also indicated by the UV spectrum. The possibility that **1** also had a branched trisaccharide at C-3 with a primary hexose was suggested by the loss of both 146 and 162 amu from $[M+H]^+$. A diglycoside **18** ($[M+H]^+$, *m/z* 611) was detected in six accessions and MS/MS of $[M+H]^+$ showed it to be formed from quercetin, hexose and deoxyhexose, with evidence for at least the hexose as a primary sugar. UV maxima at 252 and 364 nm were indicative of a quercetin 7-*O*-glycoside (Mabry et al., 1970), while the product ion spectrum of $[M-H]^-$ was characteristic of rutinosides (higher abundance of $[A-H]^-$ over $[A-2H]^-$ and only low abundance ions associated with the single loss of the rhamnose unit) rather than neohesperidosides (Grayer et al., 2000); thus **18** is likely to be quercetin 7-*O*-rutinoside. As well as containing the quercetin tetraglycoside **1**, one plant (9) produced significant amounts of a kaempferol tetraglycoside (**4**) and lower levels of several other kaempferol and quercetin triglycosides that were a feature of *S. affine* (Torrey & A. Gray) Walp. (see below). Some of these compounds were observed erratically at low levels in other samples of *S. japonicum*.

Of the nine species of *Styphnolobium* recognised (Sousa and Rudd, 1993), all except *S. japonicum* and *S. affine* are rare mesoamerican species. Only material of *S. affine*,

Table 3
Relative peak areas[a] of $[M-H]^-$ ions of flavonoids observed among samples of *Styphnolobium* and *Cladrastis*

	1	2	3	4	5	6 + 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
S. affine (1)	57	21	–	277	576	–	22	–	11	–	–	–	3	27	3	–	–	3	–	–	–
S. affine (2)	50	–	–	270	189	–	115	–	44	–	–	–	–	224	42	–	–	25	42	–	–
S. affine (3)	69	22	–	385	311	–	6	–	21	3	–	–	19	66	59	10	–	29	1	–	–
S. affine (4)	341	76	–	109	150	–	26	–	7	110	–	–	20	126	19	–	–	12	5	–	–
S. affine (5)	99	34	–	284	388	–	11	–	22	37	–	–	19	55	26	–	–	23	1	–	–
S. burseroides	–	–	–	–	–	–	–	43	–	–	6	60	–	437	–	46	–	391	17	–	–
S. japonicum (1a)	–	–	19	–	–	65	–	199	–	–	114	100	–	394	–	26	–	56	23	4	1
S. japonicum (1b)	–	–	45	–	–	66	–	249	–	–	94	98	–	336	–	15	–	42	16	38	2
S. japonicum (1c)	–	–	24	–	–	38	–	237	–	–	72	70	–	461	–	24	–	49	19	6	1
S. japonicum (1d)	–	–	28	–	–	34	–	329	–	–	69	72	–	368	–	21	–	51	23	5	–
S. japonicum (2a)	–	–	9	–	–	14	–	75	–	–	21	11	–	563	–	89	58	96	24	32	6
S. japonicum (2b)	2	–	4	–	–	16	–	41	–	–	16	7	–	724	–	48	–	87	36	13	7
S. japonicum (3a)	–	–	–	–	–	25	–	18	–	–	6	7	–	742	–	53	20	35	40	51	4
S. japonicum (3b)	–	–	1	–	–	3	–	40	–	–	6	17	–	658	–	53	89	53	40	27	11
S. japonicum (4)	17	–	23	–	–	49	9	329	–	–	186	115	–	210	–	20	5	17	7	9	3
S. japonicum (5)	–	–	23	–	–	11	–	122	–	–	24	19	–	547	–	27	96	60	64	6	–
S. japonicum (6)	–	–	3	–	–	–	–	72	–	–	7	8	–	581	–	56	126	65	31	38	12
S. japonicum (7)	–	–	–	–	–	–	–	10	–	–	–	–	–	816	–	39	–	63	70	2	1
S. japonicum (8)	–	–	1	–	–	2	–	16	–	–	5	2	–	717	–	30	94	92	42	1	–
S. japonicum (9)	10	5	6	193	69	497	2	6	63	–	106	20	–	6	9	2	–	5	–	–	–
S. japonicum (10)	1	–	110	–	–	334	2	124	–	–	70	80	–	162	–	32	–	71	12	1	–
S. japonicum (11)	–	–	46	–	–	407	–	119	–	–	145	117	–	116	–	24	–	7	8	10	1
S. japonicum (12)	–	–	–	–	–	–	–	5	–	–	917	?[b]	–	60	–	15	–	2	–	–	–
S. japonicum (13)	–	–	–	–	–	57	–	3	–	–	111	99	–	391	–	72	–	239	29	–	–
S. japonicum (14)	–	–	–	–	–	4	–	15	–	–	20	–	–	825	–	24	–	81	32	–	–
S. japonicum (15)	–	–	29	–	–	689	–	18	–	–	150	41	–	42	–	15	–	16	–	–	–
S. japonicum (16)	–	–	–	–	–	11	–	–	–	–	–	–	–	821	–	22	–	63	83	–	–
S. japonicum (17)	–	–	–	–	–	16	–	65	–	–	32	26	–	585	–	113	–	137	26	–	–
S. japonicum (18)	–	–	–	–	–	–	–	–	–	–	–	–	–	862	–	6	–	73	57	2	–
S. monteviridis	–	–	–	–	–	–	–	5	–	–	775	120	–	88	–	9	–	4	–	–	–
C. kentukea	–	–	167	83	–	537	–	58	–	–	47	46	–	49	–	3	–	9	–	–	–

[a] Relative peak areas expressed as parts per thousand of the total of all peak areas measured.
[b] **12** and **13** not resolved chromatographically due to high levels.

S. burseroides M. Sousa, Rudd & Medrano and *S. monteviridis* M. Sousa & Rudd was available for study in the Kew Herbarium. The latter two species showed glycosidic elaborations of quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside and kaempferol 3-*O*-robinobioside similar to those observed in *S. japonicum*, although the tetraglycosides were not detected. In contrast, the four specimens examined of *S. affine* presented a somewhat different profile of flavonoids, and one in which flavonol tri- and tetraglycosides were more prominent, even in specimen 2, which was sourced from herbarium material collected in 1847. The presence of the quercetin tetraglycoside **1**, noted erratically in *S. japonicum*, was one consistent feature of the flavonoids of *S. affine*, and **1** was the predominant flavonoid in one specimen (4). Three quercetin triglycosides **2**, **8** and **11** were also noted among the analyses, but in most specimens a kaempferol tetraglycoside **4** and a kaempferol triglycoside **5** were among the main flavonoids and these were accompanied by lower levels of two other kaempferol triglycosides **10** and **16**. The flavonol 3-*O*-diglycosides present contained glucose as the primary sugar rather than galactose (except for one specimen, in which **17** was detected) suggesting that the higher glycosides were elaborations of quercetin and kaempferol 3-*O*-glucosides; this assertion was also supported by negative ion mass spectral analysis as follows.

MS2 analyses of $[M-H]^-$ (at *m*/*z* 739) of **16** and a standard of the isomeric kaempferol triglycoside robinin (kaempferol 3-*O*-robinobioside-7-*O*-rhamnoside) both gave a single product ion at *m*/*z* 593. March et al. (2004) studied the fragmentation of deprotonated robinin in detail using a triple quadrupole analyser and concluded that the product ion observed at *m*/*z* 593 was due to the loss of the rhamnosyl moiety from C-7 of the aglycone, not from the terminal rhamnose of the disaccharide at C-3. In accordance with this assignment, MS3 analysis of the robinin product ion at *m*/*z* 593 using our ion trap analyser gave a spectrum matching that of the MS2 spectrum of deprotonated kaempferol 3-robinobioside, in particular in terms of the ratios of $[A-H]^-$: $[A-2H]^{\bullet -}$. The equivalent MS3 analysis of **16** gave a spectrum matching that of deprotonated kaempferol 3-*O*-rutinoside; thus **16**, which had a slightly longer retention time than robinin, is likely to be kaempferol 3-*O*-rutinoside-7-*O*-rhamnoside. The deprotonated molecule of the quercetin triglycoside **11** also showed a single loss of rhamnosyl following MS2 and this ion dissociated to produce ions at $[A-H]^-$ and $[A-2H]^{\bullet -}$ in a similar ratio to that recorded for deprotonated quercetin 3-*O*-ruti-

noside; thus **11** is likely to be quercetin 3-*O*-rutinoside-7-*O*-rhamnoside. Our studies (Kite et al., unpubl.) on the negative ion mass spectrometry of flavonols bearing a branched triglycoside at C-3 with glucose or galactose as the primary sugar, suggest that the sugar moiety at C-2″ is lost more readily than that at C-6″, and the loss is as an entire sugar molecule (i.e. 180 amu for hexose) rather than a residue. The kaempferol 3-*O*-triglycoside **10** showed a loss of 180 amu following negative ion MS2 and analysis of the resulting ion by MS3 (755 → 575) gave a spectrum more similar to the MS3 (739 → 575) of **13** (i.e. abundant ion at *m*/*z* 393, see Section 2.1) rather than **12**, thus **10** is likely to be a 3-*O*-rhamnosyl(1 → 6)-glucoside of kaempferol with a hexose substituted at C-2″, rather than a rhamnose as in **13**. The negative ion MS3 (901 → 755) spectrum of the kaempferol tetraglycoside **4** (i.e. the fragmentation of the ion resulting from the loss of rhamnosyl from C-7) was similar to the spectrum of deprotonated **10** suggesting that **4** is the 7-*O*-rhamnoside of **10**. The corresponding analysis of **5**, which also bore a C-7 rhamnose, gave a MS3 (755 → 609) spectrum that was similar to the MS2 spectrum of a standard of kaempferol 3-*O*-β-glucopyranosyl(1 → 2)-β-glucopyranoside isolated in-house; however, we did not have a standard of the galactose analogue (kaempferol 3-*O*-β-galactopyranosyl(1 → 2)-β-glucopyranoside) to compare the fragmentation and therefore we cannot infer the identity of the terminal hexose in **5**, or hence **4** and **10**. Similar MS analysis of the quercetin glycosides **1**, **2**, **8**, and **11** suggested that they are likely to show the same glycosylation pattern as the kaempferol glycosides **4**, **5**, **10**, and **16**, respectively, as indicated in Table 1.

Finally, returning to the initial question posed by this study – whether *Styphnolobium* and *Cladrastis* shared common flavonoids – it was of taxonomic interest to find that the same series of quercetin and kaempferol glycosides seen in leaves of *S. japonicum* were also detected in *Cladrastis kentukea* (Dum. Cours.) Rudd. In the latter species, the tetraglycosides **3**, **6** and **7** were the most abundant of this series. Other flavonoid glycosides were present in leaves of *C. kentukea* including **4**. The difference in leaf chemistry between *S. japonicum* and *S. affine* is noteworthy as Sousa and Rudd (1993) placed these species in Sections *Styphnolobium* and *Oresbios*, respectively, of the genus (only two Sections being recognised); however, they placed all other species with *S. affine* in Section *Oresbios*, which conflicts with the finding that *S. burseroides* and *S. monteviridis* have similar leaf flavonoid chemistry to *S. japonicum*.

3. Experimental

3.1. General

LC-PDA-MS/MS analyses were performed using a Thermo-Finnigan system consisting of 'Surveyor' autosampler, pumps and PDA connected to an 'LCQ Classic' ion trap mass spectrometer fitted with an ESI source. Chromatography of 10 μl injections was performed on a Phenomenex Luna C18(2) column (150 mm × 4.6 mm i.d., 5 μm particle size) using a 1 ml/min linear mobile phase gradient of 20–50% aq. MeOH (containing 1% HOAc) in 30 min followed by a MeOH wash. The flow to the ESI source was reduced to 0.2 ml/min by a splitter and the source was operated using the manufacturer's standard conditions. MS/MS experiments on $[M+H]^+$, $[M+Na]^+$ and $[M-H]^-$ were performed automatically in separate LC–MS analyses; spectra of the minor sodiated species were obtained by specifying a +22 *m*/*z* unit offset to the value triggered by the abundant ion ($[M+H]^+$) during data dependent selection of the ion for fragmentation. Accurate mass measurements were performed on a Finnigan MAT900 XLT mass spectrometer in positive ESI mode.

Semi-preparative HPLC was performed using a Waters system consisting of a Model 717 Plus autosampler, Model 600 pumps and controller and Model 996 PDA, fitted with a Merck LiChrospher RP-18 column (250 mm × 10 mm i.d., 10 μm particle size). The mobile phase flow rate was 4.5 ml/min and gradients were as detailed in Section 3.4. NMR spectra were acquired in CD_3OD at 30 °C on a Bruker Avance 400 MHz instrument. Standard pulse sequences and parameters were used to obtain 1D ^1H, 1D ^{13}C, 1D site selective ROE, COSY, HSQC and HMBC spectra. Chemical shift referencing was carried out with respect to TMS at 0.00 ppm.

3.2. Standards

Kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-galactopyranoside-7-*O*-α-rhamnopyranoside (robinin) and isorhamnetin 3-*O*-rutinoside were purchased from Apin. Partial hydrolysis of robinin with 1 M TFA at 80 °C for 10 min yielded kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-galactopyranoside (kaempferol 3-*O*-robinobioside) and kaempferol 7-*O*-α-rhamnopyranoside. An authentic standard of kaempferol 3-*O*-β-glucopyranosyl(1 → 2)-β-glucopyranoside (kaempferol 3-*O*-sophoroside) was available from our collection of flavonoids isolated in-house.

3.3. Plant material

For the isolation of flavonoids, leaves of *Styphnolobium japonicum* were collected on 28 June 2005 from a tree growing at the Royal Botanic Gardens, Kew [Kew Accession No. 1897-62202/BI-14276, verified by R. M. Polhill (RBG Kew), flowering herbarium voucher previously lodged as ref. G.C. Kite, BI-9414 (K)]. For comparative LC–MS analyses, leaf samples were obtained from specimens held in the Kew Herbarium (K), from plants cultivated at RBG Kew, and a tree growing over the garden of one of the authors (GCK). The non-herbarium samples were freeze-dried before extraction. Further details on the plant material are given in Table 4.

Table 4
Details of leaf material of *Styphnolobium* and *Cladrastis* studied

Species	Material	Reference[a]	Origin	Date collected (dd/mm/year)	Specimen No.	Flowering voucher reference
S. affine (1)	Herbarium	E.J. Palmer 12620 (K)	Texas, USA	24/07/1917	BI-15455	
S. affine (2)	Herbarium	F. Lindheimer 601 (K)	Texas, USA	1847	BI-15456	
S. affine (3)	Herbarium	A. Ruth 355 (K)	Texas, USA	29/04/1926	BI-15457	
S. affine (4)	Herbarium	B.F. Bush 1051 (K)	Arkansas, USA	13/10/1901	BI-15458	
S. affine (5)	Herbarium	E.J. Palmer 13460 (K)	Texas, USA	26/04/1918	BI-15459	
S. burseroides	Herbarium	Mendoza 1442 (K)	Yucunduchi, Mexico	06/07/1985	BI-15452	
S. japonicum (1a)	Living	1897–62202	RBG Kew (cult.)	13/09/2002	BI-10754	G.C. Kite/BI-9414 (K)
S. japonicum (1b)	Living	1897–62202	RBG Kew (cult.)	28/06/2005	BI-14276	G.C. Kite/BI-9414 (K)
S. japonicum (1c)	Living	1897–62202	RBG Kew (cult.)	11/08/2005	BI-14414	G.C. Kite/BI-9414 (K)
S. japonicum (1d)	Living	1897–62202	RBG Kew (cult.)	27/06/2006	BI-15413	G.C. Kite/BI-9414 (K)
S. japonicum (2a)	Living	1905–51006	RBG Kew (cult.)	26/06/2006	BI-15412	G.C. Kite/BI-15412 (K)
S. japonicum (2b)	Living	1905–51006	RBG Kew (cult.)	11/08/2005	BI-14415	G.C. Kite/BI-15412 (K)
S. japonicum (3a)	Living	1972–10834	RBG Kew (cult.)	11/08/2005	BI-14416	G.C. Kite/BI-10527 (K)
S. japonicum (3b)	Living	1972–10834	RBG Kew (cult.)	01/07/2006	BI-15417	G.C. Kite/BI-10527 (K)
S. japonicum (4)	Living	1969–13140	RBG Kew (cult.)	28/06/2006	BI-15414	
S. japonicum (5)	Living	1973–11945	RBG Kew (cult.)	29/06/2006	BI-15415	G.C. Kite/BI-15415 (K)
S. japonicum (6)	Living	1973–11942	RBG Kew (cult.)	30/06/2006	BI-15416	G.C. Kite/BI-10661 (K)
S. japonicum (7)	Living	1982–6282	RBG Kew (cult.)	02/07/2006	BI-15418	G.C. Kite/BI-15418 (K)
S. japonicum (8)	Living	1973–19403	RBG Kew (cult.)	03/07/2006	BI-15419	G.C. Kite/BI-15419 (K)
S. japonicum (9)	Living	1931–18102	RBG Kew (cult.)	04/07/2006	BI-15420	G.C. Kite/BI-15420 (K)
S. japonicum (10)	Living	1994–3561	RBG Kew (cult.)	05/07/2006	BI-15421	G.C. Kite/BI-15421 (K)
S. japonicum (11)	Living	–	Garden of G.C. Kite (cult.)	20/07/2006	BI-15542	
S. japonicum (12)	Herbarium	A.K. Schindler 130 (K)	Honan, China	07/1907	BI-15446	
S. japonicum (13)	Herbarium	W.R. Carles 509 (K)	Chia Kuing, China	08/1893	BI-15447	
S. japonicum (14)	Herbarium	C.Y. Chiao 3014 (K)	Shantung Province, China	11/09/1930	BI-15448	
S. japonicum (15)	Herbarium	C.Y. Chiao 2831 (K)	Shantung Province, China	14/07/1930	BI-15449	
S. japonicum (16)	Herbarium	A.N. Steward et al. 894 (K)	Kweichow Province, China	14/11/1931	BI-15450	
S. japonicum (17)	Herbarium	K. Yao 9113 (K)	Fenyi City, Kiangsi, China	19/08/1985	BI-15451	
S. japonicum (18)	Herbarium	L. Rico 1219 (K)	Cerro San Pedro, Bolivia (cult.)	21/01/2003	BI-15453	
S. monteviridis	Herbarium	Haber 9118	Monteverde, Costa Rica	19/02/1989	BI-15454	
C. kentukea	Living	1920–10301	RBG Kew (cult.)	22/06/2006	BI-15438	

[a] Collector reference numbers are quoted for herbarium material, and RBG Kew accession numbers for living plants.

3.4. Extraction and isolation

Freeze dried, powdered leaves (100 g) of *Styphnolobium japonicum* were extracted in 80% aq. MeOH (500 ml) and, after adjusting the volume to 200 ml, the supernatant was partitioned against hexane (2 × 100 ml). The aq. MeOH phase was dried *in vacuo* and subjected to Sephadex LH-20 CC (25 × 290 mm) eluting with MeOH–H_2O in 700 ml steps of 10%, 30%, 50%, 70% and 100% MeOH. Three fractions containing flavonol glycosides were collected: Fraction A (first 500 ml of 30% MeOH step, containing **3**, **6** and **7**), Fraction B (final 200 ml of 50% MeOH, containing **9**, **12**, **13** and **23**) and Fraction C (final 350 ml of 70% MeOH, containing **15**). Yellow crystals of **15** (150 mg) formed in Fraction C upon concentration and storage at 4 °C.

Flavonoids in Fractions A and B were further purified by repetitive semi-preparative HPLC using various mobile phases. Chlorogenic acid and related compounds were removed from Fraction A by eluting them with a mobile phase of 10% aq. MeOH for 10 min after which time **3**, **6** and **7** were eluted from the column with 100% MeOH. Flavonoids **3**, **6** and **7** were then separated using a 14 min linear gradient of 10–22% aq. MeCN to yield **3** (8 mg), **6** (4.5 mg) and a mixture of **6** + **7** (30 mg). Compound **3** and the fraction containing **6** + **7** were further purified using, respectively, a 9 min gradient of 30–65% aq. MeOH and an 11 min gradient of 35–50% aq. MeOH, both containing 0.1% HCO_2H. Impure collections containing **9** and **12** + **13** were obtained from Fraction B using an 18 min linear gradient of 25–45% aq. MeOH. Subsequent elution of the column with 100% MeOH yielded **23** (10 mg). Flavonoids **9** and **12** + **13** were further purified using isocratic elution with 25:75 MeCN–H_2O both containing 0.04% TFA to give **9** (6 mg) and a mixture of **12** + **13** (6 mg).

3.5. Quercetin 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside (*3*)

UV (LC-PDA) λ_{max} nm: 256, 356; ^{1}H and ^{13}C NMR: see Table 2; LC-ESI-MS *m/z*: 903 $[M+H]^+$; ion trap MS/MS of *m/z* 903 $[M+H]^+$, *m/z* (rel. int.): 757 $[(M+H)–Rha]^+$ (30), 611 $[(M+H)–(2\times Rha)]^+$ (30), 595 $[(M+H)–Rha–C_6H_{10}O_5]^+$ (15), 449 $[(M+H)–(2\times Rha)–Glc]^+$ (100), 303 $[(M+H)–(3\times Rha)–Glc]^+$ (50); ion trap MS/MS of *m/z* 925, $[M+Na]^+$, *m/z* (rel. int.): 779 $[(M+Na)–Rha]^+$ (100), 477 $[(M+Na)–Rha–quercetin]^+$ (30); HRESIMS *m/z*: 903.2764 $[M+H]^+$ (calc. for $C_{39}H_{51}O_{24}$, 903.2765).

3.6. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside-7-*O*-α-rhamnopyranoside (*6*)

UV (LC-PDA) λ_{max} nm: 264, 348; ^{1}H and ^{13}C NMR: see Table 2; LC-ESI-MS *m/z*: 887 $[M+H]^+$.

3.7. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside (*7*)

UV (LC-PDA) λ_{max} nm: 264, 348; ^{1}H and ^{13}C NMR: see Table 2; LC-ESI-MS *m/z*: 887 $[M+H]^+$; HRESIMS *m/z*: 887.2816 $[M+H]^+$ (calc. for $C_{39}H_{51}O_{23}$, 887.2816).

3.8. Quercetin 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside (manghaslin) (*9*)

UV (LC-PDA) λ_{max} nm: 252, 300 (sh), 352; LC-ESI-MS *m/z*: 757 $[M+H]^+$; ion trap MS/MS of *m/z* 757, $[M+H]^+$, *m/z* (rel. int.): 611 $[(M+H)–Rha]^+$ (50), 465 $[(M+H)–(2\times Rha)]^+$ (35), 449 $[(M+H)–Rha–C_6H_{10}O_5]^+$ (15), 303 $[(M+H)–(2\times Rha)–Glc]^+$ (100); ion trap MS/MS of *m/z* 779 $[M+Na]^+$, *m/z* (rel. int.): 633 $[(M+Na)–Rha]^+$ (15), 477 $[(M+Na)–quercetin]^+$ (100).

3.9. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-galactopyranoside (*12*)

UV (LC-PDA) λ_{max} nm: 264, 300 (sh), 348; LC-ESI-MS *m/z*: 741 $[M+H]^+$.

3.10. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 2)[α-rhamnopyranosyl(1 → 6)]-β-glucopyranoside (*13*)

UV (LC-PDA) λ_{max} nm: 264, 300 (sh), 348; LC-ESI-MS *m/z*: 741 $[M+H]^+$.

3.11. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-galactopyranoside (kaempferol 3-*O*-robinobioside) (*17*)

UV (LC-PDA) λ_{max} nm: 264, 300 (sh), 348; LC-ESI-MS/MS (ion trap) of *m/z* 593 $[M–H]^-$, *m/z* (rel. int.): 447 $[(M–H)–Rha]^-$ (3), 327 $[(M–H)–Rha–120]^-$ (20), 285 $[(kaempferol–H)]^-$ (100), 284 $[kaempferol–2H]^{\bullet-}$ (80), 255 (30).

3.12. Kaempferol 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranoside (kaempferol 3-*O*-rutinoside) (*19*)

UV (LC-PDA) λ_{max} nm: 264, 300 (sh), 348; LC-ESI-MS/MS (ion trap) of *m/z* 593, $[M–H]^-$, *m/z* (rel. int.): 447 $[(M–H)–Rha]^-$ (3), 327 $[(M–H)–Rha–120]^-$ (6), 285 $[kaempferol–H]^-$ (100), 284 $[kaempferol–2H]^{\bullet-}$ (10), 255 (4).

3.13. Maltol 3-*O*-(4′-*O*-*p*-coumaroyl-6′-*O*-(3-hydroxy-3-methylglutaroyl))-β-glucopyranoside (*23*)

UV (LC-PDA) λ_{max} nm: 263, 314 (sh), 348; ^{1}H NMR (CD_3OD): δ 8.01 (1H, *d*, $J = 5.6$ Hz, H-6), 7.66 (1H, *d*, $J = 15.9$ Hz, H-β), 7.46 (2H, *d*, $J = 8.7$ Hz, H-2″/6″), 6.80 (2H, *d*, $J = 8.7$ Hz, H-3″/5″), 6.46 (1H, *d*, $J = 5.6$ Hz, H-

5), 6.35 (1H, *d*, $J = 15.9$ Hz, H-α), 4.952 (1H, *d*, $J = 7.9$ Hz, Glc H-1′), 4.948 (1H, '*t*', $J = 9.4$ Hz, Glc H-4′), 4.18 (1H, *dd*, $J = 12.2$, 2.4 Hz, Glc CH_2-6′a), 4.11 (1H, *dd*, $J = 12.2$, 5.9 Hz, Glc CH_2-6′b), 3.75 (1H, *m*, Glc H-5′), 3.71 (1H, '*t*', $J = 9.3$ Hz, Glc H-3′), 3.53 (1H, *dd*, $J = 9.2$, 7.9 Hz, Glc H-2′), 2.61 (1H, br *d*, $J = 14.2$ Hz, CH_2-2‴a), 2.54 (1H, br *d*, $J = 14.1$ Hz, CH_2-2‴b), 2.52 (1H, br *m*, CH_2-4‴a), 2.44 (3H, *s*, CH_3-2), 2.39 (1H, br *m*, CH_2-4‴b), 1.28 (3H, *s*, CH_3-3‴); ^{13}C NMR (CD_3OD): δ 177.0 (C-4), 172.2 (C-1‴), 168.4 (coumaroyl CO), 164.6 (C-2), 161.5 (C-4″), 157.3 (C-6), 147.6 (C-β), 143.3 (C-3), 131.4 (C-2″/6″), 127.2 (C-1″), 117.5 (C-5), 116.9 (C-3″/5″), 114.7 (C-α), 104.8 (Glc C-1″), 75.7 (Glc C-3″), 75.5 (Glc C-2″), 73.9 (Glc C-5″), 72.1 (Glc C-4″), 70.9 (C-3‴), 63.8 (Glc C-6″), 47.5 (C-4‴), 47.0 (C-2‴), 27.8 (CH_3-3‴), 15.8 (CH_3-2) (C-5‴ not detected due to broadening); LC-ESI-MS *m/z*: 579 $[M+H]^+$; ion-trap MS/MS of *m/z* 579 $[M+H]^+$*m/z* (rel. int.): 453 $[(M+H)-\text{maltol}]^+$ (100), 309 $[(\text{coumaroyl-Glc})+H]^+$ (15), 291 $[(\text{coumaroyl-Glc})-H_2O+H]^+$ (28), 271 $[(\text{maltol-Glc})+H]^+$ (24); ion trap MS/MS of *m/z* 577 $[M-H]^-$ *m/z* (rel. int.): 515 $[(M-H)-H_2O-CO_2]^-$ (30), 475 $[(M-H)-CH_2{=}CHOHCH_2COOH]^-$ (60), 433 $[(M-H)-(\text{3-hydroxy-3-methylglutaroyl})]^-$ (100); ion trap MS3 (577 → 433) *m/z* (rel. int.): 307 [*m/z* 433 − maltol]$^-$ (100), 163 $[\text{coumaric acid}-H]^-$ (20), 145 $[(\text{coumaric acid}-H)-H_2O]^-$ (30); HRESIMS *m/z*: 601.1531 $[M+Na]^+$ (calc. for $C_{27}H_{30}O_{14}Na$, 601.1528).

Acknowledgements

We thank Dr Renée Grayer (RBG Kew) for help with the interpretation of the UV spectrum of compound **18**, Dr Gwilym Lewis (RBG Kew) for taxonomic advice, sampling of herbarium material and verification of voucher specimens, and the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea, for high resolution mass spectral data.

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