Isoflavone Diglycosides from *Glycosmis pentaphylla*

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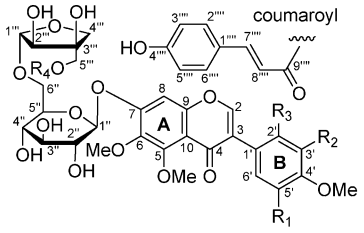
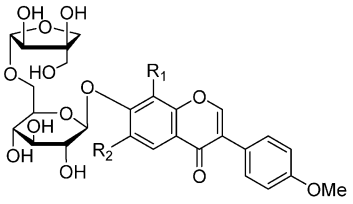
Six new apiosyl-(1→6)-glucosyl isoflavones (**1**−**6**) and four known ones were isolated from the stems of *Glycosmis pentaphylla*. The structures of the new glycosides are 3′,7-dihydroxy-4′,5,6-trimethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**), 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**4**), 7-hydroxy-4′,6-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**5**), and 4′,5-dihydroxy-3′,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**6**). Their structures were established primarily by NMR experiments and chemical methods.

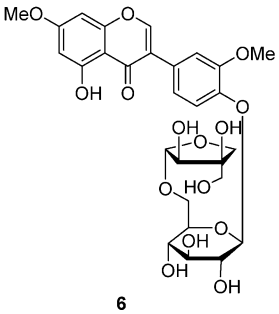
The genus *Glycosmis*, family Rutaceae, is a rich source of alkaloids[1−3] and different types of amides.[4−6] *Glycosmis pentaphylla* Retz. DC. (Rutaceae-Aurantioideae) is a small wild shrub that has been used in India as a folk medicine in the treatment of fever, liver complaints, and certain other diseases.[7] Investigations have been focused mainly on the lipophilic part of leaves, root, and stem bark, and no detailed study on glycosidic constituents of *G. pentaphylla* has been reported so far. The current report describes the isolation and structure elucidation of six new isoflavone glycosides, 3′,7-dihydroxy-4′,5,6-trimethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**), 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**4**), 7-hydroxy-4′,6-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**5**), and 4′,5-dihydroxy-3′,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**6**), from the stems of *G. pentaphylla*, together with four known compounds. The structures of **1**−**6** have been determined on the basis of the spectroscopic data including 2D NMR spectra and chemical evidence. The known compounds were identified as 7-hydroxy-4′-methoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside,[8] coromandelin,[9] 4′,5-dihydroxy-6,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside,[10] and tectorigenin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside[11] by comparison of their spectroscopic data with those reported. Compounds **1** and **2** are the first examples of a naturally occurring isoflavone glycoside with an acylated apiose unit. The aglycones of compounds **1**−**3** have not been reported before.



1 R_1 = H, R_2 = OH, R_3 = H, R_4 = Coumaroyl
2 R_1 = OMe, R_2 = H, R_3 = OH, R_4 = Coumaroyl
3 R_1 = OMe, R_2 = H, R_3 = OH, R_4 = H



4 R_1 = OMe, R_2 = H
5 R_1 = H, R_2 = OMe



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Results and Discussion

A methanolic extract of the stems of *G. pentaphylla* was suspended in H_2O and then partitioned successively with $CHCl_3$ and EtOAc. The EtOAc-soluble materials were subjected to a D101 porous resin column to give several fractions, which were each repeatedly subjected to columns of Sephadex LH-20, as well as RP-18 and preparative reversed-phase HPLC, to yield 10 isoflavone glycosides.

Compound **1** was an amorphous solid and gave a negative FABMS molecular ion at *m*/*z* 783 and an aglycone peak at *m*/*z* 343. The molecular formula, $C_{38}H_{40}O_{18}$, was inferred from negative HRESIMS (found 783.2151, calcd 783.2136), and it was supported by ^{13}C NMR and DEPT spectroscopy. The UV spectrum (λ_{max} 264 nm) of compound **1** was typical of compounds having an isoflavone skeleton.[12] A characteristic resonance for H-2 of an isoflavone was observed at δ_H 8.11 (1H, s, δ_C 155.7) in the ^1H NMR spectrum.[13,14] This assignment was confirmed by long-range connectivities to δ_C 182.5 (C-4), 154.6 (C-9), and 124.1 (C-1′) in the corresponding HMBC spectrum (Figure 1). A second singlet (1H) corresponding to H-8 of the ring A (δ_H 6.85, δ_C 95.8) and three coupled ring B protons at δ_H 7.14 (1H, br s, H-2′), 6.90 (1H, d, J = 8.0 Hz, H-5′),

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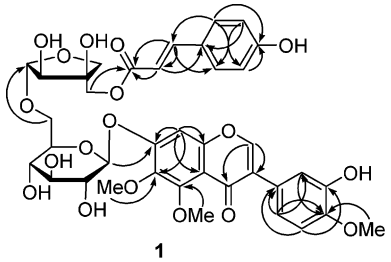

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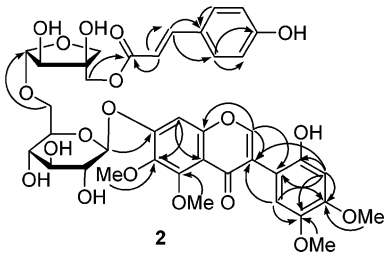

2

Figure 1. Selected HMBC correlations for compounds **1** and **2**.

and 7.00 (1H, br d, $J = 8.0$ Hz, H-6′) comprised the remaining aromatic resonances of the aglycone. The assignment of H-8 was supported by long-range HMBC connectivities to δ_C 154.6 (C-9), 157.9 (C-7), 134.1 (C-6), and 108.5 (C-10). Additionally, the ^{1}H NMR spectrum showed the resonances of three *O*-methyl singlets at δ 3.86, 3.84, and 3.83. HMBC correlations between OMe at δ 3.86 and C-5, between OMe at δ 3.84 and C-6, and between OMe at δ 3.83 and C-4′, allowed assignment to OCH_3-5, OCH_3-6, and OCH_3-4′, respectively. As expected, the C-6 methoxy resonates in the range 59−63 ppm, which is diagnostic of sterically hindered methoxy groups with substituents in both *ortho* positions; this contrasts with the range 55−58 ppm found for *O*-methyls with one unsubstituted *ortho* position or more, like that of the C-5 and C-4′ methoxy groups.[15,16] The aglycone of compound **1** was therefore confirmed to be 3′,7-dihydroxy-4′,5,6-trimethoxyisoflavone, an isoflavone that has not been reported previously in the literature.

The ^{1}H NMR signals due to aromatic and olefinic protons at δ 7.51 (1H, d, $J = 15.9$ Hz), 7.26 (2H, d, $J = 8.5$ Hz), 6.72 (2H, d, $J = 8.5$ Hz), and 6.24 (1H, d, $J = 15.9$ Hz), as well as one ester carbonyl carbon at δ 168.8, suggested the presence of a *trans-p*-coumaroyl moiety. These findings were confirmed by the negative FABMS, which gave fragment ions at *m*/*z* 621 $[M - 163]^-$, formed by elimination of one coumaric acid moiety. The ^{1}H and ^{13}C NMR spectra (Table 1) showed signals of a glucopyranosyl and an apiofuranosyl moiety, indicating that **1** is an isoflavone-*O*-diglycoside acylated with *p*-coumaric acid. Acid hydrolysis of **1** yielded glucose and apiose detected by direct co-TLC comparison with authentic samples. A large coupling constant ($J = 7.7$ Hz) for the anomeric proton (δ 4.98) of the glucose in the ^{1}H NMR spectrum suggested a β-configuration, and the β-configuration for the apiose was confirmed by the shift of its anomeric carbon in the ^{13}C NMR spectrum (δ 111.2, Table 1).[10,17] The glucose C-2″ signal appeared at δ_C 74.7, while that of C-6″ appeared at δ_C 69.5, suggesting that the interglycosidic linkage is apiosyl-(1→6)-glucose.[9,18] The location of the acyl group in **1** was determined at the C-5‴-OH of the apiosyl residue by the HMBC cross-peak from the acyl carbonyl resonance at δ 168.8 to the apiosyl C-5‴ protons at δ 4.30. The glycosidation position was unambiguously determined by a three-bond correlation between the glucosyl anomeric proton H-1 (δ 4.98) and ring A C-7 (δ 157.9) using HMBC. Therefore, compound **1** was identified as 3′,7-dihydroxy-4′,5,6-trimethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

The molecular formula of **2**, determined to be $C_{39}H_{42}O_{19}$ by HRESIMS, suggested the presence of an additional methoxy substituent compared to **1**. This was confirmed by the ^{1}H NMR spectrum, which comprised resonances for four methoxy groups at δ 3.73 (3H, s, δ_C 57.0), 3.85 (3H, s, δ_C 61.5), 3.87 (3H, s, δ_C 56.6), and 3.75 (3H, s, δ_C 57.4). Again, the UV (λ_{max} 267 nm), the ^{1}H NMR (δ 8.02 for H-2), and the ^{13}C NMR (δ 157.4 for C-2) spectra (Table 1) showed **2** to be an isoflavone derivative. The assignments of a 1H singlet at δ_H 6.87 (δ_C 95.8) to H-8 and two *O*-methyls at C-5 and C-6 were confirmed from a pattern of long-range HMBC connectivities (Figure 1) identical to those observed in **1**. The ring A of **2** was therefore characterized by 7-hydroxy-5,6-dimethoxy substitution. Of the remaining aromatic proton resonances, the appearance of two singlets at δ 6.68 and 6.87

Table 1. ^{1}H [δ_H (*J*, Hz)] and ^{13}C NMR (δ_C) Data[a] of **1** and **2**

	1		**2**	
no.	^{13}C	^{1}H	^{13}C	^{1}H
2	155.7 d	8.11 (s)	157.4 d	8.02 (s)
3	124.8 s		121.7 s	
4	182.5 s		182.5 s	
5	154.4 s		154.4 s	
6	134.1 s		134.1 s	
7	157.9 s		157.9 s	
8	95.8 d	6.85 (s)	95.8 d	6.87 (s)
9	154.6 s		154.6 s	
10	108.5 s		108.5 s	
1′	124.1 s		112.0 s	
2′	114.2 d	7.14 (br s)	153.8 s	
3′	150.2 s		99.3 d	6.68 (s)
4′	150.7 s		151.8 s	
5′	112.8 d	6.90 (d, 8.0)	144.2 s	
6′	122.7 d	7.00 (br d, 8.0)	117.3 d	6.87 (s)
Glc-1″	102.2 d	4.98 (d, 7.7)	102.1 d	5.00 (d, 7.5)
2″	74.7 d	3.56 (m)	74.7 d	3.56 (m)
3″	78.1 d	3.51 (m)	78.0 d	3.50 (m)
4″	71.9 d	3.32 (m)	71.9 d	3.31 (m)
5″	77.3 d	3.75 (t, 11.2)	77.3 d	3.74 (t, 11.2)
6″	69.5 t	3.61 (dd, 12.0, 6.0)	69.4 t	3.60 (dd, 12.0, 6.0)
		4.09 (dd, 12.0, 1.6)		4.08 (dd, 12.0, 1.6)
Api-1‴	111.2 d	5.01 (d, 2.1)	111.1 d	5.00 (d, 2.2)
2‴	78.7 d	4.03 (d, 2.1)	78.7 d	4.02 (d, 2.2)
3‴	78.8 s		78.8 s	
4‴	75.1 t	4.11 (d, 12.0),	75.1 t	4.10 (d, 12.0),
		3.85 (d, 12.0)		3.86 (d, 12.0)
5‴	67.2 t	4.30 (s)	67.2 t	4.28 (s)
coumaroyl-1⁗	126.9 s		126.9 s	
2⁗, 6⁗	131.2 d	7.26 (d, 8.5)	131.2 d	7.27 (d, 8.6)
3⁗, 5⁗	116.8 d	6.72 (d, 8.5)	116.8 d	6.71 (d, 8.6)
4⁗	161.3 s		161.3 s	
7⁗	147.1 d	7.51 (d, 15.9)	147.0 d	7.52 (d, 15.9)
8⁗	114.6 d	6.24 (d, 15.9)	114.6 d	6.24 (d, 15.9)
9⁗	168.8 s		168.8 s	
MeO-5	56.6 q	3.86 (s)	57.0 q	3.73 (s)
MeO-6	61.5 q	3.84 (s)	61.5 q	3.85 (s)
MeO-4′	56.5 q	3.83 (s)	56.6 q	3.87 (s)
MeO-5′			57.4 q	3.75 (s)

[a] Measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C).

established the presence of two *p*-coupled aromatic protons on ring B. The relative positions of groups on ring B were determined on the basis of the HMBC spectrum, which showed that the *p*-coupled aromatic protons at δ 6.68 and 6.87 were correlated to C-2′ (δ 153.8), C-4′ (δ 151.8), and C-5′ (δ 144.2); thus, these protons were assigned to H-3′ and H-6′, respectively. The remaining two *O*-methyl groups at δ 3.87 and 3.75 were assigned to C-4′ and C-5′, as they correlated to C-4′ and C-5′, respectively. This was further supported by the NOE cross-peaks observed from the *O*-methyl on C-4′ to H-3′ and from H-6′ to the C-5′ *O*-methyl group. Interestingly, the C-2 carbon resonance gave an atypical chemical shift of 157.4 ppm as compared to reported shifts less than 156.0 ppm for C-2 in other isoflavones, which may be due to the oxysubstituted position at C-2′. The aglycone of **2** was therefore confirmed to be 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone, also not reported before.

The ^{13}C NMR chemical shifts of the carbohydrate moiety of **2** were almost the same as those of compound **1**. The C-7 site of glycosidation was confirmed by an HMBC experiment, which

showed a long-range correlation between C-7 (δ 157.9) and the anomeric proton (δ 5.00) of the glucose. The observation of an NOE between H-1″ (δ 5.00) and H-8 (δ 6.87) in the NOESY spectrum supported this result. The structure of **2** is therefore 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Inspection of the NMR data (Tables 2 and 3) for compound **3** indicated that the aglycone unit was the same as that of **2**. Compound **3** was different from **2** as far as the absence of the signals of the *p*-cinnamoyl moiety and the chemical shifts of C-3‴ and C-5‴ of the apiosyl residue [C-3‴, δ 80.4 (+ 1.6); C-5‴, δ 65.6 (− 1.6)] are concerned. The ^{13}C NMR chemical shifts of the carbohydrate moiety of **3** were in accord with those reported for the isoflavone β-D-apiofuranosyl-(1→6)-β-D-glucopyranose.[19] HRESIMS of **3** displayed an ion at *m*/*z* 667.1866 [M − H]$^-$, corresponding to a molecular formula of $C_{30}H_{36}O_{17}$, 162 mass units less than **2**. Therefore, the structure of **3** was elucidated as 2′,7-dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

The HRESIMS (negative-ion mode) of **4** exhibited a pseudomolecular ion at *m*/*z* 591.1722 [M − H]$^-$ (calcd 591.1714), consistent with a molecular formula of $C_{28}H_{32}O_{14}$. Negative FABMS of **4** showed a quasi-molecular ion [M − H]$^-$ at *m*/*z* 591, and two fragment peaks at *m*/*z* 459 and 297 indicated losses of pentosyl and hexosyl moieties from the quasi-molecular ion. The ^{1}H NMR spectrum of **4** was almost identical with that of **3** in the δ_H 3.30 to 4.10 region, indicating the same identity and pattern of sugar substitution. In the aromatic region of the ^{1}H NMR spectrum, two doublets corresponding to an AA′BB′ system, δ_H 7.48 (d, J = 8.0 Hz, 2H-2′, 6′) and 6.99 (d, J = 8.0 Hz, 2H-3′, 5′), were observed, suggesting a *p*-substituted ring B. In addition, a 7,8-disubstituted ring A was evident from two *o*-coupled protons at δ 7.94 (1H, d, J = 9.0 Hz) and 7.39 (1H, d, J = 9.0 Hz) and the HMBC correlations between the proton at δ 7.94 and the carbonyl carbon at C-4. The glycosidation position was unambiguously determined by three-bond HMBC correlation between the glucosyl anomeric proton H-1 (δ 5.09) and ring A C-7 (δ 155.8). One of the *O*-methyl groups (δ 4.02) was assigned to C-8, as evidenced by the appearance of a ^{13}C NMR signal at δ 62.4, characteristic of an *O*-methyl group in an *o*-disubstituted environment.[15,16] The second *O*-methyl group at δ 3.83 was then assigned to C-4′, and **4** was determined to be 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

The molecular formula of **5**, $C_{28}H_{32}O_{14}$, determined by HRESIMS *m*/*z* 591.1706 [M − H]$^-$, was the same as that of **4**. Its FABMS (negative-ion mode) displayed a pseudomolecular ion peak [M − H]$^-$ at *m*/*z* 591 and fragment ion peaks at *m*/*z* 459 [M − H − 132]$^-$, indicating the loss of apiose, and at *m*/*z* 297 [M − H − 132−162]$^-$, which was assigned to the additional loss of the glucose (*m*/*z* 162) moiety. Its ^{1}H and ^{13}C NMR data (Tables 2 and 3) were similar to those of **4** except for the presence of the ring A signals. The ^{1}H NMR spectrum of **5** displayed two sharp 1H singlets at δ 7.50 and 7.28 assignable to two *p*-coupled aromatic protons H-5 and H-8 of ring A. The carbon at δ 177.6 (C-4) showed HMBC correlation with the proton at δ 7.50, which can only be assigned to H-5. This proton showed correlation with the OMe at δ 3.89 in the NOESY experiment, indicating the C-6 location of the second *O*-methyl group. That the disaccharide was attached to the 7-OH of the aglycone was directly deduced from the correlation between the anomeric proton of the glucose moiety and C-7 of the aglycone in the HMBC experiment. Therefore, **5** was identified as 7-hydroxy-4′,6-dimethoxyisoflavone 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

The molecular formula of **6**, determined to be $C_{28}H_{32}O_{15}$ by HRESIMS, suggested the presence of an additional hydroxy substituent at δ 13.4 (characteristic resonance for C-5-OH) compared to **5**. The aromatic region of the ^{1}H NMR spectrum showed two broad singlets at δ 6.60 and 6.53, which correlated to carbons at δ 98.8 and 92.8 in the HSQC spectrum. This is characteristic of two *m*-related H-6 and H-8 protons of ring A. The ^{1}H NMR signals at δ 7.39 (d, J = 8.4 Hz), 7.42 (br s), and 7.77 (br d, J = 8.4 Hz) suggested a 1,2,4-trisubstituted ring B. The two *O*-methyl groups at δ 3.78 and 3.76 were assigned to the 3′ and 7 position, as they correlated to C-3′ and C-7, respectively, in the HMBC experiment. This was confirmed by the observation of the NOE correlations between OMe/H-8 and OMe/H-2′. The HMBC cross-peak between C-4′ (δ 148.4) and the anomeric proton (δ 5.69) revealed the site

Table 2. ^{1}H NMR (500 MHz) Data [δ_H (J, Hz)] of **3**−**5** in CD_3OD and **6** in C_5D_5N

no.	3	4	5	6
2	8.10 (s)	8.29 (s)	8.18 (s)	8.18 (s)
5		7.94 (d, 9.0)	7.50 (s)	
6		7.39 (d, 9.0)		6.60 (br s)
8	6.88 (s)		7.28 (s)	6.53 (br s)
2′		7.48 (d, 8.0)	7.46 (d, 8.3)	7.42 (br s)
3′	6.69 (s)	6.99 (d, 8.0)	6.96 (d, 8.3)	
5′		6.99 (d, 8.0)	6.96 (d, 8.3)	7.77 (d, 8.4)
6′	6.88 (s)	7.48 (d, 8.0)	7.46 (d, 8.3)	7.39 (d, 8.4)
Glc-1″	5.04 (d, 7.5)	5.09 (d, 7.5)	5.03 d (7.5)	5.69 (d, 7.2)
2″	3.56[a]	3.55[a]	3.55[a]	4.30−4.35
3″	3.50[a]	3.50[a]	3.51[a]	4.79*
4″	3.34[a]	3.35[a]	3.36[a]	4.10 (d, 5.0)
5″	3.69 (t, 11.2)	3.63 (t, 11.1)	3.67 (t, 11.5)	4.30−4.35
6″	3.58 (dd, 12.0, 6.0)	3.58 (dd, 12.0, 6.2)	3.60 (dd, 11.6, 6.6)	4.76[a]
	4.05 (dd, 12.0, 1.6)	4.04 (dd, 12.0, 1.5)	4.03 (dd, 11.6, 1.5)	4.18 (d, 10.2, 5.6)
Api-1‴	4.96 (d, 2.1)	4.95 (d, 2.0)	4.97 (d, 2.0)	5.74 (d, 1.8)
2‴	3.93 (d, 2.1)	3.90 (d, 2.0)	3.93 (d, 2.0)	4.30−4.35
4‴	3.78 (d, 12.4)	3.74 (d, 12.4)	3.77 (d, 12.8)	4.63 (d, 9.0)
	4.00 (d, 12.4)	3.96 (d, 12.4)	4.00 (d, 12.8)	4.30−4.35
5‴	3.56 (s)	3.55 (s)	3.55 (s)	4.18 (s)
−OMe	3.75 (s) (MeO-5)	4.02 (s) (MeO-8)	3.89 (s) (MeO-6)	3.76 (s) (OMe-7)
−OMe	3.88 (s) (MeO-6)	3.83 (s) (MeO-4′)	3.81 (s) (MeO-4′)	3.78 (s) (OMe-3′)
−OMe	3.88 (s) (MeO-4′)			
−OMe	3.77 (s) (MeO-5′)			

[a] Signal pattern unclear due to overlapping.

Table 3. ^{13}C NMR (125 MHz) Data (δ_C) of **3**−**5** in CD_3OD and **6** in C_5D_5N

no.	3	4	5	6
2	157.6 d	155.1 d	155.1 d	154.3 d
3	121.7 s	125.3 s	125.1 s	123.6 s
4	182.6 s	178.1 s	177.6 s	181.2 s
5	154.4 s	122.1 d	106.2 d	163.3 s
6	134.1 s	115.9 d	149.4 s	98.8 d
7	157.9 s	155.8 s	153.4 s	166.1 s
8	95.8 d	138.0 s	105.5 d	92.8 d
9	154.7 s	152.3 s	153.4 s	158.3 s
10	108.4 s	121.3 s	119.8 s	106.8 s
1′	112.2 s	125.8 s	125.6 s	125.6 s
2′	153.9 s	131.4 d	131.4 d	114.4 d
3′	99.4 d	114.9 d	114.9 d	149.9 s
4′	151.9 s	161.3 s	161.1 s	148.4 s
5′	144.2 s	114.9 d	114.9 d	117.2 d
6′	117.5 d	131.4 d	131.4 d	122.2 d
Glc-1″	102.0 d	102.3 d	102.1 d	102.6d
2″	75.0 d	75.0 d	75.0 d	74.9 d
3″	78.0 d	78.1 d	77.9 d	77.9 d
4″	71.6 d	71.6 d	71.6 d	71.8 d
5″	77.2 d	77.4 d	77.2 d	77.5 d
6″	69.0 t	68.8 t	69.1 t	69.1 t
Api-1‴	111.2 d	111.0 d	111.2 d	111.3 d
2‴	78.1 d	78.1 d	78.2 d	78.6 d
3‴	80.4 s	80.5 s	80.4 s	80.4 s
4‴	75.0 t	75.0 t	75.0 t	75.1 t
5‴	65.6 t	65.6 t	65.7 t	65.6 t
−OMe	57.0 q (OMe-5)	62.4 q (OMe-8)	56.8 q (OMe-6)	56.1 q (OMe-7)
−OMe	61.5 q (OMe-6)	55.8 q (OMe-4′)	55.8 q (OMe-4′)	56.1 q (OMe-3′)
	56.7 q (OMe-4′)			
	57.5 q (OMe-5′)			

of glycosidation at 4′-OH. Thus, **6** was confirmed to be 4′,5-dihydroxy-3′,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

The subfamily Aurantioideae is divided into two tribes on the basis of morphological characters, Clauseneae and Citreae.[20] The tribe Clauseneae was subdivided into Micromelinae (with only *Micromelum*), Clauseninae (with *Glycosmis*, *Clausena*, and *Murraya*), and Merrilliinae (with only *Merrillia*).[20] Polyoxygenated flavonoids are characteristic of *Citrus* and are also known from *Micromelum*, *Murraya*, and *Merrillia*.[21,22] Compared with other genera of the Clauseneae, little is known about the distribution of flavonoids in *Glycosmis*.[5,23] This is the first report of polyoxygenated isoflavones from *Glycosmis*, which is not surprising in view of the disparity between the plant parts examined since previous studies on *Glycosmis trichanthera* demonstrated distinct organ-specific chemical differences.[24] Certainly additional chemical work on different plant parts of this genus would be valuable.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH solution. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. The IR (KBr) spectra were obtained on a Bio-Rad FTS-135 spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a DRX-500 spectrometer with TMS as internal standard. MS data were obtained on a VG AutoSpec 3000 spectrometer. HPLC separations were performed on a HP 1100 apparatus equipped with a diode array UV detector and XTERRA C18 (Waters, 10 μm, 15 × 200 mm, flow rate: 15 mL/min) column. GC-MS was run on a FISONS MD-800 instrument.

Plant Material. The stems of *G. pentaphylla* were collected in Xishuangbanna, Yunnan, China, in March 2003. The plant material was identified by Prof. De-Ding Tao, and a voucher specimen (BN20030412) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried material (10 kg) was finely pulverized and extracted by percolation with MeOH for one month at room temperature. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (350 g). The extract was partitioned between H_2O and $CHCl_3$, and then the H_2O layer was further extracted with EtOAc. The EtOAc-soluble fraction (25 g) was fractionated by column chromatography over D101 porous resin with a gradient from 20% to 95% EtOH to give six fractions (fractions I−VI).

Fraction I (7.2 g) was applied to a D101 porous resin column and eluted with 2−25% Me_2CO in H_2O under gradient conditions, yielding fractions I-1−I-5. Fraction I-2 (2 g) was subjected to medium-pressure chromatography (MPLC) over C18 Si gel and eluted with MeOH−H_2O (1:9−4:6) under gradient conditions, yielding fractions I-2-1−I-2-6. Fraction I-2-2 (900 mg) was applied to Sephadex LH-20 chromatography with MeOH. The major component was further purified by RP-18 preparative HPLC with MeOH−H_2O (3:7), yielding tectorigenin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (560 mg) at 20 min. Fraction I-2-4 (383 mg) was chromatographed on Sephadex LH-20 with MeOH, yielding 62 mg of yellow amorphous solid. This material was further purified by successive RP-18 preparative HPLC with 30% MeOH to give 32 mg of 4′,5-dihydroxy-6,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside at 26.0 min.

Fraction II (5 g) was subjected to column chromatography over D101 porous resin and eluted with 5−30% Me_2CO in H_2O, yielding fractions II-1−II-5. Fraction II-4 (550 mg) was chromatographed on Sephadex LH-20 with MeOH, followed by reversed-phase HPLC (RP-18, 35% MeOH) to yield 12 mg of compound **6** at 21.0 min. Fraction II-3 (3 g) was applied to RP-18 MPLC and eluted with Me_2CO−H_2O (1:9−3:7) under gradient conditions, yielding fractions II-3-1−II-3-6. Fraction II-3-1 (1.3 g) was again applied to RP-18 MPLC and eluted with MeOH−H_2O (1:9−5:5), yielding fractions II-3-1-1−II-3-1-6. Fraction II-3-1-1 (600 mg) was chromatographed on Sephadex LH-20 with MeOH and further purified by successive RP-18 preparative HPLC with 30% MeOH to obtain compound **5** (300 mg, t_R 25.0 min). Sephadex LH-20 chromatography (MeOH) of fraction II-3-1-2 (90 mg) followed by RP-18 preparative HPLC (30% MeOH) yielded **3** (14 mg, t_R 20.2 min).

Fraction IV (2.7 g) was applied to RP-18 MPLC and eluted with Me_2CO−H_2O (1: 9−3:7) under gradient conditions to give five fractions (fractions IV-1−IV-5). Fraction IV-2 (1.4 g) was applied to RP-18 MPLC and eluted with MeOH−H_2O (2:8−5:5) to yield fractions IV-2-1−IV-2−6. Fraction IV-2-2 (150 mg) was separated by RP-18 preparative HPLC in 45% MeOH to give 80 mg of compound **2** at 16 min. Fraction IV-2-3 (500 mg) was chromatographed on Sephadex LH-20 with MeOH and then subjected to HPLC on RP-18 with 43% MeOH to give 7-hydroxy-4′-methoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (200 mg, t_R 15.8 min). Sephadex LH-20 chromatography (MeOH) of fraction IV-1 (250 mg) followed by RP-18 preparative HPLC (30% MeOH) gave **4** (6 mg, t_R 34.0 min).

Fraction V (1.7 g) was applied to RP-18 MPLC and eluted with Me_2CO−H_2O (1:9−4:6) under gradient conditions, providing fractions V-1−V-6. Fraction V-3 (250 mg) was chromatographed on Sephadex LH-20 with MeOH, yielding fractions V-3-1−V-3-6. Fraction V-3-1 (50 mg) was chromatographed on Sephadex LH-20 with MeOH−$CHCl_3$ (1:1) and then purified by RP-18 preparative HPLC with 40% MeOH, yielding coromandelin (4 mg, t_R 24.4 min). Fraction V-3-2 (40 mg) was subjected to Sephadex LH-20 with MeOH−$CHCl_3$ (1:1) and then HPLC on RP-18 with 48% MeOH to afford **1** (12 mg, t_R 20.0 min).

3′,7-Dihydroxy-4′,5,6-trimethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (1): yellow amorphous powder; $[\alpha]_D^{25}$ −78.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm 209, 264, 298, 325 (sh); IR ν_{max} (KBr) 3424, 2936, 1701, 1651, 1605, 1515, 1460, 1367, 1271, 1212, 1170, 1073, 1027, 831 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; negative FABMS (glycerol matrix) *m*/*z* 783 [M − H]$^-$, 621 [M − $C_9H_7O_3$]$^-$, 343 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 783.2151 [M − H]$^-$ (calcd for $C_{38}H_{39}O_{18}$, 783.2136).

2′,7-Dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (2): yellow amorphous powder; $[\alpha]_D^{25}$ −70.4 (*c* 0.26, MeOH); UV (MeOH) λ_{max} nm: 206, 267, 298; IR ν_{max} (KBr) 3426, 2937, 1704, 1655, 1602, 1515, 1460, 1364, 1306, 1279, 1208, 1168, 1150, 1071,829 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; negative FABMS (glycerol matrix) *m*/*z* 813 [M − H]$^-$, 373 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 813.2237 [M − H]$^-$ (calcd for $C_{39}H_{41}O_{19}$, 813.2242).

2′,7-Dihydroxy-4′,5′,5,6-tetramethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (3): yellow amorphous powder; $[\alpha]_D^{25}$ −71.2 (*c* 0.20, MeOH); UV (MeOH) λ_{max} nm 209, 266, 320 (sh); IR ν_{max} (KBr) 3424, 2936, 1656, 1617, 1514, 1461, 1306, 1280, 1209, 1148, 1073, 1027, 817 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3; negative FABMS (glycerol matrix) *m*/*z* 667 [M − H]$^-$, 535 [M − H − Api]$^-$, 373 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 667.1866 [M − H]$^-$ (calcd for $C_{30}H_{35}O_{17}$, 667.1874).

7-Hydroxy-4′,8-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (4): yellow amorphous powder; $[\alpha]_D^{25}$ −74.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} nm: 210, 260, 321 (sh); IR ν_{max} (KBr) 3414, 2972, 1654, 1608, 1584, 1512, 1490, 1380, 1305, 1240, 1180, 1020 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3; negative FABMS (glycerol matrix) *m*/*z* 591 [M − H]$^-$, 459 [M − H − Api]$^-$, 297 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 591.1722 [M − H]$^-$ (calcd for $C_{28}H_{31}O_{14}$, 591.1714).

7-Hydroxy-4′,6-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (5): colorless amorphous powder; $[\alpha]_D^{25}$ −76.8 (*c* 0.88, MeOH); UV (MeOH) λ_{max} nm 208, 260, 322 (sh); IR ν_{max} (KBr) 3423, 1654, 1615, 1516, 1460, 1318, 1271, 1228, 1177, 1073, 824 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3; negative FABMS (glycerol matrix) *m*/*z* 591 [M − H]$^-$, 459 [M − H − Api]$^-$, 297 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 591.1706 [M − H]$^-$ (calcd for $C_{28}H_{31}O_{14}$, 591.1714).

4′,5-Dihydroxy-3′,7-dimethoxyisoflavone 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (6): yellow amorphous powder; $[\alpha]_D^{25}$ −68.2 (*c* 0.35, MeOH); UV (MeOH) λ_{max} nm 210, 262; IR ν_{max} (KBr) 3453, 2925, 1663, 1613, 1568, 1514, 1443, 1356, 1265, 1157, 1048, 911, 827 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3; negative FABMS (glycerol matrix) *m*/*z* 1215 [2M − H]$^-$, 607 [M − H]$^-$, 313 [Aglycone − H]$^-$; negative HRESIMS *m*/*z* found 607.1680 [M − H]$^-$ (calcd for $C_{28}H_{31}O_{15}$, 607.1663).

Sugar Composition Analysis of 1−6. Each compound (2 mg) was refluxed with 1 M HCl (dioxane−H_2O, 1:1, 2 mL) at 95 °C for 2 h. After drying under a stream of nitrogen, the residue was suspended in H_2O and extracted with EtOAc (×3). The aqueous layer was neutralized

with $NaHCO_3$ and concentrated under reduced pressure to dryness to give a residue of the sugar fraction.

The residue was compared with standard sugars by co-thin-layer chromatography ($CHCl_3$−MeOH−H_2O−HOAc, 16:9:2:2; detection with spray agent: 4% α-naphthol−EtOH−5% H_2SO_4). Hexoses gave purple spots and pentoses blue spots. The R_f values of each sugar are as follows: glucose, 0.42; apiose, 0.52.

An aliquot of the hydrolysis mixture was dissolved in 0.5 mL of dry pyridine and treated with 0.5 mL of trimethylchlorosilane, and the reaction mixture was kept at ambient temperature for 20 min. After the reaction mixture was dried in vacuo, the residue was dissolved in Et_2O and subjected to GC-MS analysis (30 m × 0.32 mm 30QC2/AC5 column; carrier N_2 gas; 180 to 240 °C, Δ 5 °C/min). In the acid hydrolysate of **1**−**6**, D-glucose and D-apiose were confirmed by comparison of the retention times of their TMS derivatives with those of D-glucose and D-apiose derivatives prepared in a similar way, which showed retention times of 6.86 and 3.08 min, respectively.

Acid Hydrolysis of 5. Compound **5** (20 mg) was hydrolyzed by 0.5 M H_2SO_4 (2 mL) at 70 °C for 1 h. The reaction mixture was then partitioned with EtOAc. The lower layer was neutralized using 0.5 M $Ba(OH)_2$ (2 mL) and filtered through glass wool. The filtrate was evaporated to dryness and subjected to silica gel chromatography [$CHCl_3$−MeOH−H_2O (6:4:1)] to give D-glucose: $[\alpha]_D^{25}$ +21.5 (*c* 0.12, H_2O) and D-apiose: $[\alpha]_D^{25}$ +7.8 (*c* 0.10, H_2O).

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Supporting Information Available: ^1H, ^{13}C NMR and DEPT spectra of **1**−**6**, HSQC and HMBC spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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