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Isoflavonoid glycosides from *Dalbergia sissoo*

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Abstract

Two isoflavone glycosides, biochanin A 7-*O*-[β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] and tectorigenin 7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside], were isolated from *Dalbergia sissoo*. Their structures were elucidated on the basis of spectral and chemical evidence.

Keywords: Leguminosae; *Dalbergia sissoo*; Isoflavone glycosides; Isoflavone apioglucosides

1. Introduction

An aqueous extract of the leaves of *Dalbergia sissoo* Roxbg. (Leguminosae) has been used for the treatment of gonorrhea in Arabic countries (El-Dagwy, 1996). Many isoflavonoids and neoflavonoids have been reported from *D. sissoo* (Seshadri, 1972; Sharma et al., 1979, 1980; Ingham, 1983). Our detailed study on the constituents of the above plant led to the isolation of two new isoflavone glycosides (**1**, **3**) together with five known isoflavone glycosides (**2**, **4**–**7**) from the leaves and stem-bark of *D. sissoo* (Kinjo et al., 1987; Ishikura et al., 1989; Rao et al., 1989; Ramesh and Yuvarajan, 1995; Mathias et al., 1998).

2. Results and discussion

The methanol extract of the leaves of *D. sissoo* collected in Egypt was successively partitioned between water and hexane, chloroform, ethyl acetate and *n*-butanol to give the corresponding solubles. The butanol and water soluble fractions were respectively separated to give a new compound **1** and a known compound **2**. The methanol extract of the stem-bark was partitioned between water and chloroform, and between water and *n*-butanol. The *n*-butanol soluble fraction was separated to give a new compound **3** and five known compounds **4**–**7**. These compounds were identified as biochanin A 7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] (**2**) (Rao et al., 1989), prunetin 4′-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] (**4**) (Ramesh and Yuvarajam, 1995), 7-methyltectorigenin 4′-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] (**5**) (Mathias et al., 1998), genistein 8-*C*-[β-D-glucopyranoside] (**6**) (Kinjo et al., 1987) and prunetin 4′-*O*-[β-D-glucopyranoside] (**7**) (Ishikura et al., 1989) by comparison of the spectral data with the reported data.

Compound **1**, $[\alpha]_D^{25}$ −106.1° (*c* 0.26, MeOH), was found by high-resolution FABMS to have the molecular formula $C_{32}H_{38}O_{18}$. The UV spectrum (λ_{max}^{MeOH} 261 and 327 nm) showed the characteristic absorption of an isoflavone, and a bathochromic shift (271 and 322 nm) on addition of $AlCl_3$ suggested the presence of free 5-OH and substituted 7-OH. The ^{1}H NMR spectrum exhibited one methoxy group [δ 3.8 (3H, *s*)], one set of *meta*-coupled aromatic protons [δ 6.47 (*d*, *J* = 1.8 Hz) and δ 6.63 (*d*, *J* = 1.8 Hz)], two sets of *ortho*-coupled aromatic hydrogens [δ 6.95 (2H, *d*, *J* = 8.4 Hz) and δ 7.46 (2H, *d*, *J* = 8.4 Hz)] and a non-coupled aromatic hydrogen [δ 8.12 (1H, s)] (Table 1). These features are characteristic of a 5,7,4′-trihydroxyisoflavone derivative (Markham and Geiger, 1994). The ^{1}H and ^{13}C NMR spectra (Table 1) revealed three anomeric signals ($\delta_{H\text{-}1''}$ 4.92 (*d*, *J* = 7.3 Hz) and $\delta_{C\text{-}1''}$ 101.5 (*d*), $\delta_{H\text{-}1'''}$ 4.97 (*d*, *J* = 2.6 Hz) and $\delta_{C\text{-}1'''}$ 110.8 (*d*) and $\delta_{H\text{-}1''''}$ 4.96 (*d*, *J* = 2.6 Hz) and $\delta_{C\text{-}1''''}$ 111.9 (*d*)) together with 13 carbon signals in the

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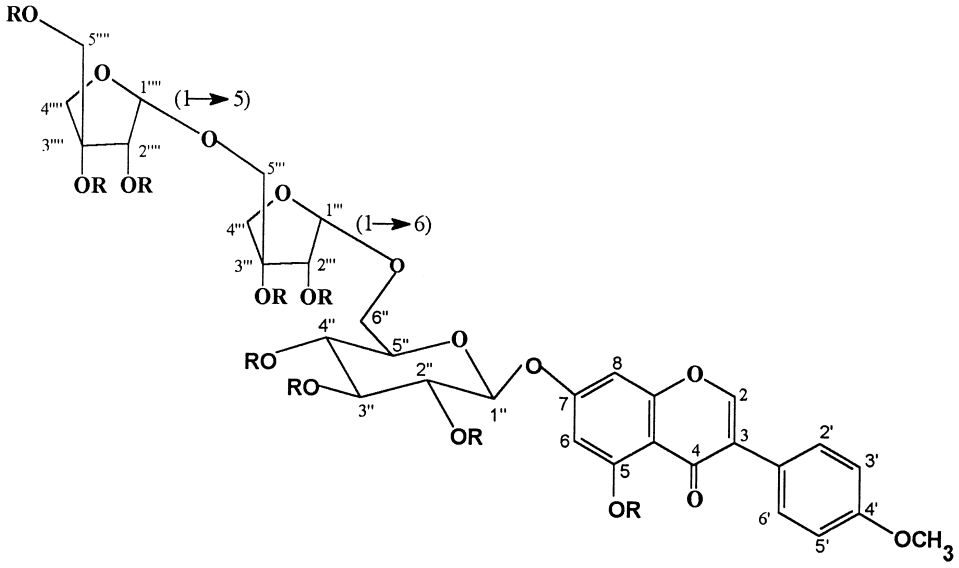
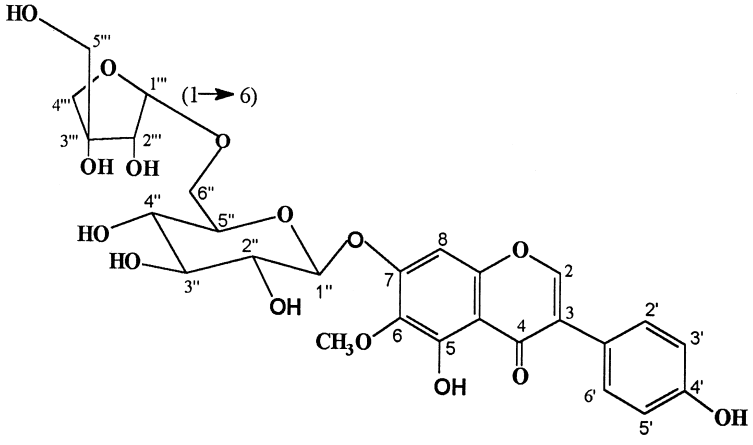


Compound 1 R= H
Compound 8 R= Ac



Compound 3

region of sugars indicated **1** to be an isoflavone-*O*-triglycoside (Agrawal, 1992). The ^{13}C NMR shifts of the three sugars are consistent with those corresponding to one glucopyranosyl and one apiofuranosyl moiety (Rao et al.,1989) with an additional apiofuranosyl moiety. The β-configuration of the glycosidic linkages was evident from the observed ^{13}C NMR chemical shifts for the anomeric carbons of glucose ($\delta_{C\text{-}1''}$ 101.5) and apiose ($\delta_{C\text{-}1'''}$ 110.8 and $\delta_{C\text{-}1''''}$ 111.9) (Ramesh and Yuvarajan, 1995; Mathias et al., 1998). The downfield shifts of C-6″ (δ_C 67.6) and C-5‴ (δ_C 69.3) suggesting that the interglycosidic linkages is apiofuranosyl (1⁗→5‴) apiofuranosyl (1‴→6″) glucopyranoside (Ramesh and Yuvarajan, 1995). The heteronuclear multiple bond correlation spectroscopy (HMBC) spectrum (Table 1) showed cross peaks between H-2′ (6′) δ_H 7.52 and C-4′ (δ_C 159.2) and between $C\underline{H}_3O$ (δ_H 3.8) and C-4′, respectively, determined the position of the methoxy group to be 4′. The identity of the three sugars and their sequence were determined by the exhaustive analysis of the NMR spectral data of the corresponding nonaacetate **8**, which was obtained by acetylation of **1** with acetic anhydride and pyridine in the presence of 4-(dimethylamino) pyridine (DMAP). The NMR data of **8** (see Experimental) are in accor-

Table 1
NMR spectral data of compounds **1** and **3** in DMSO-d_6

	1			**3**		
Positions	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
2	154.8 CH	8.42 (1H, *s*)		154.6 CH	8.35 (1H, *s*)	
3	122.2 C		2, 2′, 6′	122.0 C		2, 2′, 6′
4	180.4 C			180.8 C		2
5	161.6 C			152.9 C		
6	99.6 CH	6.48 (1H, *s*)	8	132.5 C		8, OMe
7	162.9 C		6	156.5 C		8, 1″
8	94.5 CH	6.72 (1H, *s*)		94.3 CH	6.89 (1H, *s*)	
9	157.2 C		2, 8	152.5 C		2, 8
10	106.1 C		6, 8	106.6 C		8
1′	122.7 C		3′, 5′	121.1 C		2, 3′, 5′
2′, 6′	130.1 CH	7.52 (2H, *d*, *J*=8.4)		130.1 CH	7.38 (2H, *d*, *J*=8.8)	
3′, 5′	113.7 CH	7.01 (2H, *d*, *J*=8.4)		115.1 CH	6.83 (2H, *d*, *J*=8.8)	
4′	159.2 C		2′, 3′, 5′, 6′, OMe	157.5 C		2′, 3′, 5′, 6′
1″	99.8 CH	5.05 (1H, *d*, *J*=7.3)		100.3 CH	5.04 (1H, *d*, *J*=7.3)	3″, 5″
2″	73.0 CH	3.28 (1H, *m*)		73.1 CH	3.34 (1H, *m*)	3″
3″	76.2 CH	3.29 (1H, *m*)		76.7 CH	3.33 (1H, *m*)	2″, 4″
4″	69.7 CH	3.13 (1H, *t*, *J*=8.8)		70.0 CH	3.14 (1H, *t*, *J*=8.8)	3″, 5″
5″	75.5 CH	3.61 (1H, *m*)		75.7 CH	3.62 (1H, *dd*, *J*=8.8, 7.3)	3″, 5″a,b
6″	67.6 CH_2	3.88 (1H, *d*, *J*=11)	1‴	67.8 CH_2	3.88 (1H, *d*, *J*=11)	4″, 1‴
		3.46 (1H, *dd*, *J*=11, 7)			3.47 (1H, *dd*, *J*=11, 7.3)	
1‴	109.0 CH	4.82 (1H, *d*, *J*=2.9)*[a]	6″a, 4‴b, 5‴a,b	109.5 CH	4.81 (1H, *d*, *J*=2.9)	6″a,b, 2‴, 4‴
2‴	76.3 CH	3.74 (1H, *d*, *J*=2.9)		75.9 CH	3.78 (1H, *d*, *J*=2.9)	4‴b, 5‴a
3‴	77.4 C		4‴b, 5⁗a,b	78.7 C		1‴, 5‴a,b, 4‴b
4‴	73.4 CH_2	3.89 (1H, *d*, *J*=9.2)	1‴, 5‴a,b	73.2 CH_2	3.92 (1H, *d*, *J*=9.2)	1‴
		3.62 (1H, *d*, *J*=9.2)			3.60 (1H, *d*, *J*=9.2)	
5‴	69.3 CH_2	3.53 (1H, *d*, *J*=9.9)	1‴,4‴a, 1⁗	63.3 CH_2	3.38 (1H, *m*)	2‴, 4‴a, 4‴b
		3.36 (1H, *d*, *J*=9.9)			3.34 (1H, *m*)	
1⁗	109.0 CH	4.81 (1H, *d*, *J*=2.9)*	4⁗b			
2⁗	75.6 CH	3.75 (1H, *d*, *J*=2.9)	4‴b			
3⁗	78.7 C		4⁗b,5⁗b			
4⁗	73.4 CH_2	3.83 (1H, *d*, *J*=9.5)	1⁗, 5⁗a			
		3.56 (1H, *d*, *J*=9.5)				
5⁗	63.1 CH_2	3.32 (1H, *m*)	2⁗, 4⁗a			
		3.27 (1H, *m*)				
OMe	55.1 CH_3	3.79 (3H, *s*)		60.3 CH_3	3.76 (3H, *s*)	

[a] *The assignments may be interchangeable.

dance with this interpretation. In the HMBC spectrum (see Fig. 1), the anomeric proton signal at δ_H 5.197 (6-substituted glucopyranosyl moiety), δ_H 4.956 (5-substituted apiofuranosyl moiety) and δ_H 4.986 (terminal apiofuranosyl moiety) showed long-range correlations with the ^{13}C signals at δ_C 159.9 (C-7 of aglycone), δ_C 66.3 (C-6″ of 6-substituted glucopyranosyl moiety) and δ_C 67.5 (C-5‴ of 5-substituted apiofuranosyl moiety), respectively. The other correlations are in agreement with the proposed structure. Furthermore, acid-catalysed hydrolysis of **1** gave biochanin A (5,7-dihydroxy-4′-methoxyisoflavone). From the above evidence, the structure of **1** was concluded to be biochanin A 7-*O*-[β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-glucopyranoside].

Compound **3**, $[\alpha]_D^{25}$ −67.0° (*c* 0.28, MeOH), was assigned the molecular formula C_{27} H_{30} O_{15} from its HRFABMS. The UV spectrum showed a bathochromic shift (264 and 330 nm to 274 and 330 nm) on addition of $AlCl_3$, suggesting the presence of free 5-OH and a substituted 7-OH group. The ^1H NMR spectrum exhibited signals at δ 8.35 (1H, *s*), δ 7.38 (2H, *d*, *J* = 8.8 Hz), and 6.38 (2H, *d*, *J* = 8.8 Hz), attributable to hydrogens at H-2 and two sets of *ortho*-coupled aromatic hydrogens at H-2′, 6′ and H-3′, 5′ of an isoflavone derivative as shown in Table 1. A singlet signal at δ 6.89 (1H, *s*) indicated only one hydrogen to be present on the A-ring of the isoflavone. The ^1H and ^{13}C NMR spectra (Table 1) showed two anomeric signals [$\delta_{H\text{-}1''}$ 5.04 (1H, *d*, *J* = 7.3 Hz) and $\delta_{C\text{-}1''}$ 100.3 (*d*) and $\delta_{H\text{-}1'''}$ 4.81 (1H, *d*, *J* = 2.9 Hz) and $\delta_{C\text{-}1'''}$ 109.5 (*d*)] and 9 carbon signals in the region of sugars, indicating that **3** is an isoflavone having two phenolic hydroxyl groups, one methoxy group [δ_H 3.76 (3H, *s*) and δ_C 60.3 (*q*)] and one *O*-diglycosidic group (Agrawal, 1989, 1992). The ^{13}C NMR chemical shifts were in agreement with one glucopyranosyl and one apiofuranosyl moiety (Rao et al., 1989). The chemical shifts of the anomeric carbons at $\delta_{C\text{-}1''}$ 100.3 and $\delta_{C\text{-}1'''}$ 109.5 recognized the β-configuration of the glycosidic linkages (Ramesh and Yuvarajan, 1995; Mathias et al., 1998). The interglycosidic linkage (1‴→6″) was indicated by the downfield shift of C-6″ at δ_C 67.8 (Ramesh and Yuvarajan, 1995). The identity of the two sugars and their sequence were assigned by the ^1H–^1H COSY and HMBC spectra. The HMBC spectrum (Table 1) showed that the signals due to the disaccharide moiety consisted of one glucopyranosyl and one apiofuranosyl moiety. The anomeric proton signals at [δ_H 5.04 (6-substituted glucopyranosyl moiety) and δ_H 4.81 (terminal apiofuranosyl moiety) showed the correlations with the ^{13}C signals at δ_C 156.5 (C-7 of aglycone) and δ_C 67.8 (C-6″ of 6-substituted glucopyranosyl moiety), respectively. The methoxy group was located at C-6 as follows. In the ^{13}C NMR spectrum, the chemical shift values (δ_C 130.1 and 115.1) were assigned to carbon atoms 2′/ 6′ and 3′/ 5′, respectively, suggesting that **3** contained a 4′-hydroxyphenyl group (δ_C 130.0 and 115.2) as in genistein but not a 4′-methoxyphenyl group (δ_C 131.1 and 114.5) as in biochanin (Agrawal, 1989). Also the HMBC spectrum showed cross peak between the methoxy group signal at δ_H 3.76 and the C-6 signal at δ_C 132.5. Furthermore, acid-catalysed hydrolysis of **3** gave tectorigenin (5,7,4′-trihydroxy-6-methoxyisoflavone). From the above evidence, the structure of **3** was determined to be tectorigenin7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside].

Apiose sugar is not commonly encountered in glycosides. Three isoflavone apioglucosides have been iso-

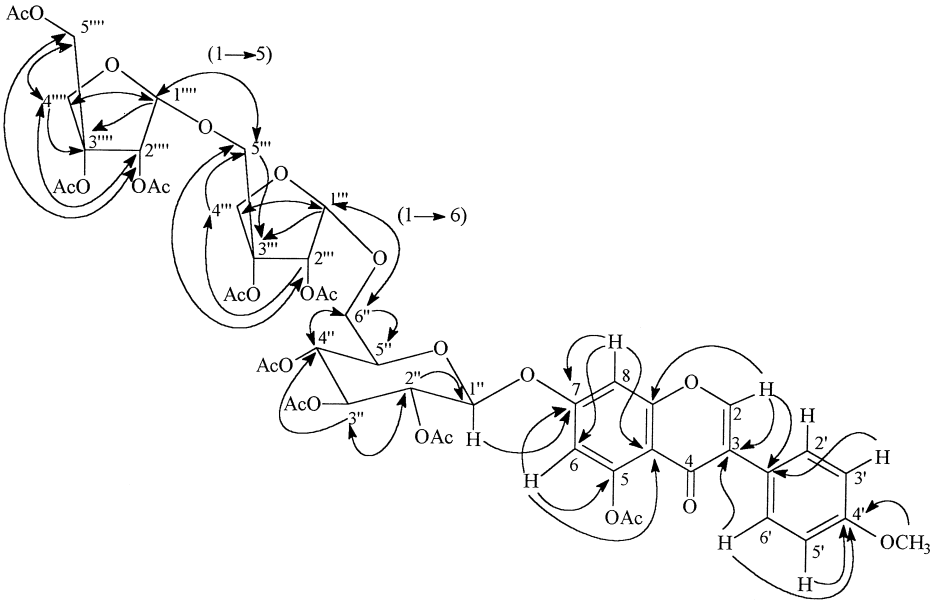


Fig. 1. Significant long range correlations observed in the HMBC spectrum of compound **8**.

lated in three species of *Dalbergia* (Rao et al., 1989; Ramesh and Yuvarajan, 1995; Mathias et al., 1998). In the course of the present work, this is the first report of the occurrence of isoflavone apioglucosides from *D. sissoo* Roxbg. which is of great importance.

3. Experimental

3.1. General

Optical rotations were taken on a JASCO P-1020 polarimeter (cell length 100 mm). UV spectra were measured on a JASCO V-560 UV/VIS spectrophotometer (cell length 10 mm). IR spectra were recorded on JASCO FT/IR-410 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on JEOL A-400 and A-600 spectrometers. FABMS was measured with a JEOL HX-110 spectrometer using *m*-nitrobenzyl alcohol as a matrix. Analytical thin layer chromatography (TLC) was conducted on Si gel GF_{254} (Type 60) (E. Merck) and on precoated aluminium sheets of RP-18 F_{254} (E. Merck). Preparative TLC was performed on Si gel GF_{254} (Type 60) (E. Merck). Column chromatography was performed with Silica gel 60 (E. Merck), Diaion HP-20 AG (75-150 μ, Mitsubishi Chemical Industries Co. Ltd.) and Develosil Lop ODS (30-50 μ, Nomura Chemicals).

3.2. Plant material

The leaves and stem-bark of *D. sissoo* were collected in the campus of Assiut University, Assiut, Egypt, in August 1997. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

3.3. Extraction and isolation

3.3.1. Extraction and isolation of the leaves

The air-dried leaves (2.0 kg) were powdered and extracted with MeOH (2×15 l) at room temperature. The MeOH extract (100 g) was partitioned between water and *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH to give the corresponding solubles (40 g), (20 g), (5.0 g), (5.0 g) and H_2O (30.0 g), respectively. The *n*-BuOH soluble fraction (5.0 g) was subjected to column chromatography on Si gel (600 g) and eluted with a stepwise gradient solvent system of $CHCl_3$–MeOH (each 500 ml), to give fractions 1–5, respectively. Fraction 3 (20 mg), eluted with $CHCl_3$–MeOH (7:3), was purified by preparative TLC (EtOAc–acetone–AcOH–H_2O/30:3:1:1) to give compound **2** (5 mg). The water soluble fraction (30.0 g) was chromatographed on Diaion HP-20 using a stepwise gradient solvent system of H_2O–MeOH (H_2O, 50% MeOH, 80% MeOH and MeOH) to give 4 fractions. The fraction eluted with 50% MeOH (8 g) was subjected to column chromatography on Si gel (240 g) and eluted with a stepwise gradient solvent system of $CHCl_3$–MeOH (each 250 ml). The fraction eluted with $CHCl_3$–MeOH (1:1) (70 mg) was purified by reversed-phase column chromatography on ODS (100 g) and eluted with a solvent system of MeOH–H_2O (2:3) to give compound **1** (25 mg).

3.3.2. Extraction and isolation of stem-bark

The air-dried stem-bark (1.0 kg) was ground and extracted with MeOH (2×10 l) at room temperature. The MeOH extract (90 g) was partitioned between $CHCl_3$ and H_2O and *n*-BuOH and H_2O, successively. The *n*-BuOH soluble material (20.0 g) was subjected to column chromatography on Diaion HP-20 using a stepwise gradient solvent system of H_2O–MeOH (H_2O, 50% MeOH, 80% MeOH and MeOH) to give 4 fractions. The second fraction (10.0 g) eluted with 50% MeOH was chromatographed on Si gel using a stepwise gradient solvent system of EtOAc–MeOH. The fraction (2.0 g) eluted with EtOAc–MeOH (7:3) was separated by reversed-phase column chromatography on ODS (250 g) and eluted with MeOH–H_2O (1:3) to give compound **3** (38 mg) and then with MeOH–H_2O (2:3) to give compound **5** (64 mg).The third fraction (1 g) eluted from Diaion HP-20 with 80% MeOH was subjected to column chromatography on Si gel (30 g) and eluted with a stepwise gradient solvent system EtOAc–MeOH (10:0–7:3) (each 100 ml). The fraction (35 mg) eluted with EtOAc–MeOH (9:1) was purified by reversed-phase column chromatography on ODS (100 g) and eluted with a solvent system of MeOH–H_2O (1:1) to give compound **4** (10 mg).

The fourth fraction (5 g) eluted from Diaion HP-20 was subjected to column chromatography on Si gel (150 g) and eluted with a stepwise gradient solvent system EtOAc–MeOH (each 250 ml). The fraction (24 mg) eluted with EtOAc–MeOH (9:1) was purified by reversed-phase column chromatography on ODS (100 g) and eluted with a solvent system of MeOH–H_2O (1:1) to give compound **6** (2.4 mg). The fraction (200 mg) eluted with EtOAc–MeOH (8:2) was purified by reversed-phase column chromatography on ODS (250 g) and eluted with a solvent system of MeOH–H_2O (1:3) to give compound **7** (70 mg).

Compound **1**: yellow amorphous powder. $[\alpha]_D^{25}$ −106.1° (*c* 0.26, MeOH). UV λ_{max}^{MeOH} nm: 261, 327; +$AlCl_3$: 271, 322; +NaOAc : 261, 327. IR (KBr) ν_{max}: 3383, 2930, 2361, 1654, 1613, 1515, 1442, 1291, 1249, 1181, 1070, 835 cm^{-1}. ^1H and ^{13}C NMR spectral data: see Table 1. HRFAB–MS *m*/*z* 711.2163 $[M+1]^+$, *m*/*z* 711.2136 calculated for $C_{32}H_{39}O_{18}$.

Compound **3**: yellow amorphous powder. $[\alpha]_D^{25}$ −67.0° (*c* 0.28, MeOH). UV λ_{max}^{MeOH} nm: 264, 330; +$AlCl_3$: 275, 325; +NaOAc: 264, 330. IR (KBr) ν_{max}: 3372, 2926, 1655, 1613, 1590, 1516, 1460, 1442, 1367, 1319, 1271, 1228,

1177, 1073, 1002, 924, 827 cm^{-1}. ^1H and ^{13}C NMR spectral data: see Table 1. HRFAB–MS m/z 595.1681 $[M+1]^+$, m/z 595.1663 calculated for $C_{27}H_{31}O_{15}$.

4. Acetylation of 1

Compound **1** (9.4 mg) was treated with 0.25 ml of acetic anhydride and 2 mg of DMAP in 0.5 ml of pyridine at room temperature for 24 h. After dilution with 5 ml of water, the reaction mixture was extracted twice with 5 ml of ethyl acetate. The organic layer was washed with water and brine and dried over magnesium sulfate. The crude product obtained by evaporation was purified by Si gel column chromatography using $CHCl_3$–MeOH (98:2) to give the corresponding nonacetate **8** (11.0 mg, yield 76%). Compound **8**: colorless amorphous powder. IR (KBr)ν_{max}: 3400, 1652, 1612, 1165, 1075, 825 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.927 (1H, *s*: H-2), 7.418 (2H, *d*, $J=8.8$ Hz: H-2′ and H-6′), 6.978 (1H, *d*, $J=2.6$ Hz: H-8), 6.930 (2H, *d*, $J=8.8$ Hz: H-3′ and H-5′), 6.634(1H, *d*, $J=2.6$ Hz: H-6), 5.332 (1H, *s*: H-2‴′), 5.312 (1H, *dd*, $J=9.5$, 9.2 Hz: H-3″), 5.303 (1H, *d*, $J=0.7$ Hz: H-2‴), 5.266 (1H, *dd*, $J=9.5$, 7.7 Hz: H-2″), 5.197 (1H, *d*, $J=7.7$ Hz: H-1″), 5.053 (1H, *dd*, $J=10.3$, 9.2 Hz: H-4″), 4.986 (1H, *s*: H-1‴′), 4.956 (1H, *br.s*: H-1‴), 4.705 (1H, *d*, $J=12.5$ Hz: H-5‴′a), 4.606 (1H, *d*, $J=12.5$ Hz: H-5‴′b), 4.371 (1H, *d*, $J=11.0$ Hz: H-5‴a), 4.242 (1H, *d*, $J=10.6$ Hz: H-4‴′a), 4.194 (1H, *d*, $J=9.9$ Hz: H-4‴a), 4.129 (1H, *d*, $J=10.6$ Hz: H-4‴′b), 4.113 (1H, *d*, $J=9.9$ Hz: H-4‴b), 3.946 (1H, *d*, $J=11.0$ Hz: H-5‴b), 3.922 (1H, *ddd*, $J=10.3$, 7.3, 2.2 Hz: H-5″), 3.807 (3H, *s*:-OC$\underline{H}_3$), 3.777 (1H, *dd*, $J=11.0$, 2.2 Hz: H-6″a), 3.552 (1H, *dd*, $J=11.0$, 7.3 Hz: H-6″b), 2.388, 2.063, 2.058, 2.045, 2.042, 2.025, 2.018, 2.002, 1.956 (each 3H, *s* :-OC$\underline{H}_3$). ^{13}C NMR (150 MHz, $CDCl_3$) : δ152.0 (CH: C-2), 125.8 (C: C-3), 174.7 (C: C-4), 151.1 (C: C-5), 102.2 (CH: C-8), 159.9 (C: C-7), 110.2 (CH: C-6), 158.7 (C: C-9), 113.6 (C: C-10), 123.9 (C: C-1′), 130.5 (CH: C-2′ (6′)), 114.7 (CH: C-3′ (5′)), 159.8 (C: C-4′), 98.3 (CH: C-1″), 71.1 (CH: C-2″), 72.78 (CH: C-3″), 68.6 (CH: C-4″), 73.8 (CH: C-5″), 66.3 (CH_2: C-6″), 106.5 (CH: C-1‴), 76.6 (CH: C-2‴), 84.5 (C: C-3‴′), 73.2 (CH_2: C-4‴), 67.5 (CH_2: C-5‴), 106.0 (CH: C-1‴′), 76.3 (CH: C-2‴′), 83.3 (C: C-3‴′), 72.82 (CH_2: C-4‴′), 63.3 (CH_2: C-5‴′), 55.4 (CH_3:-O$\underline{C}H_3$), 21.2, 20.8, 20.5 (each CH_3:-CO$\underline{C}H_3$), 21.3, 20.72, 20.66 (each $2CH_3$:-CO$\underline{C}H_3$), 170.6, 170.3, 169.9, 169.77, 169.76, 169.5, 169.3, 169.29, 169.0 (each C:-$\underline{C}OCH_3$). FAB-MS $[M+1]^+$, m/z 1089 calculated for $C_{50}H_{57}O_{27}$.

5. Acid-catalyzed hydrolysis of 1

Compound **1** (13 mg) was heated in 1 ml of 2 M HCl at 100°C for 2 h. The reaction mixture was evaporated and then dried in vacuo. The residue was separated by Si gel column chromatography using $CHCl_3$–MeOH (98:2) to give an aglycone (3.0 mg, 58% yield). The aglycone was identified as biochanin A by comparison of its ^1H NMR spectral data with those of an authentic sample (commercially available from Sigma-Aldrich Co).

6. Acid-catalyzed hydrolysis of 3

Compound **3** (17 mg) was treated in the same manner as compound **1** to give an aglycone (3.4 mg, 40% yield). The aglycone was identified as tectorigenin by comparison of its ^1H NMR spectral data with those of an authentic sample derived from tectoridin (Farag et al., 1999).

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References

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids. Elsevier Science, London, p. 196.

Agrawal, P.K., 1992. NMR spectroscopy in the structure elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.

El-Dagwy, A.A., 1996. Encyclopedia for Production of Aromatic and Medicinal Plants, Vol. 1. Imprimerie Atlas, Cairo, p. 192.

Farag, S.F., Backheet, E.Y., El-Emary, N.A., Niwa, M., 1999. Isoflavonoids and flavone glycosides from rhizomes of *Iris carthalinae*. Phytochemistry 50, 1407–1410.

Ingham, J. L., 1983. In: Ingham, J. L., Kokinen, A., Lounasmaa, M. (Eds.), Progress in the Chemistry of Natural Organic Products, Vol. 43. Springer, Wien/New York, pp. 1–200.

Ishikura, N., Watanabe, Y., Teramoto, S., 1989. The formation of flavonoids in cell suspension cultures of *Prunus* × *yedoensis* Matsum. Botanical Magazine, Tokyo 102, 547–560.

Kinjo, J., Furusawa, J., Baba, J., Takeshita, T., Yamasaki, M., Nohara, T., 1987. Studies on the constituents of *Pueraria lobata*. III. Isoflavonoids and related compounds in the roots and the voluble stems. Chemical Pharmaceutical Bulletin 35, 4846–4850.

Markham, K. R., Geiger, H., 1994. In: Harborne, J.B. (Ed.), The Flavonoids Advances in Research Since 1986. Chapman & Hall, London, p. 458.

Mathias, L., Vieira, I.J.C., Braz-Filho, R., Rodrigues-Filho, E., 1998. A new isoflavone glycoside from *Dalbergia nigra*. Journal of Natural Products 61, 1158–1161.

Ramesh, P., Yuvarajan, C.R., 1995. Coromandelin, a new isoflavone apioglucoside from the leaves of *Dalbergia coromandeliana*. Journal of Natural Products 58, 1240–1241.

Rao, P.S., Asheervadam, Y., Khalilullah, M., Murti, V.V.S., 1989. A revised structure for the isoflavone lanceolarin. Phytochemistry 28, 957–958.

Seshadri, T.R., 1972. Polyphenols of *Pterocarpus* and *Dalbergia* woods. Phytochemistry 11, 881–898.

Sharma, A., Chibber, S.C., Chawla, H.M., 1979. Caviunin 7-*O*-gentiobioside from *Dalbergia sissoo* pods. Phytochemistry 18, 1253.

Sharma, A., Chibber, S.C., Chawla, H.M., 1980. Isocaviunin 7-gentiobioside, a new isoflavone glycosides from *Dalbergia sissoo*. Phytochemistry 19, 715.