A New Isoflavone Glycoside from *Dalbergia nigra*

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The new 5-hydroxy-6,7-dimethoxy-4′-*O*-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)isoflavone (**1**) has been isolated from *Dalbergia nigra*. The structure was elucidated using extensive spectroscopic analysis (1D and 2D NMR, MS, IR, UV).

Dalbergia nigra (Vell.) Fr., a Fabaceae tree of medium size, is fairly common in southeastern Brazil, from Bahia State to São Paulo State. It is known as "jacarandá da Bahia", "caviuna", and "jacarandá preto". This tree is commonly used in carpentry.[1] There are previous phytochemical reports indicating that its wood contains dalbergin (6-hydroxy-7-methoxy-4-phenylcoumarin), caviunin (5,7-dihydroxy-2′,4′,5′,6-tetramethoxyisoflavone), (*R*)-4-methoxydalbergione and (*S*)-4,4′-dimethoxydalbergione.[2]

In this paper we report the isolation and structure determination of new 5-hydroxy-6,7-dimethoxy-4′-*O*-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)isoflavone (**1**) from the leaves of a specimen of *D. nigra* collected in Espírito Santo State, Brazil. The structure of this natural product was established by spectral analysis of the original compound and its acetyl derivative (**1a**), mainly ^{1}H and ^{13}C NMR spectra, including homonuclear ^{1}H−^{1}H COSY and heteronuclear ^{13}C detected (conventional method) ^{13}C−^{1}H COSY $^1J_{CH}$ and ^{13}C−^{1}H COSY $^nJ_{CH}$ (n = 2 and 3, COLOC) and ^{1}H-detected (reversed method) ^{1}H−^{13}C HMQC $^1J_{CH}$ and ^{1}H−^{13}C HMBC $^nJ_{CH}$ (n=2 and 3) 2D shift-correlated.

Isoflavone glycoside (**1**), an amorphous powder, was obtained from the EtOAc-insoluble portion of the MeOH extract followed by chromatography on cellulose and Sephadex LH-20. The UV spectrum [(MeOH) λ_{max} (log ϵ): 265 (4.62), 315 (sh) (3.88) nm] was consistent with an isoflavonoid aglycon, and a free hydroxyl at C-5 was suggested by shift of maxima upon addition of $AlCl_3$ [(MeOH−$AlCl_3$) λ_{max} (log ϵ): 280 (4.59), 310 (sh) (4.14), 380 (3.67)nm] unchanged in the presence of HCl.

The IR spectrum showed absorptions for a hydroxy function (ν_{max} 3426 cm^{-1}, very strong broad band), a conjugated and chelated carbonyl group (ν_{max} 1654 cm^{-1}), a conjugated double bond (ν_{max} 1618 cm^{-1}), and an aromatic ring (ν_{max} 1581 and 1509 cm^{-1}). The ^{1}H NMR (Table 1) spectrum (CD_3OD) revealed the presence of a chelated hydroxy group [δ_H 12.85 (s, HO-5)], two methoxy functions [δ_H 3.71 (s, MeO-6) and 3.90 (s, MeO-7)], and additional signals for six hydrogens attached to sp^2 carbon atoms [two singlets: δ_H 8.46 (H-2) and 6.82 (H-8); two doublets corresponding to an AA′BB′ system: δ_H 7.49 (d, J = 8.3 Hz, 2H-2′,6′) and 7.09 (d, J = 8.3 Hz, 2H-3′,5′)] and for oxymethine and oxymethylene hydrogens bound to sp^3 carbons (δ_H 5.40−3.05). These data, in combination with the comparative analysis of the proton noise-decoupled (PND) and DEPT ^{13}C NMR (Table 1) spectra and the usual chemical shift parameters and LRMS {m/z 608 ([M]$^{\cdot+}$, absent) and 609 ([M + H]$^+$, absent), 315 ($C_{17}H_{15}O_6$, [aglycon + H]$^+$, 100%), 314 ($C_{17}H_{14}O_6$, [aglycon]$^+$, 53%), 299 ($C_{16}H_{11}O_6$, [aglycon − Me$^{\cdot}$]$^+$, 21%), 163 ([$C_6H_{11}O_5$]$^+$, 9%), 133 ([$C_5H_9O_4$]$^+$, 62%) obtained by chemical ionization led to the deduction of the molecular formula C_9 (C═O) $(CH)_6$ $(HC-O)_5$ $(O-CH-O)_2$ $(CH_2O)_3$ $(OCH_3)_2$ (OH) = $C_{28}H_{32}O_{15}$, which was supported by ^{1}H and ^{13}C NMR (Table 1) spectra of the heptaacetyl derivative **1a**.

The molecular formula $C_{28}H_{32}O_{15}$ is consistent with an isoflavone ($C_6-C_3-C_6$) aglycon sustaining one hydroxy, two methoxy groups, and one oxy group at carbon C-4′[C_8O (C═O) $(CH)_6$ $(MeO)_2$ (HO) = $C_{17}H_{13}O_6$]. The subtraction of the carbon signals corresponding to this aglycon from the total number of signals observed in the ^{13}C NMR spectra of **1** allowed the assignment of the resonances due to two saccharide moieties [(C−O) $(CH-O)_7$ $(CH_2-O)_3$ = $C_{11}H_{13}O_{11}$→$C_{11}H_{13}O_9$ (two ether functions)], corresponding to one pentose and one hexose moiety. The presence of seven hydroxy groups was supported by the corresponding singlets of acetyl functions [δ_H 2.42 (AcO − 5), 2.07, 2.03, 2.03, 2.00, 1.97, and 1.97] observed in the ^{1}H NMR of **1a** (Table 1), which was confirmed by ^{13}C NMR through the signals correlated with ester carbonyls (δ_C 170.55, 170.19, 169.70, 169.43, 169.43, 169.30, and 168.99) and methyls (δ_C 21.05−20.49) of the acetoxy groups (Table 1).

A D-apio-*β*-D-furanoside moiety was recognized by chemical shift at δ_C 109.46 attributed to an anomeric dioxymethine carbon −O−CH−O− (CH-1‴) in a furanoside ring observed in the ^{13}C NMR spectrum of **1**, along with the carbinolic signals of one quaternary at δ_C 78.93 (C-3‴), two oxymethylenes at δ_C 73.35 (CH_2-4‴) and 63.18 (CH_2-5‴), and one oxymethine at δ_C 76.02 (CH-2‴) (Table 1). This deduction was confirmed by ^{13}C−^{1}H COSY$^nJ_{CH}$ [n = 2 and 3, COLOC (^{13}C-detected conventional method)] spectrum of **1** (MeOH-d_4 as solvent) through cross-peaks revealing heteronuclear coupling of quaternary C-3‴ (δ_C 78.93) and methine CH-1‴ (δ_C 109.46) and hydrogen H-4‴ (δ_H 3.65), as shown in Table 1. Additional confirmation was obtained in the

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Table 1. ^{1}H and ^{13}C NMR Spectral Data of Compound **1** (CD_3OD and DMSO-d_6) and **1a** ($CDCl_3$) Compared with Values ($CDCl_3$) Described in the Literature for **2**−**4**[a]

	1				**1a**		model compounds[c]		
	CD_3OD		DMSO-d_6		$CDCl_3$		**2**	**3**	**4**
	δ_C	δ_H, m (J)	δ_C	δ_H	δ_C	δ_H, m (J)	δ_C	δ_C	δ_C
C									
3	121.93		121.80		125.12				125.9
4	180.57		180.42		174.47				
5	153.11		152.45		151.00				
6	132.05		131.96		139.98				
7	158.96		158.80		157.65				
9	152.62		152.95		154.19				
10	105.92		105.80		112.00				
1′	124.25		124.04		126.48				
4′	157.44		157.27		157.14				156.4
3‴	78.92		78.62		83.84		83.8	82.0	84.3/84.3
AcO					170.55				
					170.19				
					169.70				
					169.43				
					169.43				
					169.30				
					168.99				
CH									
2	154.91	8.46, s	154.75	8.50	151.36	7.82, s	153.4	151.1	151.3
8	91.30	6.82, s	91.21	6.86	97.99	6.79, s			
2′,6′	130.30	7.49, d (8.3)	130.05	7.55	130.52	7.38, d (8.2)			130.4
3′,5′	116.22	7.08, d (8.3)	116.06	7.15	116.68	6.99, d (8.2)			116.6
1″	100.70	4.83	100.41	4.90	98.88	5.03, d (7.4)	98.4	98.6	100.7/100.8
2″	73.34	3.36	73.13	3.29	71.08	5.20	72.7	70.9	71.1/71.1
3″	76.70	3.95	75.84	3.78	72.69	5.30	71.1	70.6	72.7/72.9
4″	70.12	3.18	69.89	3.13	68.74	5.03	68.7	67.1	68.3/68.3
5″	75.77	3.60	76.51	3.28	73.25	3.80	73.8	71.8	71.9/71.9
1‴	109.46	4.82	109.24	4.85	105.89	5.02, s	106.0	103.9	104.5/104.5
2‴	76.02	3.86	75.59	3.54	76.02	5.34, s	76.1	74.6	76.5/76.7
CH_2									
6″	67.90	3.40	67.62	3.88, 3.47	66.37	4.18, d; 4.10, d[b]	66.4	64.7	61.8/61.8
4‴	73.35	3.65, 3.35	73.21	3.92, 3.60	72.39	4.76, d; 4.48, d[c]	72.7	69.3	72.7/73.3
5‴	63.18	5.12, 3.95	62.91	3.45−3.26	62.84		63.1	61.3	67.4/68.0
CH_3									
MeO6	60.19	3.71, s	59.94	3.74	61.49	3.82, s			
MeO7	56.61	3.90, s	56.46	3.95	56.33	3.94, s			
AcO					21.05	2.42, s			
					20.70	2.07, s			
					20.60	2.03, s (6H)			
					20.49	2.00, s			
						1.97, s (6H)			
HO5		12.85							

[a] Chemical shifts in δ (ppm). Signals described without multiplicity were assigned with aid of the homonuclear ^{1}H−^{1}H COSY and heteronuclear ^{13}C−^{1}H COSY $^{n}J_{CH}$ (n = 1; n = 2 and 3), HMQC and HMBC (**1** in DMSO-d_6, Figure 1) 2D shift-correlated spectra. [b] J = 10.6 Hz. [c] J = 12.4 Hz. [d] Only values classified as useful for comparison are described.

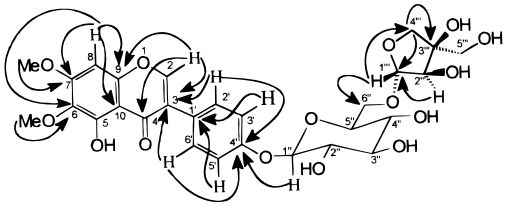
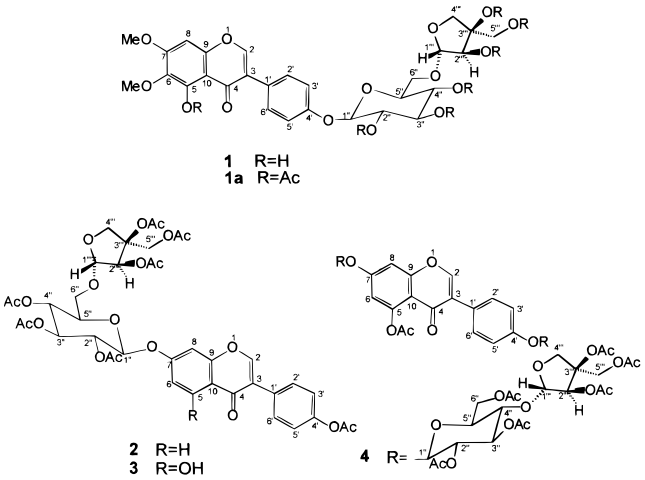


Figure 1. Significant long-range correlations observed in the ^{1}H-^{13}C HMBC $^{n}J_{CH}$ (n = 2 and 3) spectrum of **2** in DMSO-d_6.

^{1}H−^{13}C HMBC $^{n}J_{CH}$ [n = 2 and 3 (^{1}H-detected reverse method)] spectrum of **1** (DMSO-d_6 as solvent), which showed coupling of the carbons CH_2-4‴ (δ_C 73.21) and C-3‴ (δ_C 78.62) with hydrogens H-1‴ (δ_H 4.85) and H-4‴b (δ_H 3.60), respectively (Figure 1).

The remaining ^{13}C signals correlated to carbinolic carbon atoms were used to establish a β-D-glucopyranoside moiety: one anomeric dioxymethine [δ_C 100.70 (CH-1″)], six monooxymethine [δ_C 73.34 (CH-2″), 76.70 (CH-3″), 70.12 (CH-4″), and 75.77 (CH-5″)], and one oxymethylene [δ_C 67.90 (CH_2-6″)] carbons (Table 1). The chemical shift of the oxymethylene carbon CH_2-6″ (δ_C 67.90) was used to define the disaccharide linkage as apiofuranosyl(1‴→6″)glucopyranoside, because the chemical shift of a monosaccharide oxymethylene group appears at about δ_C 62.[3] The ^{1}H−^{13}C HMBC $^{n}J_{CH}$ (n = 2 and 3) spectrum (DMSO-d_6) of **1** was used to confirm this deduction, which revealed cross-peaks corresponding to long-range coupling of carbon CH_2-6″ (δ_C 67.62) and hydrogen H-1‴ (δ_H 4.85, $^{3}J_{CH}$), along with the coupling of the CH-1‴ (δ_C 109.46) and 2H-6″ [δ_H 3.88 (H-6‴a) and 3.47 (H-6‴b)] (Figure 1). The ^{1}H and ^{13}C NMR spectral data of the peracetyl derivative **1a**, along with the heteronuclear ^{13}C−^{1}H COSY $^{1}J_{CH}$ and ^{13}C−^{1}H COSY $^{n}J_{CH}$ (n = 2 and 3, COLOC) and homonuclear ^{1}H−^{1}H COSY 2D shift-correlated spectra[4] of **1** and **1a** and comparison with the model compounds **2**−**4** described in the literature[5] (Table 1), are in accordance with this interpretation.



The location of the 6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl moiety attached to the oxygenated carbon 4′ of the aglycon was suggested by the slight modification observed in the ^{13}C signals of the quaternary C-4′ and methine 2CH-3′,5′ carbon atoms revealed by comparative analysis of the ^{13}C NMR spectra of **1** [δ_C 157.44 (C-4′) and 116.22 (2CH-3′,5′)] and **1a** [δ_C 157.14 (C-4′) and 116.68 (2CH-3′,5′)] (Table 1). The HMBC spectrum of **1** (Figure 1) was also used to confirm definitively this deduction, inasmuch as it showed coupling of the carbon C-4′ (δ_C 157.27) to both hydrogens 2H-2′,6′ (δ_H 7.55, $^3J_{CH}$) and H-1″ (δ_H 4.90, $^3J_{CH}$).

The characterization of the aglycon as a 7-*O*-methyltectorigenin (4′,5-dihydroxy-6,7-dimethoxyisoflavone) derivative was first supported by chemical shifts of the hydrogen H-8 at δ_H 6.82 (MeOH-d_4) and 6.86 (DMSO-d_6) observed in the ^{1}H NMR spectra of **1**. In the alternative substitution of the ring A as 5-hydroxy-7,8-dimethoxy, the hydrogen H-6 should resonate at higher field (δ_H ca 6.3).[6] This deduction was confirmed by cross-peaks observed in the ^{13}C−^{1}H COSY $^nJ_{CH}$ ($n = 2$ and 3, COLOC) spectra of **1** and **1a** indicating spin−spin interaction of the quaternary C-9 [δ_C 152.62 (**1**) and 154.19 (**1a**)] and H-2 [δ_H 8.46 (**1**) and 7.86 (**1a**), $^3J_{CH}$] and H-8 [δ_H 6.82 (**1**) and 6.79 (**1a**), $^2J_{CH}$]. These results were also confirmed by ^{1}H−^{13}C HMBC $^nJ_{CH}$ ($n = 2$ and 3) spectrum of **1** (Figure 1). Thus, the alternative with ring A 5-hydroxy-7,8-dimethoxy substituted was also eliminated on the basis of experimental difficulty in observing the coupling of C-9 and H-6 ($^4J_{CH}$).

Thus, the isoflavone glycoside isolated from *D. nigra* was established as 5-hydroxy-6,7-dimethoxy-4′-*O*-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)isoflavone (**1**). Complete ^{1}H and ^{13}C chemical shift assignments of **1** and **1a** were secured by extensive analysis of 1D and 2D NMR spectroscopy (Table 1 and Figure 1). The 7-*O*-apiosylglucopyranosylbiochanin A has been reported in the literature as an isoflavone glycoside isolated from *Dalbergia lanceolaria*.[7]

The isoflavones, isoflavone glycosides, isoflavanones, isoflavans, and pterocarpans are frequently reported as phytoalexins, bioproduced as stress metabolites from plant tissues challenged with fungi, bacteria, or abiotic agents and biosynthesized as part of the hypersensitive reaction.[8]

Experimental Section

General Experimental Procedures. LRCIMS was obtained by chemical ionization (CI) on a VG Prospec Fisons mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Bruker AC-200 spectrometer in CD_3OD (**1**) and $CDCl_3$ (**1a**) solutions. ^{13}C, HMQC, and HMBC spectra in DMSO-d_6 were recorded using a Bruker ARX-400 spectrometer. Hydrogen chemical shifts were referenced to the residual solvent signal [δ_H 3.34 (CD_3OD) and 7.24 ($CDCl_3$)] and ^{13}C NMR to the center peak of the septet at δ_C 49.0 (CD_3OD) and triplet at δ_C 76.99 ($CDCl_3$). The ^{13}C multiplicities were deduced by comparative analysis of the PND and DEPT ^{13}C NMR spectra. Homonuclear ^{1}H connectivities were determined by ^{1}H−^{1}H COSY spectra. Heteronuclear ^{1}H and ^{13}C connectivities were deduced by ^{13}C × ^{1}H COSY $^1J_{CH}$ [spin−spin coupling of carbon and hydrogen via one bond ($^1J_{CH} \approx 138.0$ Hz)] and ^{13}C × ^{1}H COSY $^nJ_{CH}$ [$n =$ 2 and 3, spin−spin interaction of carbon and hydrogen via two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds, optimized for $^nJ_{CH}$ of 8.0 Hz]. UV spectra in MeOH were recorded on a Hitachi U 2000 spectrometer. IR spectra with KBr plates were obtained on a FTIR Perkin−Elmer 1600/1605 spectrometer.

Plant Material. The leaves of *D. nigra* were collected at Reserva Florestal de Linhares, Companhia Vale do Rio Doce (CVRD), Espírito Santo State, Brazil, during September 1995, and identified by a botanist of the Companhia Vale Rio Doce (CVRD). A voucher specimen has been deposited in the CVRD Herbarium (voucher no. CVRD-2204).

Extraction and Isolation. The air-dried and powdered leaves (161 g) of *D. nigra* were successively extracted with hexane, EtOAc, and MeOH at room temperature. The residue obtained from the MeOH solution was dissolved in EtOAc and the insoluble portion was chromatographed on a cellulose column eluting with H_2O−*n*-BuOH (saturated solution) followed by MeOH. The residue of this MeOH fraction was rechromatographed several times on a Sephadex LH-20 column eluting with MeOH to furnish **1** (95 mg).

5-Hydroxy-6,7-dimethoxy-4′-*O*-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)isoflavone (1): colorless amorphous powder, mp 150−153 °C; UV (MeOH) λ_{max} (log ϵ) 265 (4.62), 315 (sh) (3.88) nm, (MeOH−$AlCl_3$): 280 (4.59), 310 (sh) (4.14), 380 (3.67) nm, unchanged in the presence of HCl; IR (KBr) ν_{max} 3426, 1654, 1618, 1581, 1509, 1273, 1237, 1135, 1079, 1005 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD; 400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (50 MHz, CD_3OD; 100 MHz, DMSO-d_6) Table 1; LRCIMS *m/z* (rel int) 608 ([M]$^{\cdot+}$, absent), 315 (100), 314 (53), 301 (17), 300 (14), 299 (21), 285 (6), 271 (11), 181 (2), 163 (9), 153 (11), 145 (36), 143 (14), 133 (62), 127 (95), 117 (16), 115 (54), 113 (22), 107 (17) 103 (50).

Peracetyl Derivative 1A. Natural product **1** (50 mg) was treated with Ac_2O (9.0 mL) and dry pyridine (1.0 mL) at room temperature overnight. After the usual workup, the crude peracetyl derivative was chromatographed on a Si gel column eluting with $CHCl_3$ to furnish the heptaacetate **1a** (43 mg) as a white amorphous powder: IR (KBr) ν_{max} 1754, 1643, 1610, 1509,

1228, 1053 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$), Table 1; ^{13}C NMR (50 MHz, $CDCl_3$), Table 1.

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