

A New Isoflavone Glycoside from *Ceiba pentandra* (L.) GAERTNER

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From the 80% EtOH extract of the bark of *Ceiba pentandra* (L.) GAERTNER, a new isoflavone glycoside was isolated along with known isoflavones, vavain and vavain glucoside. The structure was elucidated by spectroscopic analysis as 5-hydroxy-7,4',5'-trimethoxyisoflavone 3'-O- α -L-arabinofuranosyl(1 \rightarrow 6)- β -D-glucopyranoside.

Key words *Ceiba pentandra*; Bombacaceae; vavain; vavain glycoside

Ceiba pentandra (L.) GAERTNER (Bombacaceae) is a tropical tree and the capsules are known as kapok. It is widely used in traditional medicines as a diuretic, an antidiarrheic and an emollient.¹⁾ From the root bark of *Ceiba pentandra*, naphthoquinones have been reported with antimicrobial activities.²⁾ From the bark, vavain (**1**) and its glucoside (**2**) have been isolated with their inhibitory effects on cyclooxygenase-catalyzed prostaglandin biosynthesis.³⁾ On our studies of finding inhibitory active compounds against α -glucosidase from medicinal plants, the bark of *Ceiba pentandra* which was called Samauma-verdadeira in Belém, Brazil and used for the treatment of diabetes, exhibited some inhibitory activities (data not shown). A new vavain glycoside (**3**) was isolated in addition to **1** and **2** in the EtOAc-soluble fraction and *n*-BuOH-soluble fraction although these compounds showed no activities against this enzyme.

Compound **3** showed a blue color under UV light (2537 Å), and a brown color when heated with 10% H₂SO₄ on TLC plate. The presence of phenolic hydroxyl group and carbonyl group was apparent from the absorption bands at 3566 (OH), 1655 (C=O), 1508 and 1474 (aromatic) cm⁻¹ in the IR spectrum. These observations on TLC and IR were quite similar to those of **1** and **2**. The negative secondary ion (SI)-MS of **3** revealed [M-H]⁻ at *m/z* 637.1782, which corresponded to the molecular formula C₂₉H₃₄O₁₆. This suggested that compound **3** had an additional pentose sugar to vavain glucoside (**2**: C₂₄H₂₆O₁₂). Since ¹H- and ¹³C-NMR data of **3** were similar to those of **2**, it was speculated that a pentose sugar was attached to the glucose moiety of **2**. This was proved by a correlation between the anomeric proton (δ 4.94, *J*=7.5 Hz) and C-3' carbon (δ 150.7), as well as a correlation between the anomeric proton (δ 4.70, *J*=2.0 Hz) and a primary alcohol carbon (δ 66.9), in the heteronuclear multiple bond connectivity (HMBC) spectrum. Since the anomeric carbons attached to the anomeric protons at δ 4.94 and δ 4.70 were assigned at δ 101.3 and δ 108.3 by the heteronuclear multiple quantum coherence (HMQC), respectively, the terminal pentose sugar was connected to the 6-hydroxyl group of the glucosyl moiety. This was also supported by HMBC connectivities between a hydroxyl proton (δ 5.36, *J*=5.0 Hz) and sugar carbons (δ 101.3, δ 76.6, δ 73.3) and between a hydroxyl proton (δ 5.12, *J*=5.0 Hz) and sugar carbons (δ 75.3, δ 66.9), and by the comparison with ¹³C-NMR chemical shifts of **2**, that is, a signal due to C-6 of the glucosyl moiety was displaced downfield by 6.2 ppm and a signal due to C-5 of the glucosyl moiety was shielded by 1.7 ppm, while the other glucosyl carbon remained almost unshifted.⁴⁾ Based on

HMBC connectivities between a hydroxyl proton (δ 5.17, *J*=5.5 Hz) and sugar carbons (δ 108.3, δ 81.9, δ 76.9) and between a hydroxyl proton (δ 4.95, *J*=5.5 Hz) and sugar carbons (δ 81.9, δ 76.9, δ 83.7), the terminal pentose sugar was determined as α -arabinofuranoside, whose ¹³C-NMR chemical shifts demonstrated the characteristic pattern.⁵⁾ To confirm the configuration of sugars, **3** was hydrolyzed to afford **1** and D-glucose and L-arabinose detected by the chromatography. Thus, the structure of **3** was elucidated as 5-hydroxy-7,4',5'-trimethoxyisoflavone 3'-O- α -L-arabinofuranosyl(1 \rightarrow 6)- β -D-glucopyranoside.

Experimental

General Procedures Melting points were determined on a Yanaco micro melting point apparatus and uncorrected. UV and IR spectra were recorded using a Hitachi U-3010 spectrometer in MeOH and on a Shimadzu FTIR-8200 spectrometer in KBr, respectively. Optical rotation were measured on JASCO DIP-370 digital polarimeter in EtOH or pyridine. ¹H- and ¹³C-NMR spectra were taken on a Varian VXR-500 instrument at 500 MHz, and 125 MHz, respectively with TMS as an internal standard. HMQC and HMBC spectra were obtained on a Varian VXR-500 instrument at 500 MHz. High resolution electron impact (HR-ESI)-MS and HR-SI-MS (glycerol for a matrix) were recorded with a Hitachi M-4100 instrument. Column chromatography was carried out on silica gel (Kieselgel 60, 70–230 mesh, Merck Co.). TLC was performed on Silica gel 60 F₂₅₄ plates (0.25 mm, Merck Co.), and spots were detected under UV light and colored by spraying with 10% H₂SO₄ solution followed by heating. HPLC was carried out on LiChrospher 100 NH₂ (4 \times 125 mm, Merck Co.) eluted with CH₃CN–H₂O (9 : 1), using refractive index (RI) detector (RID-10A, Shimadzu).

Plant Materials The dry bark of *Ceiba pentandra* (L.) GAERTNER was purchased in Belém, Brazil in June 1996, and was identified by Dr. Joaquim Gomes and Dr. Irenice Alves (EMBRAPA-CPATU, Belém, Brazil). A voucher specimen (No. Belém 1996-5), representing the collection has been deposited at Kobe Pharmaceutical University, Japan.

Extraction and Isolation The dry bark of *Ceiba pentandra* (560 g) were powdered and extracted with 80% EtOH (1000 ml \times 3). Each filtrate

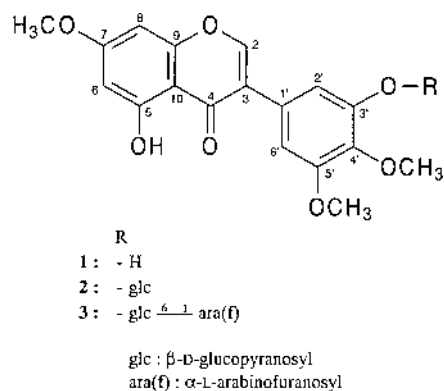


Fig. 1. Structures of Compounds 1–3

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Table 1. ¹H-NMR Spectral Data of Compounds 1—3

Proton No.	1 ^{a)}	2 ^{b)}	3 ^{b)}
2	7.88 (1H, s)	8.50 (1H, s)	8.46 (1H, s)
6	6.39 (1H, d, 2.0)	6.44 (1H, d, 2.0)	6.43 (1H, d, 2.0)
8	6.41 (1H, d, 2.0)	6.69 (1H, d, 2.5)	6.67 (1H, d, 2.5)
2'	6.70 (1H, d, 2.5)	6.99 (1H, d, 2.0)	6.96 (1H, d, 2.0)
6'	6.74 (1H, d, 2.0)	6.98 (1H, d, 2.0)	6.97 (1H, d, 2.0)
5-OH	12.82 (1H, s)	12.92 (1H, s)	12.92 (1H, s)
3'-OH	5.83 (1H, s)		
7-OMe	3.88 (3H, s)	3.88 (3H, s)	3.87 (3H, s)
4'-OMe	3.93 (3H, s)	3.75 (3H, s)	3.75 (3H, s)
5'-OMe	3.91 (3H, s)	3.81 (3H, s)	3.81 (3H, s)
Glc 1		4.91 (1H, d, 7.0)	4.94 (1H, d, 7.5)
2		3.25—3.32 ^{c)} (1H)	3.26—3.30 ^{c)} (1H)
3		3.25—3.32 ^{c)} (1H)	3.26—3.30 ^{c)} (1H)
4		3.13—3.18 (1H, m)	3.12—3.18 (1H, m)
5		3.25—3.32 ^{c)} (1H)	3.45—3.50 (1H, m)
6		3.42—3.47 (1H, td, 12.0, 6.0)	3.38—3.44 ^{c)} (1H)
		3.68—3.71 (1H, ddd, 12.0, 5.5, 2.0)	3.84—3.88 ^{c)} (1H)
Glc 2-OH		5.32 (1H, d, 5.0)	5.36 (1H, d, 5.0)
3-OH		5.09 (1H, d, 4.5)	5.16 (1H, d, 4.0)
4-OH		5.01 (1H, d, 5.5)	5.12 (1H, d, 5.0)
6-OH		4.55 (1H, t, 5.5)	
Ara 1			4.70 (1H, d, 2.0)
2			3.69—3.72 (1H, m)
3			3.55—3.59 (1H, m)
4			3.62—3.67 (1H, m)
5			3.31—3.38 ^{c)} (1H)
			3.48—3.52 ^{c)} (1H)
Ara 2-OH			5.17 (1H, d, 5.5)
3-OH			4.95 (1H, d, 5.5)
5-OH			4.67 (1H, t, 5.0)

a) Measured in CDCl₃. b) Measured in DMSO-*d*₆. c) Overlapped with other proton peaks. Glc: β-D-glucopyranosyl, Ara: α-L-arabinofuranosyl.

was combined and evaporated to obtain an extract (25.7 g). The EtOH extract was dissolved in CHCl₃ (500 ml×3, 2.4 g), EtOAc (500 ml×3, 0.8 g) and *n*-BuOH (500 ml×3, 14.5 g), successively, and further obtained residue (7.1 g). At first the EtOAc-soluble fraction (0.78 g) was chromatographed on a column of silica gel (80 g) using CHCl₃-MeOH (10 : 1) to give 4 fractions (frs. 1—4). Repeated column chromatography of fr. 2 on silica gel (20 g) using EtOAc-MeOH (10 : 1) and CHCl₃ gave **1** (15.5 mg). Similarly repeated column chromatography of fr. 3 on silica gel (80 g) using EtOAc-MeOH-H₂O (40 : 5 : 0.1) gave **2** (32.6 mg) and column chromatography of fr. 4 on silica gel (20 g) using EtOAc-MeOH-H₂O (40 : 5 : 0.1) gave **3** (10.7 mg). Next the *n*-BuOH-soluble fraction (2.0 g) was chromatographed on a column of silica gel (120 g) using stepwise gradient of CHCl₃-MeOH to give 6 fractions (frs. A—F). Repeated column chromatography of fr. C on silica gel using EtOAc-MeOH-H₂O (40 : 5 : 0.1) gave **3** (19.5 mg).

Vavain (Pentandrin⁶⁾) (**1**): Pale yellow amorphous powder (MeOH); mp 162—163 °C; HR-EI-MS (positive ion mode): *m/z* [M]⁺ 344.0092 (Calcd for C₁₈H₁₆O₇: 344.0994); IR (KBr) *v*_{max} cm⁻¹: 3425 (OH), 1661 (C=O), 1508 (aromatic); UV (MeOH) *λ*_{max} (log *ε*): 213 (4.34), 262 (4.55); ¹H- and ¹³C-NMR: see Tables 1 and 2.

Vavain 3'-*O*-β-D-glucopyranoside (Pentandrin glucoside⁶⁾) (**2**): White amorphous powder (MeOH); mp 127—128 °C; [*α*]_D²⁰ -14.5° (*c*=0.2, pyridine), -4.0° (*c*=0.1, EtOH); HR-SI-MS (negative ion mode): *m/z* [M-H]⁻ 505.1335 (Calcd for C₂₄H₂₅O₁₂: 505.1344); IR (KBr) *v*_{max} cm⁻¹: 3425 (OH), 1663 (C=O), 1510 (aromatic); UV (MeOH) *λ*_{max} (log *ε*): 215 (4.39), 263 (4.53); ¹H- and ¹³C-NMR: see Tables 1 and 2.

Table 2. ¹³C-NMR Spectral Data of Compounds 1—3

Carbon No.	1 ^{a)}	2 ^{b)}	3 ^{b)}	HMBC
2	153.2	155.2	155.3	
3	123.7	122.2	122.3	H-2, H-2', H-6'
4	180.6	180.0	180.1	H-2, H-8
5	162.8	161.7	161.7	H-6, 5-OH
6	98.3	98.1	98.2	H-8, 5-OH
7	165.6	165.3	165.3	H-6, H-8, 5-OH, 7-OMe
8	92.5	92.5	92.6	H-6
9	157.9	157.3	157.5	H-2, H-8
10	106.3	105.3	105.4	H-6, H-8, 5-OH
1'	126.7	125.7	126.0	H-2, H-2', H-6'
2'	108.5	109.9	110.4	H-6'
3'	149.4	150.6	150.7	H-2', Glc H-1
4'	135.8	138.3	138.7	H-2', H-6', 4'-OMe
5'	152.3	152.6	152.8	H-6', 5'-OMe
6'	105.5	107.4	107.8	H-2'
7-OMe	55.8	55.9	56.1	
4'-OMe	61.0	60.2	60.4	
5'-OMe	56.0	56.1	56.0	
Glc 1		100.8	101.3	Glc H-3, Glc H-5, Glc 2-OH
2		73.2	73.3	Glc H-3, Glc 2-OH, Glc 3-OH
3		76.8	76.6	Glc H-2, Glc H-4, Glc 2-OH
4		69.8	69.9	Glc H-3, Glc H-5, Glc 4-OH
5		77.0	75.3	Glc H-6, Glc 4-OH
6		60.7	66.9	Glc H-4, Glc H-5, Ara H-1
Ara 1		108.3	108.3	Ara H-2, Ara H-6, Ara 2-OH
2		81.9	81.9	Ara H-1, Ara H-3, Ara 2-OH, Ara 3-OH
3		76.9	76.9	Ara H-1, Ara H-2, Ara H-4, Ara 2-OH, Ara 3-OH
4		83.7	83.7	Ara H-1, Ara H-3, Ara H-5, Ara 3-OH
5		61.1	61.1	Ara 5-OH

a) Measured in CDCl₃. b) Measured in DMSO-*d*₆. Glc: β-D-glucopyranosyl, Ara: α-L-arabinofuranosyl.

Compound **3**: White amorphous powder (MeOH); mp 213—214 °C; [*α*]_D²⁰ -71.6° (*c*=0.1, pyridine); HR-SI-MS (negative ion mode): *m/z* [M-H]⁻ 637.1782 (Calcd for C₂₉H₃₃O₁₆: 637.1766); IR (KBr) *v*_{max} cm⁻¹: 3566 (OH), 1655 (C=O), 1508, 1474 (aromatic); UV (MeOH) *λ*_{max} (log *ε*): 216 (4.31), 263 (4.49); ¹H- and ¹³C-NMR: see Tables 1 and 2.

Acid Hydrolysis of 3 Compound **3** (3 mg) was refluxed with 5% HCl in MeOH (5 ml) for 2 h. After cooling and evaporating MeOH off *in vacuo*, the reaction mixture was partitioned between chloroform and water. The chloroform layer was applied for co-TLC with **1**. The water layer was lyophilized, and then the residue was compared with authentic sugars detected by RI on HPLC.

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