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Phenylpropanoid glucosides from leaves of *Coussarea hydrangeifolia* (Rubiaceae)

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Abstract

Phenylpropanoid glycosides, 1′-*O*-benzyl-α-L-rhamnopyranosyl-(1″ → 6′)-β-D-glucopyranoside (**1**) and α-L-xylopyranosyl-(4″ → 2′)-(3-*O*-β-D-glucopyranosyl)-1′-*O*-*E*-caffeoyl-β-D-glucopyranoside (**2**), together with the known derivatives, 1,6-di-*O*-caffeoyl-β-D-glucopyranoside (**3**), 1-*O*-(*E*)-caffeoyl-β-D-glucopyranoside (**4**) and 1-*O*-(*E*)-feruloyl-β-D-glucopyranoside (**5**), were isolated from leaves of *Coussarea hydrangeifolia*. Their structures were determined by IR, HRESIMS, and 1D and 2D NMR experiments, and their antioxidant activities, evaluated by assaying the free radical scavenging capacity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical as substrate. The antioxidant activities of **3** and **4** (IC_{50} values of 15.0 and 19.2 μM, respectively) were comparable to that of the standard positive control caffeic acid, whilst **2** and **5** were only weakly active and **1** was inactive.

Keywords: *Coussarea hydrangeifolia*; Rubiaceae; Phenylpropanoid glycosides; Antioxidant activity

1. Introduction

Although *Coussarea* is considered to be only a small genus within the taxon Rubiaceae, it is well represented in the main Brazilian biomes including the Amazônia and the Cerrado. *Coussarea hydrangeifolia*, commonly know as "falsa-quina", is characteristic of the vegetation of the Cerrado and of the semi-deciduous forests of Brazil, where it is considered to be a pioneer species (Correa, 1974). In the course of our studies concerning the bioactive principles of Brazilian plants, with special emphasis on the vegetation of the State of São Paulo (Pauletti et al., 2003; Hamerski et al., 2003), we found that the aqueous extract of the leaves of *C. hydrangeifolia* showed antioxidant activity, as determined by a preliminary test with β-carotene, and strong free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Fractionation of this extract provided five phenylpropanoid glycosides including two new compounds, **1** and **2**, which had their structures determined by spectroscopic methods.

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2. Results and discussion

Compound **1** was isolated from the aqueous extract of *C. hydrangeifolia* as an amorphous powder with an $[\alpha]_{D}^{30}$ of −44.3°. A quasimolecular ion peak $[M - H]^{-}$ for **1** was observed at *m/z* 415.1610 in the high-resolution electrospray ionisation mass spectrum (HRESIMS; negative mode) suggesting a molecular formula of $C_{19}H_{28}O_{10}$. The IR spectrum exhibited strong absorption bands at 3430 and 1042 cm^{-1} suggesting the presence of hydroxyl groups. The ^{1}H NMR spectrum (Table 1) displayed signals that could be attributed to a monosubstituted benzene ring [δ 7.54 (*d*, *J* = 7.5 Hz, H-2,6), 7.30 (*m*, H-3,5), 7.23 (*m*, H-4)], a β-D-glucosyl moiety [δ 4.90 (*d*, *J* = 7.5 Hz, H-1′), 4.06 (*dd*, *J* = 7.5, 8.0 Hz, H-2′), 4.19 (*t* like, *J* = 8.0 Hz, H-3′), 4.12 (*m*, H-4), 4.20 (*ddd*, *J* = 9.5, 7.5, 2.5 Hz, H-5′), 4.18 (*dd*, *J* = 11.0, 7.5 Hz, H – $6'_{a}$), 4.64 (*dd*, *J* = 11.0, 2.5 Hz, H – $6'_{b}$), and a rhamnosyl group [δ 5.55 (*d*, *J* = 1.0 Hz, H-1″), 4.62 (*m*, H-2″), 4.55 (*dd*, *J* = 3.5, 9.0 Hz, H-3″), 4.26 (*t* like, *J* = 9.0 Hz, H-4″), 4.97 (*m*, H-5), 1.62 (*d*, *J* = 6.0 Hz, H-6″). Additionally, the signals at δ 4.85 (*d*, *J* = 11.5 Hz) and 5.15 (*d*, *J* = 11.5 Hz) could be ascribed to benzylic oxymethylene hydrogens, thus characterising the aromatic portion of **1** as a benzylic system. The ^{13}C NMR spectrum (Table 1) showed signals at δ 137.8, 128.2 (2×), 127.9 (2×), 127.2 and 70.9, which were consistent with the presence of a monosubstituted benzylic ring. Two anomeric methine carbons at δ 103.7 and 102.6, several hydroxymethines, one hydroxymethylene carbon at δ 68.4 and one methyl group at δ 18.7 resembled those of glucose and rhamnose, respectively, and clearly suggested that **1** possessed glucopyranosyl and rhamnopyranosyl units. Data from COSY and TOCSY experiments revealed the hydrogen sets belonging to the glucosyl and rhamnosyl moieties. The complete assignment of hydrogen and carbon signals and the positions of the bonds of the sugar moieties in **1** were determined by an HMBC experiment which showed long-range correlations between H-6′ and C-4′ and the anomeric C-1″, between H-1″ and the methylene C-6′, and between H-7 of the benzylic system and C-1′

Table 1
^{1}H and ^{13}C NMR spectroscopic data of hydrangeifolin I (**1**), hydrangeifolin II (**2**) and 1,6-di-*O*-caffeoyl-β-D-glucopyranose (**3**) in pyridine-d_5

Position	1		2		3	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}H	δ ^{1}H	δ ^{13}C
1	–	137.8 (*s*)	–	126.6 (*s*)	–	127.6 (*s*)
2	7.54 *d* (7.5)	128.2 (*d*)	7.53 *d* (2.0)	115.8 (*d*)	7.05 *m*	111.7 (*d*)
3	7.30 *m*	127.9 (*d*)	–	147.0 (*s*)	–	148.8 (*s*)
4	7.23 *m*	127.2 (*d*)	–	148.7 (*s*)	–	149.5 (*s*)
5	7.30 *m*	127.9 (*d*)	7.16 *d* (8.0)	116.7 (*d*)	6.80 *m*	116.2 (*d*)
6	7.54 *d* (7.5)	128.2 (*d*)	7.10 *dd* (2.0; 8.0)	122.2 (*d*)	6.99 *m*	124.1 (*d*)
7	4.85 *d* (11.5) 5.15 *d* (11.5)	70.9 (*t*)	7.97 *d* (15.5)	145.5 (*d*)	6.64 *d* (16.0)	114.8 (*d*)
8			6.56 *d* (15.5)	114.4 (*d*)	7.93 *d* (16.0)	147.4 (*d*)
9			–	165.3 (*s*)	–	169.6 (*s*)
1′	4.90 *d* (7.5)	103.7 (*d*)	6.20 *d* (8.0)	96.1 (*d*)	4.48 *d* (7.4)	104.3 (*d*)
2′	4.06 *dd* (7.5)	75.1 (*d*)	4.27 *t* (9.0)	78.8 (*d*)	4.04 *t* (9.0)	74.4 (*d*)
3′	4.19 *t* (8.0)	77.2 (*d*)	4.34 *m*	78.5 (*d*)	3.95 *t* (9.0)	76.9 (*d*)
4′	4.12 *m*	71.9 (*d*)	4.36 *m*	71.1 (*d*)	3.93 *t* (9.0)	71.0 (*d*)
5′	4.20 *ddd* (9.5, 7.5, 2.5)	78.5 (*d*)	4.08 *m*	79.5 (*d*)	4.10 *m*	77.5 (*d*)
6′	4.18 *dd* (11.0, 7.5) 4.64 *d* (11.0, 2.5)	68.4 (*t*)	4.40 *d* (12.0) 4.60 *d* (12.0)	62.3 (*t*)	4.29 *m* 4.39 *m*	66.5 (*t*)
1″	5.55 *d* (1.0)	102.6 (*d*)	5.90 *d* (3.5)	94.2 (*d*)	–	127.5 (*s*)
2″	4.62 *m*	72.3 (*d*)	4.20 *dd* (3.5; 9.0)	74.6 (*d*)	7.05 *m*	111.7 (*d*)
3″	4.55 *dd* (3.5; 9.0)	72.8 (*d*)	4.08 *m*	72.7 (*d*)	–	148.8 (*s*)
4″	4.26 *t* (9.0)	74.1 (*d*)	4.75 *t* (9.0)	75.5 (*d*)	–	149.4 (*s*)
5″	4.97 *m*	69.8 (*d*)	4.54 *dd* (2.5; 11.5) 4.42 *dd* (5.5; 11.5)	63.1 (*t*)	6.80 *m*	116.2 (*d*)
6″	1.62 *d* (6.0)	18.7 (*d*)			6.99 *m*	124.1 (*d*)
7″					6.52 *d* (15.5)	114.7 (*d*)
8″					7.87 *d* (15.5)	147.1 (*d*)
9″					–	169.7 (*s*)
1‴			5.35 *d* (7.5)	99.6 (*d*)		
2‴			4.29 *t* (8.5)	74.3 (*d*)		
3‴			4.14 *t* (8.5)	76.9 (*d*)		
4‴			4.25 *t* (8.5)	72.2 (*d*)		
5‴			4.03 *ddd* (2.5; 5.5; 9.0)	78.6 (*d*)		
6‴			4.39 *d* (11.5) 4.58 *d* (11.5)	63.3 (*t*)		

of the glucosyl moiety and C-2/C-6 of the aromatic system. Consequently, the structure of **1** was established to be 1′-*O*-benzyl-α-L-rhamnopyranosyl-(1″ → 6′)-β-D-glucopyranoside, and this new compound has been named hydrangeifolin I.

Compound **2** exhibited a quasimolecular ion peak $[M-H]^-$ at *m*/*z* 635.1828 in the HRESIMS (negative mode) suggesting a molecular formula $C_{26}H_{36}O_{18}$. MS/MS analysis of the quasimolecular ion revealed a significant fragment ion at *m*/*z* 502.1321 assigned to $[M - H - \text{deoxyhexose}]^-$. The ^{13}C NMR spectrum (Table 1) clearly showed the presence of three sugar moieties together with nine additional signals that could be attributed to the aglycone moiety. Data from the ^1H NMR spectrum (Table 1) indicated the presence of an (*E*)-caffeoyl system [1H, δ 7.97 (*d*, $J = 15.5$ Hz, H-7), 6.56 (1H, *d*, $J = 15.5$ Hz, H-8), 7.53 (1H, *d*, $J = 2.0$ Hz, H-2), 7.16 (1H, *d*, $J = 8.0$ Hz, H-5), 7.10 (1H, *dd*, $J = 2.0$, 8.0 Hz, H-6)] together with three anomeric protons, two of which [5.35 (*d*, $J = 7.5$ Hz) and 6.20 (*d*, $J = 8.0$ Hz)] were assigned to the two β-glucose units, and one [δ 5.90 (*d*, $J = 3.5$ Hz)], to an α-xylose moiety. From the ^{13}C NMR spectrum it could be established that the aromatic unit was an ester of (*E*)-caffeic acid [δ 165.3 (C-9), 145.5 (C-7), 114.4 (C-8), 126.6 (C-1), 115.8 (C-2), 147.0 (C-3), 148.7 (C-4), 116.7 (C-5), 122.2 (C-6)]. Complete assignment of the protons in each sugar system was achieved by considering the 1D and 2D TOCSY, 1D and 2D NOESY, and the ^1H–^1H COSY spectra, while the carbons were assigned from HMQC and HMBC data. A 1D TOCSY experiment obtained by irradiating at the well-resolved anomeric proton at δ 5.35 (1H, *d*, $J = 7.5$ Hz) revealed a set of coupled oxymethine protons (δ 4.29, 4.14, 4.25 and 4.03) and a methylene proton (δ 4.39). These data, when analysed with the correlated ^{13}C NMR signals in the HMQC spectrum, led to the identification of a β-D-glucosyl moiety. Its position at C-3 of the caffeic acid moiety was established unambiguously by HMBC correlations observed between the anomeric proton H-1‴ at δ 5.35 and C-3 (δ 147.0), and from the proton at δ 7.10 (H-6) to C-2 (δ 115.8) and C-4 (δ 148.7). A 1D TOCSY experiment obtained by irradiating at the well-resolved anomeric proton at δ 6.20 (1H, *d*, $J = 8.0$ Hz) showed a set of coupled oxymethine protons (δ 4.27, 4.34, 4.36 and 4.08) and oxymethylene protons (δ 4.40 and 4.60). Analysis of the correlated ^{13}C NMR signals in the HMQC spectrum led to the identification of an additional β-D-glucosyl moiety. From the HMBC correlation observed between the signals at δ 6.20 (H-1′) and δ 165.3 [the carbonyl of the (*E*)-caffeoyl group], the site of esterification with the (*E*)-caffeoyl acid unit was determined to be at C-1′ of the glucopyranosyl. The downfield shift of C-2′ (δ 78.8) together with the upfield shift of C-1′ (δ 96.1) of the β-glucopyranosyl unit also suggested that the α-xylopyranosyl unit was attached to C-2′ of the glucopyranosyl moiety. The cross peak in the HMBC experiment between δ 4.27 (*t*, $J = 9.0$ Hz, H-2′) and δ 75.5 (C-4″), and the correlation in the NOESY experiment between the signals at δ 4.27 (*t*, $J = 9.0$, H-2′) and at δ 4.75 (*t*, $J = 9.0$, H-4″), confirmed the (4″ → 2′) linkage of the disaccharide moiety. Acid hydrolysis of **2** afforded glucose and xylose, which were identified by TLC by comparison with authentic samples. Consequently, the structure of **2** was established as α-L-xylopyranosyl-(4″ → 2′)-(3-*O*-β-D-glucopyranosyl)-1′-*O* *E*-caffeoyl-β-D-glucopyranoside, and this new compound has been named hydrangeifolin II (see Fig. 1).

Compound **3** was identified as 1,6-di-*O*-caffeoyl-β-D-glucopyranose from physical and spectroscopic data (Akhmedov and Litvinenko, 1968). Although this compound was first isolated from *Scrophularia grossheimi* (a traditional medicinal plant used in Russia for the treatment of jaundice, hepatitis and rheumatism), to the best of our knowledge its ^{13}C NMR spectroscopic data remain unreported. The assignment of the ^{13}C NMR signals of **3**, based mainly on 1D and 2D NMR experiments, is provided in Table 1. Compounds **4** and **5** were identified as 1-*O*-(*E*)-caffeoyl-β-D-glucopyranose and 1-*O*-(*E*)-feruloyl-β-D-glucopyranose, respectively (Birkofer et al., 1961, 1969).

Preliminary TLC autographic test using β-carotene as revealing agent (Silva et al., 2001) evidenced several antioxidant compounds in the aqueous extract from leaves of *C. hydrangeifolia*. Compounds **1**–**5**, isolated from this extract, were evaluated for their free radical scavenging activity towards the stable free radical DPPH (Pauletti et al., 2003), and compared to standard antioxidants

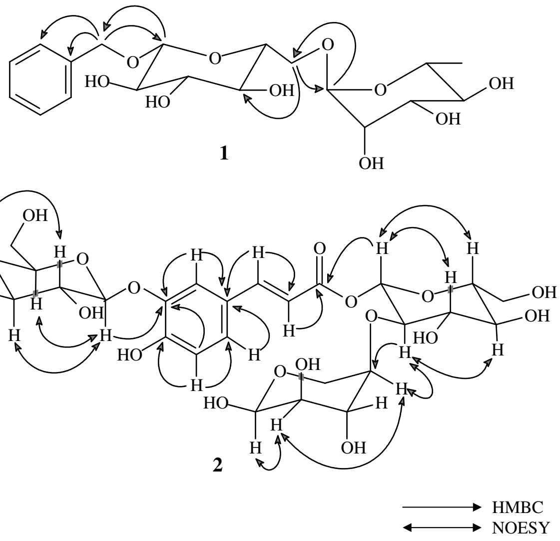


Fig. 1. Selected HMBC correlations (H → C) for hydrangeifolin I (**1**) and hydrangeifolin II (**2**), and NOESY correlations for **2**.

Table 2
Antioxidant activities of phenylpropanoids isolated from leaves of *C. hydrangeifolia* and standard compounds

Compound	DPPH (IC_{50}, μM)
Hydrangeifolin I (**1**)	n.a.[a]
Hydrangeifolin II (**2**)	33.2
1,6-Di-*O*-caffeoyl-β-D-glucopyranose (**3**)	15.0
1-*O*-(*E*)-caffeoyl-β-D-glucopyranose (**4**)	19.2
1-*O*-(*E*)-feruloyl-β-D-glucopyranose (**5**)	35.5
Caffeic acid[b]	13.7
p-Coumaric acid[b]	27.7

[a] n.a., not active.

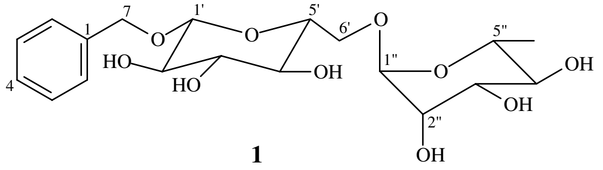
[b] Standard compounds employed as positive controls.

caffeic and *p*-coumaric acids, used as positive controls. As shown on Table 2, compounds **3** and **4** exhibited strong antioxidant activities, comparable with that of caffeic acid, probably due to the presence of the 3,4-dihydroxy (catechol) moiety in their structures. Compounds **2** and **5**, which bear only one free hydroxy group, showed weak free radical scavenging activities towards DPPH and compound **1** was inactive.

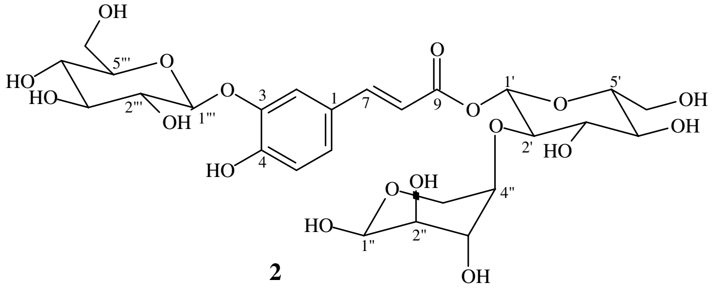
3. Experimental

3.1. General

IR and UV spectra were obtained using Perkin–Elmer model 1600 series FTIR and Lambda 14P UV/VIS spectrometers, respectively. ES-MS were measured on a VG Platform Fisons instrument and HRESIMS on a VG Autospec (40 eV) spectrometer. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) and 2D NMR spectra were obtained using a Varian Inova model 500 spectrometer. The NMR data were measured in pyridine-d_5 or DMSO-d_6 and chemical shifts are expressed in δ (ppm) values with reference to TMS. Preparative HPLC was conducted using a Phenomenex Luna ODS column (250 × 21.20 mm i.d.; 10 μm) coupled to a Varian PrepStar S0-1 pump with flow rate of the mobile phase set at 10 mL/min; and a Varian ProStar 320 UV/VIS detector set at 254 nm. Sephadex LH-20 (Pharmacia), C-18 (Merck) and Si-gel (Merck) were used as stationary phases in CC fractionations. A Varian Star 9090 electrochemical detector (ElCD) composed of a glassy carbon



1



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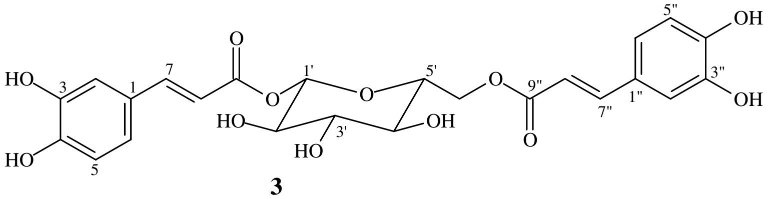

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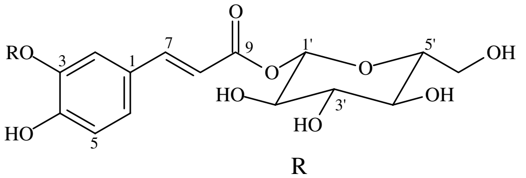

R
4 H
5 Me

working-electrode and an Ag/AgCl reference electrode was employed in the DPPH$^{\cdot}$ assay.

3.2. Plant material

Leaves of *C. hydrangeifolia* were collected in Mogi-Guaçu (São Paulo State, Brazil) in December 1997 and identified and authenticated by Dr. Ines Cordeiro (Instituto de Botânica, São Paulo, SP, Brazil). A voucher specimen was deposited in the Herbarium of the Botanic Garden, São Paulo (number SP 167423).

3.3. Antioxidant assay

The β-carotene test was performed on TLC plates according to a previously described method (Pratt and Miller, 1984;Silva et al., 2001). The stable radical DPPH$^{\cdot}$ was used for the determination of free radical scavenging activity: a methanolic solution (200 μM, 2 mL) of DPPH was added to methanol (1 mL) containing an appropriate amount of each test compound in order to yield a final concentration of 100, 80, 40, 20, 10 or 5 μM. Caffeic and *p*-coumaric acids were used as standard positive controls, whilst a solution of DPPH (200 μM, 2 mL) in methanol (1 mL) served as blank. All samples were evaluated as triplicates ($n = 3$). Each reaction mixture was shaken thoroughly and incubated for 30 min at room temperature in the dark. The amount of the reduced form of the radical (DPPH-H) generated in situ was determined from the measured decrease in current (I) in the electrochemical cell of the ElCD (Son and Lewis, 2002). The area measured on each chromatogram was normalised and compared with that obtained for the blank. The radical scavenging activity of each sample was expressed in terms of IC_{50} (the concentration in μM of test compound required to produce a 50% decrease in I caused by the formation of DPPH-H) (Pauletti et al., 2003).

3.4. Extraction and isolation

Dried and powdered leaves (2.70 kg) of *C. hydrangeifolia* were extracted with EtOH (3.0 L) for 24 h at room temperature, followed by solvent evaporation under reduced pressure to yield the crude extract (65.80 g). This extract was suspended in water (3.0 L) and partitioned with *n*-BuOH (3.0 L): the aqueous solution was evaporated to dryness and the resulting residue (2.0 g) subjected to gel filtration on a Sephadex LH-20 column (3.0 × 120 cm) eluted with MeOH. Fractions (10 mL each) were collected and analysed by TLC ($CHCl_3$/MeOH 6:4). On the basis of their TLC profiles, fractions 6–10 were combined (380.8 mg) and further subjected to over Sephadex LH-20 CC with MeOH as eluent. Fractions 8–12 from this second column fractionation were combined (54.3 mg) on the basis of their TLC profiles and purified by HPLC on an ODS column eluted with MeCN/H_2 O (1:9) to yield **1** (12.0 mg; t_R 12.54 min). Fractions 13–14 from the first Sephadex column were combined (500 mg) and subjected to CC over C-18 eluted with a MeOH/H_2O step gradient (in ratios 1:19, 3:17 and 3:2, successively) to yield fractions A, B and C. Fraction A (132.7 mg) was further purified by HPLC on an ODS column eluted with MeCN/H_2O (7:93) to yield **2** (35.8 mg: t_R 10.25 min). Fraction B (67.5 mg) was subjected to HPLC on an ODS column eluted with MeCN/H_2O (1:9) to yield, **3** (6.1 mg; t_R 22.38 min) and **4** (16.9 mg; t_R 14.64 min). Fraction C (145.9 mg) was purified under the same conditions and afforded **5** (8.4 mg, t_R 18.91 min).

3.4.1. Hydrangeifolin I. 1′-O-benzyl-α-L-rhamnopyranosyl-(1′ → 6′)-β-D-glucopyranoside (*1*)

Amorphous powder, $[\alpha]_D^{30} - 44.3°$ ($c = 0.003$, MeOH). UV λ^{MeOH} 264, 204. IR (film) cm^{-1} 3450, 2920, 1600, 1550, 1460, 1100, 1020. ES-MS $[M+Na]^+$ *m/z* 439. HRESIMS $[M - H]^-$ *m/z* 415.1610 (Calcd. for $C_{19}H_{28}O_{10}$, 415.1604). ^1H and ^{13}C NMR (pyridine-d_5), see Table 1.

3.4.2. Hydrangeifolin II. α-L-xylopyranosyl-(4″ → 2′)-(3-O-β-D-glucopyranosyl)-1′-O-E-caffeoyl-β-D-glucopyranoside (*2*)

Amorphous powder, $[\alpha]_D^{30} + 6.5°$ ($c = 0.0039$, MeOH). UV λ^{MeOH} 282, 209. IR (film) cm^{-1} 3445, 2940, 1650, 1560, 1520, 1098, 1010. ES-MS $[M + Na]^+$ *m/z* 659. HRESIMS $[M - H]^-$ *m/z* 635.1858 (Calcd. for $C_{26}H_{36}O_{19}$, 635.1821). ^1H and ^{13}C NMR (pyridine-d_5), see Table 1.

3.4.3. 1,6-Di-O-caffeoyl-β-D-glucopyranose (*3*)

Amorphous powder, $[\alpha]_D^{30} - 24.6°$ ($c = 0.0058$, MeOH). UV λ^{MeOH} 334, 299, 265, 209. ES-MS $[M+Na]^+$ *m/z* 529. HRESIMS $[M - H]^-$ *m/z* 505.1355 (Calcd. for $C_{24}H_{24}O_{13}$, 505.1345).^{1} H and ^{13}C NMR (pyridine-d_5) (see Table 1).

3.4.4. 1-O-(E)-caffeoyl-β-D-glucopyranose (*4*)

Amorphous powder, $[\alpha]_D^{30} - 16.5°$ ($c = 0.003$, MeOH). UV λ^{MeOH} 332, 300, 264, 211. The IR, ^1H and ^{13}C NMR, and ES-MS data were in agreement with literature values (Birkofer et al., 1961).

3.4.5. 1-O-(E)-feruloyl-β-D-glucopyranose (*5*)

Amorphous powder, $[\alpha]_D^{30} - 13.5°$ ($c = 0.0028$, MeOH). UV λ^{MeOH} 281, 201. IR, ^1H and ^{13}C NMR, and ES-MS data were in agreement with the literature values (Birkofer et al., 1961).

3.4.6. Acid hydrolysis of **2**

A solution of **2** (10 mg) in 2 M HCl (10 mL) was heated at 90 °C for 30 min and the mixture separated by CC over Si-gel eluted with 15–70% MeOH to yield caffeic acid (3.5 mg), glucose and xylose. The sugars were identified by TLC using authentic standards for comparison.

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