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Article

A New Flavonoid Derivative from Leaves of *Oxandra sessiliflora* R. E. Fries

Elcilene A. de Sousa,[a] Armenio A. C. A. da Silva,[a] Alberto J. Cavalheiro,[b] João Henrique G. Lago[c] and Mariana H. Chaves*,[a]

[a]*Departamento de Química, Universidade Federal do Piauí, 64049-550 Teresina-PI, Brazil*

[b]*Instituto de Química, Universidade Estadual Paulista, CP 355, 14800-900 Araraquara-SP, Brazil*

[c]*Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, 09972-270 Diadema-SP, Brazil*

A fração em acetato de etila (EtOAc) obtida a partir da partição do extrato de etanol (EtOH) das folhas de *O. sessiliflora* R. E. Fries (Annonaceae) foi submetida a diversos procedimentos cromatográficos, incluindo cromatografia líquida de alta eficiência (HPLC), o que resultou no isolamento dos flavonóides: quercetina-3-O-α-L-ramnopiranosil-(1→4)-β-D-glucopiranosídeo (**1**), inédito na literatura, canferol-3-O-α-L-ramnopiranosil-(1→4)-β-D-glucopiranosídeo (**2**), rutina (**3**) e canferol-3-O-rutinosídeo (**4**). As estruturas foram definidas através da análise dos espectros de ressonância magnética nuclear (NMR) de ^{1}H e de ^{13}C (1D e 2D) e espectrometria de massas.

The ethyl acetate (EtOAc) phase obtained from the partition of the ethanol (EtOH) extract from leaves of *O. sessiliflora* R. E. Fries (Annonaceae) was subjected to several chromatographic steps, including high efficiency liquid chromatography (HPLC), to afford the flavonoids: quercetin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (**1**), unprecedented in the literature, kaempferol-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (**2**), rutin (**3**), and kaempferol-3-O-rutinoside (**4**). The structures were elucidated by analysis of their ^{1}H and ^{13}C nuclear magnetic resonance (NMR) (1D and 2D) spectra and mass spectrometry.

Keywords: *Oxandra sessiliflora,* flavonoids, quercetin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside

Introduction

The genus *Oxandra* (Annonaceae) consists of about 22 species, 14 of these being found in Brazil and distributed in North, Northeast, Midwest and Southeast regions.[1,2] This genus has native origin and phytogeographic domains in the Amazon, Caatinga, Cerrado, and Atlantic Forest.[3] There are few articles reporting the chemical composition and pharmacological activity of plants of the genus *Oxandra*. Alkaloids, triterpenes, monoterpenes, and steroids with anti-inflammatory and antioxidant activities were isolated from *O. xylopioides*,[4-12] while trypanocidal and antileishmanial monoterpenes have been reported from *O. espintana*.[13] Additionally, alkaloids, sesquiterpenes and triterpenes have been isolated from *O. asbeckii*.[14]

Oxandra sessiliflora R. E. Fries, popularly known as "conduru-preto",[3,15,16] is a species endemic to Brazil in which only the chemical composition of essential oil from leaves have previously been reported in the literature.[17] In continuation with our studies on *O. sessiliflora*, the present work describes the isolation and characterization of four flavonoids from ethyl acetate (EtOAc) phase from ethanol (EtOH) extract from leaves: quercetin-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**1**), kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**2**), quercetin-3-O-β-D-glucopyranosyl-(1→6)-α-L-rhamnopyranoside (rutin, **3**), and kaempferol-3-O-β-D-glucopyranosyl-(1→6)-α-L-rhamnopyranoside (**4**). The structures of the isolated compounds were established based on the analysis of their ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra, including HMQC, HMBC and COSY experiments, and comparison with literature data. This is the first occurrence of flavonoid **1** and assignment of ^{13}C NMR data of flavonoid **2**.

*e-mail: mariana@ufpi.edu.br

Results and Discussion

The EtOH extract of the leaves of *O. sessiliflora* was partitioned between MeOH:H_2O 2:1 and hexane, CH_2Cl_2 and EtOAc successively. The EtOAc fraction was subjected to column chromatography on reverse phase (C_{18}) and Sephadex LH-20, followed by purification of the obtained fractions by high performance liquid chromatography (HPLC) to afford compounds 1-4 (Figure 1).

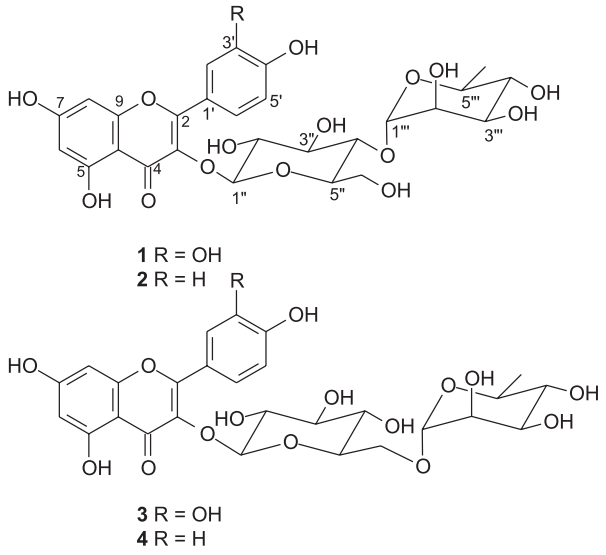

Figure 1. Structures of isolated flavonoids from *Oxandra sessiliflora* R. E. Fries.

The ^{1}H NMR spectrum of compound **1** showed five signals in the aromatic hydrogen region, consistent with the replacement pattern of the flavonol quercetin: two broad singlets at δ_H 6.21/6.40, assigned to H-6/H-8, two doublets at δ_H 6.88 (d, 1H, *J* 8.0 Hz, H-5') and 7.58 (d, 1H, *J* 8.0 Hz, H-6') as well as one broad singlet at δ_H 7.71, assigned to H-2'. This spectrum displayed also signals at δ_H 3.20-3.72 (H-2''-H-6''), which in association to the presence of one doublet at δ_H 5.24 (d, 1H, *J* 7.5 Hz, H-1''), assigned to the anomeric hydrogen *trans*-diaxial position with H-2, characterize the β-D-glucoside unit. In this spectrum was also observed a broad singlet at δ_H 5.22 (H-1''') assigned to an anomeric di-equatorial hydrogen, which, associated to the doublet at δ_H 1.25 (d, 3H, *J* 6.0 Hz, H-6''') suggests the presence of α-L-rhamnose.[18]

The negative HRESIMS of **1** revealed a pseudo-molecular ion at *m/z* 609.1411 $[M-H]^-$, consistent with the molecular formula $C_{27}H_{30}O_{16}$. ^{13}C NMR spectra, including DEPT 90° and 135°, displayed 27 carbon signals being one methyl, one methylene, 15 methyne and 10 non-hydrogenated carbons. Oxymethine carbon signals ranging from δ_C 84 to 69, mainly those at δ_C 62.5 (C-6''), 17.9 (C-6'''), 102.7 (C-1''') and 104.3 (C-1''), confirmed the presence of glucose and rhamnose in the molecule of **1**.[18,19]

Hydrogen signals of each sugar unit were assigned by analysis of the 1D TOCSY spectrum. Irradiation of the anomeric hydrogen from rhamnose (δ_H 5.22, H-1''') allowed the attribution of signals at δ_H 3.99 (H-2''/H-5'''), 3.72 (H-3'''), 3.41 (H-4''') and 1.25 (H-6''') to rhamnose unit and those at δ_H 3.59 (H-2''/ H-4''/ H-6''a), 3.25 (H-3''), 3.41 (H-5''), and 3.72 (H-6'' b) to glucose unit (Table 1). HMQC, HMBC and DQF-COSY spectra displayed important correlations between hydrogens and carbons of **1** (Figure 2), mainly that of H-1'' (δ_H 5.24) with C-3 (δ_C 135.6), which showed that glucose is linked to the aglycone at C-3, and that of H-1''' (δ_H 5.22) with C-4'' (δ_C 84.4), which indicated that rhamnose is linked at C-4 of glucose. Therefore, analysis of the obtained data was consistent with the new structure quercetin-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**1**).

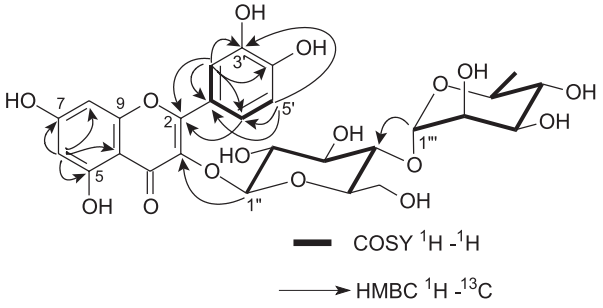

Figure 2. HMBC and COSY correlations in the structure of **1**.

The ^{1}H NMR spectrum of **2** showed similarities to that recorded to flavonoid **1**, with two broad singlets at δ_H 6.19/6.38, assigned to H-6/H-8 of ring A. This spectrum displayed also signals at range from δ_H 3.20 to 5.21 (oxymethine hydrogens) and one doublet at δ_H 1.24 (*J* = 6.0 Hz), suggesting the presence of rhamnose in the molecule. The signals superimposed at δ_H 5.21 (2H) have been assigned to the anomeric protons H-1'' and H-1'''. The main observed difference in the ^{1}H NMR spectrum of **2** is associated to the substitution pattern of kaempferol (1,4-disubstituted B ring), due to the presence of two doublets at δ_H 6.88 and 8.03 (d, *J* = 8.0 Hz) integrated to two hydrogens each and thus assigned to H-3'/H-5' and H-2'/H-6', respectively. ^{13}C NMR spectra of **2**, including DEPT 90° and 135°, showed one carbonyl carbon signal at δ_C 179.4 (C-4) and aromatic carbon signals at range δ_C 166-95, to confirm the kaempferol aglycone moiety. Oxygenated carbons at range δ_C 84-70, mainly methylene carbons and methyl at δ_C 62.5 (C-6'') and 17.9 (C-6'''), respectively, as well as anomeric carbons at δ_C 104.2 (C-1'')

Table 1. NMR data of compounds **1** and **2** (500 MHz and 125 MHz, δ in ppm, J in Hz, CD_3OD)

C	**1**					**2**
	δ_c	δ_H	HMBC		COSY	δ_c
			$^2J_{CH}$	$^3J_{CH}$	^{1}H-^{1}H	
2	158.5	–	–	H-2', H-6'	–	158.4
3	135.6	–	–	H-1''	–	135.5
4	179.5	–	–	–	–	179.4
5	163.0	–	H-6	–	–	162.9
6	100.0	6.21 (br s)	–	–	–	100.0
7	166.1	–	H-6	–	–	166.1
8	94.8	6.40 (br s)	–	H-6	–	94.8
9	159.0	–	–	–	–	159.4
10	105.6	–	–	H-6	–	105.6
1'	123.0	–	H-2'	H-5'	–	122.7
2'	117.6	7.71 (br s)	–	H-6'	–	132.3
3'	145.9	–	H-2'	H-5'	–	116.1
4'	149.9	–	–	H-2', H-6'	–	161.6
5'	116.0	6.88 (d, *J* 8.0)	H-6'	–	H-6'	116.1
6'	123.2	7.58 (d, *J* 8.0)	H-5'	H-2'	H-2', H-5'	132.3
Glucose[a]						
1''	104.3	5.24 (d, *J* 7.5)	H-2''	–	H-2''	104.2
2''	76.2	3.59 (m)	–	H-4''	H-3''	76.2
3''	78.3	3.25 (m)	–	H-5''	H-4''	78.2
4''	84.4	3.59 (m)	H-5''	H-2'', H-6'', H-1'''	H-5''	84.3
5''	69.8	3.41 (m)	H-5''	H-3'', H-1''	H-4''	69.9
6''	62.5	3.59/3.72 (m)	–	–	–	62.5
Rhamnose[a]						
1'''	102.7	5.22 (s)	–	H-4''	H-2'''	102.7
2'''	72.3	3.99 (m)	–	H-4'''	H-3'''	72.3
3'''	72.3	3.72 (m)	H-2'''	H-5'''	H-4'''	72.3
4'''	74.0	3.41 (m)	H-3''', H-5'''	H-2'''	H-5'''	74.0
5'''	70.1	3.99 (m)	H-4'''	H-1'''	H-6'''	70.1
6'''	17.9	1.25 (d, *J* 6.0)	–	H-4'''	H-5'''	17.9

[a]Assignments were based in analysis of TOCSY 1D NMR spectra.

and 102.7 (C-1'''), confirming the presence of glucose and rhamnose. These information, associated with literature data for flavonoids with the same aglycone,[19] allowed the identification of **2** as kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, previously isolated from *Acacia pennata* Willd (Mimosaceae).[20] However, this is the first occurrence in Annonaceae family and the first description of its assigned ^{13}C NMR data.

The structures of flavonoids **3** and **4** were identified by analysis of ^{1}H and ^{13}C NMR as well as HRESIMS and comparison with data described in the literature.[18,19]

Conclusion

This study contributed to the expansion of the chemical constituents of the *Oxandra* genus since the compound kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**2**) is being described for the first time in Annonaceae while quercetin-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**1**) is a new compound.

Experimental

General procedures

^{1}H and ^{13}C NMR spectra were obtained on Varian spectrometer-model INOVA, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C using CD_3OD as a solvent and tetramethylsilane (TMS) as internal reference. HRESIMS spectrum (negative mode) was recorded on a Bruker Daltonics UltrOTOFq-ESI-TOF spectrometer. Silica gel (70-230 mesh, Merck) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC), whereas silica gel 60 GF_{254} was employed for analytical

thin layer chromatography (TLC) (0.50 mm). HPLC analyses were performed on Varian Pro Star with ternary system pumps Model 240, UV-Vis Diode Array Detector (DAD) model 330 and injector model 410 (analytical), and Varian Star Model Prep SD-1 with UV-Vis detector model 320, manual injector Rheodyne model 7725i with sample loop of 2.5 mL (preparative). Phenomenex Gemini C-18 columns (250 × 4.6 mm, 5 μm and 250 × 21 mm, 10 μm) were used to these analyses. Solvents and reagents used were of analytical purity grade and HPLC.

Plant material

The leaves of *O. sessiliflora* were collected in the Environmental Park of Teresina-PI, in June 2009. The species was identified by Professor Roseli Farias Melo Barros and a voucher specimen with number TEPB 27870 was deposited in the Herbarium Graziela Barroso do Amaral (UFPI).

Extraction and isolation

The leaves of *O. sessiliflora* were dried at room temperature and then grinded. The obtained material (779 g) was subjected to exhaustive maceration with EtOH at room temperature. After concentration on reduced pressure, 109 g of EtOH extract were obtained (14%). Part of the EtOH extract (86 g) was suspended in MeOH-H_2O (2:1) and extracted with hexane, CH_2Cl_2 and EtOAc successively to afford 21 g (24%), 30 g (35%) and 14 g (17%) of organic phases, respectively.

Part of the EtOAc phase (3.5 g) was suspended in 10 mL of H_2O-MeOH 1:1 and the soluble portion was applied in a Stracta column (C_{18}, 10 g), which was eluted with MeOH:H_2O 1:1, MeOH and chloroform ($CHCl_3$) successively. The fraction eluted with MeOH-H_2O 1:1 (FA1; 1380 mg) was chromatographed on Sephadex LH-20 eluted with MeOH to afford 5 groups (A-E). Group D (345 mg) was analyzed by reverse phase HPLC-UV DAD eluted with exploratory gradient H_2O + 0.2% AcOH-MeOH (5% → 100%; 200-600 nm, 1 mL min^{-1}; 50 min) and then subject to a isocratic elution mode. The improved separation of the constituents was achieved with the mobile phase (MeOH-ACN 1:1) / (H_2O + 0.2% AcOH) (3:7), resulting in the isolation of flavonoids **1** (20 mg), **2** (21 mg) **3** (11 mg) and **4** (8 mg).

Quercetin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (**1**)

Yellow amorphous solid; HRESIMS: 609.1411 $[M-H]^-$ (calculated to $C_{27}H_{29}O_{16}$: 609.1455); NMR data: see Table 1.

Kaempferol-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (**2**)

Yellow amorphous solid; ^1H NMR (CD_3OD, 500 MHz) δ 6.19 (br s, H-6), 6.38 (br s, H-8), 8.03 (d, *J* 8.0 Hz, H-2'/H-6''), 6.88 (d, *J* 8.0 Hz, H-3'/H-5'), 5.21 (d, *J* 7.5 Hz, H-1''), 5.21 (br s, H-1'''), 1.24 (d, *J* 6.0 Hz, H-6'''), 3.20-4.00 (H-2'' to H-6'', H-2''' to H-5'''); ^{13}C NMR: see Table 1.

Quercetin 3-O-β-D-glycopyranosil-(6→1)-α-L-rhamnopyranoside (rutin, **3**)

Yellow amorphous solid; HRESIMS: 609.1616 $[M-H]^-$ (calculated to $C_{27}H_{29}O_{16}$: 609.1455) and 301.0851 [M-glucose unit]$^-$; ^1H NMR (CD_3OD, 500 MHz) δ 6.21 (d, *J* 2.0 Hz, H-6), 6.40 (d, *J* 2.0 Hz, H-8), 7.66 (d, *J* 2.0 Hz, H-2'), 6.86 (d, *J* 8.5 Hz, H-5'), 7.60 (dd, *J* 8.5 and 2.0 Hz, H-6'), 5.11 (d, *J* 7.5 Hz, H-1''), 4.52 (d, *J* 1.5 Hz, H1'''), 1.18 (d, *J* 6.0 Hz, H-6'''), 3.20-3.90 (H-2'' to H-6'', H-2''' to H-5'''); ^{13}C NMR (CD_3OD, 125 MHz) δ 158.5 (C-2), 135.9 (C-3), 179.5 (C-4), 163.0 (C-5), 100.0 (C-6), 166.1 (C-7), 94.9 (C-8), 159.0 (C-9), 105.6 (C-10), 123.0 (C-1'), 117.9 (C-2'), 145.8 (C-3'), 150.0 (C-4'), 116.1 (C-5'), 123.6 (C-6'), 104.7 (C-1''), 75.7 (C-2''), 77.2 (C-3''), 71.4 (C-4''), 78.2 (C-5''), 68.6 (C-6''), 102.4 (C-1'''), 72.1 (C-2'''), 72.3 (C-3'''), 73.1 (C-4'''), 69.7 (C-5'''), 18.0 (C-6''').

Kaempferol-3-O-rutinoside (**4**)

Yellow amorphous solid; HRESIMS: 593.1639, $[M-H]^-$ (calculated to $C_{27}H_{29}O_{15}$: 593.1506), 284.0652 [M-glucose unit]$^-$; ^1H NMR (CD_3OD, 500 MHz) δ 6.21 (br s, H-6), 6.40 (br s, H-8), 8.06 (d, *J* 9.0 Hz, H-2'/H-6'), 6.90 (d, *J* 9.0 Hz, H-3'/H5'), 5.11 (d, *J* 7.5 Hz, H-1''), 4.52 (br s, H-1'''), 1.12 (d, *J* 6.0 Hz, H-6'''), 3.27-3.80 (H-2'' to H-6'', H-2''' to H-5'''); ^{13}C NMR (CD_3OD, 125 MHz) δ 158.7 (C-2), 135.5 (C-3), 179.4 (C-4), 163.1 (C-5), 100.0 (C-6), 166.2 (C-7), 95.0 (C-8), 159.4 (C-9), 105.6 (C-10), 122.8 (C-1'), 132.4 (C-2'/C-6'), 116.2 (C-3'/C-5'), 161.5 (C-4'), 104.6 (C-1''), 76.8 (C-2''), 78.2 (C-3''), 71.5 (C-4''), 77.2 (C-5''), 68.6 (C-6''), 102.4 (C-1'''), 72.1 (C-2'''), 72.3 (C-3'''), 74.0 (C-4'''), 69.7 (C-5'''), 17.9 (C-6''').

Supplementary Information

Supplementary information (NMR and LRESIMS for compounds **1**-**4**) is available free of charge at http://jbcs.sbq.org.br as PDF file. (Figures S1 to S26).

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