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Chemical constituents of *Distictella elongata* (Vahl) Urb. (Bignoniaceae)

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ABSTRACT

Pectolinarin, a flavone heteroside, was isolated from *Distictella elongata* (Vahl) Urb. leaves ethanol extract, along with a mixture of ursolic, pomolic and oleanolic acids, besides β -sitosterol. Their structures were established on the basis of spectral analysis (¹H and ¹³C NMR, ¹D and ²D) and they were compared with literature. This is the first report on the occurrence of this flavonoid in a species of the Bignoniaceae family.

Key words: Bignoniaceae, *Distictella elongata*, pectolinarin, triterpenes.

INTRODUCTION

Bignoniaceae is a large and diverse clade of neotropical lianas. The group is widely distributed in the neotropics, occurring in Central America, Amazonia, the Atlantic forests of eastern Brazil, and the open dry forests and savannahs of Argentina, Bolivia, Brazil, and Paraguay. Bignoniaceae contains all the lianas of the Brazilian Bignoniaceae and most of the species (approximately 250 of the 350) are in the four large genera, *Arrabidaea*, *Adenocalymma*, *Anemopaegma* and *Memora* (Lohmann 2006). *Distictella* Kuntze is a genus of 18 species in this tribe. The species are lianas or, less frequently, shrubs (Pool 2009). *Distictella elongata* (Vahl) Urb. appears as *Distictis elongata* Bureau & K. Schum. in the FLORA BRASILIENSIS and its actual name is *Amphilophium elongatum* (Vahl) L.G. Lohmann (Cipriani et al. 2007, Bedir et al. 2009, Lohmann 2010).

Bignoniaceae is characterized by the presence of terpenoids, flavonoids, alkaloids, and special aromatic derivatives of the shikimic acid pathway (Cipriani et al. 2007). A β -lapachone derivative was also previously obtained from the roots of *D. elongata* (Bedir et al. 2009).

In the present study, leaves ethanol extract (LEE) from *D. elongata* were phytochemically investigated affording the flavonoid di-O-glycoside pectolinarin (1) along with a mixture of the triterpenoids: ursolic, pomolic and oleanolic acids and β -sitosterol.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

Optical rotation was measured on a Bellingham + Stanley Ltda ADP 220 polarimeter, whereas Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer. Melting

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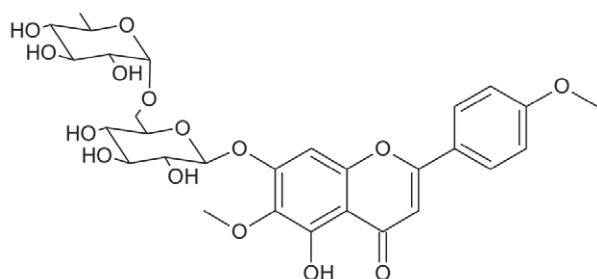


Fig. 1 - Structure of pectolinarin.

point was determined on an electrothermal digital apparatus (model MQAPF-30; Microquímica, Brazil), without correction. UV spectra were measured in a UV-2900 UV-VIS recording spectrophotometer (HITACHI, Japan). NMR spectra were obtained in DMSO- d_6 with TMS as internal standard and they were recorded on a Bruker Avance DRX-400 equipment, (DQ/ICEX/UFGM). The MS system used was a quadrupole time-of-flight instrument (UltrTOF-Q, Bruker Daltonics, Billerica, MA, U.S.A) equipped with an ESI positive and negative ion source. The analyses were performed with the mass spectrometer in positive mode. The following settings were applied throughout the analyses: capillary voltage 4500 V; dry gas temperature 150 °C; dry gas flow 4 L/min.; nebulizer gas nitrogen.

PLANT MATERIAL

The leaves of *Distictella elongata* (Vahl) Urb. were collected in 2008 in the UFMG Campus Pampulha, Belo Horizonte, Minas Gerais State, Brazil. The species was identified by Dr. Lúcia Lohmann, Universidade de São Paula (USP), São Paulo, SP, and a voucher sample is deposited at the BHCB, UFMG, Belo Horizonte, Brazil, under the number 21.862.

EXTRACTION AND ISOLATION

Dried, powdered leaves (481 g) of *D. elongata* were extracted by percolation with EtOH 95%. After removing the solvent by evaporation under

reduced pressure at 40°C, the leaves ethanol extract (LEE) (130.6 g; 27.2%) was obtained. Fractionation of LEE (7.4 g) was carried out by column chromatography (CC) on silica gel (740 g, 70-320 mesh - Merck®) eluting with *n*-hexane, *n*-hexane- CH_2Cl_2 (1:1), CH_2Cl_2 , CH_2Cl_2 -EtOAc (1:1), EtOAc, EtOAc-MeOH (1:1), MeOH and H_2O successively. The EtOAc fraction (500 mg) was further chromatographed over a flash silica gel column chromatography (15 g, 200-300 mesh - Merck®) eluted with *n*-hexane, *n*-hexane-EtOAc (1.7:0.3; 7:3; 1:1), and EtOAc. The *n*-hexane-EtOAc 7:3 fraction 21-35 (26 mg) was identified by TLC, 1H and ^{13}C NMR as β -sitosterol in comparison with literature (Castilho and Kaplan 2008). The *n*-hexane-EtOAc 7:3 fraction 51-73 (99 mg) was submitted to preparative TLC using CH_2Cl_2 -EtOAc (3:2) as eluent affording a mixture (17 mg) of ursolic, pomolic and oleanolic acids that were identified by 1H and ^{13}C NMR. A portion of the EtOAc:MeOH (1:1) fraction (230 mg) was further purified by preparative reverse phase HPLC separation to give the flavone pectolinarin (17 mg).

Pectolinarin (1). Pale yellow amorphous powder; mp 248-351 °C; $[\alpha]_D^{22.4}$ (MeOH, 0.5 mg/mL): -0.06; UV λ_{max} nm (MeOH): 273.5, 328.5, λ_{max} nm (MeOH + NaOMe): 295, 370.5; λ_{max} nm (MeOH + NaOAc): 273.5, (321-*sh*); λ_{max} nm (MeOH + $AlCl_3$): 349, 298, 281.5; (MeOH + $AlCl_3$ + HCl): 348, 298, 281.5; IR ν_{max} (cm^{-1}): 3353 (OH), 1656 (C=O), 1608, 1583 (aromatic C=C), 1068 (C-O); ESI-MS m/z : 623 $[M + H]^+$, 645 $[M + Na]^+$, 661 $[M + K]^+$.

PREPARATIVE HPLC

Pectolinarin was purified on a Shimadzu HPLC system (Japan) composed of pump LC-8A, UV-Vis detector SPD-GAV, controller system SCL-8A and integrator C-R4A. An ODS column (250 × 20 mm I.D., 10 mm; Shimadzu, Japan) was employed at room temperature, at a flow rate of 4.0 mL/min and UV220 detection. The mobile phase was consisted

of H₂O (solvent A) and MeOH (solvent B). The following segmented gradient was used: A-B (95:5, v/v) to A-B (40:60, v/v), in 2 min; A-B (40:60, v/v) to A-B (0:100, v/v), in 40 min; A-B (0:100, v/v) to A-B (95:5, v/v) in 20 min. HPLC grade solvents (Tédia, Brazil) were used and were degassed by sonication before use. Samples were dissolved in MeOH, in an ultrasonic bath for 20 min (25 mg/mL). After filtration through 0.45 µm membrane syringe filter, the sample solutions (200 µL) were injected into the apparatus.

RP-HPLC-DAD PROFILE

RP-HPLC-DAD analyses were carried out on a Waters alliance 2695 HPLC system composed of a quaternary pump, an auto sampler, a photodiode array detector (DAD) 2996 and a Waters Empower pro data handling system (Waters Corporation, Milford, USA). An ODS column (125 x 4.0 mm i.d., 5 mm; Merck, Darmstadt, Germany) was employed for the analysis. The profiles were performed employing a linear gradient of H₂O (A) and MeOH (B), A-B (95:5, v/v) to A-B (5:95, v/v) in 60 min; followed by 5 min of isocratic elution of A-B (5:95, v/v), at a temperature of 40°C and flow rate of 1.0 mL/min. The injection volume was 20 µL. The chromatograms were obtained at λ 220 nm UV spectra from λ 220 to 400 nm were recorded online. Samples were dissolved in MeOH, in ultrasonic bath for 20 min, and then filtrated at 0.45 µm membrane syringe filter, giving a final concentration of 10 mg/mL for extract and fractions, and 4 mg/mL for the isolated substance.

TLC ANALYSES

TLC plates (Merck, Silica gel 60 F254 – 0.25 mm); plates spots were detected under UV light (λ 365 nm), after spraying with anisaldehyde-H₂SO₄ reagent (general), Liebermann-Burchard reagent (terpenoids and steroids) or aluminium chloride (flavonoids) followed by heating.

HYDROLYSIS

The isolated flavone (2 mg) was hydrolysed in 2 M HCl in 20% aqueous methanol, by heating in a water bath at 80°C for 4 h (Hertog et al. 1992). The final solution was extracted with CH₂Cl₂. The aqueous layer was used for identification of the sugars by comparison with standards (β-D-glucose, α-L-rhamnose, β-D-galactose and α-L-arabinose; Sigma-Aldrich) in TLC with CHCl₃-MeOH-H₂O (70:30:4) as mobile phase and anisaldehyde-H₂SO₄ reagent as spraying reagent.

RESULTS AND DISCUSSION

The RP-HPLC profile of the LEE from *D. elongata* has shown the major peaks in the range of 19.0 to 31.0 min. The UV spectra registered online are characteristic of cinnamoyl derivatives: peaks with Rt 19.6 to 20.5 min; and flavones: peaks with Rt 26.5 to 31.0 min (Mabry et al. 1970). TLC phytochemical screening carried out for LEE indicated the presence of phenols, including flavonoids, terpenoids and/or steroids (Wagner et al. 1984).

Fractionation of LEE by column chromatography (CC) on silica gel yielded eight fractions. TLC analysis of the EtOAc fraction has shown mainly the presence of terpenoids and/or steroids. It was further chromatographed over a flash silica gel chromatography column to give 17 mg of a mixture of triterpenoids characterized by ¹H and ¹³C NMR spectroscopy.

The ¹H NMR spectrum showed many signals at region δ 0.66-2.00 ppm corresponding to methyl and methylene hydrogens. The signal at δ 5.13 was attributed to the olefin hydrogen bonded to C-12 of ursane/oleanane triterpenoids (Castilho and Kaplan 2008). ¹³C NMR spectra have shown the chemical shift of the carbinol and olefin carbons that led to the identification of the three terpenoids: ursolic, pomolic and oleanolic acids (Mahato and Kundu 1994).

TLC analysis of the EtOAc:MeOH (1:1) fraction has shown that it contained phenols only (Wagner et al. 1984). RP-HPLC-DAD analyses of the EtOAc and EtOAc:MeOH (1:1) fractions have shown a peak with retention time of approximately 31.0 min. The substance corresponding to this peak was isolated from the fraction EtOAc:MeOH (1:1), by preparative RP-HPLC, and was identified by spectrometric analyses.

The UV spectrum of the isolated substance was typical of a flavone (λ_{max} 273.5 and 328.5 nm). The presence of a free hydroxy group at C-5 and the absence of free hydroxy at C-4' and C-7 was indicated by the effects of AlCl_3 and NaOAc in the UV curve. The difference in band I in the methanol spectra (λ 328.5 nm) and after addition of NaOMe (λ 370.5 nm) is 42 nm with a decrease in intensity, indicating the absence of free OH at C-7 and C-4'. No modification of the UV curve, relatively to the AlCl_3 curve, after addition of HCl, indicated the presence of a free 5-hydroxyl group (Mabry et al. 1970, Markham 1982).

The 1D ^1H NMR spectrum showed an AA'XX' system of spins at δ 8.04 and 7.17 ppm (2H each, J = 8.0 Hz, 2' and 6'; 3' and 5', respectively) due to hydrogens in a 4'-oxygenated B ring. The doublets at 4.57 ppm (J = 1.1 Hz, H-1'') and δ 5.12 ppm (J = 4.00 Hz, H-1'') are typical of anomeric hydrogens. The singlet at δ 12.96 ppm for the hydroxy proton indicate the formation of a hydrogen bond with the neighboring oxygen. H-8 and H-3 appeared as singlets at δ 6.94 and 6.93 ppm, respectively. The doublet at δ 1.06 (3H, J = 4.0 Hz, H-6'') was related to a rhamnose methyl group. Two methoxy singlets at δ 3.78 (4'-OCH₃) and 3.97 ppm (6-OCH₃) were observed and they were confirmed by the 1D ^{13}C NMR signals at δ 55.6 (4'-OCH₃) and δ 60.3 ppm (6-OCH₃). Nine signals were observed in the ^{13}C NMR in the range of δ 66.0-76.5 ppm, corresponding to two sugar units: D-glucose and L-rhamnose as confirmed by 2D HMBC and HSQC-TOCSY experiments. The methyl group of rhamnose gave

a signal at δ 17.8 ppm in the ^{13}C spectrum. Acid hydrolysis of the isolated substance yielded an aglycone, pectolarigenin, besides D-glucose and L-rhamnose which were confirmed by TLC. According to the coupling constant observed in the ^1H NMR spectrum, the configurations at the anomeric carbons of D-glucose and L-rhamnose were determined as β - (J = 1.1 Hz) and α -linkages (J = 4.00 Hz), respectively.

The chemical shifts of the carbon signals for D-glucose and L-rhamnose, indicated L-rhamnose as the terminal sugar. The C-6 glucose signal at δ 66.0 ppm indicated that the disaccharide should be rutinose (Moccelini et al. 2009). The DEPT experiment confirmed this signal for a methylene group. The linkage of the rutosyl moiety to the oxygen atom attached to C-7 was confirmed by the HMBC correlation between the glucosyl H-1 (δ 5.12) and C-7 (δ 157.3). The ^{13}C NMR data also supported the attachment of the rutosyl moiety to the oxygen at the 7-position of a flavone (Table I and Figure 1).

Direct correlations observed in the 2D HMBC maps confirmed the identification of this compound as pectolarin (Figure 2). H-3 signal (δ 6.93 ppm) is recognized by 2J coupling with C-2 and C-4 (δ 164.3 and 182.9 ppm, respectively) and by 3J coupling with C-10 and C-1' (δ 105.4 and 122.8 ppm, respectively). H-8 (δ 6.94 ppm) is determined by 2J coupling with C-7 and C-9 (δ 157.3, 152.9 ppm, respectively), 3J with C-6 (δ 133.2 ppm) and 4J with C-5 (δ 152.7) while H-6' H-2' (δ 8.04 ppm) showed 3J coupling with C-2 and C-4' (δ 164.3, 162.8, respectively). Similarly, H-3' H-5' (δ 7.17 ppm) showed 2J coupling with C-4' (δ 162.8) and 3J with C-1' (δ 122.8). The direct coupling between H-2' H-6' and H-3' H-5' was not observed in the HMBC spectrum. The direct correlation between the OCH₃ hydrogens (δ 3.78 ppm) and C-4' (δ 162.8 ppm) confirmed the position of this methoxyl group at C-4'. Similar correlations were observed between the methoxyl group signal at 3.87 ppm

TABLE I
 ^{13}C and ^1H NMR data for pectolinarin in DMSO- d_6 .

Position	Type	δC ppm	δH ppm (M, J)
2	C	164.3	-
3	CH	103.5	6.93 (s)
4	C	182.9	-
5	C	152.7	-
6	C	133.2	-
7	C	157.3	-
8	CH	94.3	6.94 (s)
9	C	152.1	-
10	C	105.4	-
-	-	-	12.96 (s, 5-OH)
1'	C	122.8	-
2'	CH	128.4	8.04 (d, $J = 8.0$ Hz)
3'	CH	114.8	7.17 (d, $J = 8.0$ Hz)
4'	C	162.8	-
5'	CH	114.8	7.17 (d, $J = 8.0$ Hz)
6'	CH	128.4	8.04 (d, $J = 8.0$ Hz)
6-OCH ₃	CH ₃	60.3	3.87 (s)
4'-OCH ₃	CH ₃	55.6	3.78 (s)
1''	CH	100.4	5.12 (d, $J = 4.0$ Hz)
2''	CH	73.2	3.33*
3''	CH	76.5	3.35*
4''	CH	69.5	3.18*
5''	CH	75.8	3.63*
6''	CH ₂	66.0	3.89; 3.48 (dd)
1'''	CH	100.4	4.57 (d, $J = 1.1$ Hz)
2'''	CH	70.8	3.46*
3'''	CH	70.4	3.67*
4'''	CH	72.0	3.16*
5'''	CH	68.3	3.41*
6'''	CH ₃	17.8	1.06 (d, $J = 4.0$ Hz)

M: Multiplicity, J : coupling constant

*Approximate shifts obtained by HSQC experiment.

and C-6 (down field at δ 133.2 ppm) (Figure 1) (Silverstein et al. 2007, Yim et al. 2003).

The ESI-MS spectrum showed an ion peak at m/z 623 $[\text{M} + \text{H}]^+$ and ion peaks at 645 $[\text{M} + \text{Na}]^+$ and 661 $[\text{M} + \text{K}]^+$, which are coherent with the molecular formula $\text{C}_{29}\text{H}_{34}\text{O}_{15}$ for the flavone, pectolinarin.

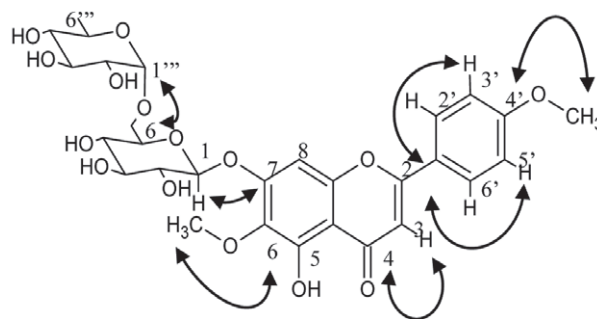


Fig. 2 - Main $^1\text{H} \rightarrow ^{13}\text{C}$ correlations inferred from HMBC pectolinarin spectra.

This is the first report on the occurrence of pectolinarin, a flavone, in the Bignoniaceae family. Earlier studies indicated some bioactivities of this flavone.

The antioxidant potential of seven Korean thistles rich in pectolinarin was evaluated via the peroxynitrite. The DPPH free radical assays exhibiting strong activity (Jeong et al. 2008). Pectolinarin isolated of the leaves of *Cirsium setidens* (Compositae), demonstrated hepatoprotective efficacy in a rat model of hepatic injury caused by D-galactosamine. It was suggested that the activity occurs mainly *via* SOD (superoxide dismutase) antioxidant mechanism (Yoo et al. 2008).

However, in the present study, pectolinarin has not presented any radical scavenging activity on the DPPH assay, although the LEE and the EtOAc:MeOH (1:1) fraction presented antioxidant activity by the DPPH assay (data not show). These results might be indicative of the presence of other phenols in LEE and EtOAc:MeOH (1:1) fraction which would be responsible for their antioxidant activity.

In vivo studies have demonstrated that oral administration of pectolinarin and a fraction rich in pectolinarigenin isolated from aerial parts of *Cirsium chanroenicum* at 20-100 mg/kg in several animal models resulted in inhibitory activities of inflammation/allergy: arachidonic acid-induced mouse ear edema, carrageenan-induced mouse

paw edema and passive cutaneous anaphylaxis. All of these results suggest that pectolinarigenin and pectolinarin possess anti-inflammatory activity and that they may inhibit eicosanoid formation in inflammatory lesions. These activities certainly contribute to the anti-inflammatory effects of *C. chanroenicum* (Lim et al. 2008).

The promising application of pectolinarin in the osteogenesis imperfecta (OI) type I pharmacological therapy was shown by *in vitro* tests. The flavonoid normalized collagen synthesis in OI cells. It was suggested that it exerts its effects through β 1-integrin-mediated signaling (Galicka and Nazarruk 2007).

In vitro studies have demonstrated that leaves ethanol extract of *D. elongata* and pectolinarin isolated from the same species have antiviral activity against vaccinia virus Western Reserve (VACV-WR) and dengue virus 2 (DENV-2) (Simões et al. 2011).

Although no ethnomedical use is reported for *D. elongata*, it might be considered useful as a source of pectolinarin for its disclosed antiviral, anti-inflammatory and collagen inducing synthesis effects.

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RESUMO

Pectolinarina, uma flavona heterosídica, foi isolada do extrato etanólico das folhas de *Distictella elongata* (Vahl) Urb., além de uma mistura de ácidos ursólico, pomólico e oleanólico, além de β -sitosterol. Suas

estruturas foram estabelecidas com base em análise espectral (RMN de ^1H e ^{13}C 1D e 2D) em comparação com a literatura. Esta é a primeira vez em que se relata a ocorrência deste flavonoide em uma espécie da família Bignoniaceae.

Palavras-chave: Bignoniaceae, *Distictella elongata*, pectolinarina, triterpenos.

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