

## Short communication

New glycosylated biscoumarins from *Hymenaea courbaril* L. seeds

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## ABSTRACT

*Hymenaea courbaril* L. seeds are widely used by the population for the treatment of several ailments. Despite its popular use, no other phytochemical studies had been carried out about “Jatobá” seeds. For this reason, this is the first study on the matter and it was carried out with the purpose of finding and identifying the molecules that constitute this seed. This work presented the isolation and elucidation of two new biscoumarins from *H. courbaril* L. seeds. The new compounds: hymenain 7-O-β-glucopyranosyl-(1''' → 2'')-O-α-apiofuranosyl-(1'''' → 2''')-O-α-galactopyranoside (**1**) and hymenain 7-O-β-glucopyranosyl-(1''' → 2'')-O-α-apiofuranoside (**2**). Their structures were elucidated by extensive 1D and 2D NMR experiments, IR and electrospray high resolution mass spectrometry (ESI-HRMS).

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## 1. Introduction

Coumarins represent chemical classes that are present in different plant parts such as roots, flowers and fruits. They are widely distributed in plants, predominantly in angiosperms, where simple structures are the most frequent. Coumarins are biogenetically derived from Shikimic Acid pathways, but a significant number comes from a mixed biosynthetic route (Shikimic Acid and Acetate pathway) (Simões et al., 2007). The *Hymenaea* genus belongs to the family Fabaceae and subfamily Caesalpinioideae. It is an important representative of the Brazilian flora with over 30 species. The *Hymenaea courbaril* L. var. *courbaril* species, popularly known as “Jatobá”, is used as a carminative, astringent and sedative medicinal plant (Martins et al., 2007). The fruit has a hard, dark colored shell, and it contains a hard and woody seed, coated with an ample supply of fresh yellow powder. They are widely consumed by the population due to its powdery pulp, which is rich in fiber, sugar and omega-3-fatty acids (Jayaprakasam et al., 2007). The tree bark has terpenes and phenolic compounds (Sasaki et al., 2009), as demonstrated by several studies, what validates its use in

folk medicine. It is used for stomachache (Marsaioli et al., 1975), treatment of ulcers, diarrhea, inflammation (Orsi et al., 2012), influenza, bronchitis, cough, weakness, intestinal cramping, bladder infections and prostate cancer (Silva, 2006). Despite its popular use, phytochemical studies had been carried out about “Jatobá” seeds. For this reason, this study on the matter, with the purpose of finding and identifying the molecules that constitute this seed. This paper reports the occurrence of two new biscoumarin seeds of *H. courbaril* L. var. *courbaril*: hymenain 7-O-β-glucopyranosyl-(1''' → 2'')-O-α-apiofuranosyl-(1'''' → 2''')-O-α-galactopyranoside (**1**) and hymenain 7-O-β-glucopyranosyl-(1''' → 2'')-O-α-apiofuranoside (**2**) (Fig. 1).

## 2. Results and discussion

2.1. Identification of **1**

Compound **1** was isolated as an amorphous yellow solid (M.p. 226–228 °C) and it was found to have a molecular formula of C<sub>37</sub>H<sub>42</sub>O<sub>22</sub>Na by using HRESIMS spectrum, which showed a molecular ion peak at *m/z* 861.2063 [M+Na]<sup>+</sup> (calcd. for C<sub>37</sub>H<sub>42</sub>O<sub>22</sub>Na, 861.2065). The FTIR spectrum exhibited an absorption stretch at 3300 cm<sup>-1</sup> of the OH group. It has also presented a strong absorption stretch at 1700 cm<sup>-1</sup>, which suggested the

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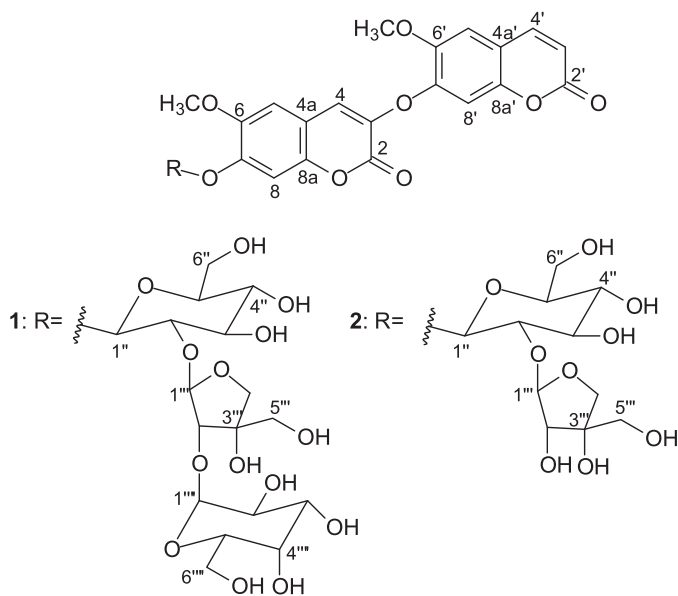


Fig. 1. Biscoumarins isolated from *Hymenaea courbaril* L.

binding C=O, stretching band at  $1050\text{ cm}^{-1}$  and  $1250\text{ cm}^{-1}$  of C–H and C–O, respectively, as well as at  $1500\text{ cm}^{-1}$  representing aromatic moiety C=C.

The structural formula of compound **1** was attributed by NMR analysis and compared to hymenain isolated from *H. courbaril* L. and *Erycibe hainanensis* (Table 1) (Simões et al., 2009; Song et al., 2010).

The  $^1\text{H}$  NMR spectrum showed two methoxyl singlet signals at  $\delta$  3.92 (3H, s) and 3.89 (3H, s) and two doublet signals at  $\delta$  7.97 (1H, d,  $J = 9.6\text{ Hz}$ ) and  $\delta$  6.40 (1H, d,  $J = 9.6\text{ Hz}$ ), which are characteristic *ortho* coupling between H–4' and H–3'. Based on correlations observed in the HSQC contour maps, five singlet signals at  $\delta$  7.43, 7.37, 7.20, 7.14 and 7.15 were attributed to H–4, H–5', H–8', H–8 and H–5 respectively, and five carbon signals at  $\delta$  125.2, 111.6, 104.4, 107.4 and 110.0 were assigned to C–4, C–5', C–8', C–8, and C–5, also respectively.

The HSQC and HMQC spectra, along with comparisons to chemical shifts literature data (Niassy et al., 2004; Santos et al., 2005; Breitmaier and Voelter, 1987) as well as assisted correlations in the glycoside units, allowed the determination of the two coumarin units (Fig. 2). The  $^{13}\text{C}$  NMR spectrum of compound **1** showed two signals at  $\delta$  157.8 and 161.7, that were assigned to the carbons C–2 and C–2', characteristic to the C=O lactone rings. The chemical shift of C–3 and C–7' quaternary carbons at  $\delta$  139.6 and 147.9 suggests that the two units are connected by a coumarin C–O–C bond. The  $^{13}\text{C}$  NMR spectrum also showed signals at  $\delta$  101.0 (C–1''), 109.2 (C–1''') and 92.4 (C–1'''). For that reason, the anomeric carbon was attributed according to the observed HSQC correlation and literature data (Song et al., 2010).

The HSQC correlation from the anomeric protons at H–1'' (5.01 d,  $J = 7.8\text{ Hz}$ ), H–1''' (4.97 d,  $J = 2.8\text{ Hz}$ ) and H–1'''' (5.41 d,  $J = 4.0\text{ Hz}$ ) suggested the three glycosidic units, O– $\beta$ -glucopyranosyl, O– $\alpha$ -apiofuranosyl and O– $\alpha$ -galactopyranosyl. It was possible to confirm their positions and structure based on HMBC contour map correlation, which showed the correlation of anomeric protons H–1', H–1''' and H–1'''' with carbons C–7, C–2' and C–2'''.

The ESI-HRMS  $[\text{M}+\text{Na}]^+$  mass spectrum generated representative fragment of  $m/z$  699.1513  $[\text{M}-162+\text{Na}]^+$ , indicating the loss of galactose (162).

Table 1

$^1\text{H}$  and  $^{13}\text{C}$  (400 and 75 MHz,  $\text{CD}_3\text{OD}$ ) spectroscopic data for **1** and **2** with  $J$  values (in Hertz) in parentheses.

H	1	2	C	1	2
2			2	157.8	158.4
3			3	139.6	139.5
4	7.43 s	7.43 s	4	125.4	125.2
4a			4a	110.8	110.7
5	7.15 s	7.15 s	5	110.0	110.0
6			6	146.4	146.4
7			7	149.1	149.1
8	7.14 s	7.14 s	8	107.4	107.4
8a			8a	147.4	147.4
2'			2'	161.7	161.7
3'	6.40 d (9.6)	6.40 d (9.6)	3'	115.8	115.8
4'	7.97 d (9.6)	7.97 d (9.6)	4'	144.1	144.1
4a'			4a'	113.3	113.3
5'	7.37 s	7.37 s	5'	111.6	111.0
6'			6'	147.6	147.8
7'			7'	147.9	148.9
8'	7.20 s	7.20 s	8'	104.4	104.3
8a'			8a'	149.1	149.1
1''	5.02 d (7.2)	5.01 d (7.8)	1''	101.0	101.0
2''	3.72 m	3.72 m	2''	76.0	76.0
3''	3.57 m	3.57 d (12.0)	3''	73.9	73.9
4''	3.55 m	3.55 d (7.8)	4''	70.5	70.5
5''	3.46 m	3.46 d (14.4)	5''	76.8	76.8
6''	3.63–4.06 m	3.63–4.06 m	6''	67.8	67.8
1'''	4.97 d (2.8)	4.97 d (2.4)	1'''	109.2	109.2
2'''	3.99 m	3.99 m	2'''	77.1	77.1
3'''			3'''	79.3	79.3
4'''	3.80–4.08 m	3.80–4.08 m	4'''	73.5	73.5
5'''	3.58–3.62 m	3.58–3.62 m	5'''	64.6	64.6
1''''	5.41 d (3.6)		1''''	92.4	
2''''	3.42 m		2''''	72.0	
3''''	3.78 m		3''''	82.6	
4''''	3.65 m		4''''	70.2	
5''''	3.56 m		5''''	73.2	
6''''	3.50–3.63 m		6''''	62.1	
6-OCH <sub>3</sub>	3.92 s	3.92 s		56.0	55.9
6'-OCH <sub>3</sub>	3.89 s	3.89 s		56.2	55.9

## 2.2. Identification of 2

Compound **2** was isolated as an amorphous yellow solid, with the following specifications: M.p. 228–230 °C, ESI-HRMS  $m/z$  699.1522  $[\text{M}+\text{Na}]^+$  (calcd. for  $\text{C}_{31}\text{H}_{32}\text{O}_{17}\text{Na}$ , 699.1537). The FTIR absorption indicated the existence of  $3353\text{ cm}^{-1}$  OH,  $1650\text{ cm}^{-1}$  C=O,  $1050\text{ cm}^{-1}$  C–H and  $1250\text{ cm}^{-1}$  C–O stretch bindings, and a  $1500\text{ cm}^{-1}$  C=C aromatic moiety stretch binding band.

The structure of compound **2** was assigned by analysis of NMR data and compared to hymenain isolated from *H. courbaril* L. and *E. hainanensis* (Table 1) (Simões et al., 2009; Song et al., 2010).

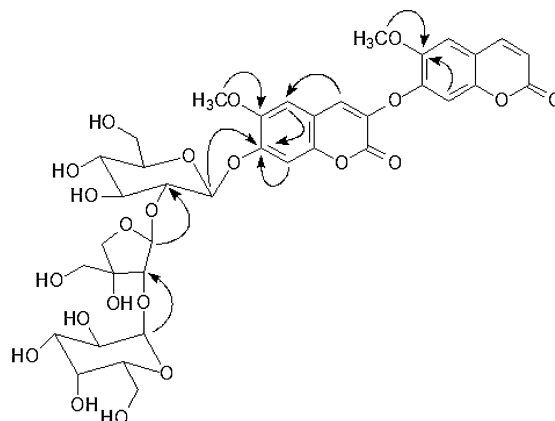


Fig. 2. Important HMBC ( $^1\text{H}$ – $^{13}\text{C}$ ) correlations observed for **1**.

The  $^1\text{H}$  NMR spectrum of compound **2** showed two singlet signals at  $\delta$  3.92 and 3.89, indicative of two methoxyl groups, and two doublet signals at  $\delta$  7.97 (1H, d,  $J = 9.6$  Hz) and 6.40 (1H, d,  $J = 9.6$  Hz), which are characteristic *ortho* coupling between H-4' and H-3'. Five singlet signals at  $\delta$  7.43, 7.37, 7.20, 7.14 and 7.15 were assigned to H-4, H-5', H-8', H-5 and H-8, and five carbon signals at  $\delta$  125.2, 111.0, 104.3, 107.4 and 110.0 were assigned to C-4, C-5', C-8', C-8 and C-5, based on correlations observed in the HSQC contour maps.

The  $^{13}\text{C}$  NMR spectrum showed two signals at  $\delta$  158.4 and 161.7, that were assigned to the carbons C-2 and C-2', characteristic to C=O lactone rings. The chemical shift of quaternary carbons at  $\delta$  139.5 C-3 and at  $\delta$  148.9 C-7', suggested that the two coumarin units are connected by a C–O–C bond. The NOESY 1D spectrum presented peaks relating at  $\delta$  7.15 H-5,  $\delta$  3.92 C-6-OMe,  $\delta$  7.37 H-5' and ( $\delta$  3.89 C-6'-OMe, indicating that a methoxyl was linked to C-6 and another was connected at C-6'.

The TOCSY spectrum was obtained by irradiation on the glucose H-1'' ( $\delta$  5.01, d,  $J = 7.8$  Hz), which exhibited correlations with signals at  $\delta$  3.72, 3.57, 3.55, 3.43, 3.63 and 4.06, assigned to H-2', H-3', H-4', H-5' and H-6' of the O- $\beta$ -glucopyranosyl unit. Irradiation at H-1''' ( $\delta$  4.97, d,  $J = 2.4$  Hz) exhibited a single correlation signal at  $\delta$  3.99 assigned to H-2''' of the O- $\alpha$ -apiofuranosyl unit. The only correlation occurred due to interruption of quaternary carbon (C-3'''), thus confirming apiose unit presence. The assignments of glycosidic units connected to biscoumarins (Fig. 3) were performed using TOCSY, HMQC and HSQC spectra and comparisons to the literature (Niassy et al., 2004; Santos et al., 2005; Breitmaier and Voelter, 1987). The ESI-HRMS  $[\text{M}+\text{Na}]^+$  spectrum revealed representative fragment at  $m/z$  550.6276  $[\text{M}-149+\text{Na}]^+$ , indicating the loss of apiose (149).

### 3. Experimental

#### 3.1. Plant material

Fruits were collected in October 2011 in the Tapaihuna community, located in the municipality of Nova Canaã do Norte, state of Mato Grosso. A voucher specimen (4800) was deposited in the Herbarium of Acervo Biológico da Amazônia Meridional da Universidade Federal de Mato Grosso (ABAM/UFMT). The seeds of the fruits were removed, screened, washed and dried under forced air circulation at 40 °C for seven days and powdered in a Wiley mill, yielding 3.9 kg.

#### 3.2. Solvents

All solvents were purchased from Sigma–Aldrich (St. Louis, MO, USA), and Merck for HPLC analysis. The chromatography column was carried out on silica gel columns (200–300 mesh) and Sephadex LH-20 obtained from Merck (Darmstadt, Germany).

#### 3.3. Extraction and isolation

The dried powdered seed of *H. coubaril* L. (3.9 kg) was extracted with ethyl acetate (EtOAc, 5 L) for 144 h at room temperature followed by extraction with EtOH (5 L) for another 144 h. The two extracts were concentrated to give 140.1 g of dark brown residue of EtOAc extract and 430.6 g of dark brown residue of EtOH extract. The EtOAc crude extract produced 1.6 g of yellow precipitate. A 161.4 mg portion was chromatographed in a Sephadex LH-20 column (20  $\times$  300 mm) and eluted with MeOH to yield 1–10 fractions, where fractions 6 and 8 had their compounds **1** (7.3 mg) and **2** (5.1 mg) isolated.

#### 3.4. Spectral data

NMR experiments were acquired using a 300 MHz Varian ( $^{13}\text{C}$ ), 400 MHz Bruker ( $^1\text{H}$ , HSQC, HMBC and COSY for Compound **1**) and 600 MHz Bruker ( $^1\text{H}$ , NOESY, HMBC, HSQC and TOCSY for Compound **2**). IR spectra were recorded using a Shimadzu, Irtaffinity 1 model (Kyoto, Japan), in KBr disks. ESI-HRMS spectra was carried out using a BRUKER MicroTOF spectrometer, with high-resolution electrospray ionization, performed in the positive mode with 0.180 stream/h, scan range of 160–1600.

### 4. Conclusions

In this study of *H. courbaril* L. var *courbaril* seeds, we isolated and identified two new biscoumarins: hymenain 7-O- $\beta$ -glucopyranosyl-(1'''  $\rightarrow$  2'')-O- $\alpha$ -apiofuranosyl-(1''''  $\rightarrow$  2''')-O- $\alpha$ -galactopyranoside and Hymenain 7-O- $\beta$ -glucopyranosyl-(1'''  $\rightarrow$  2'')-O- $\alpha$ -apiofuranoside, confirmed by 1D and 2D NMR spectra, IR, mass spectrometry and literature data.

#### Author contributions

HPF (PPGQ student) carried out laboratory work as part of her final year research project. VCS, MGC and GMVJ obtained the NMR and MS data and contributed to compound identification. APS and VDGs supervised this project, provided intellectual input and prepared the manuscript. All the authors have read the final manuscript and approved the submission.

#### Conflicts of interest

The authors declare no conflict of interest of disclose.

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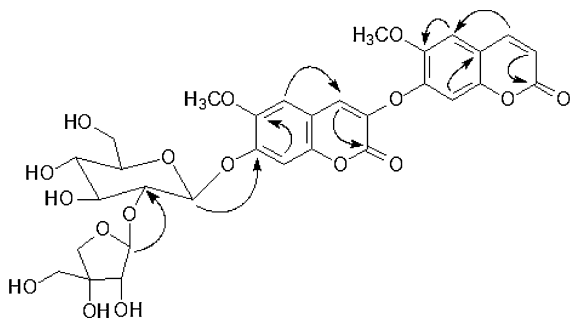


Fig. 3. Important HMBC ( $^1\text{H}$ - $^{13}\text{C}$ ) correlations observed for **2**.

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