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Artigo

FLAVONOIDS AND A NEOLIGNAN GLUCOSIDE FROM *Guarea macrophylla* (MELIACEAE)

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This work describes the phytochemical study of the methanol extract obtained from leaves of *Guarea macrophylla,* leading to the isolation and identification of three flavonoid glycosides (quercetin 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-galactopyranoside, kaempferol 7-*O*-β-D-glucopyranoside) and a neolignan glucoside, dehydrodiconiferyl alcohol-4-β-D-glucoside. All compounds were identified by a combination of spectroscopic methods (^{1}H, 1D, 2D NMR, ^{13}C and UV), ESI-MS and comparison with the literature data. This is the first report of flavonoids in the genus *Guarea* and of a neolignan glucoside in the Meliaceae family.

Keywords: *Guarea macrophylla;* dehydrodiconiferyl alcohol-4-β-D-glucoside; flavonoid glycosides.

INTRODUCTION

In recent years, the Meliaceae family, with nearly 1400 species, has attracted considerable attention as an important source of limonoides and tetranortriterpenoids with insecticidal and antifeedant activities.[1,2] *Guarea* is a distinctive Neotropical member of the Meliaceae family widely distributed in Central and South America.[3] Some species have been used in folk medicine for the treatment of rheumatism,[4] as an emetic and hemostatic remedy.[3] Furthermore, some biological activities, particularly antiprotozoal, have been demonstrated experimentally.[4,5] Several phytochemical studies on the genus *Guarea* have been published and a wide variety of secondary metabolites including coumarins,[4] sesquiterpenes,[6] diterpenes,[4,7] and triterpenes have been identified.[8]

Guarea macrophylla is a tall tree found in the south and southeast of Brazil and in the Amazon region.[8] Previous phytochemical investigations have shown the presence of sesqui-, di- and triterpenes in its leaves and barks, besides the chemical composition of volatile oil.[9] Despite the large number of chemical studies conducted on the Meliaceae family, there are few reports concerning the identification of phenolic compounds in this family and no reports on this type of secondary metabolites in *Guarea* are available.[10] Therefore, in order to contribute to the study of the phenolic composition in the Meliaceae family, we report the phytochemical analysis of the *n*-BuOH extract from leaves of *G. macrophylla* and the identification of flavonoids glycosides (quercetin 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-galactopyranoside, kaempferol 7-*O*-β-D-glucopyranoside) for the first time in the genus *Guarea* plus a neolignan glucoside (dehydrodiconiferyl alcohol 4-β-D-glucoside) for the first time in the Meliaceae family.[11-13] All compounds were identified by a combination of spectroscopic methods (^{1}H, 1D, 2D NMR, ^{13}C, UV and MS).

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EXPERIMENTAL

General experimental procedures

^{1}H and ^{13}C 1D and 2D NMR were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) on a Varian® spectrometer using TMS as the internal standard with DMSO-d_6 and CD_3OD as solvents. The high resolution ESI mass spectra were obtained from a Bruker Apex III 70e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, USA). The instrument was calibrated both in the positive and negative ion mode by using the ES tuning Mix (Agilent G2421A). Column chromatography procedures were performed on Sephadex LH-20 (Pharmacia®) and Amberlite XAD-2 (Sigma-Aldrich) devices. For thin layer chromatography (TLC), Merck silica gel 60 F_{254} chromatoplates (20 x 20 and 0.30 mm thickness) were used. TLC was observed under a UV lamp (254 and 365 nm) and visualized using NP/PEG reagent (diphenylboric acid 2-aminoethyl ester/polyethylene glycol) under 365 nm. The reversed phase analytical HPLC method was run on a Shimadzu LC 10 AD system, using a RP-18 column (0.5 mm x 250 x 4.6 mm - Merck, Darmstadt, Germany) with a diode array detector (SP-M10A) and a gradient solvent elution with MeOH:H_2O.

Plant material

Plant sample comprising the flowering branches of *Guarea macrophylla* were collected in Jacarepagua, Rio de Janeiro, Rio de Janeiro State, Brazil, in October 2008. The specimen was identified by Dr. C. M. Sakuragui and a voucher deposited at the Herbarium of the Botanic Garden of Rio de Janeiro, Brazil with the collection number RB 466067.

Extraction and isolation of chemical constituents

The air-dried leaves (700 g) were extracted with 80% MeOH at room temperature by static maceration for 10 days. After concentration under reduced pressure, the methanol extract (10 g) was

suspended in water, and successively extracted with CH_2Cl_2 (4 g), EtOAc (0.5 g) and *n*-BuOH (2 g). One gram of the *n*-BuOH extract was introduced into a XAD-2 column (id/h = 3 cm/50 cm) and chromatographed in a stepwise gradient with H_2O:MeOH (9:1/0:10, v/v) (Procedure 1). Each combination of solvents was eluted through the column and fractions of 200 mL were collected. Ten fractions were obtained and analyzed by TLC with BuOH:AcOH:H_2O (40:10:50, v/v) as the solvents system. The fractions eluted with 60 to 90% MeOH were taken together (163 mg) and re-chromatographed on Sephadex LH-20 (id/h = 3 cm/50 cm) with 100% MeOH as the mobile phase (Procedure 2). The second fraction from Procedure 2 was re-chromatographed on a Sephadex LH-20 device with 100% water to obtain 5 mg of the neolignan glucoside (dehydrodiconiferyl alcohol-4-*β*-D-glucoside). The fractions eluted with 70 to 50% water from Procedure 1 were re-chromatographed on a Sephadex LH-20 device with 100% water as mobile phase to obtain the flavonoids hyperin and isoquercitrin (5.8 mg) and an unusual kaempferol 7-*O*-glycoside (kaempferol 7-*O*-*β*-D-glucopyranoside (2.5 mg)**.** All compounds were identified by a combination of spectroscopic methods (^{1}H, ^{13}C NMR, ESI-MS) and comparison with the literature data.

Neolignan (dehydrodiconiferyl alcohol-4-*β*-D-glucoside): Amorphous powder. HMRS *m/z*: 559. 1583 [M+ K]$^+$. ^{1}H NMR and ^{13}C NMR data (CD_3OD), Table 1.

Flavonoids (quercetin 3-*O*-*β*-D-galactopyranoside, quercetin 3-*O*-*β*-D-glucopyranoside and kaempferol 7-*O*-*β*-D-glucopyranoside) were identified after application of spectroscopic methods and comparison with the literature data. ^{1}H 1D and 2D NMR data (DMSO-d_6), Table 1.

RESULTS AND DISCUSSION

The main *n*-butanol extract obtained from *G. macrophylla* leaves was fractionated by a classical chromatography methodology. Columns packed with XAD-2 and Sephadex LH-20 adsorbents were efficient for the isolation of phenolic compounds (1a, 1b, 1c, 1d).

Compounds **1a** (quercetin 3-*O*-*β*-D-galactopyranoside) and **1b** (quercetin 3-*O*-*β*-D-glucopyranoside) were obtained as a yellow powder mixture which reacted positively for flavonoids using NP/PEG reagents. The UV spectra obtained from the HPLC/DAD chromatogram showed λ maxima at 353 nm (band I) and 254 nm (band II) characteristic of the quercetin type.[14] The molecular formula for quercetin 3-*O*-*β*-D-galactopyranoside (Figure 1a) and quercetin 3-*O*-*β*-D-glucopyranoside (Figure 1b) were established as $C_{21}H_{20}O_{12}$ based on HRMS data [M- H]$^-$ at 463.0877.

The ^{1}H NMR spectrum of the mixture showed signal at δ_H 12.59 which is characteristic of a chelated C-5 hydroxyl group in a flavonol unit and two meta-coupled protons of H-6 and H-8, typical of A ring, at δ_H 6.17 (2H, d, *J* = 1.71 Hz) and δ_H 6.39 (2H, d, *J* = 1.71 Hz). Furthermore six signals, typical of B ring, were observed at δ_H 6.81 (*J* = 8.47 Hz, H-5'), δ_H 6.83 (*J* = 8.91 Hz, H-5'), δ_H 7.63 (dd, *J* = 2.16/8.47 Hz, H-6'), δ_H 7.56 (m, H-6´and H-2') and at δ_H 7.54 (d, *J* = 2.16 Hz, H-2'). These groups of signals suggest two polyphenolic compounds from quercetin derivatives. The sugar moieties were identified as glucopyranose and galactopyranose with chemical shifts for H-1" and C-1" at δ_H 5.43 (*J* =7.48 Hz), δ_c 101.2 and δ_H 5.34 (*J* = 7.70 Hz) δ_C 102.2 respectively. Their linkage position, in both cases at C-3, was confirmed on the basis of chemical shifts

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1a**, **1b, 1c** (DMSO- d_6) and **1d** (CD_3OD)

Pos.	1a δ ^{13}C	1a δ ^{1}H	1b δ ^{13}C	1a δ ^{1}H	1c δ ^{13}C	1c δ ^{1}H	1d δ ^{13}C	1d δ ^{1}H
1	-	-	-	-	-	-	138.0	-
2	156.5	-	156.5	-	147.5	-	112.1	7.02 (br) d (2.0)
3	133.9	-	133.9	-	136.1	-	150.9 150.9	-
4	177.6	-	177.5	-	176.1	-	147.7	-
5	161.2	5- OH 12.29, s	161.2	5- OH 12.29,s	160.4	5- OH 12.85 s	118.0 118.0	7.14 d (8.4)
6	99.1	6.17 d (1.7)	99.1	6.17 d (1.7)	103.0	6.44 d (1.7)	119.3	6.93 dd (8.4/2.0)
7	165.1	-	165.1	-	157.9	-	88.8/ 88.7	5.58 d (5.9)
8	94.0	6.38 d (1.7)	94.0	6.38 d (1.7)	98.0	6.81 d (1.7)	55.4	3.47
9	156.8	-	156.8	-	155.8	-	64.9	3.84;3.77 dd (11.1/7.4)
10	103.9	-	103.9	-	104.7	-	-	-
1'	121.3	-	122.9	-	121.5	-	132.7	-
2'	116.4	7.54 d (2.1)	116.4	7.56	130.5	7.96 d (8.8)	111.1	6.94 (br) s
3'	145.1	-	144.9	-	116.2	6.80	145.5	-
4'	148.8	-	148.7	-	159.4	-	149.2	-
5'	115.5	6.80 d (8.4)	115.5	6.83 d (8.9)	116.2	6.80	130.0	-
6'	122.2	7.63 dd (8.5/ 2.1)	121.8	7.56	130.5	7.96 d (8.8)	116.5	6.95 (br) s
7'	-	-	-	-	-	-	131.9	6.53 dt (15.8/1.5)
8'	-	-	-	-	-	-	127.6	6.22 dt (15.8/5.9)
9'	-	-	-	-	-	-	63.8	4.19 dd (5.9/1.5)
1"	102.2	5.34 d (7.7)	101.2	5.43 d (7.5)	96.9	5.50 d (7.6)	102.7	4.88 d (7.4)
2"	71.4	3.56	74.3	3.23	73.1	3.36	74.9	3.48
3"	73.5	3.30	76.7	3.23	76.4	3.30	77.8	3.45
4"	68.2	3.65	70.2	3.08	69.6	3.65	71.3	3.38
5"	76.1	3.32	77.8	3.08	77.2	3.32	78.2	3.39
6"	60.2	3.37	61.0	3.57	60.6	3.37	62.5	3.67/3.67
3-OMe	-	-	-	-	-	-	56.7	3.83 s / 3.82 s
3'OMe	-	-	-	-	-	-	56.7	3.88 s

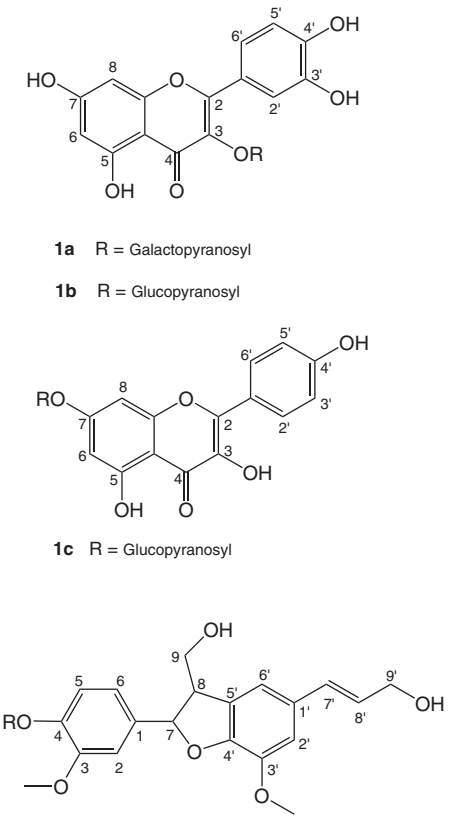

Figure 1. *Structures of compounds isolated from G. macrophylla) - 1a: Hyperin (quercetin 3-O-β-D-galactopyranoside); 1b: Isoquercitrin (quercetin 3-O-β-D-glucopyranoside); 1c: Kaempferol 7-O-β-D-glucopyranoside; 1d: Dehydrodiconiferyl alcohol 4-β-D-glucoside*

of the anomeric protons, HMBC correlations and comparison with the literature data.[11]

Based on HRMS, a molecular formula $C_{21}H_{20}O_{11}$ ([M- H•]$^-$ at 447.0931) was found for kaempferol 7-*O*-*β*-D-glucopyranoside (Figure 1c). ^{1}H NMR and HMBC correlations (H-1"→C-7) were important to identify the linkage between sugar moiety and the aglycone at C-7. Furthermore, the observed HMBC correlations between H1" and C-6 and C-8 were further proof of the above assertion. This is an unusual situation, since for flavonols, the glycosylation occurs commonly at C-3 or at C-3 and C-7 simultaneously. This occurrence (glycosylation at C-7 only) is more usual for flavones.[15,16] The chemical shifts for B ring at δ_H 7.96 (d, J = 8.8 Hz, H-2′ and H-6') and at δ_H 6.88 (H-3' and H-5') were typical of kaempferol. The *β* configuration for the sugar unit was determined based on the J value information for the anomeric proton (J = 7.6 Hz). These data strongly suggest the presence of the sugar unit at C-7 position in the *β* configuration. Chemical shifts values at δ_H 6.44 for H-6 and δ_H 6.81 for H-8 are in accordance with values previously reported in the literature for 7-*O*-glycosylated flavonoids.[16] The other chemical shifts were also similar to the literature data.[12]

The molecular formula for the neolignan glucoside (Figure 1d) was established as $C_{26}H_{32}O_{11}$ based on HRMS data [M+ K]$^+$ at 559.1583 and on analyses of the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR data showed the presence of five protons in the aromatic region, two methoxyl groups, and an anomeric proton (δ_H 4.88) with large coupling constant (J = 7.4 Hz) typical of *β* configuration for the sugar moiety. The presence of signals at δ_H 6.22 (dt, 5.9/15.8 Hz, H-8'), δ_H 6.54 (dt, 15.8/ 1.5 Hz, H-9') and δ_H 4.19 (dd, 5.9/1.5 Hz, H-9') allowed the identification of a *trans* configuration for the propenyl group. To confirm the linkage position of the sugar moiety at C-4, the HMBC was measured and a long range carbon proton correlation for H-1"→C-4 observed. All the other signals were similar to those reported in the literature for the dehydrodiconiferyl alcohol-4-*β*-D-glucoside.[13]

From a phytochemical standpoint, the Meliaceae family of the *Melia* and *Azadirachta* genera has been widely investigated worldwide leading to the isolation of a number of limonoids and other terpenoids with a variety of biological effects.[1,17] However, chemical studies concerning the isolation and identification of phenolic compounds in Meliaceae remain scarce where catechins have been previously reported in *Toona* and *Cedrela* genera,[18] flavalignans in *Trichillia*,[19] flavonols glycosides in *Melia* and lignans in *Walsura* and *Aglaia*.[10,20] The phenolic profile for *Guarea* species has not yet been described. Information about the occurrence of kaempferol 7-*O*-glycoside, a neolignan glucoside and quercetin derivatives described here for the first time in *G. macrophylla*, could prove helpful in the chemosystematic investigation of polar constituents for *Guarea* species.

SUPPLEMENTARY MATERIAL

^{1}H, 1D, 2D NMR, ^{13}C, UV and MS spectra of compounds **1a**-**1d** are available free of charge at http://quimicanova.sbq.org.br as a PDF file.

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