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A C-glucoside benzoic acid derivative from the leaves of *Peltophorum dubium*

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ABSTRACT

From the methanolic extract of the leaves of *Peltophorum dubium* Taub (Leguminosae) was isolated after successive chromatographic procedures a new C-glucoside benzoic acid derivative, 3αC-glucopyranosil-4,5-dihydroxy-2-methoxy-benzoic acid. The structure of this compound was determined by 1D and 2D NMR and MS data analysis. The new compound showed moderate antioxidant activity in the assay and the auto-oxidation of β-carotene in a linolenic acid suspension method.

1. Introduction

The species *Peltophorum dubium* Taub is a tree belonging to the Leguminosae family (Caesalpinoideae). It occurs in Brazil from Bahia to Rio de Janeiro states, especially in the Atlantic forest complex, and also in other Brazilian regions as Minas Gerais, Goiás, Mato Grosso do Sul, Paraná and Sâo Paulo in deciduous forests, usually found in clay soils, deep and well common in riparian habitats (Lorenzi, 2000). In Brazil it is popularly known as "amendoim bravo", "faveiro", "pau vermelho", "angico" and "canela de veado". Its wood is employing in constructions, roof and the wood power is used for extraction of a red dye (da Silva, 2004). To date there is no records in literature regarding its chemical composition. Previously it was reported the antimicrobial activity (Salvat et al., 2004) and trypsin inhibition (Trancoso et al., 2003) of extracts of *P. dubium*.

However from *P. africanum* was previously isolated catechins and derivatives of gallic acid (Bam et al., 1988; Ferreira et al., 2005). The MeOH extract of this species showed inhibition of HIV-1 (Bessong et al., 2005), bactericidal (Samie et al., 2005) and antioxidant activities (Bizimenyera et al., 2007). Beside these, the ethanol extract of *P. pterocarpum* showed antimicrobial activity (Voravuthikunchai and Limsuwan, 2006).

This work describes the isolation of a new unusual C-glucoside benzoic acid derivative (Fig. 1) besides known triterpenes from the AcOEt extract of leaves of *P. dubium*.

2. Results and discussion

Compound **1a** was obtained as an amorphous white powder [m.p 198 °C (dec.)] and its structure was elucidated by spectrometric data analysis of the pure compound and the peracetylated derivative (**1b**). The ESIMS of **1a** showed a negative *pseudo*-molecular ion at *m/z* 345.0834 and, the combination with hydrogen and carbon counts obtained by ^{1}H, ^{13}C and DEPT NMR spectra permitted to propose the molecular formulae as $C_{14}H_{18}O_{10}$ to this compound (requires 345.0822). The IR spectra permitted to identify presence of hydroxyl groups and conjugate carboxyl group (ν 1702 cm^{-1}). The analysis of ^{1}HNMR spectrum permitted to observe a singlet at δ 7.75 of a pentasubstituted aromatic ring besides the presence of methoxyl group and hydrogens of sugar moiety (Table 1). The ^{13}CNMR spectra, including DEPT experiments (135° and 90°) permitted to corroborate with the previous statements and, moreover pointed the observed peak for the methoxyl group (δ 60.4) was indicative it was ortho-disubstituted. The comparison of oxymethine and oxymethylene carbons with the NMR data of sugars (Agrawal et al., 1989) permitted to identify the presence of glucose and, the anomeric carbon at δ 75.6 was indicative of that **1** was a C-glucoside derivative of benzoic acid. The correlations of H-6 (δ 7.75) and carboxyl carbon (δ 164.6), C-2 (δ 142.0) and C-4 (δ 152.7) observed in the HMBC spectra as well as the correlation of the peaks at δ 4.5 (anomeric H), methoxyl hydrogens and C-2 (δ 142.0) permitted to locate the glucose at C-5. The $\Delta\delta$ of ^{13}CNMR signals of **1** and the acetylated derivative **1a** corroborated with the presence of two vicinal hydroxyl groups at C-3 and C-4. Thus the new compound **1** could be identified as 3αC-glucopyranosil-4,5-dihydroxy-2-methoxy-benzoic acid.

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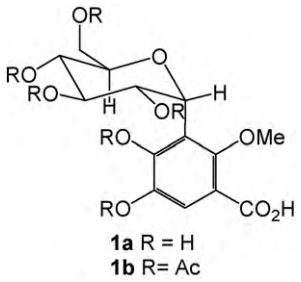

Fig. 1. New glucoside and derivative from *Peltophorum dubium*.

This work is the first report of **1**, however, compounds with close structures, like norbergenin and bergenin were isolated from *P. africanum* (Mebe and Makuhunga, 1992) besides catechins esterified with gallic acid (Bam et al., 1988; Ferreira et al., 2005). Larreagenin-A was previously isolated from *Larrea divaricata* (Habermeeh and Christ, 1974) and *Guaiacum officinale* (Ahmad et al., 1984), two species of Zygophyllaceae family but this is the first occurrence in Leguminosae.

The extracts and the isolates were submitted to brine shrimp test employing *Artemia salina* nauplii and compound **1** was submitted to antioxidant tests (quenching of DPPH and inhibition of co-oxidation of β-carotene in a suspension of linolenic acid). The AcOEt extract showed IC_{50} 320 μg/ml in the BST indicating moderate activity. However, the hexane extract as well as the isolates were inactive ($IC_{50} > 500$ μg/ml). The IC_{50} values (μg/ml) for 50% of DPPH scavenging radicals were calculated by linear extrapolation of values obtained from curve of antioxidant activity *versus* concentration. The results show that compound **1** ($IC_{50} > 120$) did not present antioxidant activity when compared with gallic acid (IC_{50} 32.2) and quercetin (IC_{50} 11.2). However, in relation to inhibition of auto-oxidation of β-carotene method the data with 95% of confidence indicated that compound **1** (AA = 10.52; 11.16%) presented the highest antioxidant activity than gallic acid (AA = 8.75; 9.2%) but lower than quercetin (AA = 95.09; 100%).

3. Experimental

3.1. Plant material

The leaves of *P. dubium* Taub were collected at Ondina *Campus*, Universidade Federal da Bahia, Salvador, BA, Brazil. A voucher of A voucher is deposited at Herbarium Alexandre Leal da Costa of Instituto de Biologia da UFBA under number 69237.

Table 1
^{13}C NMR chemical shifts (75 MHz) of compound **1** [C_5D_5N, δ (ppm)] and the peracetyl derivative **1a** [$CDCl_3$, δ (ppm)].

C	1	1a
1	119.6	118.6
2	142.0	141.3
3	152.8	144.1
4	152.7	144.1
5	116.7	129.5
6	111.1	124.0
7	164.6	161.5
1′	75.6	72.1
2′	72.2	68.2
3′	81.4	72.9
4′	73.9	68.2
5′	83.6	76.7
6′	62.7	61.8
OCH_3	60.4	61.4

3.2. Extraction and isolation

The dried leaves (1000 g) of *P. dubium* were powdered and submitted to extraction with MeOH at room temperature. The solution obtained was partitioned between hexane furnished the hexanic extract (2.0 g). Sequentially, the MeOH phase was diluted with H_2O and then submitted to liquid extraction with $CHCl_3$ and EtOAc. The EtOAc extract (1.2 g) was chromatographed in a polyamide 6 CC eluted with mixtures of MeOH:H_2O. The fraction (300 mg) obtained after elution with MeOH:H_2O (1:4) was submitted to CC on Sephadex LH-20 employing $CHCl_3$:MeOH (1:4) mixtures. This procedure permitted to isolate **1** (62 mg). The Si gel CC of the hexane extract eluted with mixtures of hexane:ETOAc permitted to obtain a mixture (43.4 mg) of lupeol and larreagenin-A. These compounds were identified by direct comparison of MS and NMR spectral data (^1H, ^{13}C and DEPT) with the literature (Mahato and Kundu, 1994; Ahmad et al., 1984).

3αC-Glucopyranosil-4,5-dihydroxy-2-methoxy-benzoic acid (**1**): IV ν_{max} (cm^{-1}): 3408, 3090, 1702, 1613, 1528, 1071, 1044; ^1H NMR (C_5D_5N, 300 MHz): 7.75 (s, 1H, H-2), δ 4.50 (d, J = 4.5 Hz, H-1″), 4.41–4.18 (m, 7H, H-1′-H6′, glucose moiety), δ 3.98 (s, 3H, OMe); ^{13}C NMR (C_5D_5N, 75 MHz): Table 1. HRESIMS m/z: 345.0834 $[M-H]^+$

Preparation of derivative: Compound **1** (20.0 mg) was added to a solution of pyridine (0.5 ml), acetic anhydride (0.5 ml) and dimethylaminepyridine (DMAP) for 24 h at room temperature and the peracetyl derivative (**1a**, 23 mg) was extracted with $CHCl_3$. ^1H NMR (C_5D_5N, 300 MHz): δ 7.76 (s, 1H, H-2), 3.91 (s, 3H, OMe); ^{13}C NMR (C_5D_5N, 75 MHz): Table 1.

3.3. Brine shrimp and antioxidant tests

The hexane and AcOEt extracts and isolated compounds were submitted to a *Artemia salina* lethality test (David et al., 2001). The antioxidant activities were evaluated by the ability of the new compound (**1**) and the gallic acid (Sigma) and quercetin (Sigma) scavenging the 1,2-diphenyl-2-picryl-hydrazyl (DPPH, Sigma) free radical and was carried out according to established protocol (Barreiros et al., 2004). The inhibition of the AA of the isolates was measured using the method of auto-oxidation of β-carotene (Merck) in a suspension of linolenic acid (Aldrich) (Barreiros et al., 2000). The results were compared to those from the commercial antioxidant gallic acid and BHT (Merck).

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