



Phenylpropanoids from *Paspalum atratum* (Poaceae)



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1. Subject and source

Paspalum L. (Poaceae) is a genus of grasses comprising ca. 400 species distributed mainly in tropical to warm-temperate areas worldwide, of which nearly 220 species occur naturally in Brazil. *Paspalum* is recognized for its ecological and agronomic importance, derived from its forage and ornamental attributes and constitutes a taxonomically complex group of plants (Cidade et al., 2010, 2013). *Paspalum atratum* Swallen is a perennial, apomitic, cespitose grass native to Central and South America. *P. atratum* cv. Pojuca was released in 2000 for commercial use in Brazil and, under other cultivar names, in several other countries as well. It is well adapted to wetlands and low fertility soils, and presents good forage and seed production potentials. In addition, this species is rarely attacked by pests and tolerates fire (Ramos et al., 2002).

Mature leaves and leaf sheaths of *Paspalum atratum* Swallen cv. Pojuca (Poaceae) were collected in Embrapa Cattle – Southeast (São Carlos, SP, Brazil) and identified by Dr. F. H. D. de Souza. Accessions of this species have been maintained *in vivo* at a bank (Brazilian Register Code BRA 009610) of Embrapa Cattle-Southeast, located in São Carlos, SP, Brazil.

2. Previous work

No previous report is available on the chemical composition of *P. atratum*.

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3. Present study

The material was separated into roots and aerial parts, dried (ca. 45 °C) and ground. The aerial parts (443.0 g) were extracted successively at room temperature with *n*-hexane (3 × 600 mL) and EtOH (3 × 600 mL), and the extracts were individually concentrated to give Hex extract (3.0 g) and EtOH extract (10.1 g), respectively. The crude EtOH extract (10.0 g) was washed with CHCl₃ and the insoluble part (3.0 g) was subjected to column chromatography (C18, H₂O/MeOH gradient, 19:1–100% MeOH) to give 10 fractions (Fr.1–10). Fr.4 gave compound **1** (329.4 mg). Fr.2 was subjected to HPLC (C18, MeOH–H₂O 4:21, with 0.5% AcOH) to give compounds **2** + **8** (8.3 mg), **3** + **4** (4.1 mg), **5** (17.3 mg), **6** (4.8 mg), and **7** (4.3 mg).

The structures of the compounds **1–8** (Fig. 1) were elucidated based on analyses of their MS, ¹H and ¹³C NMR (including COSY, TOCSY, HMBC, and HSQC) and on comparisons of their data with those reported in the literature. Fraction 2 (**2** + **8**) were also analysed by DOSY NMR. These compounds were identified as (*R*)-rosmarinic acid (**1**) (Moharram et al., 2012; Murata et al., 2009), 3-*O*-caffeoylquinic acid (**2**, neochlorogenic acid) (Nakatani et al., 2000; Wei et al., 2010), 5-*O*-caffeoylquinic acid (**5**, chlorogenic acid) (Dahmani-Hamzaoui et al., 2012; Wei et al., 2010), 4-*O*-caffeoylquinic acid (**6**, cryptochlorogenic acid) (Nakatani et al., 2000; Wei et al., 2010), caffeic acid (**7**) (Xu et al., 2013), and phenylalanine (**8**) (Wang et al., 2012; Yasukawa and Asano, 2012).

Although 3-*O*-coumaroylquinic acids (**3** and **4**) have been known for some time, and have even been identified, for example, from species belonging to Rubiaceae, Asteraceae, Poaceae, and Solanaceae families, most of spectroscopic data of **3** and **4** still remain to be described. These compounds were isolated as a mixture of *cis* and *trans*-3-*O*-coumaroylquinic acids (2:3), as determined by the integral of the ¹H NMR signals at δ 5.9–6.1, 6.4–6.5, 7.0–7.1, and 7.7–7.8 referent to the olefinic hydrogens and peak areas of chromatogram obtained by HPLC-DAD. The *cis* isomer showed a coupling constant of 12.3 Hz, whereas the *trans* showed characteristic coupling constant of 16.0 Hz. The UV absorption suggested the presence of *p*-coumaroyl moiety (λ_{max} at 312 nm). A detailed analysis of ¹H–¹H gCOSY, TOCSY, gHMBC, and gHMBC data of these compounds enabled us to assign their chemical shifts to carbons and hydrogens in the structures and helped to determine their relative configurations. Moreover, the chemical shifts of one quaternary (δ_C ~77) and three methine (δ_C 66.9, 72.9 and 73.4) oxygenated carbons and two methylene carbons (δ_C 35.6 and 40.6) were in accordance with a 3-substituted quinic acid unity (Wei et al., 2010). In addition, the coupling constants (*J*) values determined for the hydrogens of this unity were confirmed by selective proton irradiation, being its relative configuration identical to that determined for **2**.

cis-3-*O*-Coumaroylquinic acid (**3**). ¹H NMR (600 MHz, D₂O): δ 1.93–2.13 (2H, *m*, 2H-6), 2.18–2.25 (2H, *m*, 2H-2), 3.71–3.78 (1H, *m*, H-4), 4.08 (1H, *dt*, *J* = 4.2, 10.1 Hz, H-5), 5.35 (1H, *bd*, *J* = 2.8 Hz, H-3), 6.00 (1H, *d*, *J* = 12.3 Hz, H-8'), 6.90 (2H, *d*, *J* = 8.3 Hz, H-3', 5'), 7.08 (1H, *d*, *J* = 12.3 Hz, H-7'), 7.52 (2H, *d*, *J* = 8.3 Hz, H-2', 6'). ¹³C NMR (151 MHz, D₂O): δ 35.6 (C-2), 40.6

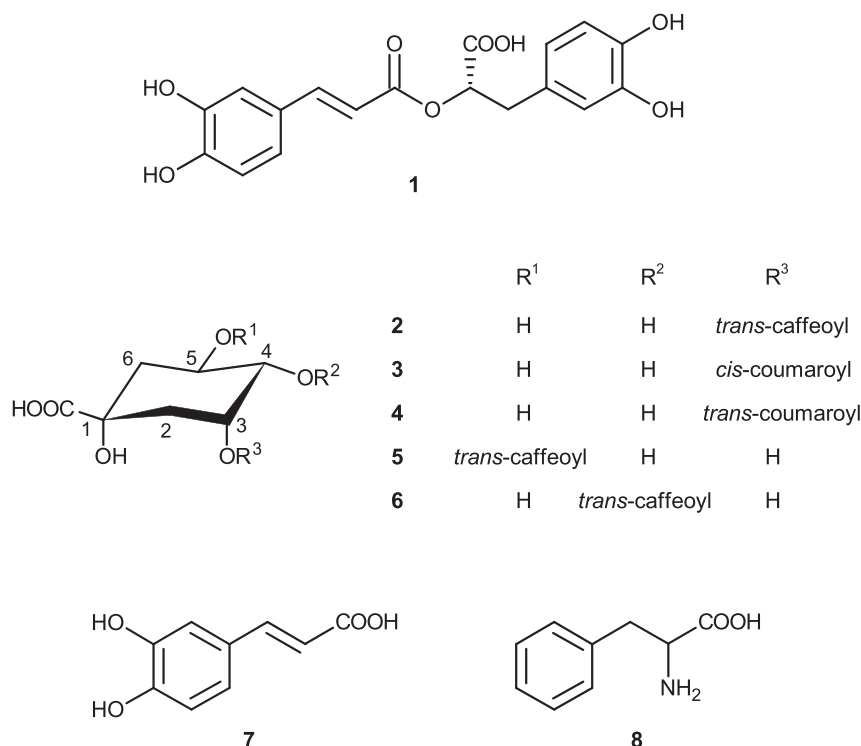


Fig. 1. Structures of compounds **1–8**.

(C-6), 66.9 (C-5), 72.9 (C-3), 73.4 (C-4), 77.7 (C-1), 115.0 (C-3'/5'), 116.9 (C-8'), 126.9 (C-1'), 131.6 (C-2'/6'), 144.3 (C-7'), 156.7 (C-4'), 168.3 (C-9').

trans-3-*O*-Coumaroylquinic acid (**4**). ¹H NMR (600 MHz, D₂O): δ 1.93–2.13 (2H, *m*, 2H-6), 2.18–2.25 (2H, *m*, 2H-2), 3.71–3.78 (1H, *m*, H-4), 4.18 (1H, *dt*, *J* = 3.9, 9.3 Hz, H-5), 5.41 (1H, *bd*, *J* = 2.3 Hz, H-3), 6.49 (1H, *d*, *J* = 16.0 Hz, H-8'), 6.96 (2H, *d*, *J* = 8.2 Hz, H-3', 5'), 7.61 (2H, *d*, *J* = 8.2 Hz, H-2', 6'), 7.73 (1H, *d*, *J* = 16.0 Hz, H-7'). ¹³C NMR (151 MHz, D₂O): δ 35.6 (C-2), 40.6 (C-6), 66.9 (C-5), 72.9 (C-3), 73.4 (C-4), 76.6 (C-1), 114.9 (C-8'), 115.9 (C-3'/5'), 126.8 (C-1'), 130.4 (C-2'/6'), 146.1 (C-7'), 158.3 (C-4'), 168.6 (C-9').

4. Chemotaxonomic significance

Paspalum has a vast genetic and morphological diversity within and among its species. Because of this degree of complexity, the division of the genus *Paspalum* into subgenera, sections or informal groups has been proposed by many authors and has been extensively discussed (Cidade et al., 2013). Currently, four subgenera are recognized based on morphological similarities: *Paspalum* subg. *Anachyris* Chase, *P.* subg. *Ceresia* (Pers.) Rchb., *P.* subg. *Harpostachys* (Trin.) S. Denham, and *P.* subg. *Paspalum* (Cidade et al., 2013).

Although the genus *Paspalum* comprises ca. 400 species, up to now, only three species of *Paspalum* have been chemically studied: *Paspalum dilatatum*, *Paspalum conjugatum*, and *Paspalum scrobiculatum*. From *P. dilatatum* and *P. conjugatum* lupeolmethylether and luteolin have been isolated, respectively (Li et al., 2002; Ohmoto et al., 1970). From *P. scrobiculatum*, cultivated in India as a cereal crop ('Kodo millet'), *n*-alkanes were isolated along with fatty alcohols, fatty acids, sterols, and δ-palmitin (Gupta et al., 2014; Misra and Siddiqi, 2000; Sharma et al., 1972; Shukla et al., 2000). Flavonoids, hydroxybenzoic and hydroxycinnamic acids and derivatives were also identified from *P. scrobiculatum*, by HPLC-MS (Chandrasekara and Shahidi, 2011).

This study describes the phytochemical investigation of *P. atratum* and reports the isolation and characterization of eight phenylpropanoids, six of which (**1–4**, **6**, and **8**) were isolated for the first time from the genus. Rosmarinic acid (RA, **1**) and chlorogenic acids (CGAs, **2–6**) are widely found in the plant kingdom, particularly in some crop plants, and presumably accumulated as defence compounds against pathogens and herbivores (Petersen, 2013; Petersen and Simmonds, 2003; Upadhyay and Rao, 2013). The majority of CGAs occurs as a series of esters belonging to three classes: feruloylquinic acids (FQA), coumaroylquinic acids (3-CQA, 4-CQA, and 5-CQA), and di-CQAs (3,4diCQA, 3,5diCQA, and 4,5diCQA). RA and ca. 80 CGAs have been identified from angiosperms, mainly from plants belonging to Boraginaceae family and to Asparagales, Malpighiales, Fabales, Rosales, Gentianales, Solanales, Lamiales, Aquifoliales, Apiales, Dipsacales, and Asterales orders (Petersen, 2013; Upadhyay and Rao, 2013). The occurrence of RA and CGAs has also been observed in Poales (Campos et al., 2014; Chandrasekara and Shahidi, 2011; Kowalska et al., 2014; Kweon et al., 2001; Lee et al., 2010; Míka et al., 2005; Parveen et al., 2008; Petersen, 2013; Petersen et al., 2009; Quan et al., 2013).

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