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Chemical profile of the polar extract of *Paepalanthus microphyllus* (Guill.) Kunth (Eriocaulaceae)

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From the ethanolic extract of the capitulae of Paepalanthus microphyllus, *one caffeic acid derivative (**1**) was isolated. The structure of the compound was characterized by spectroscopic methods, mainly 1D and 2D NMR experiments, as well as ESMS spectrometry. In addition, three flavonoids of taxonomic relevance were isolated and identified by comparison to literature data.*

Uniterms:
- Caffeic acid derivative
- Eriocaulaceae
- *Paepalanthus*

INTRODUCTION

The Eriocaulaceae family encompasses around 1200 species, many of which are ornamental and endemic of Brazil (Giulietti *et al*., 2000). However, few of these plants have been chemically studied. Among the investigated species, those belonging to *Paepalanthus* genus are known for the production naphthopyranone and flavonoid glycosides (Vilegas *et al*., 1998; Vilegas *et al*., 1999a,b; Santos *et al*., 2001a,b; Piacente *et al.,* 2001). The isolated compounds have shown a number of biological activities, like mutageniticity (Tavares *et al*., 1999; Coelho *et al*., 2000; Moreira *et al*., 2000).

This work deals with the chemical investigation of *P. microphyllus* Giull. Kunth., that belongs to subgenus *Paepalocephalus* section *Eriocaulopsis*, that grows wild at Serra do Cipó, Espinhaço Chain, Minas Gerais State, Brazil.

MATERIALS AND METHODS

Plant material

Capitula of *P. microphyllus* (Guill.) Kunth. (Eriocaulaceae) were collected in February 1997, at Serra do Cipó, in Espinhaço Chain, Minas Gerais State, Brazil. The specimen were determined by Prof. Paulo Takeo Sano from Instituto de Biociências, USP, São Paulo. A voucher specimen (CFCR 5610) has been deposited at the Herbarium SPF (Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Brazil).

General experimental procedures

Capitulae of *Paepalanthus microphyllus* (300 g) were powdered and extracted successively with *n*-hexane, methylene choride an EtOH (1 week each). Solvents were evaporated under vacuum. The EtOH-extract (2.0 g) was chromatographed on a Sephadex LH-20 collumn (100x5cm), with MeOH as eluent. Fractions (8 mL) were collected and checked by TLC [Si gel plates, BAW (*n*-BuOH/AcOH/H_2O 12:3:5, v/v/v)]. Fractions were further purified by HPLC on a Waters 590 system equipped with a Waters R401 refractive index detector and with a Waters (*m*-Bondapak RP-18) column (30 cm x 7.6 mm i.d) using MeOH-H_2O (1:1, v/v) as eluent equiped with a Photo Diode-Array Detector set at 254 nm. We obtained the pure compounds 1,3-di-*E*-caffeoylglycerol **1**, and the flavonoids

3',4',5,6,7,8-hexahydroxyflavone **2**, 3',4',5,6,7-pentahydroxyflavone **3** and 3',4',5,6-tetra-hydroxy-7-O-β-D-glucopyranosilflavone, **4** (Figure 1). NMR spectra in CD_3OD were obtained using a Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^1H and 150.86 MHz for ^{13}C. 2D experiments: ^1H-^1H-COSY (Chemical shift correlation spectroscopy), inverse-detected ^1H-^{13}C HSQC (heteronuclear single quantum coherence), HMBC (heteronuclear multiple bond connectivity). ESMS were performed in a Fisons Platform spectrometer in the positive mode (70 V). The sample were dissolved in MeOH and injected directly.

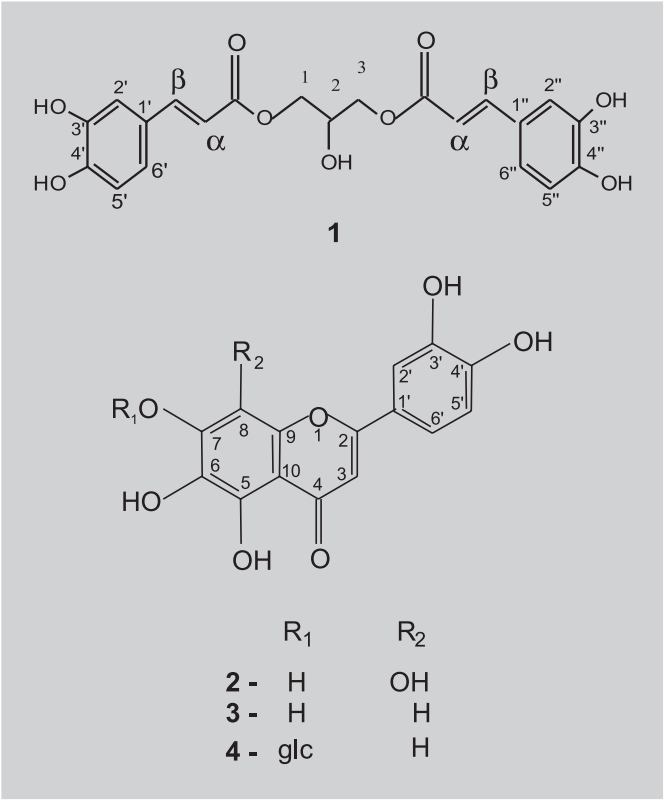

FIGURE 1 - Compounds from *P. microphyllus*.

RESULTS AND DISCUSSION

Compound **1** was colourless and amorphous. The IR spectra showed hydroxy groups (3400 cm^{-1}), an α,β-unsaturated ester ($\nu_{C=O}$ 1700 cm^{-1}), an alkene conjugated with an aromatic ring ($\nu_{C=C}$ 1620 cm^{-1}), and an aromatic ring (1590, 1505 cm^{-1}). The ESMS (70V, positive mode) gave the quasi-molecular ion $[M + H]^+$ at *m/z* 417, corresponding to the molecular formula $C_{21}H_{20}O_9$, and fragment at *m/z* 180, corresponding to the formula protonated $[C_9H_7O_4 + H]^+$, and *m/z* 238, corresponding to the fragment $[C_{12}H_7O_{13} + H]^+$. Loss of the two units of the caffeoyl moiety of **1** led to the glycerol unit observed at *m/z* 60.

In the ^1H NMR spectrum, the signals for the primary alcoholic functions and the secondary alcoholic function of a glycerol unit were evident at δ 4.29 (4H, m) for the two OCH_2 groups of glycerol and the signal of an OCH group at δ 4.16 (1H, m). Also evident were two doublets typical of a *trans* double bond at δ 6.32 (2H, d, *J* = 16.0 Hz) and 7.57 (2H, d, *J* = 16.0 Hz) and signals at δ 6.56 (2H, dd, *J* = 1.5 and 8.0 Hz), 6.77 (2H, d, *J* = 8.0 Hz) and 7.07 (2H, d, *J* = 1.5 Hz). The above signals suggested the occurrence of two caffeoyl moieties (Birkofer *et al*., 1968) which should be linked to C-1 and C-3 of the glycerol unit in a symmetrical position in agreement with the ^1H NMR and ^{13}C NMR signals. The HMBC spectrum showed connectivities for H-1 and H-3 of the glycerol unit (δ 4.29) and (C=O) of the caffeoyl units (δ 169.0). Thus, **1** was identified as 1,3-*O*-di-*E*-caffeoylglycerol.

TABLE I - ^1H and ^{13}C data of 1,3-*O*-di-*E*-caffeoylglycerol **(1)** in 600 MHz (*J* in HZ; attribute values accordingly to HSQC, HMBC experiments)

Position	^{13}C	^1H
1	66.0	4.29 m
2	68.3	4.16 m
3	66.0	4.29 m
1',1''	127.4	-
2',2''	115.1	7.07 d (1.5)
3',3''	147.8	-
4',4''	149.5	-
5',5''	116.6	6.77 d (8.0)
6',6''	122.9	6.56 dd (1.5, 8.0)
α	114.6	6.32 d (16.0)
β	147.2	7.57 d (16.0)
C = O	169.0	-

The structures of the flavonoids **2-4** compounds were unambiguously determined by means of spectroscopic methods (IR, ESMS, ^1H, ^{13}C and 2D NMR experiments COSY, HSQC, HMBC) and compared to those previously reported (Agrawal, 1989; Harborne, Mabry, 1982; Harborne, 1998).

Caffeic acid and other hydroxycinnamic derivatives are of major importance for the protection of plants against herbivores and pathogens (Bazzalo *et al*., 1985). They also have important biological activities. Braca *et al*. (2003) reported the antioxidant and free radical scavenging activities of caffeoyl acid derivatives from different *Aconitum* species. Baset *et al*. (1996) reported the *in vitro*

hepatoprotective activity of four di-*O*-caffeoyl quinic acid derivatives from propolis. Kwon *et al*. (2000) reported the inhibitory activity against human immunodeficiency virus-1 presented by the caffeoyl quinic acid isolated from the aerial parts of *Aster scaber* Thunb. (Asteraceae).

Natural bitter phenolic glycerol derivatives have been isolated from a number of *Lilium* species (Liliaceae) as well as from plants of the Gramineae, Bromeliaceae and Salicaceae families (Shimomura *et al*, 1986; Shimomura *et al*., 1989).

CONCLUSION

The presence of compound **1** in *P. microphyllus* is the first report of a natural phenolic glyceride in Eriocaulaceae. Since it is reported the bitter taste of this kind of substance, this could explain the fact that species from this family are hardly attacked by insect in the region where they occur. Further studies are in progress to check this possibility.

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RESUMO

Perfil químico do extrato polar de *Paepalanthus microphyllus*(Guill.) Kunth (Eriocaulaceae)

*Do extrato etanólico dos capítulos de Paepalanthus microphyllus, isolou-se um derivado do ácido cafeico (**1**). Sua estrutura foi caracterizada por métodos espectroscópicos (RMN mono e bi-dimensionais) e por espetrometria de massas Electrospray. Foram, também, isolados outros três flavonóides (**2-4**) de interesse taxonômico, os quais foram identificados por métodos espectroscópicos e comparados com dados da literatura.*

UNITERMOS: Derivado do ácido cafeico. Eriocaulaceae. Paepalanthus.

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