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Acyl glucosylated flavonols from *Paepalanthus* species

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Abstract

Two acylated flavonoids, 6-methoxykaempferol-3-O-β-D-6″(p-coumaroyl)glucopyranoside and 6-methoxyquercetin-3-O-β-D-6″(p-coumaroyl)glucopyranoside have been isolated from the capitulae of *Paepalanthus polyanthus*, *P. hilairei*, *P. robustus, P. ramosus* and *P. denudatus*. Their structures were determined by spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Paepalanthus polyanthus*; *Paepalanthus hilairei*; *Paepalanthus robustus*; *Paepalanthus ramosus*; *Paepalanthus denudatus*; Eriocaulaceae; Flavonoids

1. Introduction

The genus *Paepalanthus* (Eriocaulaceae) is endemic to the Serra do Cipó — MG — Brazil and comprises ca. 400 species, most of them herbaceous. The total number of species is not known with certainty because of the complex taxonomy of the family (Giulietti, & Pirani, 1988; Salatino, Pereira, Salatino, & Giuletti, 1988; Giulietti, & Hensold, 1990; Hensold, & Giulietti, 1991; Sano, 1994). We have previously reported the presence of naphthopyrone and flavonoid derivatives as the chemical constituents of this genus (Vilegas, Roque, Salatino, Giesbrecht, & Davino, 1990; Vilegas et al., 1998; Vilegas, Dokkedal, Rastrelli, Piacente, & Pizza, 1998). In order to contribute to the chemotaxonomy of the Eriocaulaceae, we have examined the capitulae of five species: *Paepalanthus polyanthus*, *P. hilairei*, *P. robustus, P. ramosus* and *P. denudatus*. We report here the isolation and characterization of two new taxonomically relevant acyl glucosylated flavonoids from these plants.

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2. Results and discussion

Chromatographic fractionation of the extracts of five *Paepalanthus* species afforded the substances presented in Table 1. Yields were estimated based on the isolated amounts from each plant. When revealed with NP-PEG reagent, compounds **1**–**9** showed yellow or orange spots, characteristic of flavonoids (Wagner, Bladt, & Zgainski, 1984). Compounds **3**–**11** were determined as being quercetagetin **3**, quercetagetin-7-*O*-glucopyranoside **4**, patuletin **5**, patuletin-3-*O*-glucopyranoside **6**, patuletin-3-*O*-rutinoside **7**, 6-methoxykaempferol **8**, 6-methoxykaempferol 3-*O*-glucopyranoside **9**, paepalantine-9-*O*-glucopyranoside **10** and paepalantine-9-*O*-allopyranosylglucopyranoside **11** by their spectrometric data (Markham, 1982; Harborne, & Mabry, 1982; Agrawal, 1989; Vilegas et al., 1998) and also by TLC with authentic standards from a collection in our laboratory.

Compound **1** showed UV absorption bands at 262 and 358 nm with a shoulder at 278 nm. The IR spectrum presented bands at 3425 cm^{-1} (OH) and at 1656 cm^{-1} (C=O) and 1604 cm^{-1} (C=C). The ^{13}C NMR and DEPT spectra of (**1**) showed 29 signals, six of which

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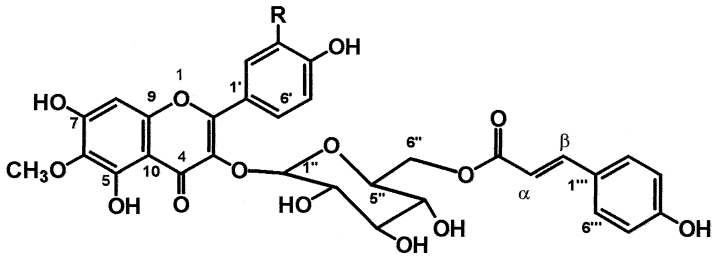

	R
1	OH
2	H

could be assigned to a β-glucopyranosyl moiety. Sixteen other signals were similar to those of 6-methoxyquercetin (Agrawal, 1989). The remaining seven signals are compatible with a *p*-coumaroyl moiety (Agrawal, 1989). The ^{1}H NMR of (**1**) clearly indicated a singlet for 1 OH group at δ 12.71, due to hydrogen bonding to the C4 carbonyl. A doublet at δ 7.55 ($J=2.0$), a double doublet at δ 7.51 ($J=8.0$; 2.0) and a doublet at δ 6.76 ($J=8.0$) are related to the B-ring of the aglycone moiety. The singlet at δ 6.47 was assigned to H8. Two other doublets at δ 6.83 ($J=8.0$) and δ 7.35 ($J=8.0$) were attributed to H3‴/H5‴ and H2‴/H6‴ of the *p*-coumaroyl moiety, respectively. The two doublets ($J=16.0$) at δ 6.15 and 7.33 were assigned to Hα and Hβ of the *p*-coumaroyl moiety with *trans* stereochemistry, respectively. The signal at δ 5.48 ($J=7.0$) was assigned to a D-glucose with β-configuration. The singlet at δ 3.69 (3H) indicated the presence of the OMe group.

The assignment of each signal was based on 2-D ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and ^{13}C–^{1}H RELAY spectra and are shown in Table 2.

The signal at δ 63.1 (CH_2) shows that the *p*-coumaroyl linkage was at C-6 of the glucose unit (Agrawal, 1989). The deshielding of C2 when compared to patuletin (in which C2 is observed at δ 147.1) indicated that position 3 should be substituted by the *p*-coumaroylglucose moiety (Agrawal, 1989).

The ES-MS (100V, positive ion) mass spectrum gave the pseudomolecular ion $[M+H]^+$ at *m/z* 641, corresponding to a molecular formula $C_{31}H_{28}O_{15}$. The adduct with sodium $[M+Na]^+$ appeared at *m/z* 663 and the adduct with potassium $[M+K]^+$ appeared at *m/z* 679. The fragment at *m/z* 333 corresponds to the protonated aglycone $[A+H]^+$. Loss of the methyl group led to the ion $[A\text{-}15+H]^+$ at *m/z* 317. The signal corresponding to the loss of the *p*-coumaroyl moiety $[M\text{-}147+H]^+$ was clearly observed at *m/z* 494. The retro-Diels–Alder fragmentation of (**1**) led to the adduct $[C_9H_5O_5+glc+p\text{-coumaroyl}+Na]^+$ at *m/z* 409 and the corresponding adduct with potassium $[C_9H_5O_5+glc+p\text{-coumaroyl}+K]^+$ at *m/z* 445. Acid hydrolysis of (**1**) afforded D-glucose and *p*-coumaric acid, identified by TLC with authentic standards. Thus (**1**) was 6-methoxyquercetin-3-O-β-D-6″(*p*-coumaroyl)glucopyranoside. An analogue of this molecule is tiliroside, (kaempferol-3-O-β-D-6″(*p*-coumaroyl)glucopyranoside (Kumar, Bhan, Katia, & Dhar, 1985).

Substance (**2**) was quite similar to (**1**) with a UV absorption maxima at 238 and 371 nm, with shoulders at 274 and 312 nm. The IR spectrum showed absorptions at 3420 cm^{-1} (O–H), 1652 cm^{-1} (C=O) and at 1600 cm^{-1} (C=C). In the ^{1}H NMR spectrum, the main difference is due to the B-ring of the flavonoid skeleton: the spectrum showed two signals, both integrating for two protons, with $J=8.0$ Hz at δ 7.98 (2H, H2′/H6′) and at δ 6.85 (2H, H3′/H5′), clearly indicating a kaempferol derivative. The singlet at δ 6.49 was ascribed to H8. The remaining signals are similar to

Table 1
Distribution of the compounds in the extracts of the *Paepalanthus* species. – absent, + trace amount, ++ intermediary concentration, +++ majority

	1	2	3	4	5	6	7	8	9	10	11
P. polyanthus	+++	–	++	++	++	+	++	–	–	–	–
P. hilairei	–	+	–	++	–	–	++	+++	+++	++	++
P. robustus	++	++	++	–	–	+	–	–	+++	+	+
P. ramosus	–	+	++	++	–	–	–	+++	+++	++	++
P. denudatus	+	+++	–	–	–	–	–	++	++	+	+

Table 2
^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** (DMSO-d_6, 200MHz, 50MHz) (*J* in Hz). b: broad

Position	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
Aglycone				
2	156.6		156.9	
3	132.7		132.8	
4	177.6		177.8	
5	151.5		151.8	
6	131.3		131.5	
7	157.4		157.9	
8	93.8	6.47 s	94.2	6.49 s
9	152.4		152.4	
10	104.3		104.3	
1′	121.2		121.0	
2′	116.2	7.55 d (2.0)	131.0	7.98 d (8.0)
3′	144.6		115.3	6.85 d (8.0)
4′	148.5		160.2	
5′	115.2	6.76 d (8.0)	115.3	6.85 d (8.0)
6′	121.6	7.51 dd (8.0;2.0)	131.0	7.98 d (8.0)
OMe	59.9	3.69 s	60.1	3.70 s
OH-5		12.71 s		12.50 s
Glucose				
1″	100.8	5.48 d (7.0)	101.2	5.43 d (7.0)
2″	74.0	3.2–3.9[a]	74.3	3.2–3.9[a]
3″	76.3	3.2–3.9[a]	76.3	3.2–3.9[a]
4″	69.9	3.2–3.9[a]	70.1	3.2–3.9[a]
5″	74.2	3.2–3.9[a]	74.3	3.2–3.9[a]
6a″	63.1	4.26 bd (10.0)	63.1	4.20 bd (10.0)
6b″		4.05 m		4,00 m
Acyl moiety				
1‴	124.9		125.0	
2‴/6‴	130.2	7.35 d (8.0)	130.6	7.37 d (8.0)
3‴/5‴	115.9	6.83 d (8.0)	116.0	6.78 d (8.0)
4‴	159.8		159.9	
α	113.7	6.15 d (16.0)	113.8	6.14 d (16.0)
β	144.9	7.33 d (16.0)	144.8	7.35 d (16.0)

[a] Overlaped signals.

those of **1** Table 2. The ^{13}C NMR spectrum presented two intense signals at δ 131.0 and at δ 115.3, assigned to H2′/H6′ and to H3′/H5′, respectivelly. The signals at δ 121.0 and at δ 160.2 are typical of an unsubstituted B-ring of a kaempferol-like structure (Agrawal, 1989). The other signals are similar to those of **1** Table 2.

The ES-MS of (**2**) gave a pseudomolecular ion $[M+H]^+$ at *m/z* 625 and a molecular formula of $C_{31}H_{28}O_4$. The adducts with sodium $[M+Na]^+$ and with potassium $[M+K]^+$ occurred, respectively, at *m/z* 647 and *m/z* 663. We observed the protonated aglycone $[A+H]^+$ at *m/z* 317, as well as its adducts with sodium $[A+Na]^+$ at *m/z* 339 and with potassium $[A+K]^+$ at *m/z* 355. Loss of the *p*-coumaroyl moiety led to the ion $[M-147+H]^+$ at *m/z* 478. The retro-Diels–Alder fragmentation was also observed, leading to the ion $[C_9H_5O_4+glc+p\text{-coumaroyl}]^+$ at *m/z* 390 and to the adducts $[C_9H_5O_4+glc+p\text{-coumaroyl}+Na]^+$ at *m/z* 413 and $[C_9H_5O_4+glc+p\text{-coumaroyl}+K]^+$ at *m/z* 429. Acid hydrolysis of **2** released D-glucose and *p*-coumaric acid, both of which were identified by TLC with authentic standards. Therefore, **2** was identified as being 6-methoxykaempferol-3-O-β-D-6″-(*p*-coumaroyl)-glucopyranoside.

Compounds **1** and **2** have never been described before. The presence of acyl glucosylated flavonoids in the five *Paepalanthus* species investigated represents a unique chemical characteristic in the Eriocaulaceae, since such compounds have not been described in plants belonging to any other genus of Eriocaulaceae previously investigated, namely *Syngonanthus* (Ricci, 1993; Bomfim, 1993), *Leiothrix* (Dokkedal, & Salatino, 1992) and *Eriocaulon* (Bate-Smith, & Harborne, 1969; Gibbs, 1974). It is interesting to note that **1** and **2** were found in the five species that belong to the subgenus *Actinocephalus*, but they were not detected in the

capitulae of *P. bromelioides, P. vellozioides* and *P. latipes*, that belong to the subgenus *Platycaulon* (Vilegas et al., 1990; Garcia, 1997; Vilegas et al., 1998).

The chemistry of these five species seem to be qualitatively similar. Differences among the species arise from the amounts of the substances isolated from each plant (Table 1). Compound **1** is a major flavonoid in *P. polyanthus*, but is also present in *P. robustus* and in minor amounts in *P. denudatus*. On the other hand, **2** has not been detected only in *P. polyanthus*, but it is the major flavonoid in *P. denudatus*. *P. robustus* is the only specie that contains almost equal amounts of **1** and **2**. *P. hilairei* and *P. ramosus* display a similar chemical pattern, the unique difference being the occurrence of **7** in *P. hilairei*. Therefore, these flavonoids may play an important role in the chemosystematics of Eriocaulaceae. It is also remarkable that only *P. polyanthus* does not contain the naphtopyrone derivatives **10** and **11**, present in all *Paepalanthus* species investigated up to now. This may be a distinctive characteristic of this species. Other investigations are in progress in order to obtain more chemical data from this family.

3. Experimental

3.1. General

IR: KBr. UV: MeOH. TLC: silica gel 60H (Merck, 10–40 μm). Detection of the flavonoids with NP-PEG reagent (Wagner et al., 1984). Detection of glucose and *p*-coumaric acid with 80% soln of H_2SO_4 in EtOH followed by heating at 110°C for 2–3 min. ^1H and ^{13}C NMR: Brucker AC200, 200 and 50 MHz, respectively, in DMSO-d_6, TMS as int. standard. ES-MS Fisons VG Platform with one quadrupole. 100 V, PI.

3.2. Plant material

All plants were collected at Serra do Cipó — State Minas Gerais — Brazil and identified by Professor Paulo Takeo Sano. Voucher specimens have been deposited at the herbarium of Departamento de Botânica do Instituto de Biociências — USP. *P. polyanthus* (Bong.) Kunth CFSC 13849, *P. robustus* Silveira CFSC 13840, *P. hilairei* Koern. CFSC13843.

3.3. Extraction and isolation

Capitula were sepd, dried in an oven at 60°C for 1 week and powdered. The resulting material was separately macerated at room temp. sequentially with hexane, methylene chloride and EtOH for 1 week with each solvent. After filtration and evaporation under red. pres of the solvents EtOH extracts were obtained. Approximately 1.0 g of each extract was fractionated by gel permeation CC (Sephadex LH-20, Pharmacia) eluted with MeOH. The substances obtained were further purified by repeated CC either on polyvinylpolypyrrolidone (Sigma) eluted with MeOH or on a Lobar RP-8 column (Merck, 40–63 μm) eluted with MeCN/water 1:1 v:v. The distribution of the isolated compounds is given in Table 1.

3.4. Acid hydrolysis of *1* and *2*

A soln of **1**–**2** (10 mg each) in 10% HCl was refluxed for 1 h. The reaction mixture was neutralized with 5% NaOH and extracted with Et_2O. The Et_2O layer was evaporated to give the aglycones of **1** and **2**. The aqueous layer was evapd and examined by TLC, as determined by TLC comparison with sugar standards and cinamic acid derivatives, affording D-glucose and *p*-coumaric acid.

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References

Agrawal, P. K. (1989). *Carbon 13 NMR of flavonoids*. Amsterdam: Elsevier.

Bate-Smith, E. C., & Harborne, J. B. (1969). *Phytochemistry*, *8*, 1025.

Bomfim, M. C. P. (1993). *Flavonoid pattern of the sections Eulepsis Bong. and Thysanocephalus Koern. and the taxonomy of Syngonanthus Ruhl. (Eriocaulaceae)*. São Paulo, MS Dissertation (pp. 102).

Dokkedal, A. L., & Salatino, A. (1992). *Biochemical Systematics and Ecology*, *20*, 31.

Garcia, A. C. L. (1997). *Application of analytical techniques to the determination of the chemical constituents from 'everlasting' plants*. Araraquara, MS Dissertation (pp. 73).

Gibbs, R. (1974). *Chemotaxonomy of flowering plants*. Montreal: McGill-Queen's University Press.

Giulietti, A. M., & Hensold, N. C. (1990). *Acta Botanica Brasileira*, *4*, 135.

Giulietti, A. M., & Pirani, J. R. (1988). *Patterns of geographic distribution of some plant species from the Espinhaço* range Minas Gerais and Bahia, Brazil. Rio de Janeiro, Brazil: Academia Brasileira de Ciências.

Harborne, J. B., & Mabry, T. J. (1982). *The flavonoids: advances in research*. New York: Van Nostrand.

Hensold, N. C., & Giulietti, A. M. (1991). *Miss. Bot. Gard.*, *7*, 441.

Kumar, R., Bhan, S., Katia, A. K., & Dhar, K. L. (1985). *Phytochemistry*, *24*, 1124.

Markham, K. R. (1982). *Techniques of flavonoid identification*. London: Academic Press.

Ricci, C. V. (1993). *Flavonoid profile of the species belonging to the sections Carpocephalus Koern. and Dimorphocaulon Ruhl. from Syngonanthus Ruhl. (Eriocaulaceae)*. São Paulo, MS Dissertation (pp. 100).

Salatino, M. L. F., Pereira, H. A. B., Salatino, A., & Giulietti, A. M. (1988). *Boletim de Botanica da Universidade de São Paulo*, *10*, 55.

Sano, P. T. (1994). *The genus Paepalanthus Kunth, section Actinocephalus Koern at Serra do Cipó, Minas Gerais, Brazil: taxonomy and fenology*. São Paulo, MS dissertation (pp. 120).

Vilegas, W., Roque, N. F., Salatino, A., Giesbrecht, A. M., & Davino, S. (1990). *Phytochemistry*, *29*, 2299.

Vilegas, W., Santos, L. C., Alécio, A. C., Pizza, C., Piacente, S., DePauw, E., & Sano, P. T. (1998). Phytochemistry, in press.

Vilegas, W., Dokkedal, A. L., Rastrelli, L., Piacente, S., & Pizza, C. (1998). *Journal of Natural Products*, submitted for publication.

Wagner, H., Bladt, S., & Zgainski, E. M. (1984). *Plant drug analysis*. Berlin: Springer.