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DISTRIBUTION OF COUMARINS IN THE TRIBE PLUCHEEAE, GENUS *Pterocaulon*

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The genus *Pterocaulon* (Asteraceae) is included in the tribe Plucheeae [1]. About half of the *Pterocaulon* species have been analyzed previously and many compounds have been isolated: caffeoylquinic acids from *P. virgatum* [2]; thiophene acetylenes and flavanols from *P. virgatum* [3]; polyacetylenes from *P. alopecuroides, P. balansae, P. lanatum,* and *P. rugosum* [4]; hydroxy-α-caryophyllene from *P. serrulatum* [5]; flavonoids from *P. sphacelatum* [6], *P. purpurascens* [7], and *P. alopecuroides* [8]; and coumarins from *P. balansae, P. lanatum* [4], *P. purpurascens* [9–11], *P. redolens* [12], *P. virgatum* [13–16], *P. polystachyum* [17], and *P. alopecuroides* [8]. Several species of the genus are used in folk medicine as insecticides and agents against snake bites [15].

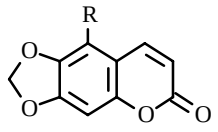
In this work we investigate the occurrence of coumarins in exudates of the species of *Pterocaulon* native to Brazil and isolate the most abundant compounds. The taxonomic significance of these compounds is discussed.

In Plucheeae the only genus that contains coumarins is *Pterocaulon* in which 40 different coumarins were previously found in ten species. In this work we analyzed five other species native to South Brazil, and coumarins were found in all of them. These data indicate that these compounds could be considered chemotaxonomic markers for the genus *Pterocaulon*.

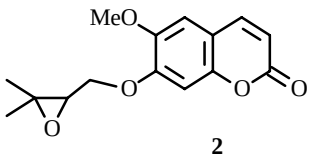
Although the species of *Pterocaulon* exhibit other classes of compounds, coumarins are characteristic of the genus. In *Pterocaulon*, coumarins can be di-, tri-, or tetraoxygenated, and all of them are 6,7 oxygenated.

Pterocaulon alopecuroides and *P. polystachyum* were studied previously, but in this work we investigated the exudate instead of the extract of the powdered plant. The exudate of *P. alopecuroides* afforded 5-methoxy-6,7-methylenedioxycoumarin (**1**) and 7-(2,3-epoxy-3-methyl-3-butyloxy)-6-methoxycoumarin (**2**), previously found in *P. virgatum* [13, 18]. To our knowledge this is the first report on the occurrence of coumarins **1** and **2** in *Pterocaulon alopecuroides.* From this plant, the compounds 7-(2,3-dihydroxy-3-methylbutyloxy)-6-methoxycoumarin and 7-(2,3-dihydroxy-3-methylbutyloxy)-5-hydroxy-6-coumarin were previously obtained [8]. This difference could be due to the extraction method employed and to the fact that the plants were collected in different places. Nevertheless, it is worthwhile to state that the plant exudate contained several coumarins of which only the most abundant were isolated.

The exudate of *P. polystachyum*, besides 5-methoxy-6,7-methylenedioxycoumarin (**1**), afforded three other coumarins, namely ayapin (**3**), prenyletin (**4**), and prenyletin methyl-ether (**5**). These compounds were previously isolated from the chloroform extract of the powdered plant material [17, 19].



1, 3



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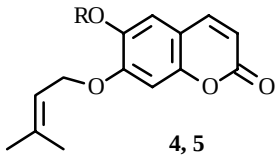

4, 5

1: R = OMe; **3:** R = H; **4:** R = H; **5:** R = Me

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TABLE 1. ^{1}H NMR Data of Compounds **1-5** (coupling constants in Hz)

Atom	**1***	**2***	**3**	**4**	**5**
H-3	6.22 (d, J = 9.7)	6.27 (d, J = 9.4)	6.27 (d, J = 9.5)	6.20 (d, J = 9.4)	6.22 (d, J = 9.4)
H-4	7.95 (d, J = 9.7)	7.62 (d, J = 9.4)	7.58 (d, J = 9.5)	7.83 (d, J = 9.4)	7.77 (d, J = 9.4)
H-5	-	6.90 (s)	6.82 (s)	7.07 (s)	6.93 (s)
H-8	6.54 (s)	6.87 (s)	6.83 (s)	6.89 (s)	6.88 (s)
5-OMe	4.15 (s)	-	-	-	-
6-7-OCH_2O-	6.00 (s)	-	6.07 (s)	-	-
6-OH	-	-	-	5.50 (t)	-
6-OMe	-	3.90 (s)	-	-	3.82 (s)
H-1′a	-	4.12 (dd, J = 11.4; 6.0)	-	4.62 (d, J = 6.7)	4.67 (d, J = 6.4)
H-1′b	-	4.34 (dd, J = 11.4; 4.0)	-	4.62 (d, J = 6.7)	4.67 (d, J = 6.4)
H-2′	-	3.19 (dd, J = 6.0; 4.0)	-	5.46 (t)	5.51 (t)
H-4′	-	1.39 (s)	-	1.76 (s)	1.78 (s)
H-5′	-	1.38 (s)	-	1.78 (s)	1.78 (s)

Experimental data (400 MHz; $CDCl_3$). *Experimental data (500 MHz; $CDCl_3$).

TABLE 2. ^{13}C NMR Data of Compounds **1-5**

Atom	**1***	**2***	**3**	**4**	**5**
C-2	161.27	161.22	161.13	163.82	164.00
C-3	111.72	113.79	113.36	112.89	113.40
C-4	138.76	143.17	143.40	145.87	145.80
C-5	138.04	108.65	105.05	110.05	108.15
C-6	131.74	146.67	144.89	139.97	145.50
C-7	152.65	151.82	151.25	150.15	152.47
C-8	92.36	101.89	98.35	102.11	101.75
C-9	151.51	149.77	151.25	148.36	148.36
C-10	106.60	111.97	112.67	112.98	112.98
5-OCH_3	59.92	-	-	-	-
6-7-OCH_2O-	101.80	102.31	-	-	-
6-OCH_3	-	56.44	-	-	56.83
C-1′	-	68.51	-	67.20	67.20
C-2′	-	60.83	-	120.11	120.27
C-3′	-	58.25	-	139.76	139.76
C-4′	-	24.53	-	25.84	25.84
C-5′	-	19.07	-	18.28	18.28

Experimental data (100 MHz; $CDCl_3$). *Experimental data (125 MHz; $CDCl_3$).

The presence of high amounts of coumarins in the exudate of these plants suggests that the compounds are accumulated in the epicuticular wax or in the trichomes or in both. In order to determine the exact localization of the compounds, a morpho-anatomical study is being carried out.

The presence of some secondary metabolites can be a useful character in the revision of taxonomic systems. Nevertheless sometimes the available data are not sufficient. As until now the only chemical character used in cladistic analysis of the tribe Plucheeae was the presence or absence of the sesquiterpene cuauhtematl (cuauhtemon) and its derivatives, the data presented here could be useful.

Some coumarins such as ayapin isolated from several species are described as phytoalexins, protecting the plant against microorganisms attach to it, especially fungus. In the case of *Pterocaulon* the method employed for the extraction of the compounds could indicate that the substances, if not all, the majority, are located in the surface of the leaves and branches where they could act as phytoalexins. Interestingly enough, native species of *Pterocaulon* are used in some rural areas in South Brazil

to treat animal skin diseases popularly diagnosed as mycoses. In a recent study our group investigated the antifungal activity of crude extracts obtained from *P. alopecuroides*, *P. balansae*, and *P. polysthachyum*, finding significant results [20].

Plant Material. Aerial parts of the plants were collected in the Rio Grande do Sul state, in March, 2003. The voucher specimens are deposited in the Herbarium of the Federal University of Rio Grande do Sul (ICN) (*Pterocaulon alopecuroides* (Lam.) DC. (136583); *P. angustifolium* DC. (136587); *P. balansae* Chodat (136589); *P. cordobense* O.K. (136588); *P. lorentzii* Malme (136586); *P. polypterum* (DC.) Cabrera (136585); *P. polystachyum* DC. (136584) and *P. rugosum* (Vahl) Malme (136590).

Chemical Methods. Whole leaves and branches of the plants *(*ca. 50 g) were carefully dried and extracted twice by immersion in hexane:dichloromethane (1:1) for 15 s. This short extraction period proved sufficient for obtaining the compounds that coated the twigs and leaves without extracting other components such as chlorophyll from those parts of the plant. The extracts were evaporated to dryness under reduced pressure and analyzed by TLC on silicagel using elution with CH_2Cl_2 100% up to CH_2Cl_2–MeOH (95:5). From *P. alopecuroides* and *P. polystachyum*, the main products in each case were isolated by column chromatography on silica gel using a chloroform-methanol gradient system followed by preparative TLC on silica gel with chloroform–methanol (98:2), to afford 5-methoxy-6,7-methylenedioxycoumarin (**1**) and 7-(2,3-epoxy-3-methyl-3-butyloxy)-6-methoxycoumarin (**2**) from *P. alopecuroides*. From *P. polystachyum*, besides compound **1**, ayapin (**3**), prenyletin (**4**), and prenyletin methyl-ether (**5**) were isolated. The products were identified by ^{1}H NMR and ^{13}C NMR spectroscopy (Tables 1, 2) and comparison with literature data [14, 18, 21, 22].

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