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COUMARINS AND A FLAVONOID FROM *PTEROCAULON ALOPECUROIDES*

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Abstract—From a CH_2Cl_2 extract of the aerial parts of *Pterocaulon alopecuroides*, two oxyprenyl coumarins, 7-(2,3-dihydroxy-3-methylbutyloxy)-6-methoxy coumarin and 7-(2,3-dihydroxy-3-methylbutyloxy)-5-hydroxy-6-methoxy coumarin and one flavononol, 3,5,3′,4′-tetrahydroxy-7-(2,3-en-3-methylbutyloxy)-2,3-dihydroflavonol, were isolated.

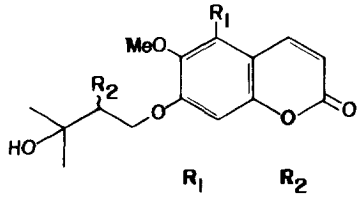
INTRODUCTION

The genus *Pterocaulon* has about 20–25 species distributed throughout America and Oceania [1]. Early works report the isolation of monoterpenes, sesquiterpenes, coumarins, flavonoids and polyacetylenes from *P. sphacelatum*, *P. balansae*, *P. lanatum*, *P. virgatum*, *P. alopecuroides* and *P. rugosum* [2–6]. The present work deals with the isolation of coumarins and flavononoids from the aerial parts of *P. alopecuroides*, a shrub collected at Serra do Taboão, State Minas Gerais, Brazil.

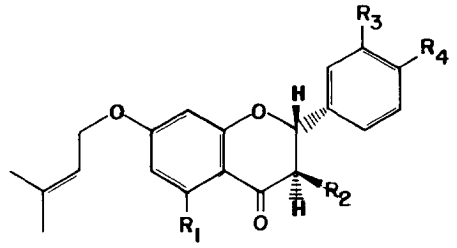
RESULTS AND DISCUSSION

After chromatographic fractionation, the CH_2Cl_2 extract of *P. alopecuroides* afforded UV-absorbing substances which exhibited signals of aromatic, as well as aliphatic protons in the NMR spectra. Compound **1a** has already been isolated from *P. balansae*, *P. lanatum*, *P. virgatum* and *P. rugosum* [3–5]. The spectral data are in full accordance with those already reported [7].

The ^1H NMR spectra of **1b** (Table 1) were very similar to those of **1a**, the main difference being the presence of only one aromatic proton at 6.43 ppm. Its ^{13}C NMR spectrum (Table 2) showed only 15 peaks; this clearly indicates a trioxygenated aromatic moiety. The mass spectrum furnished a $[M]^+$ at *m/z* 310, with a molecular formula of $C_{15}H_{18}O_7$. Hence, we deduced that **1b** has one further hydroxyl group bonded to the aromatic moiety. H-4 absorbs at 7.97 ppm, suggesting oxygenation at C-5 [8]. The 2D ^1H–^1H COSY spectrum showed interaction between 7.97 ppm (H-4) and 6.43 ppm, this being assigned to H-8, as the literature reports a W-like coupling between H-4 and H-8 [9]. The UV spectra of **1b** showed a bathochromic shift on addition of NaOAc, thus indicating that the phenolic hydroxyl group should be placed either at C-5 or at C-7. Acetylation of **1b** with Ac_2O–pyridine also led us to the same conclusion, because of the downfield shift of H-8 in the NMR spectra of **1c**. The ^{13}C NMR chemical shift of the methoxyl group in



	R_1	R_2
1a	H	OH
1b	OH	OH
1c	OAc	OAc
1d	OMe	OH



	R_1	R_2	R_3	R_4
2a	OH	OH	OH	OH
2b	OH	OH	OMe	OMe
2c	OMe	OH	OMe	OMe
2d	OAc	OAc	OAc	OAc

Table 1. ^{1}H NMR spectral data of **1a–1d**

H	1a	1b	1c	1d
3	6.18 *d*	6.23 *d*	6.17 *d*	6.29 *d*
4	7.52 *d*	7.97 *d*	7.86 *d*	7.62 *d*
5	6.75 *s*	—	—	—
8	6.72 *s*	6.43 *s*	6.55 *s*	6.77 *s*
1′	4.08 *dd*	4.35 *dd*	4.20 *dd*	4.30 *m*
2′	3.77 *m*	4.10 *dd*	4.04 *dd*	4.20 *m*
4′	1.27 *s*	1.39 *s*	1.26 *s*	1.33 *s*
5′	1.25 *s*	1.35 *s*	1.19 *s*	1.30 *s*
OMe	3.79 *s*	4.07 *s*	3.79 *s*	3.78 *s*
OAc	—	—	—	2.03 *s*
				2.11 *s*
				2.12 *s*

J[Hz]: 3,4 10.0; 1′, 2′ 9.7 (*gem*), 7.2 (*vic*), 2.2 (*vic*).

Table 2. ^{13}C NMR spectral data of **1a–1d**

C	1a	1b	1c	1d
2	162.1	161.3	161.1	168.2
3	114.2	111.6	112.9	114.1
4	144.1	138.8	138.6	137.4
5	108.7	146.1	146.9	160.4
6	146.9	131.8	136.6	138.3
7	152.2	154.7	155.9	155.4
8	101.7	93.0	96.8	99.1
9	150.2	151.6	151.4	150.9
10	112.4	103.2	107.8	107.3
1′	71.8	70.5	71.1	68.2*
2′	75.7	75.8	75.2	81.2
3′	72.5	71.9	71.8	67.8*
4′	26.9	26.3	26.6	26.3
5′	26.1	25.1	26.6	23.0
OMe	56.6	61.5	61.7	60.9
			61.3	
OAc	—	—	—	20.3
				22.0
				22.2

*Interchangeable signals.

Table 3. 2D ^{1}H–^{13}C long range COSY of **1b**

^{13}C signals	Correlated ^{1}H signals
103.2 (C-10)	6.23 (H-3)
	6.43 (H-8)
131.8 (C-6)	4.07 (OMe)
	6.43 (H-8)
146.1 (C-5)	7.97 (H-4)
151.6 (C-8)	7.97 (H-4)
154.7 (C-7)	6.43 (H-8)
162.1 (C-2)	7.97 (H-4)

1b showed that it must be *ortho*-disubstituted and placed either at C-5 or C-6. Methylation of **1b** with CH_2N_2 afforded **1d**, the OMe group resonating near 61 ppm in the ^{13}C NMR spectrum. All these data are compatible with the structure, 5-hydroxy-6-methoxy-7-prenyloxy-coumarin for **1b**. This proposal was confirmed by long-range couplings of carbons and protons in 2D ^{1}H–^{13}C COSY experiments (Table 3). It would appear that **1b** is a new coumarin. Compound **2a**, the major compound, has already been isolated from *P. virgatum* and *P. rugosum* [4, 5]. It was identified through comparison of its ^{1}H NMR data (Table 4) with those reported [4]. ^{13}C NMR data assignment (Table 5) was done

Table 4. ^{1}H NMR spectral data of **2a–2d**

H	2a	2b	2c	2d
2	4.94 *d*	4.95 *d*	5.41 *d*	4.88 *d*
3	4.53 *d*	4.50 *d*	5.18 *d*	4.30 *m*
6	6.12 *d*	6.05 *d*	6.22 *d*	6.07 *bs*
8	6.05 *d*	6.00 *d*	6.11 *d*	6.06 *bs*
2′	7.10 *d*	6.86 *d*	7.09 *bs*	7.09 *m**
5′	6.94 *d*	7.00 *d*	7.02 *d*	6.86 *m**
6′	6.98 *d*	7.03 *d*	7.22 *bd*	6.94 *m**
1″	4.51 *d*	4.45 *d*	4.46 *bd*	4.38 *bd*
2″	5.44 *m*	5.36 *m*	5.39 *m*	5.33 *m*
4″	1.72 *s*	1.65 *s*	1.61 *s*	1.67 *s*
5″	1.79 *s*	1.72 *s*	1.69 *s*	1.74 *s*
OMe	—	3.86 *s*	3.85 *s*	—
		3.83 *s*	3.85 *s*	
			3.86 *s*	
OCOMe	—			1.88 *s*
				2.10 *s*
				2.11 *s*
				2.19 *s*
OH	11.16 *s*	11.12 *s*	—	—

J[Hz]: 2,3 12.0; 6,8 2.0; 2′,6′ 1.5; 5′, 6′ 8.0; 1″, 2″, 7.0.
*Overlapped broad bands.

Table 5. ^{13}C NMR spectral data of **2a–2d**

C	2a	2b	2c	2d
2	83.0	83.4	83.0	79.9
3	72.3	72.3	72.6	72.5
4	195.5	195.7	190.8	183.3
5	163.4	163.5	164.9	164.6
6	96.1	96.1	94.2	105.5
7	168.2	168.1	166.3	167.6
8	95.2	95.2	93.4	99.5
9	162.7	162.0	162.0	162.0
10	102.0	102.0	100.0	106.5
1′	128.4	128.4	129.0	133.6
2′	115.4	111.0	110.5	122.7
3′	144.7	149.6	146.0	142.4
4′	143.7	149.1	146.0	141.7
5′	114.5	110.1	109.8	123.3
6′	120.5	120.3	121.0	124.6
1″	65.5	65.5	65.3	65.1
2″	118.2	118.3	118.3	118.9
3″	139.2	139.0	139.0	137.8
4″	18.2	18.2	18.2	18.0
5″	25.7	25.7	25.7	25.6
OMe	—	55.9	56.1	—
			55.9	

OCOMe: 167.5; 166.4; 166.3; 164.6; 20.5; 20.1; 20.1; 19.8.

through NOISE, DEPT and 2D ^1H–^{13}C COSY experiments.

Derivatives **2b** and **2c** were prepared, and their NMR data were registered for the first time (Tables 4 and 5). Comparison of ^{13}C NMR data of **2b** with those of **2a** shows significant changes only at ring B (Table 3). The ^1H NMR spectrum of **2b** shows a signal at 11.12 ppm from OH-5, showing that this was not methylated, because it is hydrogen bonded to the carbonyl.

Compound **2c** was methylated at OH-5, OH-3′ and OH-4′, as clearly shown from the ^{13}C NMR data of ring C and those from rings A and B when compared to **2a**. The ^{13}C NMR of **2d** shows downfield shifts for the carbons *ortho* and *para* to hydroxyls, as expected. The signal for C-3 was moved upfield due to the shielding effect of the acetyl group.

EXPERIMENTAL

Plant material. Aerial parts of *P. alopecuroides* D. C. Kuntz were collected at Serra do Taboão, Campo do Meio, State Minas Gerais, Brazil.

Isolation of constituents. Dried and powdered aerial parts (250 g) were extracted (Soxhlet) with hexane and then CH_2Cl_2. The soln was concd under vacuum, affording 20 g of CH_2Cl_2 extract. The extract was subjected to CC (silica gel, hexane–EtOAc gradient); frs of 125 ml were collected and monitored by TLC. Fr. 85 afforded pure **1a** [3]. Frs 77–79 were combined and submitted to flash CC (silica gel, hexane–EtOAc–HoAc, 4:6:1) affording the oil, **1b**. Frs. 55–56 were purified by HPLC (μBondapak 25 × 10, hexane–EtOAc 1:1, 1 ml min^{-1}, 300 nm) affording **2a** [6].

General. Ms: 70 eV. ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz), TMS at int. standard. IR: KBr. UV: MeOH. CC: silica gel (70–130 mesh, Merck). Flash CC: silica gel 60 H, Merck. Molecular formulae were determined by low resolution MS in combination with hydrogen and carbon counts by NMR.

Compound **1b**. Semi-solid. UV λ_{max} nm (log ε): 231 (4.05), 254 sh, 324 (3.83). IR ν_{max} cm^{-1}: 3383 (OH), 1699 (C=O). MS *m/z* (rel. int.) 310 $[M]^+$ (18, $C_{15}H_{18}O_7$), 208 (100, $C_{10}H_8O_5$), 193 (92, $C_9H_5O_5$). ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) Tables 1 and 2.

Methylation of **1b**. Compound (10 mg) **1b** was treated with CH_2N_2–Et_2O to give **1c**. Oil. UV λ_{max} nm (log ε): 227 (3.89), 254 sh, 324 (3.83). MS *m/z* (rel. int.) 394 $[M]^+$ (31, $C_{19}H_{22}O_9$), 352 (20, $C_{17}H_{20}O_8$), 208 (60, $C_{10}H_8O_5$), 193 (39, $C_9H_5O_5$), 59 (18, C_3H_7O). ^1H and ^{13}C NMR: Tables 1 and 2.

Acetylation of **1b**. Compound (10 mg) **1b** was acetylated with Ac_2O–pyridine to give **1d**. Oil. UV λ_{max} nm (log ε): 224 (3.76), 250 sh, 329 (3.66). MS *m/z* (rel. int.): 324 $[M]^+$ (58, $C_{16}H_{20}O_7$), 222 (100, $C_{11}H_{10}O_5$), 207 (93, $C_{10}H_7O_5$), 59 (20, C_3H_7O).

Compound **2a**. Solid, mp 175°. UV λ_{max} nm (log ε) 290 (4.2), 330 sh; + NaOH 291 (4.2), 359 (3.8). IR ν_{max} cm^{-1}: 3375 (OH), 1640 (C=O). ^1H and ^{13}C NMR: Tables 4 and 5.

Methylation of **2a**. Compound **2a** (50 mg) was treated with CH_2N_2–Et_2O to give **2b** and **2c**. They were sepd by flash CC. Compound **2b**. Yellowish oil. UV λ_{max} nm (log ε) 289 (4.1), 340 sh; + NaOH 290 (4.0), 360 (3.6). ^1H and ^{13}C NMR: Tables 4 and 5. Compound **2c**. Yellowish oil. UV λ_{max} nm (log ε) 291 (4.3), 340 sh; + NaOH 290 (4.3), 359 (3.8). ^1H and ^{13}C NMR: Tables 4 and 5.

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REFERENCES

1. Cabrera, A. L. and Ragonese, A. M. (1978) *Darwiniana* **21**, 185.
2. Johns, S. R., Lamberton, J. A., Price, J. R. and Sioumis, A. A. (1968) *Aust. J. Chem.* **21**, 3079.
3. Magalhães, A. F., Magalhães, E. G., Leitão Filho, H. F., Frighetto, R. T. S. and Barros, S. M. G. (1981) *Phytochemistry* **20**, 1369.
4. Bohlmann, F., Abraham, W.-R., Xing, R.-M. and Robinson, H. (1981) *Phytochemistry* **20**, 825.
5. Magalhães, A. F., Magalhães, E. G., Petrucci, M. I. F. and Leitão Filho, H. F. (1987) *Ci. e Cult.* **39** Suppl., 537.
6. Magalhães, A. F., Magalhães, E. G., Leitão Filho, H. F. and Nunes Jr, V. (1987) *Ci. e Cult* **39** Suppl., 525.
7. Bohlmann, F. and Jakupovic, J. (1979) *Phytochemistry* **18**, 1367.
8. Debenedetti, E. L., Nadinic, E. L., Coussio, J. D., Kimpe, N. D., Ferreau-Dupont, J. and Declercq, J. (1979) *Phytochemistry* **30**, 2757.
9. Murray, R. D. H., Méndez, J. and Brown, S. A. (1982) *The Natural Coumarins*, John Wiley, New York.