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Flavonoids from *Pterocaulon alopecuroides* with Antibacterial Activity

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Key words
- *Pterocaulon alopecuroides*
- Asteraceae
- Flavonoids
- antibacterial activity

Abstract

Three flavonoids, (2*R*,3*R*)-5,4′-dihydroxy-3′-*O*-methyl-7-(γ,γ-dimethylallyloxy)dihydroflavonol **1**, (2*R*,3*R*)-5,7,4′-trihydroxy-3′-*O*-methyl-6-(α,α-dimethylallyl)dihydroflavonol **2**, and (2*R*,3*R*)-5,7,4′-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol **3**, together with three known flavonoids (**4–6**), were isolated from the aerial parts of *Pterocaulon alopecuroides*. The structures of the compounds were determined by mass and by 1 D and 2 D NMR spectroscopy. Screening of the antibacterial activity of all six compounds was conducted by a disc diffusion test against *Bacillus cereus*, *Bacillus subtilis*, *Salmonella typhimurium* and *Proteus mirabilis*. The minimum inhibitory concentration (MIC) of the active compounds (**2**, **3**, **4**, **6**) was determined by a microdilution assay. These compounds were active only against both Gram (+) bacteria with MIC values ≤ 200 µg/mL.

Introduction

The genus *Pterocaulon* (Asteraceae) comprises at least 25 species growing in America and Oceania [1]. The chemistry of several of them has been studied; their common metabolites include flavonoids and coumarins [2], [3], [4], [5], [6], [7], [8], [9]. Plants from the genus *Pterocaulon* are used in traditional medicine in different zones of the world and antibiotic [9], antiviral [10], cytotoxic [11] and antifungal [12], [13] activities have been reported for them. As part of our continuing search for biologically active compounds from Argentinian medicinal plants, we have examined the aerial parts of *Pterocaulon alopecuroides* Lamarck and report here the isolation and structural elucidation of three new flavonoids (**1**, **2** and **3**) as well as the anti-bacterial activity of the new and known isolated compounds.

Material and Methods

General experimental procedures

The NMR spectra were recorded on a Bruker AC 200 (^{1}H at 200 MHz and ^{13}C at 50 MHz) or a Bruker Avance 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometer with TMS as internal reference. MS were performed on a VG-ZAB SEQ4F spectrometer. IR spectra were taken on an IR-FT Bruker model IFS-88 spectrometer. UV spectra were recorded on a Shimadzu UV-260 instrument. Optical rotation was measured on a Jasco P-1010 polarimeter, and CD spectra were obtained on a Jasco 810 spectropolarimeter. CC were performed on silica gel 230–400 mesh (Fluka), RPCC on C-18 silica gel (Merck), TLC was carried out on precoated silica gel 60 F_{254} plates (Fluka). Detection was achieved by UV light and spraying with 10% vanillin in EtOH followed by heating.

Plant material

P. alopecuroides aerial parts were collected in December 2003, Cuesta del Gallinato, La Caldera (Province of Salta, Argentina). The identification was carried out by Ing. Julio Tolaba. A voucher specimen (No. 3399) is on deposit at the Museo de la Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina.

Extraction and isolation

Air-dried aerial parts (300 g) of *P. alopecuroides* were macerated in EtOH for 7 days at room temperature. The organic solution was distilled under reduced pressure at 40 °C to obtain 70.0 g of crude extract. This extract was suspended in H_2O (500 mL) and then extracted with CH_2Cl_2 (4 × 200 mL).

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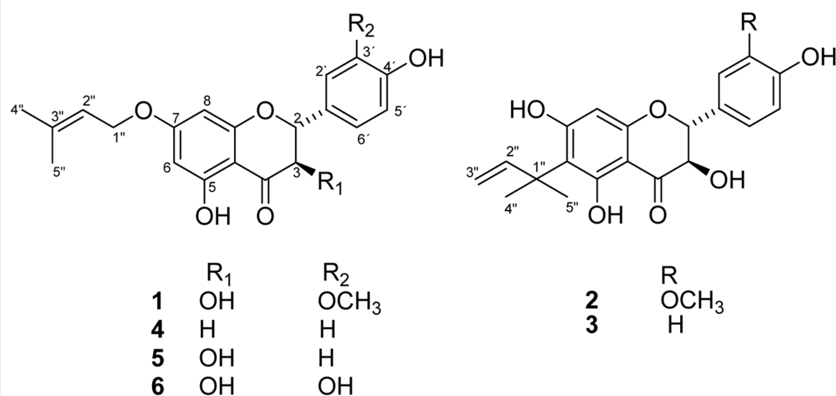

The CH_2Cl_2-soluble extract (20.0 g) was divided into 2 fractions by flash column chromatography on silica gel C-18 (70 g) (7 × 15 cm), eluting with MeOH : H_2O (9 : 1, 500 mL) and MeOH (500 mL). The first fraction (6.5 g) was subjected to VLC using a Büchner type funnel with fibrous glass frit (disc diam. 150 mm, capacity 400 mL) filled with 250 g of silica gel, each subfraction (250 mL) was eluted with hexane (F_1), hexane-EtOAc 7 : 3 (F_2), hexane-EtOAc 1 : 1 (F_3), hexane-EtOAc 3 : 7 (F_4), EtOAc (F_5).

F_2 (107 mg) was chromatographed on a silica gel column (2 × 30 cm, 40 g) using hexane containing increasing amounts Et_2O (0 – 100 %); a total of 100 fractions (10 mL each) were collected. Frs 30 – 33 (hexane-Et_2O 7 : 3) afforded 20 mg of **4**.

F_3 (780 mg) was first purified by CC on silica gel (3 × 30 cm, 60 g) eluting with CH_2Cl_2 (200 mL), CH_2Cl_2-Me_2CO 9.5 : 0.5 (200 mL), CH_2Cl_2-Me_2CO 9 : 1 (200 mL) and CH_2Cl_2-Me_2CO 8 : 2 (200 mL); fractions of 10 mL were collected. Subfractions 41 – 54 (400 mg) were combined and chromatographed by CC on silica gel (3 × 30 cm, 60 g) using mixtures (100 mL each) of hexane-Et_2O of increasing polarity (2 %) to yield **1** (6 mg), **2** (3.5 mg) and **5** (250 mg).

F_4 (1.11 g) was first chromatographed on a silica gel column (4 × 30 cm, 90 g) eluted with mixtures of CH_2Cl_2-Me_2CO of increasing polarities (1 %) from 100 : 0 to 80 : 20 (200 mL, each). The subfraction eluted with CH_2Cl_2-Me_2CO (9.3 : 0.7) contained a mixture of **1** and **2** (8 mg). The subfraction eluted with CH_2Cl_2-Me_2CO (88 : 12) was subjected to silica gel column chromatography (2 × 30 cm) using a gradient solvent system hexane-EtOAc (100 : 0 – 60 : 40) to give **3** (8 mg) and **6** (300 mg).

Characterization of the compounds

5,4′-Dihydroxy-3′-O-methyl-7-(γ,γ-dimethylallyloxy)dihydroflavonol (**1**): Amorphous solid; $[\alpha]_D^{24}$: + 127 (*c* 0.50, MeOH), UV (MeOH): λ_{max} = 290, 338 (sh); +NaOMe: 290, 360; +NaOAc: 290, 338; +$AlCl_3$: 380; +$AlCl_3$/HCl: 380 nm; CD (MeOH, *c* 0.05): $\Delta\varepsilon_{332}$ + 0.70; IR (KBr): ν_{max} = 3411, 2962, 2937, 1645, 1520, 1298, 1196, 1090 cm^{-1}; for ^1H-NMR and ^{13}C-NMR data, see **Table 1** and **Table 2**; HR-MS: 386.3928 (calcd. 386.3952); EI-MS (70 eV): *m/z* = 386 [M^+] (2), 149 (40), 69 (34), 41 (100).

5,7,4′-Trihydroxy-3′-O-methyl-6-(α,α-dimethylallyl)dihydroflavonol (**2**). Amorphous solid; $[\alpha]_D^{24}$: + 219 (*c* 0.35, MeOH); UV (MeOH): λ_{max} = 294, 346 (sh); +NaOMe: 336; +NaOAc: 296, 336; +$AlCl_3$: 314, 392; +$AlCl_3$/HCl: 314, 390 nm; CD (MeOH, *c* 0.01): $\Delta\varepsilon_{333}$ + 5.15; IR (KBr): ν_{max} = 3444, 2958, 2929, 2850, 1631, 1275, 1124, 1072 cm^{-1}; for ^1H-NMR and ^{13}C-NMR data, see **Table 1** and **Table 2**; HR-MS: *m/z* = 386.3944 (calcd. 386.3956); EI-MS (70 eV): *m/z* = 149 (100), 71 (26), 57 (45), 43 (53).

5,7,4′-Trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol (**3**). Amorphous solid; UV (MeOH): λ_{max} = 296, 346 (sh); +NaOMe: 338; +NaOAc: 298, 336; +$AlCl_3$: 314, 392; +$AlCl_3$/HCl: 312, 388 nm; CD (MeOH, *c* 0.06): $\Delta\varepsilon_{330}$ + 6.65; IR (KBr): ν_{max} = 3433, 2958, 2929, 1619, 1286, 1126, 1074 cm^{-1}; for ^1H-NMR and ^{13}C-NMR data, see **Table 1** and **Table 2**; HR-MS: *m/z* = 356.3613 (calcd. 356.3692); EI-MS (70 eV): *m/z* = 356 [M^+] (2), 149 (100), 71 (30), 57 (55), 43 (66).

5,4′-Dihydroxy-7-(γ,γ-dimethylallyloxy)flavanone (**4**). Amorphous solid; UV (MeOH): λ_{max} = 288, 338 (sh); +NaOMe: 288, 330; +NaOAc: 288, 330; +$AlCl_3$: 310, 380; +$AlCl_3$/HCl: 310, 380 nm.

5,4′-Dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol (**5**). White solid powder; UV (MeOH): λ_{max} = 290, 335 (sh); +NaOMe: 292, 344; +NaOAc: 290, 335; +$AlCl_3$: 299, 378; +$AlCl_3$/HCl: 299, 378 nm. Data unreported: ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 83.2 (C-2), 72.3 (C-3), 196.0 (C-4), 163.0 (C-5), 96.0 (C-6), 168.1 (C-7), 95.1 (C-8), 162.9 (C-9), 100.8 (C-10), 127.5 (C-1′), 129.1 (C-2′), 115.6 (C-3′), 157.3 (C-4′), 115.6 (C-5′), 129.1 (C-6′), 65.5 (C-1″), 118.4 (C-2″), 139.3 (C-3″), 25.7 (H-4″), 18.2 (H-5″).

5,3′,4′-Trihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol (**6**). White solid powder; UV (MeOH): λ_{max} = 292, 335 (sh); +NaOMe: 292, 334; +NaOAc: 292, 331; +$AlCl_3$: 314, 377; +$AlCl_3$/HCl: 314, 377 nm.

Antibacterial assays

Microorganisms: The microorganisms used in this work were two Gram-positive bacteria: *Bacillus cereus* and *Bacillus subtilis* and two Gram-negative strains: *Salmonella thyphimurium* and *Proteus mirabilis* all of them isolated in Área de Microbiología-UNSL. All strains tested were maintained at 4 °C in Tripticase Soy Agar (TSA) and were subcultured every month.

Disc diffusion method: To determine the antimicrobial activity, the paper disc diffusion technique was used. A population of approximately 10^6 CFU/mL of each strain was inoculated on a duplicate plate containing Mueller Hinton Agar (Britania) by using sterile cotton swabs. Sterilized paper discs of 6 mm diameter were used. They were soaked with 50 μg of each compound. Commercial gentamicin discs (10 μg; Britania) were used as standard. The discs were then placed aseptically over inoculated plates and incubated at 37 °C for 24 h. After incubation, the inhibition halos around of paper discs were measured accurately using a metric ruler. The experiment was replicated twice.

Determination of minimum inhibitory concentration (MIC): The inocula of microorganisms were prepared from 18-h broth cultures, and serial dilutions were made to achieve a suspension of approximately 10^4 CFU/mL. The test compounds initiallity dis-

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Table 1 ^{1}H-NMR and NOESY spectral data for compounds **1 – 3**

Position	1			2			3	
	δ [1]H†	δ [1]H‡	NOESY‡	δ [1]H†	δ [1]H‡	NOESY‡	δ [1]H†	δ [1]H‡
2	5.01 d (12.0)	5.11 d (12.0)	H-3	4.96 d (12.0)	5.06 d (11.7)	H-3	4.96 d (12.0)	5.07 d (11.5)
3	4.55 m	4.73 d (12.0)	H-2	4.51 d (12.0)	4.69 d (11.7)	H-2	4.51 d (12.0)	4.64 d (11.5)
6	6.12 d (2.2)	6.09 d (2.2)	H-1″	–	–		–	–
7	–	–		–	–		–	–
8	6.97 d (2.2)	6.06 d (2.2)		5.95 s	6.00 s		5.95 s	6.00 s
2′	6.97 – 7.09 m	7.23 d (1.7)	OCH_3	6.80 – 7.10 m	7.22 d (1.7)	OCH_3	7.37 d (8.8)	7.42 d (8.4)
3′	–	–		–	–		6.88 d (8.8)	6.90 d (8.4)
4′	–	–		–	–		–	–
5′	6.97 – 7.09 m	6.89 d (8.0)	H-6′	6.80 – 7.10 m	6.88 d (8.0)	H-6′	6.88 d (8.8)	6.90 d (8.4)
6′	6.97 – 7.09 m	7.05 dd (8.0, 1.7)	H-5′	6.80 – 7.10 m	7.04 dd (8.0, 1.7)	H-5′	7.37 d (8.8)	7.42 d (8.4)
1″	4.55 m	4.64 d (6.7)	H-6	–	–		–	–
2″	5.42 t,br	5.46 t (6.7)		6.42 dd (17.7, 10.5)	6.31 dd (17.5, 10.6)		6.41 dd (17.8, 10.6)	6.31 dd (17.4, 10.7)
3″	–	–		5.41 d,br (17.7) 5.32 dd (10.5, 1.1)	4.93 dd (17.5, 1.5) 4.84 dd (10.6, 1.5)		5.39 d,br (17.8) 5.31 dd (10.6, 1.1)	4.93 dd (17.4, 1.5) 4.84 dd (10.7, 1.5)
4″	1.80 s	1.78 s	–	1.59 s	1.58 s	–	1.58 s	1.58 s
5″	1.73 s	1.76 s	–	1.58 s	1.58 s	–	1.57 s	1.58 s
OCH_3	3.95 s	3.89 s	H-2′	3.93 s	3.89 s	H-2′	–	–
5-OH	11.19 s	11.7 s		12.27 s	12.7 s		12.20 s	12.7 s

† At 200 MHz in $CDCl_3$.
‡ At 400 MHz in Me_2CO-d_6.
Coupling constant values (in parentheses) are in Hz.

solved in DMSO were diluted in phosphate saline (PBS) to give a concentration of 800 μg/mL, and then serial two-fold dilutions were made in concentration ranges from 800 to 25 μg/mL. The nutrient broth was tripticase soy broth (TSB) pH 7.2 supplemented with 0.01 % (w/v) of 2,3,5-triphenyltetrazolium chloride as a visual indicator of bacterial growth. Microplate method (microwell dilution) was used to determinate the MICs. The 96-well plates were prepared by dispensing into each well 95 μL of nutrient broth, 5 μL of inoculum and 100 μL of test solutions. The final volume in each well was 200 μL. The plate was covered with sterile plate sealer and then incubated at 37 °C for 24 h. Gentamicin (Schering-Plough; 80 mg/mL) was used as positive control. All tests were performed in duplicate. The MIC was recorded as the lowest concentration of compound at which no bacterial growth was observed after incubation.

Results and Discussion

The CH_2Cl_2 soluble extract of the aerial parts of *P. alopecuroides* yielded three new flavonoids: 5,4′-dihydroxy-3′-*O*-methyl-7-(γ,γ-dimethylallyloxy)dihydroflavonol **1**; 5,7,4′-trihydroxy-3′-*O*-methyl-6-(α,α-dimethylallyl)dihydroflavonol **2** and 5,7,4′-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol **3**. The known compounds 5,4′-dihydroxy-7-(γ,γ-dimethylallyloxy)flavanone **4** [14], [15]; 5,4′-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol **5** [3] and 5,3′,4′-trihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol **6** [2] were also isolated.

The UV spectra of the compounds showed similar features, with maxima at λ = 288 – 296, and 335 – 346 (sh) nm, corresponding to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions matching flavanone skeletons [16]. The IR spectra of the isolated compounds showed a similar series of absorption bands at ν_{max} = 3500 – 3400 cm^{-1}, corresponding to OH vibrations; 2950 – 2850 cm^{-1}, corresponding to CH vibrations, which are not usually so apparent in flavonoid spectra; and 1639 – 1619 cm^{-1}, assigned to the C = O vibration of the carbonyl group [17].

Compound **1** was isolated as an amorphous solid. Its molecular formula was determined as $C_{21}H_{22}O_7$ based on the HR-MS and NMR spectroscopic data. Its ^{1}H-NMR spectrum (**Table 1**, Me_2CO-d_6) exhibited the typical AB system due to H-2 and H-3 of a 2,3-*trans*-dihydroflavonol at δ = 5.11 (1H, d, J = 12.0 Hz) and 4.73 (1H, d, J = 12.0 Hz), respectively [18], [19]. Two *meta*-coupled doublets (J = 2.2 Hz) at δ = 6.09 and 6.06, each integrating for one proton, were assigned to H-6 and H-8, respectively. Two vinylic methyl groups at δ = 1.76 and 1.78 (each 3H, s), an olefinic proton at δ = 5.46 (t, J = 6.7 Hz) and two oxymethylene protons at δ = 4.64 (d, J = 6.7 Hz) indicated the presence of a γ,γ-dimethylallyloxy (prenyloxy) substituent. The spectrum of ^{1}H-NMR also showed signals for a chelated phenolic group at δ = 11.7 (s) and

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Table 2 ^{13}C-NMR and HMBC spectral data for compounds **2–5**

Position	1			2		3	
	δ ^{13}C†	δ ^{13}C‡	HMBC‡	δ ^{13}C‡	HMBC‡	δ ^{13}C‡	HMBC‡
2	83.5	83.8	H-3, H-2′, H-6′	83.2	H-3, H-6′	83.3	H-3, H-2′, H-6′
3	72.4	72.3	H-2	72.2	H-2	72.2	H-2
4	195.8	198.1	H-2, H-3	198.5	–	197.7	H-2
5	163.6	163.8	H-6, 5-OH	163.2	5-OH	163.2	5-OH
6	96.2	95.5	5-OH, H-8	113.1	H-8, H-2″, H-4″, 5-OH	113.1	H-8, H-2″, H-4″, 5-OH
7	168.2	167.7	H-8, H-6, H-1″	165.7	H-8	165.8	H-8
8	95.2	94.4	H-6	96.0	–	95.9	–
9	162.9	162.9	H-8	160.7	H-8	160.8	H-8
10	100.7	101.5	5-OH, H-6, H-8	100.5	5-OH, H-8	100.5	5-OH, H-8
1′	127.9	128.6	H-2, H-3, H-2′, H-5′	128.6	H-2, H-3, H-5′	128.2	H-3, H-3′, H-5′
2′	109.7	111.5	H-2, H-6′	111.6	H-2, H-6′	129.3	H-2
3′	146.7	147.2	H-2′, OCH_3	147.2	H-2′, OCH_3	115.0	–
4′	146.7	147.0	H-5′, H-6′	147.0	H-5′, H-6′	158.0	H-2′, H-3′, H-5′, H-6′
5′	114.6	114.6	H-6′	114.8		115.0	–
6′	121.1	121.3	H-2, H-2′, H-5′	121.4	H-5′, H-2′	129.3	H-2
1″	65.5	65.3	–	40.5	H-2″, H-3″, H-4″	40.5	H-2″, H-3″, H-4″
2″	118.4	119.1	H-1″	149.8	H-3″, H-4″	149.8	H-3″
3″	139.4	138.2	H-1″	107.7	–	107.3	–
4”	25.8	24.9	H-2″	28.5	H-2″	28.5	H-2″
5″	18.2	17.3	H-2″	28.5	H-2″	28.5	H-2″
OCH_3	56.0	55.5	–	55.6	–	–	–

† At 50 MHz in $CDCl_3$.
‡ At 100 MHz in Me_2CO-d_6.

a methoxy group at 3.89 (s). Resonances at δ = 6.89, 7.05 and 7.23 and their coupling patterns indicated a 3′,4′-disubstituted B ring. A NOESY experiment (**Table 1**) performed on **1** showed an interaction between the OCH_3 and H-2′, indicating that they were located on adjacent carbons. An HMBC experiment (**Table 2**) showed correlations from C-3′ to OCH_3 and H-2′, and C-4′ to H-5′ and H-6′, indicating the placements of the 3′-OCH_3 and 4′-OH groups. The 5-OH proton signal correlated with carbons at δ = 163.6 (C-5), 96.2 (C-6), and 100.7 (C-10). The attachment of the γ,γ-dimethylallyloxy moiety was determined to be at C-7 by the detection of HMBC correlations from H-1″ to C-2″, C-3″ and C-7. In the CD spectrum a positive Cotton effect at 332 nm ($\Delta\varepsilon$ + 0.7) indicated the configuration 2*R*,3*R* [20]. Therefore, this new compound was characterized as (2*R*,3*R*)-5,4′-dihydroxy-3′-*O*-methyl-7-(γ,γ-dimethylallyloxy)dihydroflavonol.

The molecular formulae of the new compounds **2** and **3** were established in turn as $C_{21}H_{22}O_7$ and $C_{20}H_{20}O_6$ by HR-EI mass spectrometry. Analysis of their ^{1}H-NMR, ^{13}C-NMR and mass spectral data indicated that they were closely related dihydroflavonol structures containing a α,α-dimethylallyl functionality, differing only in their B-ring pattern.

The ^{1}H-NMR spectrum of **2** (**Table 1**, Me_2CO-d_6) showed the presence of a chelated hydroxy group at δ = 12.7, an isolated aromatic proton at δ = 6.00 (1H, s) and signals due to a α,α-dimethylallyl group at δ = 1.58 (6H, s), 4.84 (1H, dd, *J* = 10.6 and 1.5 Hz), 4.93 (1H, dd, *J* = 17.5 and 1.5 Hz) and 6.31 (1H, dd, *J* = 17.5 and 10.6 Hz). This spectrum also exhibited a methoxy group at δ = 3.89 and signals of a 3′,4′-disubstitued B-ring at δ = 7.22 (1H, d, *J* = 1.7 Hz, H-2′), 7.04 (1H, dd, *J* = 8.0 and 1.7 Hz, H-6′) and 6.88 (1H, d, *J* = 8.0 Hz, H-5′). A NOESY experiment (**Table 1**) showed an interaction between OCH_3 and H-2′, indicating that they were located on adjacent carbons. The position of the methoxy group was confirmed by HMBC correlations (**Table 2**) from the methoxy protons to C-3′.

In the HMBC spectrum of **2** (**Table 2**), the chelated 5-hydroxy proton at δ = 12.7 caused cross-peaks with three quaternary aromatic carbons at δ = 163.2 (C-5), 113.1 (C-6) and 100.5 (C-10). HMBC correlations of H-4″ and H-5″ to C-6 indicated that the α,α-dimethylallyl moiety was attached to C-6. The one proton singlet at δ = 6.00 was assigned to H-8 due its HMBC connectivities with C-7, C-9, C-10, C-6 (**Table 2**).

The ^{1}H-NMR spectrum of **3** (**Table 1**, Me_2CO-d_6) was similar to **2** except for the disappearance of the methoxy group proton and the appearance of signals at δ = 6.90 (2H, d, *J* = 8.4 Hz) and 7.42 (2H, d, *J* = 8.4 Hz) typical for a 4′-monosubstituted B-ring (**Table 1**). These protons, in the HMBC experiment, showed correlations with a quaternary carbon at δ = 158.0 which indicated that the 4′-position was substituted with a hydroxy group. A 2*R*,3*R*-configuration was assigned to compounds **2** and **3** by analysis of the CD spectrum, in which a positive Cotton effect was observed at 333 nm ($\Delta\varepsilon$ + 5.15) and 330 nm ($\Delta\varepsilon$ + 6.65), respectively [20]. Therefore, compounds **2** and **3** were structurally assigned as (2*R*,3*R*)-5,7,4′-trihydroxy-3′-*O*-methyl-6-(α,α-dimethylallyl)dihydroflavonol and (2*R*,3*R*)-5,7,4′-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol, respectively.

To the best of our knowledge, the ^{13}C-NMR data of **5** have not been previously reported (see Material and Methods).

The antibacterial activity of the isolated compounds **1–6** was analyzed by the paper disc diffusion method using *Bacillus cereus*, *Bacillus subtilis*, *Salmonella typhimurium*, and *Proteus*

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Table 3 Antibacterial activity of compounds **1–6** against Gram (+) and Gram (–) bacteria.

Compound	Diameter of inhibitory zones (mm)				MICs (µg/mL)	
	B. cereus	*B. subtilis*	*S. typhimurium*	*P. mirabilis*	*B. cereus*	*B. subtilis*
1	–	–	–	–	–	–
2	12	12	–	–	< 25	50
3	16	–	–	–	200	200
4	–	9	–	–	< 50	< 25
5	–	–	–	–	–	–
6	11	9	–	–	< 50	< 50
gentamicin	27	27	25	26	5	5

mirabilis as the test bacteria (**Table 3**). None of the flavonoids tested had inhibitory activity against Gram (–) bacteria. Also, compounds **1** and **5** were inactive on *B. subtilis* and *B. cereus* (there was no zone of inhibition at the concentration of 50 µg/disk).

The flavonoids which exhibited anti-bacterial effects in the disc diffusion assay (**2**, **3**, **4**, **6**) were further tested to determine their MICs using a colorimetric broth microdilution technique (**Table 3**). Compounds **2** and **4** were quantitatively the most active against *B. cereus* and *B. subtilis* (MICs < 25 µg/mL). Compound **3** displayed moderate activity against both Gram (+) bacteria, with MIC = 200 µg/mL.

Our results showed that **6** has a moderate antibacterial activity on *B. subtilis* and *B. cereus* with MICs < 50 µg/mL. This compound has been previously isolated from *Pterocaulon* genus but no information has been published on its biological activity so far.

This work contributes information on the chemistry and antibacterial activity of *P. alopecuroides*. This is the first report of the isolation of **4** from genus *Pterocaulon*.

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