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Article

Modified Eremophilanes and Anti-Inflammatory Activity of *Psacalium cirsiifolium*

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Quatro novos eremofilanos modificados, juntamente com dez derivados conhecidos de cacalol, dois cariofilenos, um aromadendreno e um flavonoide foram purificados a partir de *Psacalium cirsiifolium*. As estruturas destes compostos foram elucidadas por análise espectroscópica. A atividade anti-inflamatória dos extratos e de sete dos compostos isolados foi avaliada no modelo de 12-*O*-tetradecanoilforbol-13-acetato (TPA) de inflamação aguda induzida. O composto inédito 2α-hidroxiadenostin B (**4**) mostrou uma atividade dependente da dose (IC_{50} 0,41 μmol por orelha) e um efeito de inibição de neutrófilos medido pelo teste de mieloperoxidase (MPO) semelhante ao efeito da indometacina, 0,31 e 1,0 μmol por orelha.

Four new modified eremophilanes, together with ten known cacalol derivatives, two caryophyllenes, one aromadendrene and one flavonoid were isolated from *Psacalium cirsiifolium*. The structures of these compounds were elucidated by spectroscopic analysis. The anti-inflammatory activity of extracts and of seven of the isolated compounds was evaluated on 12-O-tetradecanoylphorbol-13-acetate (TPA) model of induced acute inflammation. The new compound 2α-hydroxyadenostin B (**4**) showed a dose dependent activity (IC_{50} 0.41 μmol *per* ear) and a neutrophil inhibition effect as measured by the myeloperoxidase (MPO) assay similar to that of indomethacin at 0.31 and 1.0 μmol *per* ear.

Keywords: *Psacalium*, modified eremophilanes, anti-inflammatory activity, TPA, myeloperoxidase

Introduction

Psacalium cirsiifolium is one of the 40 species of perennial herbs grouped into the genus *Psacalium* (Asteraceae, Senecioneae, Tussilagininae).[1] They are disseminated from the south of the United States to Guatemala and some of them are used in folk medicine to cure diabetes and renal, hepatic, gastrointestinal and dermatological problems.[2,3] The hypoglycemic, anti-inflammatory and antioxidant activities of *P. decompositum*,[4-6] *P. peltatum*[7,8] and *P. radulifolium*[9] extracts have been reported. The antimicrobial effects of *P. radulifolium*[10] and the anti-inflammatory properties of *P. sinuatum*[11] have also been determined. Sesquitepenes mainly of eremophilane and modified eremophilane types are the main secondary metabolites isolated from the eight species of the genus chemically studied so far: *P. decompositum*[4-6] (also studied as *Cacalia decomposita*),[12,13] *P. tussilaginoides* (studied as *Cacalia ampulacea*),[13] *P. peltatum*,[7,8] *P. sinuatum*,[11] *P. radulifolium*,[9,10] *P. paucicapitatum*,[14] *P. megaphyllum*[15] and *P. beamanii*.[16] Cacalol has been identified as the major active compound in these species with antioxidant,[9] antimicrobial[10] and anti-inflammatory[17] activities. Cacalone, *epi*-cacalone, maturinone, and radulifolin D have also shown anti-inflammatory properties.[17,18] As continuation of our research on Senecioneae we studied the chemical composition of *P. cirsiifolium* which to the best of our knowledge has no previous studies. We report the isolation of fourteen modified eremophilane derivatives (**1**-**14**), of which four (**1**-**4**) are described for the first time. Two known caryophyllenes (**16**, **18**), one aromadendrene (**17**) and one flavonoid (**15**) were also isolated (Figure 1). The 12-*O*-tetradecanoylphorbol-13-acetate (TPA) model of induced acute inflammation was used to evaluate the anti-inflammatory activity of extracts and of the isolated compounds non-evaluated previously. The most active compound (**4**) was tested on the myeloperoxidase (MPO) assay to determine its effect on the recruitment of inflammatory cells, such as neutrophils.

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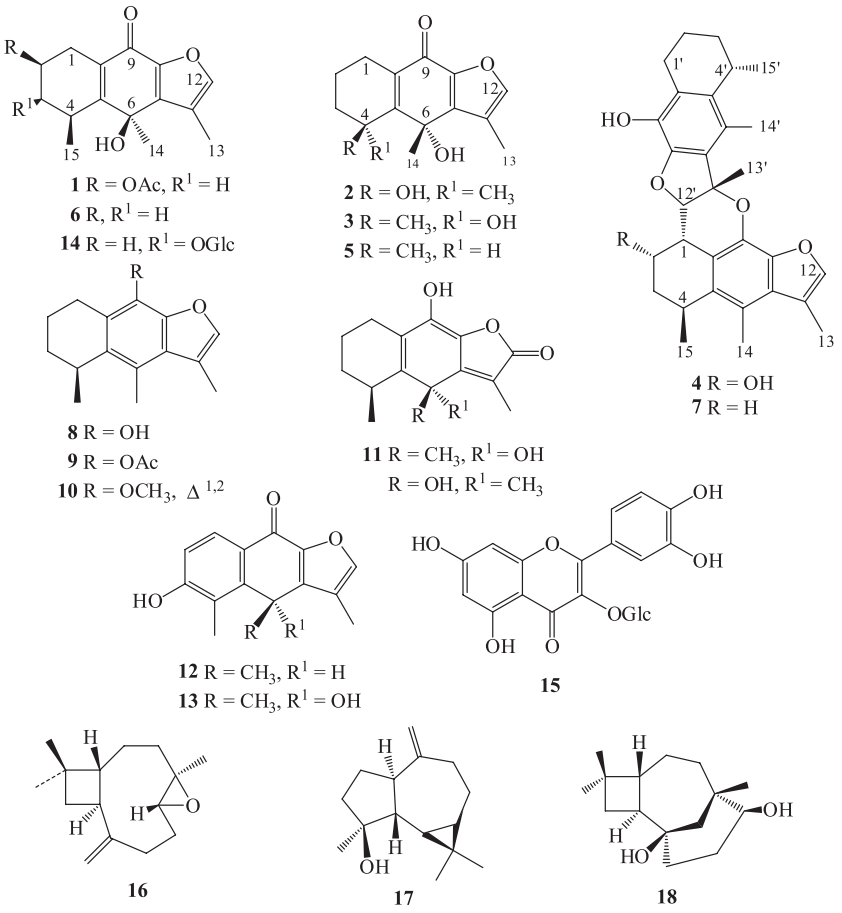

Figure 1. Chemical structures of compounds **1**-**18**.

Results and Discussion

Compound **1** was obtained as a colorless oil. The IR spectrum indicated hydroxyl, ester and conjugated ketone groups (3390, 1743, 1661 cm^{-1}). The molecular formula $C_{17}H_{20}O_5$, determined by HRESIMS, showed eight degrees of insaturation. The ^1H NMR spectrum (Table 1) was similar to those of cacalone (**5**) and *epi*-cacalone (**6**)[6] with two additional signals at δ_H 5.13 (tt, 1H, *J* 6.0, 5.0 Hz) and 2.05 (s, 3H). The first one was attributed to H-2 by its correlations with the H_2-1 and H_2-3 methylene groups observed in the COSY experiment. The downfield chemical shift of H-2 indicated that an acetate group, whose methyl group appeared at δ_H 2.05 was attached to this position, and, in addition, H-2 showed an interaction with the carbonyl at δ_C 170.5 in the HMBC experiment. The NOESY spectrum showed interactions between H_3-15 and the acetate methyl, therefore this group should have a β-pseudoaxial orientation, since on biogenetic grounds H_3-15 is β.[19] Likewise, the coupling constant of H-2 (*J* 6.0, 5.0 Hz) suggested its α-pseudoequatorial orientation. Moreover, a CD (circular dichroism) analysis of compound **1** showed a similar profile to that of *epi*-cacalone (**6**), consequently, the hydroxyl group at C-6 (δ_C 72.1) in **1** should be β-oriented as the one in **6**. Therefore, **1** was identified as (2*R*, 4*S*, 6*S*)-2-acetoxy-*epi*-cacalone.

Compounds **2** and **3** exhibited the same molecular formula $C_{15}H_{18}O_4$ (HRESIMS) and very similar spectroscopic data, with evidence of hydroxyl (3400 cm^{-1}) and conjugated carbonyl groups (1660 cm^{-1}) in the IR spectra. The ^1H and ^{13}C NMR spectroscopic data of these compounds also resembled those of cacalone (**5**) and *epi*-cacalone (**6**),[6] and were indicative of the presence of an additional hydroxyl group. Position of this additional group at C-4 (δ_C75.5 in **2** and 74.6 in **3**) was supported by the correlations observed in the HMBC experiments between H_3-15 and C-4 in both **2** and **3**. Differences between the two compounds were however observed in their ^1H NMR spectra (Table 1) where methyl groups 14 and 15 appeared at δ_H 1.91 (s, H-14) and 1.70 (s, H-15) in compound **2**, and at δ_H 1.85 (s, H-14) and 1.59 (s, H-15) in **3**; and in the ^{13}C NMR spectrum of **2** (Table 2), the signals of C-14 and C-15 appeared at δ_C 28.5 and 29.6, respectively, while in compound **3** the signals of the same atoms were observed at δ_C 32.2 and 29.7. At this point, it was evident that **2** and **3** should have different stereochemistry. The CD spectroscopy of compounds **2** and **3**

Table 1. ^{1}H NMR (500 MHz) spectroscopic data of compounds **1**-**4** in $CDCl_3$. δ (m, *J*/Hz)

Position[a]	1	2	3	4	
1α [1a']	3.08 (dd *J* 18.0, 6.0)	2.44 (ddd *J* 19.0, 8.0, 4.5)	2.52 (m)		[2.67 (br, d *J* 17.0)]
1β [1b']	2.43 (ddd *J* 18.0, 5.0, 1.5)	2.63 (dt *J* 19.0, 8.0)	2.52 (m)	3.80 (dd *J* 9.0, 3.0)	[2.35 (br, dd *J* 17.0, 9.0)]
2a [2a']	5.13 (tt *J* 6.0, 5.0)	1.86 (m)	1.95 (m)	4.78 (td *J* 9.0, 7.0)	[1.66 (m)]
2b [2b']		1.86 (m)	1.83 (m)		[1.66 (m)]
3α [3a']	1.99 (ddd *J* 14.0, 6.0, 5.5)	1.81 (m)	1.93 (m)	2.06 (m)	[1.66 (m)]
3β [3b']	1.84 (ddd *J* 14.0, 6.0, 5.0)	1.91 (m)	1.81 (m)	2.06 (m)	[1.66 (m)]
4 [4']	3.01 (qdd *J* 7.0, 5.5, 5.0)			3.26 (dqd *J* 11.0, 7.0, 3.5)	[3.01 (dqd *J* 10.5, 7.0, 4.0)]
12 [12']	7.38 (q *J* 1.0)	7.37 (q *J* 1.5)	7.35 (q *J* 1.0)	7.50 (q *J* 1.5)	[5.34 (d *J* 3.0)]
13 [13']	2.25 (d *J* 1.0)	2.25 (d *J* 1.5)	2.24 (d *J* 1.0)	2.28 (d *J* 1.5)	2.03 (s)
14 [14']	1.67 (s)	1.91 (s)	1.85 (s)	2.46 (s)	2.50 (s)
15 [15']	1.42 (d *J* 7.0)	1.70 (s)	1.59 (s)	1.25 d (7.0)	[0.92 (d *J* 7.0)]
–OAc	2.05 (s)				

[a]Arbitrary atom numbering used in the literature.[21]

was comparable with that of cacalone (**5**), indicating that they have the same stereochemistry at C-6 and, therefore, **2** and **3** are epimers at C-4. On the other hand, the fact that in compound **2**, H_3-14 and H_3-15 resonated at lower field ($\Delta\delta$ 0.06 and 0.11, respectively) than the same groups in **3**, indicated that in compound **2**, each of these methyl groups is feeling a deshielding effect due to its *syn* orientation with a hydroxyl group, which is not the case in compound **3**. Additionally, in the carbon resonance of CH_3-14 in **3** a downfield shift ($\Delta\delta$ 3.7) was observed with respect to that in **2** as a result of a change from a pseudoaxial orientation in compound **2** to a pseudoequatorial in **3**, in order to release steric crowding.[20] In the case of CH_3-15, this showed almost the same chemical shifts in **2** and **3** ($\Delta\delta$ 0.01) since it adopted in both pseudoaxial orientation which was α in **2** and β in **3**. This last was evident by the NOESY interaction observed between H_3-15 and H-1α in compound **2**, and by the coupling in "M" between H_3-15 and H-3α observed in the COSY experiment of **3**. Therefore the absolute configuration of compounds **2** and **3** should be 4*S*, 6*R* and 4*R*, 6*R*, respectively.

Compound **4**, obtained as a white amorphous powder, exhibited in the IR spectrum bands of hydroxyl groups (3300 cm^{-1}) and aromatic rings (1630, 1478, 1317, 1113, 961 cm^{-1}). The molecular formula $C_{30}H_{34}O_5$ was deduced from HRESIMS and in the EIMS, besides the molecular ion peak at *m/z* 474, two fragments at *m/z* 230 $[C_{15}H_{18}O_2]^+$ and 245 $[C_{15}H_{17}O_3]^+$ indicated the presence of two sesquiterpene moieties. The NMR spectroscopy (Tables 1 and 2) indicated that **4** had the same structure as adenostin B[21] (**7**) but bearing an additional hydroxyl group

Table 2. ^{13}C NMR (125 MHz) spectroscopic data of compounds **1**-**4** in $CDCl_3$ δ (m)

Position[a]	1	2	3	4	
1 [1']	27.8 (t)	21.2 (t)	21.0 (t)	36.9 (d)	[22.9 (t)]
2 [2']	68.3 (d)	17.8 (t)	18.4 (t)	61.5 (d)	[16.3 (t)]
3 [3']	36.6 (t)	41.0 (t)	39.3 (t)	38.9 (t)	[29.6 (t)]
4 [4']	29.8 (d)	75.5 (s)	74.6 (s)	30.0 (d)	[28.5 (d)]
5 [5']	160.0 (s)	155.2 (s)	160.9 (s)	137.8 (s)	[134.7 (s)]
6 [6']	72.1 (s)	72.7 (s)	74.3 (s)	121.7 (s)	[125.0 (s)]
7 [7']	140.6 (s)	141.5 (s)	141.7 (s)	127.7 (s)	[124.2 (s)]
8 [8']	144.9 (s)	144.6 (s)	144.8 (s)	144.3 (s)	[144.6 (s)]
9 [9']	173.8 (s)	174.6 (s)	174.7 (s)	134.2 (s)	[134.2 (s)]
10 [10']	128.8 (s)	132.2 (s)	130.2 (s)	118.7 (s)	[124.5 (s)]
11 [11']	120.3 (s)	120.3 (s)	120.0 (s)	116.3 (s)	[89.1 (s)]
12 [12']	144.8 (d)	145.0 (d)	144.8 (d)	141.5 (d)	[90.8 (d)]
13 [13']	8.8 (q)	9.0 (q)	8.8 (q)	11.2 (q)	[26.3 (q)]
14 [14']	26.4 (q)	28.5 (q)	32.2 (q)	13.7 (q)	[12.5 (q)]
15 [15']	21.8 (q)	29.6 (q)	29.7 (q)	20.3 (q)	[20.8 (q)]
–OAc	170.5 (s)				
	21.4 (q)				

[a]Arbitrary atom numbering used in the literature.[21]

whose gem proton appeared at δ_H 4.78. This proton was identified as H-2 by its correlations with H-1 and H_2-3 in the COSY experiment and by its cross peaks observed in the HMBC experiment, with C-3 and C-12'. The position of the hydroxyl group was also supported by the deshielding effect in C-1 ($\Delta\delta$ 6.44) and C-3 ($\Delta\delta$ 10.14), as well as the shielding in C-12' ($\Delta\delta$ –5.61), as compared to the corresponding carbon resonances in adenostin B.[21] The NOESY experiment showed interactions of H-12' with H-1 and H_3-13', and of H-2 with H_3-15 and H-1, suggesting that they are in the β side of the molecule as H_3-15, and therefore being indicative of an α-orientation the hydroxyl group at C-2.

Structures of the known compounds cacalone (**5**),[6] *epi*-cacalone (**6**),[6] adenostin B (**7**),[21] cacalol (**8**),[21] cacalol acetate (**9**),[22] cacalohastine (**10**),[23] adenostylide (**11**),[24] radulifolin C (**12**),[10] radulifolin D (**13**),[9] *epi*-radulifolin F (**14**),[9] hyperin (**15**),[25] β-caryophyllene-(8*R*,9*R*)-oxide (**16**),[26] spathulenol (**17**),[27] and caryolane-1,9β-diol (**18**),[26] were determined by comparison of their physical and spectroscopic features with those reported in the literature. The absolute stereochemistry of cacalone (**5**) and *epi*-cacalone(**6**) has been determined[6] but, since their CD data were not available in literature, they were obtained in the present work.

The anti-inflammatory activity of the methanolic extracts of roots and aerial parts, and that of compounds **1**-**4**, and **16**-**18** was evaluated using the TPA model of induced acute inflammation.[11] Since the anti-inflammatory properties of compounds **5**-**15** had been previously reported,[11,17,18] they were not tested in the present work. As shown in Table 3, the extracts exhibited moderate activities (48.14 and 46.76% for roots and aerial parts, respectively). Compound **4**, with 76.67% of edema inhibition, was the most active compound showing a dose dependent activity with IC_{50}0.41 μmol *per* ear while that of the reference compound, indomethacin, was 0.24 μmol *per* ear (Table 4).

Table 3. Effect of extracts and compounds **1**-**4**, **16**-**18** on TPA-induced mouse edema

Sample	Dose / (μmol *per* ear)	Edema / mg	Inhibition / %
Methanolic extract of roots[b]	1.0[a]	7.53 ± 1.16[f]	48.14[f]
Methanolic extract of aerial parts[b]	1.0[a]	7.73 ± 1.07[f]	46.76[f]
(2*R*,4*S*,6*S*)-2-acetoxy-*epi*-cacalone (**1**)[c]	1.0	5.65 ± 1.25[g]	58.04[g]
(4*S*,6*R*)-4-hydroxycacalone (**2**)[c]	1.0	5.63 ± 0.44[g]	58.17[g]
(4*R*,6*R*)-4-hydroxycacalone (**3**)[c]	1.0	11.98 ± 0.81	11.08
2α-hydroxyadesnostin B (**4**)[d]	1.0	2.80 ± 0.17[f]	76.67[f]
Caryophyllene-(8*R*,9*R*)-oxide (**16**)[d]	1.0	9.63 ± 0.63	19.72
Spathulenol (**17**)[d]	1.0	8.78 ± 0.48[f]	26.88[f]
Caryolane-1,9β-diol (**18**)[d]	1.0	7.47 ± 0.37[f]	37.78[f]
Indomethacin[e]	1.0[a]	2.06 ± 0.30[g]	87.61[g]
Indomethacin[e]	1.0	1.99 ± 0.69[f]	83.73[f]

Each value represents the mean of three animals ± standard error; [a]dose: mg *per* ear; control: [b]methanol, 14.53 ± 0.38, [c]acetone-CH_2Cl_2 1:1, 13.47 ± 0.38, [d]acetone-CH_2Cl_2 1:1, 12.00 ± 1.50, [e]ethanol-acetone 1:1, 15.00 ± 0.47; [f]$p \leq 0.05$; [g]$p \leq 0.01$.

Table 4. Dose response evaluation of 2α-hydroxyadesnostin B (**4**)

Sample	Dose / (μmol *per* ear)	Edema / mg	Inhibition / %	IC_{50} / (μmol *per* ear)
Control		16.54 ± 0.34		
2α-hydroxyadesnostin B (**4**)	0.031	15.74 ± 0.68	4.84	0.41
	0.1	13.34 ± 0.83[a]	19.35[a]	r = 0.99
	0.31	9.34 ± 1.14[b]	43.53[b]	
	1.0	5.12 ± 0.55[b]	69.04[b]	
Indomethacin[c]	0.031	12.78 ± 1.21	13.36	0.24
	0.1	10.74 ± 1.13[a]	27.19[a]	r = 0.983
	0.31	5.62 ± 0.89[b]	61.90[b]	
	1.0	2.88 ± 0.73[b]	78.76[b]	

Each value represents the mean of five animals ± standard error; [a]$p \leq 0.05$; [b]$p \leq 0.01$; [c]control 14.75 ± 1.13.

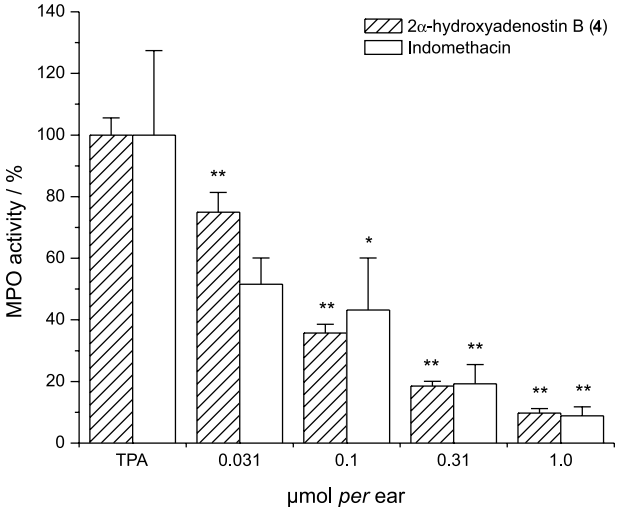

Figure 2. Comparative effect of 2α-hydroxyadesnostin B (**4**) and indomethacin on myeloperoxidase levels in the mouse ear edema induced by TPA. Data represent means ± standard error of five animals. *$p \leq 0.05$ and **$p \leq 0.01$.

Myeloperoxidase (MPO) is a biochemical marker for tissue content of polymorphonuclear leukocytes because MPO activity is well correlated with the number of infiltrated cells in inflamed regions.[28,29] In the MPO activity test compound **4** attenuated, in a dose-dependent manner, the activity of MPO and showed a similar effect to that of indomethacin at 0.31 and 1.0 μmol *per* ear (Figure 2). The Pearson's correlation analysis between skin weight and the MPO activity of all biopsies of **4** showed a positive correlation ($r = 0.83$, $p < 0.001$), indicating that edema inhibition of **4** is associated with the inhibition of infiltrated neutrophils in the ear biopsy.

Experimental

General procedures

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. Circular dichroism was obtained on a Jasco J-720 spectropolarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. 1D and 2D NMR spectra were obtained on a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. EIMS were determined on a Bruker Daltonics Analysis 3.2 mass spectrometer. HRESIMS were performed on a Bruker micrOTOF II mass spectrometer with mass resolution of 16.500 FWHM, mass interval 50-20,000 *m/z*, and speed 40 Hz. Column chromatography was carried out under vacuum (VCC) on silica gel G 60 (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was performed on silica gel 230-400 (Macherey-Nagel, Germany). Sephadex column chromatography was developed with Sephadex LH 20 (Amersham Pharmacia Biotech AB, Sweden). Analytical TLC was carried out on silica gel 60 GF_{254} or RP-18W/UV_{254} (Macherey-Nagel, Germany) and preparative TLC on Si gel GF_{254} layer thickness 2.0 mm or RP-18W/UV_{254} layer thickness 1.0 mm.

Plant material

Psacalium cirsiifolium (Zucc.) H. Rob. & Brettell was collected in Coatepec Harinas, Estado de México, México, in July 2008. A voucher specimen (MEXU 954570) was identified by Dr. José Luis Villaseñor and deposited at the Herbario del Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and isolation

Dried and ground roots (130 g) and aerial parts (180 g) were macerated separately with MeOH (three times a day for seven days each) at room temperature. The extract of roots (15 g) was fractioned by VCC eluted with a hexane-EtOAc-MeOH gradient system. The hexane eluates afforded fraction A. Fraction B was obtained with EtOAc-MeOH 19:1 and fraction C grouped the mixtures obtained with EtOAc-MeOH 9:1 and 4:1. Purification of fraction A (8.5 g) by VCC eluted with hexane-EtOAc gradient system afforded fractions A1 (hexane-EtOAc 19:1), A2 (hexane-EtOAc 9:1), A3 (hexane-EtOAc 4:1) and A4 (hexane-EtOAc 7:3). Fraction A1 (1.5 g) was purified by VCC, eluted with hexane-EtOAc 49:1, to give cacalol[21] (**8**, 220 mg), cacalol acetate[22] (**9**, 205 mg) and cacalohastine[23] (**10**, 18 mg). Fraction A2 (900 mg) produced, after a FCC eluted with hexane-EtOAc 9:1, adenostylide[24] (**11**, 350 mg) and 252 mg of a mixture which (50 mg) was purified by preparative RPTLC (MeOH-H_2O 2:3 × 4) to obtain cacalone[6] (**5**, 12 mg) and *epi*-cacalone[6] (**6**, 9 mg). Fraction A3 (380 mg) was purified by FCC eluted with hexane-acetone 7:3 to obtain fractions A31 and A32. Purification of A31 (100 mg) by preparative RPTLC (MeOH-H_2O 1:1) produced compounds **1** (12 mg) and radulifolin C[10] (**12**, 8 mg). Fraction A32 (48 mg) by preparative RPTLC (MeOH-H_2O 3:2 × 4) produced compounds **2** (10 mg) and **3** (8 mg). Fraction A4 (466 mg) purified by FCC (30 × 2 cm) eluted with hexane-acetone 7:3 followed by preparative RPTLC (MeOH-H_2O 1:1 × 3) produced radulifolin D[9] (**13**, 6 mg), adenostin B[21] (**7**, 10 mg) and compound **4** (12 mg). Fraction B (2.2 g) purified by VCC eluted with CH_2Cl_2-MeOH gradient system produced

a fraction (150 mg obtained with CH_2Cl_2-MeOH 9:1 mixture) which was submitted to a preparative RPTLC (MeOH-H_2O 1:1) to give *epi*-radulifolin F[9](**14**, 58 mg). Fraction C (1.2 g) was purified through a sephadex LH 20 column eluted with MeOH-H_2O 1:1 to afford hyperin[25] (**15**, 30 mg). The methanolic extract of the aerial parts (28 g) was worked out by VCC (30 × 10 cm) eluted with EtOAc-MeOH gradient system. Fractions eluted with EtOAc (4.5 g) were purified by VCC eluted with hexane-EtOAc gradient system to yield, from the hexane factions, β-caryophyllene-(8*R*,9*R*)-oxide[26] (**16**, 45 mg) and from the hexane-EtOAc 19:1 eluates a mixture (800 mg) which by FCC eluted with hexane-EtOAc 9:1, produced spathulenol[27](**17**, 12 mg), caryolane-1,9β-diol[26] (**18**, 10 mg), cacalol (**8**, 180 mg), cacalone-*epi*-cacalone mixture (**5** and **6**, 250 mg), **1** (5 mg), and **4** (9 mg). Fractions eluted with EtOAc-MeOH 4:1 (1.0 g) afforded hyperin(**15**, 25 mg), by purification through a sephadex LH 20 column eluted with MeOH-H_2O 1:1.

Evaluation of anti-inflammatory activity

Animals

Male NIH mice weighing 25-30 g were maintained in standard laboratory conditions in the animal house (temperature 27 ± 1 °C) in a 12/12 h light-dark cycle, being fed laboratory diet and water *ad libitum*, following the Mexican official norm MON-062-Z00-1999.

TPA-induced edema model

The TPA-induced ear edema assay in mice was performed as previously reported,[11] Tables 3 and 4.

Myeloperoxidase assay

Tissue MPO activity was measured in biopsies taken from ears 4 h after TPA administration using an adapted method of Bradley *et al.*[28] and Suzuki *et al.*[29] Each mouse ear biopsy was placed in 1 mL of 80 mmol L^{-1} phosphate-buffered saline (PBS) pH 5.4 containing 0.5% hexadecyltrimethylammonium bromide (HTAB). Each sample was homogenized for 30 s at 4 °C with a small sample laboratory Tissue Tearor Homogenizer (OMNI International, model 125). The homogenate was freeze-thawed at room temperature 3 times, sonicated 20 s and centrifuged at 12,000 rpm for 15 min at 4 °C. The resulting supernatants (10 µL in quadruplicate) were poured into 96 well microplate and 180 µL of 80 mmol L^{-1} PBS (pH 5.4) without HTAB were added. Microplate was heated at 37 °C then, 20 µL of 0.017% hydrogen peroxide were added to each well. For the MPO assay, 20 µL of 18.4 mmol L^{-1} 3,3',5,5'-tetramethylbenzidine in 50% aqueous dimethylformamide were added to start the reaction. Microliter plates were incubated at 37 °C for 5 min. The reaction was stopped with 20 µL of 2 mol L^{-1} H_2SO_4. MPO enzyme activity was assessed colorimetrically using a BioTekMicroplate Reader (EL × 808) at an absorbance wavelength of 450 nm. MPO activity test results were expressed as percent of the maximal activity, Figure 2.

Statistical analysis

All data were represented as percentage mean ± standard error of mean (SEM). The statistical analysis was done by means of Student's t-test, whereas analysis of variance ANOVA followed by Dunnett test were used to compare several groups with a control. P values $p \leq 0.05$ and $p \leq 0.01$ were considered to be significant. Pearson's correlation coefficient was calculated for the edema and MPO results of compound **4**.

(2R,4S,6S)-2-Acetoxy-epi-cacalone (**1**)

Colorless oil; $[\alpha]_D^{25}$ +40.0° (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (4.35), 262 (3.06) nm; CD (*c* 6.6 × 10^{-5} mol L^{-1}, MeOH) $\Delta\varepsilon_{210\ nm}$ −838, $\Delta\varepsilon_{226\ nm}$ +593, $\Delta\varepsilon_{263\ nm}$ −134; IR ($CHCl_3$) ν_{max} /cm^{-1}: 3390, 1743, 1661; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 244 $[M\text{-}CH_3COOH]^{+\cdot}$ (100), 229 (75), 215 (70); HRESIMS *m/z* 327.1202 $[M + Na]^+$ (calcd. for $C_{17}H_{20}NaO_5$, 327.1202).

(4S,6R)-4-Hydroxycacalone (**2**)

Colorless oil; $[\alpha]_D^{25}$ −6.6° (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (3.53), 286 (2.97), 318 (3.23) nm; CD (*c* 2.7 × 10^{-4} mol L^{-1}, MeOH) $\Delta\varepsilon_{227\ nm}$ −31, $\Delta\varepsilon_{254\ nm}$ +41, $\Delta\varepsilon_{280\ nm}$ −0.02, $\Delta\varepsilon_{316\ nm}$ +8; IR ($CHCl_3$) ν_{max}/cm^{-1}: 3400, 1660; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 262 $[M]^{+\cdot}$ (8), 244 (12), 229 (100); HRESIMS *m/z* 269.1356 $[M + Li]^+$ (calcd. for $C_{15}H_{18}LiO_4$, 269.1360).

(4R,6R)-4-Hydroxycacalone (**3**)

Colorless oil; $[\alpha]_D^{25}$ −18.7° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.45), 293 (2.83), 317 (2.91) nm; CD (*c* 6.9 × 10^{-3} mol L^{-1}, MeOH) $\Delta\varepsilon_{220\ nm}$ −26, $\Delta\varepsilon_{254\ nm}$ +0.03, $\Delta\varepsilon_{273\ nm}$ −11; IR ($CHCl_3$) ν_{max} /cm^{-1}: 3400, 1660; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 262 $[M]^{+\cdot}$ (5), 244 (15), 229 (100); HRESIMS *m/z* 269.1366 $[M + Li]^+$ (calcd. for $C_{15}H_{18}LiO_4$, 269.1360).

2α-Hydroxyadenostin B (**4**)

White amorphous powder; $[\alpha]_D^{25}$ −158.3° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (4.64), 255 (3.88) nm;

CD (*c* 3.8 × 10^{-5} mol L^{-1}, MeOH) $\Delta\varepsilon_{212\ nm}$ −838, $\Delta\varepsilon_{230\ nm}$ +578, $\Delta\varepsilon_{267\ nm}$ −111; IR ($CHCl_3$) ν_{max}/cm^{-1}: 3300, 1630, 1478, 1317, 1113, 961; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 474 $[M]^{+\cdot}$ (15), 245 (40), 230 (100), 215 (45); HRESIMS *m/z* 497.2294 $[M + Na]^+$ (calcd. for $C_{30}H_{34}NaO_5$, 497.2298).

Cacalone(**5**)

CD (*c* 8.1 × 10^{-5} mol L^{-1}, MeOH) $\Delta\varepsilon_{227nm}$ −175, $\Delta\varepsilon_{251\ nm}$ +116, $\Delta\varepsilon_{275\ nm}$ −54, $\Delta\varepsilon_{319\ nm}$ +68.

Epi-cacalone (**6**)

CD (*c* 9.8 × 10^{-5} mol L^{-1}, MeOH) $\Delta\varepsilon_{234\ nm}$ +845, $\Delta\varepsilon_{254\ nm}$ −16, $\Delta\varepsilon_{275\ nm}$+38, $\Delta\varepsilon_{310\ nm}$ −5.

Conclusions

This study shows that the modified eremophilanes are the main secondary metabolites in *Psacalium cirsiifolium* in agreement with the chemotaxonomy of the genus *Psacalium* reported so far. The study of the anti-inflammatory properties of seven of the isolated metabolites, using the TPA-induced mouse edema model, revealed that the new eremophilane derivative 2α-hydroxyadenostin B (**4**) was the most active compound and that this activity is associated with the inhibition of infiltrated neutrophils in the ear biopsy.

Supplementary Information

Comparative CD of *epi*-cacalone (**6**) and compound **1**, and of cacalone (**5**) and compounds **2** and **3**, ^1H NMR spectra of compounds **1**-**7**, ^{13}C and 2D NMR experiments of compounds **1**-**4** are available free of charge at http://jbcs.sbq.org.br as PDF file.

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