Chemical Composition and Biological Activity of *Laennecia schiedeana*

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The chemical study of *Laennecia schiedeana* afforded three sterols, five diterpenes, five flavonoids, three caffeoyl derivatives of quinic acid, and two triterpenes. Evaluation of the cytotoxic activity of the extracts and isolated metabolites showed that 15-methoxy-16-oxo-15,16*H*-strictic acid was the most active compound [(15.05 ± 2.2) μg/mL against U-251 cells]. In antibacterial assays the acetonic extract of leaves was the only active extract exhibiting its highest effect against the multiresistant *Staphylococcus epidermidis* (MIC 0.25 mg/mL). The anti-inflammatory activity observed was mild in the extracts and not relevant in the isolated compounds.

Key words: Diterpenes, Cytotoxic Activity, Anti-Inflammatory Activity, Antibacterial Activity

Introduction

The genus *Laennecia* (Asteraceae: Astereae) groups about 15 species of annual herbs restricted to the highlands of North, Central, and South America (Nesom, 1990). They have been segregated from the genus *Conyza* whose species are used around the world in folk medicine to treat gastrointestinal diseases (Martinez, 1959; Mata *et al.*, 1997), chronic bronchitis (Su *et al.*, 2003), gout (Kong *et al.*, 2001), and rheumatism (Torrenegra *et al.*, 1994), and as antipyretic, anti-inflammatory, and sedative agents (Yang *et al.*, 1989; Cifuente *et al.*, 2001). *L. sophiifolia*, the only species of the genus chemically studied so far, afforded mainly diterpenes of neoclerodane and acyclic types (Simirgiotis *et al.*, 2000), which are in close structural relation with the metabolites isolated from species of the genus *Conyza* (Bohlmann and Wegner, 1982; Galal *et al.*, 1998; Jolad *et al.*, 1988; Mahato *et al.*, 1981; Pandey *et al.*, 1984; Zdero *et al.*, 1991). The aim of the present work was to study the chemical composition of *Laennecia schiedeana* and to evaluate the cytotoxic, anti-inflammatory, and antibacterial effects of its extracts and isolated metabolites.

Results and Discussion

The chemical study of *Laennecia schiedeana* afforded three sterols (Fig. 1), spinasterol (**2**, CAS 481-18-5) (Sucrow *et al.*, 1976; Akihisa *et al.*, 1986) and a mixture of sitosterol and stigmasterol; five diterpenes, centipedic acid (**1**, CAS 72943-98-7) (Bohlmann and Mahanta, 1979), conyzaleucolide A (**3**, CAS 134037-70-0) (Zdero *et al.*, 1991), *ent*-15,16-epoxy-1,3,13(16),14-clerodatetraen-18-oic acid (**4**, CAS 90761-02-7) (Pandey *et al.*, 1984), 15-methoxy-16-oxo-15,16*H*-strictic acid (**5**) (Singh *et al.*, 1988), and 15-deoxypulic acid (**6**, CAS 80441-03-8) (Muhammad *et al.*, 1992; Singh *et al.*, 1985); five flavonoids, 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone (**7**, CAS 20921-12-1) (Roitmann and James, 1985), 5,7,3'-trihydroxy-3,8,4'-trimethoxyflavone (**8**, CAS 14965-08-3) (Horie *et al.*, 1998), astragalin (**9**, CAS 480-10-4) (Arciniegas *et al.*, 2004), nicotiflorin (**11**, CAS 17650-84-9) (Calzada *et al.*, 2001), and rutin (**12**, CAS 153-18-4) (Li *et al.*, 2008); three caffeoyl derivatives of quinic acid, 3,4-dicaffeoylquinic acid (**10**, CAS 14534-61-3) (Basnet *et al.*, 1996; Martino *et al.*, 1979), chlorogenic acid (**13**, CAS 327-97-9) (Barnes *et al.*, 1950), and 3,5-dicaffeoylquinic

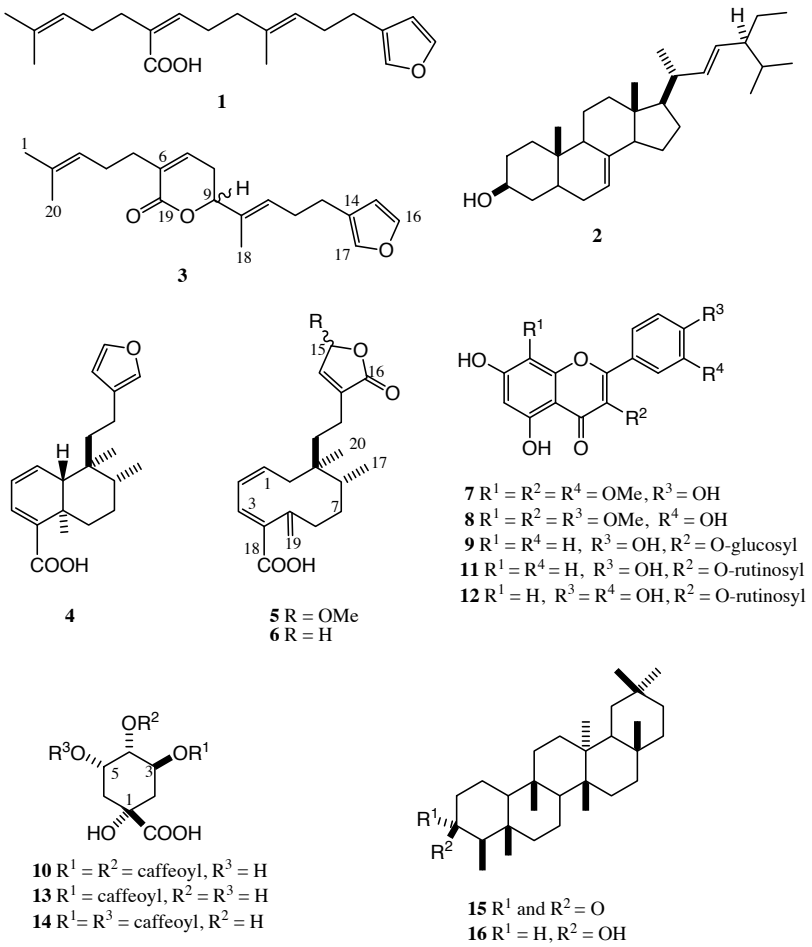

Fig. 1. Chemical structures of compounds **1**–**16**.

acid (**14**, CAS 2450-53-5) (Basnet *et al*., 1996); and two triterpenes, friedelin (**15**, CAS 559-74-0) (Hisham *et al.*, 1995) and friedelinol (**16**, CAS 16844-71-6) (Salazar *et al.*, 2000). The structures of compounds **1**–**4** and **6**–**15** were determined by comparison of their physical and spectroscopic data with those reported in the literature. The ^{13}C NMR data of compounds **1**, **3** and **4** are included (cf. Materials and Methods) since they were not available in the literature. Compound **5** was isolated from *Grangea maderaspatana* (Singh *et al.*, 1988) as methyl ester; therefore, we herein report the spectroscopic data of the free acid. Copies of the original spectra are available from the author for correspondence.

The cytotoxic activity of the extracts and isolated compounds was determined in six cancerous human cell lines. In a primary screen, the extracts were non-active or exhibited moderate activities (Table I). Among the isolated compounds, **3** inhibited 84.8% of K-562 (human chronic myelogenous leukemia) cells, **5** inhibited 84.1% of U-251 (human glioblastoma), 86.4% of HCT-15 (human colorectal adenocarcinoma), and 100% of SKLU-1 (human lung adenocarcinoma) cells, and the flavonoids **7** and **8** were active in all cell lines (Table I). The IC_{50} evaluation of these compounds in the respective cell lines (Table II) showed that **5** was the most active compound [(15.05 ± 2.2) μg/mL against U-251 cells]. The IC_{50} values of compounds **7** and **8**, evaluated only in SKLU-1 cells (due to the small quantity of material available), showed that the activity is affected by the position of the methoxy group in ring B (Table II).

Table I. Inhibition of cellular growth (%) by extracts (50 μg/mL) and isolated compounds (50 μM) from *L. schiedeana*.

Sample	Inhibition (%)					
	U-251	PC-3	K-562	HCT-15	MCF-7	SKLU-1
Leaf hexanic extract	9.6	6.3	NA	NA	NA	22.1
Leaf acetonic extract	4.8	0.4	NA	NA	NA	18.6
Leaf methanolic extract	NA	NA	NA	4.7	23.0	42.3
Root hexanic extract	5.1	20.3	NA	27.8	17.0	4.8
Root methanolic extract	16.6	33.9	NA	48.1	10.8	3.4
Centipedic acid (**1**)	35.1	29.0	36.5	29.8	43.8	53.0
Spinasterol (**2**)	NA	2.0	NA	NA	NA	3.2
Conyzaleucolide A (**3**)	22.6	13.8	84.8	NA	27.9	43.9
ent-15,16-Epoxy-1,3,13(16),14-clerodatetraen-18-oic acid (**4**)	44.9	28.8	NA	31.8	15.9	73.4
15-Methoxy-16-oxo-15,16*H*-strictic acid (**5**)	84.1	53.2	68.4	86.4	22.5	100
15-Deoxypulic acid (**6**)	36.2	29.9	NA	NA	NA	61.3
5,7,4'-Trihydroxy-3,8,3'-trimethoxyflavone (**7**)	85.9	89.9	65.3	93.0	81.9	75.5
5,7,3'-Trihydroxy-3,8,4'-trimethoxyflavone (**8**)	82.2	71.0	74.9	90.2	80.0	100
Astragalin (**9**)	4.6	5.0	12.2	6.0	NA	NA
Nicotiflorin (**11**)	10.8	24.4	54.7	NA	NA	NA
Rutin (**12**)	13.3	30.5	61.1	NA	NA	NA
Chlorogenic acid (**13**)	2.8	11.2	34.5	49.7	66.9	61.4
3,5-Dicaffeoylquinic acid (**14**)	4.9	6.8	27.8	NA	29.3	13.7
Friedelin (**15**)	NA	7.7	5.8		11.7	NA
Friedelinol (**16**)	37.1	19.0	NA	0.3	1.5	NA

NA, not active.

Table II. IC_{50} values (μg/mL ± S.E.) of active compounds isolated from *L. schiedeana*.

Sample	IC_{50} ± S.E. [μg/mL]			
	U-251	K-562	HCT-15	SKLU-1
Conyzaleucolide A (**3**)	ND	21.53 ± 4.1	ND	ND
15-Methoxy-16-oxo-15,16*H*-strictic acid (**5**)	15.05 ± 2.2	22.72 ± 2.0	54.68 ± 0.39	35.82 ± 0.80
5,7,4'-Trihydroxy-3,8,3'-trimethoxyflavone (**7**)	ND	ND	ND	26.95 ± 2.1
5,7,3'-Trihydroxy-3,8,4'-trimethoxyflavone (**8**)	ND	ND	ND	16.40 ± 1.3
Cisplatin	9.09 ± 0.88	15.20 ± 1.4	13.83 ± 0.79	7.13 ± 0.25

ND, not determined.

The anti-inflammatory activity of the extracts and compounds **1**–**6**, **15**, and **16** was tested in the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) model of acute inflammation. The activity of the leaf extracts was higher than that of the respective extracts of roots (Table III). However, no relevant activity was observed for the tested compounds, in comparison with the reference compound, indomethacin.

The antibacterial activity of the extracts of *L. schiedeana* is shown in Table IV. The bioassays were carried out against the Gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Streptococcus pneumoniae*, and the Gram-negative bacteria: *Pseudomonas aeruginosa* and *Enterobacter faecalis*. The leaf acetonic extract was the only active extract, exhibiting MIC values between 0.25 and 1.5 mg/mL against the Gram-positive bacteria and of 2.0 mg/mL against the Gram-negative ones. However, compounds **2**, **7**, and **8**, isolated from this extract, were not active (MIC > 2 mg/mL). The strain more sensible to the acetonic extract was the multiresistant *Staphylococcus epidermidis*. This is important since this strain can cause infections which are difficult to heal with commercial antibiotics.

Table III. Anti-inflammatory activity tested in the TPA-induced ear edema assay.

Sample	Dose [μmol/ear]	Edema [mg]	Edema inhibition (%)
Leaf hexanic extract[b]	0.31[a]	5.17 ± 0.86**	61.82**
Leaf acetonic extract[b]	0.31[a]	5.37 ± 1.73**	60.34**
Leaf methanolic extract[c]	1.0[a]	7.73 ± 1.46**	45.67**
Root hexanic extract[d]	1.0[a]	4.93 ± 0.41**	55.42**
Root methanolic extract[c]	1.0[a]	10.80 ± 0.80	24.12
Centipedic acid (**1**)[b]	1.0	5.83 ± 1.48*	56.47*
Spinasterol (**2**)[b]	1.0	8.27 ± 0.03*	38.31*
Conyzaleucolide A (**3**)[b]	1.0	7.33 ± 1.01*	45.27*
ent-15,16-Epoxy-1,3,13(16),14-clerodatetraen-18-oic acid (**4**)[b]	1.0	4.43 ± 0.34	67.24
15-Methoxy-16-oxo-15,16*H*-strictic acid (**5**)[b]	1.0	7.93 ± 0.34**	41.37**
15-Deoxypulic acid (**6**)[b]	1.0	10.20 ± 1.06*	24.63*
Friedelin (**15**)[d]	1.0	8.57 ± 1.84	26.64
Friedelinol (**16**)[e]	1.0	12.00 ± 0.91	5.06
Indomethacin[f]	1.0[a]	2.06 ± 0.30**	87.61**
Indomethacin[f]	1.0	1.99 ± 0.69*	83.73*

Each value represents the mean of three animals ± S.E. Results were analysed by the *t* Student test; *$p \leq 0.05$; **$p \leq 0.01$. [a] Dose in mg/ear.
Solvent and ear-induced edema in control animals (mg): [b] acetone/CH_2Cl_2 (1:1), 13.53 ± 0.93; [c] methanol, 14.23 ± 0.50; [d] CH_2Cl_2, 11.07 ± 0.66; [e] CH_2Cl_2/DMSO (19:1), 12.64 ± 0.57; [f] ethanol/acetone (1:1), 15.00 ± 0.47.

Table IV. Antibacterial activity of extracts of *L. schiedeana*.

Strain	Positive control (chloramphenicol) [mm]	Leaf acetonic extract [mm]	Leaf methanolic extract [mm]	Root methanolic extract [mm]	MIC (leaf acetonic extract) [mg/mL]
Staphylococcus aureus	24.5 ± 0.5	11 ± 0.0	NA	NA	1.5
Staphylococcus epidermidis	23.5 ± 0.7	10 ± 0.0	8 ± 0.5	NA	0.25
Streptococcus pneumoniae	21 ± 0.5	9.0 ± 0.0	10 ± 0.0	9 ± 0.0	0.5
Pseudomonas aeruginosa	20.5 ± 0.5	10.5 ± 0.5	9 ± 0.0	NA	2.0
Enterobacter faecalis	22.5 ± 1.8	10.5 ± 0.5	NA	NA	2.0

Zones of inhibition are expressed in mm. Each group represents the mean of three independent experiments ± S.E. Doses: 2.0 mg for extracts and 25 μg for chloramphenicol. NA, not active.

Materials and Methods

General experimental procedures

Melting points were determined on a Fisher Johns (Pittsburgh, PA, USA) melting point apparatus and are uncorrected. Optical rotations were carried out on a Perkin-Elmer 343 Plus polarimeter (Norwalk, CT, USA). IR spectra were recorded on a Bruker Tensor 27 spectrometer (Bremen, Germany). EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer (Tokyo, Japan) at 70 eV. 1D and 2D NMR spectra were obtained on a Bruker Avance 300 MHz or a Varian Unity Inova 500 MHz spectrometer (Palo Alto, CA, USA) with TMS (δ 0 ppm) as internal standard. Vacuum column chromatography (VCC) was performed using silica gel 60 G (Merck, Darmstadt, Germany) and flash column chromatography (FCC) over silica gel 230–400 (Macherey-Nagel, Düren, Germany). TLC was carried out on silica gel GF_{254} (Macherey-Nagel, 0.2 mm thick) plates, and preparative TLC was performed on 20 × 20 cm × 2.0 mm plates.

Plant material

Laennecia schiedeana (Less.) Nesom was collected in Ozumba, State of México, Mexico in September 2004. A voucher specimen (MEXU 95988) was deposited at the Herbarium of the Instituto de Biología, UNAM México, D. F., México.

Extraction and isolation of compounds

Dried and ground aerial parts (1870 g) were extracted with *n*-hexane, acetone, and methanol successively. Dried and ground roots (410 g) were extracted with *n*-hexane and methanol. Solvents were removed under reduced pressure, and the respective extracts were further processed by chromatographic methods.

The hexanic extract of the aerial parts (14.8 g) was fractioned by VCC (30 × 7.0 cm, 150 g) to obtain fraction A eluted with *n*-hexane, fraction B eluted with *n*-hexane/acetone (49:1), and fraction C eluted with *n*-hexane/acetone (19:1, 9:1, 4:1, and 1:1). Fraction A (900 mg) was again submitted to VCC (30 × 1.5 cm, 10 g) using *n*-hexane/EtOAc mixtures of increasing polarity. Fractions eluted with *n*-hexane/EtOAc (19:1) (60 mg of an oily product) were purified by preparative TLC [benzene/acetone (9:1), × 2] to yield centipedic acid (**1**; yellow oil; $[\alpha]_D^{25}$ –1.7°, *c* 0.11 $CHCl_3$; 25 mg). Fraction B (5.1 g) afforded 25 mg of spinasterol (**2**) as colourless needles from *n*-hexane/EtOAc (m.p. 170–172 °C; $[\alpha]_D^{25}$ –1.9°, *c* 0.20 $CHCl_3$). Purification of its mother liquors by VCC (20 × 3.0 cm, 55 g), using as eluent *n*-hexane/acetone mixtures of increasing polarity, afforded fractions B1 and B2. Fraction B1 was purified by VCC eluted with *n*-hexane/CH_2Cl_2 (9:1) followed by preparative TLC [benzene/acetone (24:1)] to produce conyzaleucolide A (**3**; amber oil; $[\alpha]_D^{25}$ +1.7°, *c* 0.11 $CHCl_3$; 63.5 mg). Fraction B2 (272 mg) yielded by preparative TLC [CH_2Cl_2/*n*-hexane (4:1)] *ent*-15,16-epoxy-1,3,13(16),14-clerodatetraen-18-oic acid (**4**; amber needles; m.p. 81–83 °C; $[\alpha]_D^{25}$ –131.0°, *c* 0.18 $CHCl_3$; 22.2 mg). Fraction C (5.75 g) submitted to VCC (20 × 3.0 cm, 60 g) using mixtures of *n*-hexane/acetone of increasing polarity as eluent afforded spinasterol (**2**; 23.6 mg) and fraction C1. Purification of C1 (785 mg) by VCC (20 × 2.0, 10 g) eluted with an *n*-hexane/acetone gradient system yielded a mixture (114 mg) which was purified by preparative TLC [benzene/acetone (85:15), × 3] to obtain 15-methoxy-16-oxo-15,16*H*-strictic acid (**5**; yellow oil; $[\alpha]_D^{25}$ –8.3°, *c* 0.21 $CHCl_3$; 10 mg) and 15-deoxypulic acid (**6**; yellow oil; $[\alpha]_D^{25}$ –125.0°, *c* 0.11 $CHCl_3$; 18 mg).

The acetonic extract of the aerial parts (30 g) was submitted to VCC (30 × 8 cm, 300 g). Elution was carried out with *n*-hexane/acetone mixtures of increasing polarity. Spinasterol (**2**; 15.8 mg) was obtained from fractions eluted with *n*-hexane/acetone (49:1), and from those eluted with *n*-hexane/acetone (9:1) a mixture of sitosterol and stigmasterol (20 mg) was isolated. Fractions eluted with *n*-hexane/acetone (4:1 and 7:3) (3.3 g) were purified by successive FCC eluted with *n*-hexane/acetone (9:1) and CH_2Cl_2/acetone (49:1), respectively, to produce 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone (**7**; yellow powder; m.p. 220–222 °C; 30.6 mg) and 5,7,3'-trihydroxy-3,8,4'-trimethoxyflavone (**8**; yellow needles from *n*-hexane/EtOAc; m.p. 222–223 °C; 9.1 mg).

The methanolic extract of the aerial parts (170 g) was fractioned by VCC (30 × 10 cm, 500 g) to obtain fraction D eluted with EtOAc/MeOH (4:1, 7:3, 1:1, and 3:7) mixtures and fraction E eluted with methanol. Fraction D (114.8 g) was submitted to VCC (30 × 10 cm, 500 g) eluted with EtOAc/MeOH mixtures of increasing polarity to obtain fractions D1 and D2. Fraction D1 (2.6 g) was purified with a Sephadex LH 20 column eluted with MeOH/H_2O (3:1) followed by FCC eluted with EtOAc/MeOH (19:1) to produce astragalin (**9**; m.p. 173–175 °C; $[\alpha]_D^{25}$ –15.1°, *c* 0.20 MeOH; 21.7 mg). Fraction D2 (2.34 g) was submitted to a Sephadex LH 20 column eluted with MeOH/H_2O (3:1) to obtain fractions D21 and D22. Fraction D21 (144 mg) was purified by preparative TLC [EtOAc/MeOH/H_2O (8:1:1)] to obtain 3,4-dicaffeoylquinic acid (**10**; m.p. 204–206 °C; $[\alpha]_D^{25}$ –295.0°, *c* 0.27 MeOH; 10.2 mg). Fraction D22 after a preparative TLC [EtOAc/MeOH/H_2O (8:1:1)] produced nicotiflorin (**11**; m.p. 183–184 °C; $[\alpha]_D^{25}$ –14.9°, *c* 0.20 MeOH; 5.4 mg) and rutin (**12**; m.p. 198–200 °C; $[\alpha]_D^{25}$ +10.2°, *c* 0.18 $CHCl_3$; 5.2 mg). Fraction E (32 g) was purified using a Diaion HP 20 column eluted with H_2O/MeOH mixtures of decreasing polarity to yield chlorogenic acid (**13**; m.p. 201–203 °C; $[\alpha]_D^{25}$ –30.0°, *c* 0.25 MeOH; 26.6 mg) from fractions eluted with water, and from those eluted with H_2O/MeOH (9:1) 3,5-dicaffeoylquinic acid (**14**; m.p. 200–203 °C; $[\alpha]_D^{25}$ –180.2°, *c* 0.20 MeOH; 9.6 mg) was obtained.

The hexanic extract of roots (1.7 g) was worked up by VCC (30 × 2.5 cm, 17 g) using *n*-hexane/

EtOAc mixtures as elution systems followed by FCC eluted with *n*-hexane/EtOAc (49:1) to afford friedelin (**15**; m.p. 260–262 °C; $[\alpha]_D^{25}$ +34.2°, *c* 0.2 $CHCl_3$; 35.2 mg), friedelinol (**16**; m.p. 279–281 °C; $[\alpha]_D^{25}$ +10.2°, *c* 0.18 $CHCl_3$; 52 mg), and spinasterol (**2**; 10 mg).

The methanolic extract of roots (30 g) was fractioned by VCC (30 × 8 cm, 300 g) using EtOAc/MeOH mixtures of increasing polarity as elution system. Fractions eluted with EtOAc/MeOH (4:1, 1:1, and 1:4) (9.5 g) were purified using a Sephadex LH-20 column eluted with MeOH/H_2O (9:1) to obtain chlorogenic acid (**13**; 13 mg), 3,4-dicaffeoylquinic acid (**10**; 7 mg), and 3,5-dicaffeoylquinic acid (**14**; 9 mg).

Centipedic acid (**1**): ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.5 (C-19), 145.4 (C-7), 142.6 (C-16), 138.8 (C-17), 134.9 (C-10), 132.3 (C-6), 130.6 (C-2), 124.9 (C-14), 124.5 (C-11), 123.5 (C-3), 111.0 (C-15), 39.1 (C-9), 34.6 (C-5), 28.5 (C-13), 28.2 (C-8), 27.9 (C-4), 25.6 (C-20), 25.0 (C-12), 17.7 (C-1), 15.9 (C-18).

Conyzaleucolide A (**3**): ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 165.5 (C-19), 142.7 (C-16), 138.9 (C-10), 138.5 (C-7), 138.5 (C-17), 132.4 (C-2), 132.3 (C-6), 128.5 (C-11), 124.4 (C-14), 123.3 (C-3), 110.9 (C-15), 82.7 (C-9), 30.9 (C-8), 28.7 (C-4), 28.1 (C-13), 26.7 (C-5), 25.7 (C-20), 24.4 (C-12), 17.8 (C-1), 12.2 (C-18).

Ent-15,16-Epoxy-1,3,13(16),14-clerodatetraen-18-oic acid (**4**): ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.2 (C-18), 142.7 (C-15), 139.5 (C-4), 133.8 (C-1), 138.4 (C-16), 136.1 (C-3), 125.4 (C-13), 124.5 (C-2), 110.9 (C-14), 48.4 (C-10), 38.8 (C-5), 38.5 (C-9), 38.3 (C-6), 35.7 (C-8), 34.6 (C-11), 27.3 (C-7), 19.4 (C-19), 18.2 (C-12), 15.6 (C-20), 15.4 (C-17).

15-Methoxy-16-oxo-15,16H-strictic acid (**5**): ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.38 (1H, s, H-3), 6.68 (1H, s, H-14), 5.94 (1H, brd, *J* = 11.5 Hz, H-2), 5.73 (1H, brs, H-15), 5.42 (1H, ttd, *J* = 2.5, 4.0, 10.5 Hz, H-1) 5.08 (1H, brs, H-19a), 4.86 (1H, brs, H-19b), 3.57 (3H, s, OMe), 2.64–2.66 (1H, m, H-6a), 2.28 (2H, t, *J* = 8.5 Hz, H-12), 2.25 (1H, d, *J* = 13 Hz, H-10a), 2.09 (1H, td, *J* = 2.5, 14 Hz, H-6b), 1.79 (1H, brd, *J* = 12 Hz, H-10b), 1.56 (1H, brt, *J* = 14 Hz, H-7b), 1.47 (2H, m, H-11), 1.34–1.39 (1H, m, H-8), 0.83 (1H, m, H-7a), 0.76 (3H, d, *J* = 6.5 Hz, Me-17), 0.72 (3H, s, Me-20). – ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 171.4 (C-18), 170.1 (C-16), 144.6 (C-4), 143.4 (C-3), 141.4 (C-14), 139.4 (C-13), 136.1 (C-5), 127.6 (C-2), 127.4 (C-1), 118.3 (C-19), 102.5 (C-15), 56.9 (OMe), 37.9 (C-9), 35.8 (C-10), 35.6 (C-8), 34.9 (C-11), 33.8 (C-6), 29.1 (C-7), 20.3 (C-12), 18.5 (C-20), 13.8 (C-17).

Animals

Male NIH mice, weighing 25–30 g, were provided by the Instituto de Fisiología Celular, UNAM, México, D. F., México, and approved by the Animal Care and Use Committee (No. NOM-06ZZ00 1999). All animals were held under standard laboratory conditions in the animal house at (27 ± 1) °C in a 12 h/12 h light-dark cycle. They were fed laboratory diet and water *ad libitum.* All experiments were carried out using 4–8 animals per group.

Cytotoxicity assays

Compounds were screened *in vitro* against six human cancer cell lines which were supplied by the National Cancer Institute (Bethesda, MD, USA): HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), K-562 (human chronic myelogenous leukemia), U-251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), and SKLU-1 (human lung adenocarcinoma). The tumour cells were treated with the test compounds whose cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in a microculture assay to measure cell viability and cell growth (Monks *et al.*, 1991). The cells were removed from the tissue culture flasks by treatment with trypsin, and diluted with fresh media. One hundred μL containing 5000 or 7500 cells per well were placed into 96-well microtiter plates. The material was incubated at 37 °C for 24 h in a 5% CO_2 atmosphere. Subsequently, 100 μL of a solution of the test compounds, obtained by diluting the stocks, were added to each well. The cultures were exposed for 48 h to the drug at concentrations ranging from 0.001 to 10 μM. After the incubation period, cells were fixed to the plastic substratum by addition of 50 μL of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4 °C for 1 h, washed with tap water, and air-dried. The trichloroacetic acid-fixed cells were stained by addition of 0.4% SRB. Free SRB solution was removed by washing with 1% aqueous acetic acid. The plates were air-dried, and the bound dye was dissolved by addi-

tion of 10 mM unbuffered Tris base (100 μL). The plates were placed on a shaker for 5 min, and the absorption was determined at 515 nm using an ELISA plate reader.

Anti-inflammatory tests

The TPA-induced ear edema assay in mice was performed as previously reported (Pérez-Castorena *et al.*, 2002). A solution of TPA (2.5 μg) in EtOH (10 μL) was applied topically to both faces (5 μL on each face) of the right ear of the mice. Solutions of the test substances in their respective solvents were applied 10 min after (10 μL on each face). The left ear received ethanol (10 μL) first and 20 mL of the respective solvent subsequently. Four hours later the mice were killed by cervical dislocation. A 7-mm diameter plug was removed from each ear. The swelling was assessed as the difference in weight between the left and the right ear. Control animals received TPA and the correspondent solvent in each case. Edema inhibition (*EI* %) was calculated by the equation: $EI = 100 - (B \cdot 100/A)$, where A is the edema induced by TPA in control animals, and B is the edema induced by TPA plus sample. Indomethacin was used as reference compound.

Antibacterial assays

The following strains of bacteria were used: *Staphylococcus aureus* (ATCC 12398), *Staphylococcus epidermidis* (ampicillin-, cephotaxim-, and dicloxacillin-resistant, wild type, register number 317), *Streptococcus pneumoniae* (wild type, register number 392), *Enterobacter faecalis* (wild type, register number 110), and *Pseudomonas aeruginosa* (wild type, register number 215). Wild-type strains were donated by the Clinical Analysis Laboratory of University Hospital, FES-Iztacala, UNAM, Tlanepantla, México.

The antibacterial activity was measured by the disc diffusion method. The microorganisms were grown overnight at 37 °C in 10 mL of Mueller-Hinton broth (Bioxon, D. F., México). The cultures were adjusted with sterile saline solution to obtain a turbidity comparable to that of McFarland No. 0.5 standard (Lennette *et al.*, 1987). Petri dishes containing Mueller-Hinton agar (Bioxon) were inoculated with these microbial suspensions. Discs of filter paper (Whatman No. 5) of 5 mm diameter were impregnated with 10 μL (2.0 mg) of each extract and then placed on the agar surface plates. Discs impregnated with *n*-hexane, acetone, and methanol were used as negative controls. Discs with chloramphenicol (25 μL) were used as positive controls. The plates were incubated overnight at 37 °C, and the diameters of the resulting zones of inhibition (mm) of growth were measured. Each experiment was made three times.

The evaluation of the minimal inhibitory concentration (MIC) was carried out by the broth dilution method. Dilutions of each extract from 2.0 to 0.075 mg/mL were used. A test bacteria culture was used at the concentration of 10^5 CFU/mL. MIC values were taken as the lowest of extract concentration that prevents visible bacterial growth after 24 h of incubation at 37 °C. Each experiment was carried out three times (Marín-Loaiza *et al.*, 2008).

Statistical analysis

The statistical analysis was performed by means of *t* Student test, whereas the analysis of variance ANOVA and Dunnett test were used to compare several groups with a control. The IC_{50} values were estimated by means of a linear regression equation.

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