Anti-inflammatory Constituents of *Mortonia greggii* Gray

Amira Arciniegas, Ma. Teresa Ramírez Apan, Ana L. Pérez-Castorena, and Alfonso Romo de Vivar*

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D. F., México. Fax: +5255-56162217.
E-mail: alrovi@correo.unam.mx

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 237–243 (2004); received August 26/October 1, 2003

A new phytochemical study of *Mortonia greggii* (Celastraceae) afforded four friedelan derivatives (**1**–**4**), three lupanes (**5**–**7**), retusine (**8**), two esterified polyhydroxyagarofurans (**9**–**10**), mortonin C (**11**) and photomortonin C (**12**). The anti-inflammatory activity on carrageenan and 12-*O*-tetradecanoylphorbol-13-acetate induced models of inflammation, as well as the ability to inhibit the nitric oxide (NO) produced by lipopolysaccharide-stimulated mouse peritoneal macrophages were evaluated for the main metabolites. Our results showed that the friedelan dehydrocanophyllic acid methyl ester (**1**) exhibits an anti-inflammatory effect which could be related to an inhibition of prostaglandin and NO production. The activity of lupeol (**5**), 29-hydroxylupeol (**6**) and 29-hydroxylupenone (**7**) might be involved with the prostanoid synthesis. The presence of the hydroxy groups in **6** appears to be important for activity. The edema inhibition capacity of retusine (**8**) could be related to a reduction of the prostaglandin production. The agarofuran derivative **10** is an NO inhibitor whose activity is probably not involved in the synthesis of prostaglandins.

Key words: Mortonia greggii, Anti-inflammatory Activity, Nitric Oxide

Introduction

The genus *Mortonia* (Celastraceae) is formed by five species endemic to the arid lands of northern Mexico and southern United States. Previous studies of this genus have shown the presence of triterpenes (Martínez *et al.*, 1988) and polyhydroxyagarofuran esters as its main secondary metabolites (Rodríguez-Hahn *et al.*, 1988). The anti-inflammatory activity of a large number of terpenoids has been described and many of them have been identified as the active principles of plants used in folk medicine (Mahato *et al.*, 1992; Safayhi and Sailer, 1997). On the other hand, some pentacyclic triterpenes had been reported as important inhibitors of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes (Fernández *et al.*, 2001). These inducible enzymes are implicated in the overproduction of prostaglandins and nitric oxide (NO), which play important roles in the modulation of the inflammatory response (Rioja *et al.*, 2000; Sautebin *et al.*, 1995). The nature of the triterpenoid receptor remains unknown and, therefore, it is necessary to study the structure-activity relationships, which at present are poorly understood, to elucidate their mechanism of action (Suh *et al.*, 1998).

The aim of the present study of *M. greggii* was to evaluate the anti-inflammatory effects of its main metabolites in two models of acute inflammation as well as to elucidate their influence on the NO generated by lipopolysaccharide (LPS) activated mouse peritoneal macrophages (Mϕ).

Materials and Methods

Plant material

Mortonia greggii Gray was collected in Presa Maltos, Nuevo Leon, Mexico in November 1998. A voucher specimen (IZTA 27517) was deposited at the Izta Herbarium, UNAM, Campus Iztacala.

Isolation of terpenoid compounds

Dried and ground roots (265 g) and leaves (630 g) were extracted with hexane and acetone successively. Solvents were removed under reduced pressure and extracts submitted to vacuum column chromatography (VCC) over silica gel (60G Merck) or preparative TLC on 20 cm × 20 cm, 2.0 mm plates.

The hexanic root extract (8.0 g) was purified by VCC (30 × 6.0 cm, 120 g). Elution was performed by taking 125 ml fractions starting with hexane (frs

0939–5075/2004/0300–0237 $ 06.00

1–10) followed by hexane/EtOAc 97:3 v/v (frs 11–40), hexane/EtOAc 95:5 v/v (frs 41–60), hexane/EtOAc 9:1 v/v (frs 61–75), hexane/EtOAc 8:2 v/v (frs 76–95), hexane/EtOAc 1:1 v/v (frs 96–150), and EtOAc. Fractions 65–75 (1.6 g) were submitted to a new VCC (30 × 2.5 cm, 24 g) eluted with CH_2Cl_2/EtOAc 9:1 v/v to obtain dehydrocanophyllic acid (**4**) (m.p. 308 – 310 °C, α_D – 38°, *c* 0.20 $CHCl_3$, 400 mg) (Ahmad and Atta-ur-Rahman, 1994) and fraction A. Further purification of A (500 mg) by VCC (20 × 1.5 cm, 7.5 g), using CH_2Cl_2/EtOAc 9:1 v/v as eluent afforded **3** (m.p. 270–273 °C, α_D – 22.5°, *c* 0.11 $CHCl_3$, 50 mg) (Betancor *et al.*, 1980) and fraction B. Purification of B (105 mg) by preparative TLC eluted with hexane/Me_2CO 95:5 v/v yielded **10** (colorless needles from hexane, m.p. 60–62 °C, α_D 40.7°, *c* 0.18 $CHCl_3$, 80 mg) (Rózsa and Pelczer, 1989). Fractions obtained with hexane/EtOAc 8:2 v/v (1.0 g) were purified by VCC (20 × 2.0 cm, 15 g) using hexane/EtOAc 9:1 v/v as eluent afforded **3** and fraction C. Purification of C (125 mg) by preparative TLC eluted with hexane/EtOAc 9:1 v/v produced **9** (white needles from hexane, m.p. 160–162 °C, α_D 58.8°, *c* 0.21 $CHCl_3$, 95 mg) (González *et al.*, 1993).

The hexanic leaf extract (75 g) was purified by VCC (30 × 10 cm, 700 g). Elution was performed by taking 1000 ml fractions as follows: hexane (frs 1–20), hexane/EtOAc 98:2 v/v (frs 21–35), hexane/EtOAc 97:3 v/v (frs 36–45), hexane/EtOAc 95:5 v/v (frs 46–60), hexane/EtOAc 9:1 v/v (frs 61–80), hexane/EtOAc 8:2 v/v (frs 81–100), hexane/EtOAc 1:1 v/v (frs 101–150), and EtOAc. Fractions eluted with hexane/EtOAc 97:3 v/v afforded 29-hydroxylupenone (**7**) (m.p. 180–182 °C, α_D 15.4°, *c* 0.18 $CHCl_3$, 80 mg) (Tinto *et al.*, 1992). Fractions 63–75 (5.3 g) produced, after a VCC (30 × 5.0 cm, 75 g) in hexane/EtOAc 9:1 v/v, **3** (325 mg) and retusine (**8**) (yellow needles from hexane/EtOAc, m.p. 160–162 °C, 160 mg) (Vidari *et al.*, 1971). Compound 29-hydroxylupeol (**6**) (m.p. 230–232 °C, α_D – 13°, *c* 0.18 $CHCl_3$, 820 mg) (Betancor *et al.*, 1980) was separated from fractions 72–76.

The acetonic leaf extract (80 g) was purified by VCC (30 × 10 cm, 700 g). Elution was performed by taking 1000 ml fractions as follows: hexane (frs 1–3), hexane/EtOAc 9:1 v/v (frs 4–80), hexane/EtOAc 8:2 v/v (frs 81–105), hexane/EtOAc 7:3 v/v (frs 106–120), hexane/EtOAc 1:1 v/v (frs 121–140), and EtOAc. From fractions 6–8, compound lupeol (**5**) (m.p. 211–213 °C, α_D 22.2°, *c* 0.20 $CHCl_3$, 330 mg) (Nicollier *et al.*, 1979) was isolated as white needles from EtOAc. Fractions 9–10 afforded dehydrocanophyllic acid methyl ester (**1**) (m.p. 243–245 °C, α_D – 30.0°, *c* 0.27 $CHCl_3$, 96 mg) (Govindachari *et al.*, 1967). Fraction 11 produced **2** (m.p. 213–215 °C from $CHCl_3$/MeOH, α_D – 13.5°, *c* 0.20 $CHCl_3$, 15.1 mg) (Betancor *et al.*, 1980). Fractions 34–48 (5.1 g) were submitted to VCC (30 × 5 cm, 75 g) with hexane/EtOAc 9:1 v/v to produce **4** (20 mg). From fractions 49–80 (8.2 g) mortonin C (**11**) (white crystals from EtOAc, m.p. 205–207 °C, α_D 58.7°, *c* 0.18 $CHCl_3$, 987 mg) (Rodríguez-Hahn *et al.*, 1988), **3** (1.37 g) and **4** (305 mg) were isolated after a VCC (30 × 6.0 cm, 120 g) eluted with hexane/EtOAc 9:1 v/v. Fractions 81–111 produced by VCC **11** (1.2 g), **3** (230 mg) and photomortonin C (**12**) (white prisms from hexane/EtOAc, m.p. 178–180 °C, α_D 47.8°, *c* 0.25 $CHCl_3$, 140 mg) (Sánchez *et al.*, 1984).

The acetonic root extract (9.0 g) was worked up as above to afford compounds **3** (500 mg), **4** (384 mg), **8** (300 mg) and **6** (800 mg). All mentioned compounds were identified by comparison of their spectral features with those reported in literature. Copies of the original spectra are available from the author of correspondence.

Reagents

Carrageenan (carrageenan kappa type III), 12-*O*-tetradecanoylphorbol-13-acetate (TPA), indomethacin, dexamethasone, aminoguanidine, and lipopolysaccharide (LPS) (*Escherichia coli*, Serotipe 055:B5) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified essential medium (DMEM) and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY, USA). Brewer thioglycolate medium was from DIFCO (Detroit, MI, USA).

Animals

Male NIH mice and Wistar rats, weighing 25–30 g and 150–200 g, respectively, were provided by the Instituto de Fisiología Celular, UNAM, and approved by the Animal Care and Use Committee (No. NOM-06ZZ00 1999). All animals were conditioned in standard laboratory conditions in the animal house (temperature 27 ± 1 °C) in a 12/12 h light-dark cycle. They were fed laboratory diet and water *ad libitum.* All experiments were carried out using 4–8 animals per group.

Anti-inflammatory tests

The carrageenan-induced paw edema test in rats was carried out on compounds **1**, **4**–**11** as previously described (Martínez-Vázquez *et al.*, 1997). Compounds were dissolved in DMSO/methylcellulose (1:10) and administered at 20 mg/kg and the average volume of the back-paw was determined by means of three or four measurements 1, 3 and 5 h after the injection of the inflammatory agent. The cumulative anti-inflammatory effect during the whole observation period was determined by the area under the curve (AUC). Percentages of inhibition (% I) were obtained for each group and at each record from the equation:% I = (AUC control – AUC treat)·100/AUC control. Indomethacin was used as reference compound (Table I).

The TPA-induced ear edema assay in mice was performed as previously reported (Pérez-Castorena *et al.*, 2001). Compounds **1**, **4**, **7**, and **8** were dissolved in CH_2Cl_2, **5** and **6** in EtOAc/hexane 1:1 v/v mixture, **9** and **3** in methanol and **11** in acetone. Control animals received only the correspondent solvent in each case. Edema inhibition (EI%) was calculated by means of the equation: EI% = 100 – (B × 100/A), where A is the edema induced by TPA alone and B the edema induced by TPA plus sample. Indomethacin, dissolved in ethanol, was the reference compound (Table II).

Assay of NO production in activated macrophages (Mϕ)

To obtain primary Mϕ, NIH mice were injected with 1 ml of 3% (w/v) thioglycolate broth (Fushiya *et al.*, 1999). After three days, the peritoneal exudate cells (PEC) were washed out with DMEN through the anterior abdominal wall. The PEC were then suspended in culture medium at 1×10^6 cells/ml and incubated for 2 h in 24 well tissue culture plates in a humidified CO_2 incubator. Non-adherent cells were gently washed out twice with a fresh medium. Remaining adherent cells were cultured in the same medium containing 10 μg/ml LPS with or without test sample for 24 h. Compounds **1**, **4**, **5**, **6**, **8**, and **11** were dissolved in DMSO, and compounds **7**, **9**, and **10** were applied in ethanolic solution. The amount of nitrite, an indicator of NO synthesis in the culture supernatants, was measured by the Griess reaction (Dirsch *et al.*, 1998) by adding 100 μl of Griess reagent (1% sulfanilamide/0.1% naphthylethylenediamine dihydrochloride/5% H_3PO_4) to 100 μl of supernatant. After the chromophore was formed at room temperature for 10 min, absorbance was determined at 515 nm using a microplate reader (Bio-Tek instruments, Vermont, USA) and the nitrite concentration was determined by interpolation using a calibration curve of standard sodium nitrite concentration vs. absorbance. All assays were performed in four replicates and repeated in at least three independent experiments. Inhibition of nitrite production by tested compounds was calculated by the equation: I% = 100 – (C × 100/D). C represents the nitrite production by LPS alone, and D the nitrite production by LPS plus sample. Aminoguanidine and dexamethasone were used as reference compounds. (Tabel III).

Cell respiration, as indicator of cell viability, was analyzed in the remaining Mϕ by the MTT method (Mosmann, 1983). MTT solution (10 μl) of 5 mg/ml in PBS was added to each well, and the mixture was incubated for 4 h at 37 °C. The reduced MTT-formazan was dissolved in 100 μl dimethylsulfoxide (DMSO), absorbance of this solution at 570 nm was read using a microplate reader. Results were expressed in absolute absorbance readings and a decrease of absorbance indicates reduction in cell viability. Data were expressed as cytotoxicity percentage.

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) and Dunnett's test were used to compare several groups with a control. The IC_{50} values were estimated by means of a linear regression equation.

Results

The anti-inflammatory tests, the ability to inhibit NO and the cell viability tests, were determined for compounds **1** and **4**–**11** (Fig. 1).

In the carrageenan model (Table I) the friedelan dehydrocanophyllic acid methyl ester (**1**) was the most active of the tested compounds, its highest effect was observed 3 h after the carrageenan application. This action decreased considerably in the free acid **4**. The lupane derivative 29-hydroxylupeol (**6**) showed the highest percentage of edema inhibition with a maximum after 5 h; this activity lowered in lupeol (**5**) and in 29-hydroxylu-

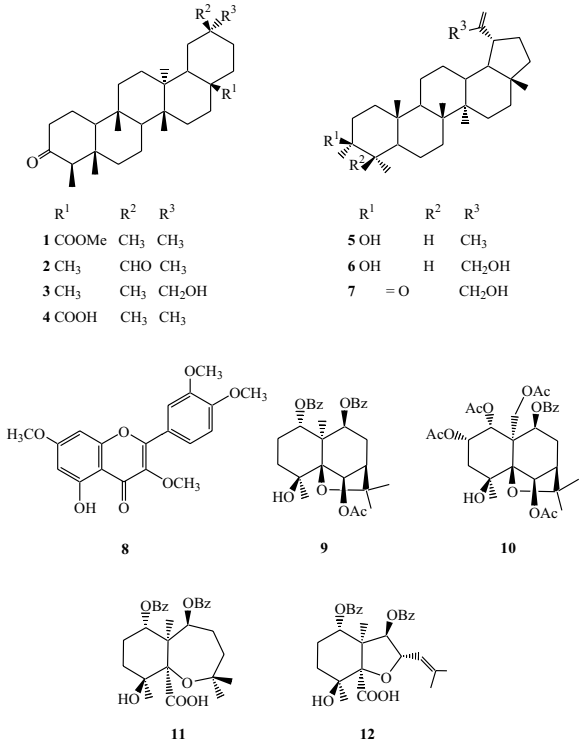

Fig. 1: Compounds isolated from *M. greggii*. Dehydrocanophyllic acid methyl ester (**1**), 3-oxofriedelan-29-al (**2**), 30-hydroxyfriedelan-3-one (**3**), dehydrocanophyllic acid (**4**), lupeol (**5**), 29-hydroxylupeol (**6**), 29-hydroxylupenone (**7**), retusine (**8**), 6-acetoxy-1,9-dibenzoyloxy-4-hydroxydihydro-β-agarofuran (**9**), 1,2,6,14-tetraacetoxy-9-benzoyloxydihydro-β-agarofuran (**10**), mortonin C (**11**), photomortonin C (**12**).

penone (**7**). Retusine (**8**) was an active compound with the maximum effect after 3 h. The agarofuran **9** showed mild anti-inflammatory capacity. Compounds **10** and **11** presented no activity.

All tested compounds affected the TPA-induced edema in a dose-dependent manner except for compounds **6** and **11** which did not reduce inflammation (Table II). The friedelan **4** was the most potent of the tested compounds; this activity was considerably diminished for its methyl ester **1**. Lupeol (**5**) showed significant anti-inflammatory action, as already reported (Recio *et al.*, 1995). This effect lowered for 29-hydroxylupenone (**7**). TPA tests on 29-hydroxylupeol (**6**) could not be satisfactorily performed due to its lack of solubility. Retusine (**8**) presented significant activity and the agarofuran derivatives **9** and **10** exhibited moderate IC_{50} values.

In the case of NO produced by activated Mϕ (Table III), the dehydrocanophyllic acid methyl ester (**1**) showed a weak inhibition and no cytotoxicity, in contrast to the free acid **4** which showed high cytotoxicity and a slight activity. Lupeol (**5**), in agreement with the reported data (Fernández *et al.*, 2001), and 29-hydroxylupeol (**6**) showed a modest activity, in contrast 29-hydroxylupenone (**7**) exhibited NO inhibition capacity similar to aminoguanidine, although its cytotoxicity was also enhanced. The flavonoid **8** and mortonin (**11**) were weak active non-toxic compounds. The two agarofuran derivatives **9** and **10** presented NO inhibition values similar to that of aminoguanidine but a different degree of cytotoxicity.

Table I. Inhibition of carrageenan-induced paw edema.

Compound	Dose	Edema inhibition (% ± SEM)			AUC ± SE	Inhibition (%)
	[mg/kg]	1 h	3 h	5 h		
1	20	41.0 ± 7.5*	60.6 ± 6.6*	44.2 ± 3.8*	67.0 ± 6.0*	53.87
4	20	26.8 ± 9.2*	34.8 ± 7.8*	43.0 ± 6.8*	84.0 ± 22*	32.35
5	20	19.2 ± 5.2	32.6 ± 7.4*	44.8 ± 9.2*	119.0 ± 38	21.19
6	20	41.5 ± 5.8**	48.7 ± 6.2**	59.0 ± 6.6**	82.5 ± 15*	45.47
7	20	12.0 ± 4.6	27.7 ± 5.0*	26.2 ± 4.9*	217.0 ± 10*	20.22
8	20	38.20 ± 4.7*	56.0 ± 1.8*	40.3 ± 5.6*	72.0 ± 4*	50.65
9	20	26.5 ± 6.5*	34.1 ± 6.5*	43.0 ± 6.7*	186.0 ± 16*	31.83
10	20	na	na	na	na	na
11	20	na	na	24.5 ± 8.4	150. ± 21	0.66
Indomethacin	7.5	40.1 ± 6.7*	49.4 ± 8.6*	64.2 ± 13.4*	56.9 ± 17*	46.9

* $p < 0.05$. ** $p < 0.01$. na: nonactive.

Table II. Inhibition of TPA-induced ear edema (% ± SEM).

Compound	0.05 mg/ear	0.1 mg/ear	0.5 mg/ear	1.0 mg/ear	IC_{50} [mg/ear]
1	0.0	15.8 ± 2.7*	45.1 ± 5.7**	55.8 ± 10.6*	0.69
4	23.7 ± 10.9	30.0 ± 16*	63.4 ± 7.7**	82.8 ± 4.4**	0.25
5	7.2 ± 4.2	16.6 ± 5.9*	49.1 ± 1.5**	66.3 ± 8.5*	0.48
6	4.7 ± 3.6	23.6 ± 10.9	15.5 ± 4.3*	nd	nd
7	6.7 ± 1.8	18.2 ± 6.7*	37.3 ± 4.2*	76.2 ± 5.6**	0.65
8	10.6 ± 4.3	20.8 ± 4.2*	56.6 ± 7.5*	88.9 ± 4.0**	0.43
9	24.8 ± 9.2	37.5 ± 10.8*	44.5 ± 13.5*	92.0 ± 1.3**	0.61
10	35.1 ± 6.9*	46.2 ± 8.4**	48.1 ± 6.8**	78.8 ± 3.2**	0.51
11	14.1 ± 4.1	3.6 ± 3.02	45.3 ± 8.3*	22.3 ± 8.3	nd
Indomethacine	15.9 ± 6.9	22.1 ± 8.4*	62.6 ± 4.2**	73.9 ± 6.3**	0.36

* $p < 0.05$. ** $p < 0.01$. nd: non determined.

Table III. Inhibition of nitrite production (%) and cytotoxicity (%) of tested compounds.

Compound, 100 μM	Inhibition of nitrite (% ± SEM)	Cytotoxicity (% ± SEM)
1	19.2 ± 7.1	< 10
4	28.0 ± 9.7*	93.2 ± 0.9
5	20.4 ± 2.1*	36.7 ± 3.3
6	17.0 ± 2.5*	68.0 ± 3.8
7	33.8 ± 4.6**	95.8 ± 0.5
8	14.2 ± 2.3*	< 10
9	31.0 ± 1.7**	85.9 ± 4.6
10	32.5 ± 2.6**	< 10
11	18.3 ± 2.8**	< 10
Aminoguanidine	33.8 ± 4.2**	< 10
Dexamethasone 10 μM	53.2 ± 3.5**	< 10

* $p < 0.05$. ** $p < 0.01$.

Discussion

The TPA has been reported to produce a long lasting inflammatory response associated with a marked cellular influx and a moderate eicosanoid production (Rao *et al.*, 1993). It is a powerful protein kinase C activator (Huguet *et al.*, 2000) whose edema is inhibited by both COX and 5-LOX inhibitors (Hara *et al.*, 1992).

The carrageenan model, which has been described as the best predictor of human doses (Mukherjee *et al.*, 1996), is characterized by the production of a biphasic edema. In the relatively rapid early phase (up to 2 h) the edema formation is governed by the presence of histamine, bradykinin and 5-hydroxytryptamine. In the late phase (2 h to 6 h) prostanoid synthesis induced by COX-2 and NO synthesized by iNOS are reported to contribute to the edema (Di Rosa, 1972; Omote *et al.*, 2001).

Murine Mϕ when stimulated by bacterial products (*i. e.* LPS or cytokines) produced by action of iNOS a large amount of NO, which is an important agent in the edema development. Therefore, the inhibitory effect on NO production in LPS activated Mϕ is an useful way of evaluation of the anti-inflammatory activity (Fushiya *et al.*, 1999)

Based on the results, the activity of dehydrocanophyllic acid methyl ester (**1**) could be related to an inhibition of prostaglandin and NO production. No cytotoxic effects on Mϕ were observed. For the free acid **4** it might be deduced that its high effect on the TPA edema obey to a different mechanism, it was also clear an increment of its cytotoxicity in relation with its methyl ester **1**. In the lupanes, the presence of the two hydroxy groups in 29-hydroxylupeol (**6**) appears to be related to its activity in the carrageenan model, since lupeol (**5**) and 29-hydroxylupenone (**7**) exhibited a modest edema inhibition. On the edema produced by TPA **5** was more active than **7**, showing that the hydroxy group on position 3 enhances the activity. On the other hand, cytotoxicity followed an increasing tendency, **5** < **6** < **7**, showing again the effect of the presence of hydroxy groups at positions 3 and 29. In summary, the activity of the lupane skeleton compounds **5**–**7** could be involved

with the prostanoid synthesis and their possible role in the NO inhibition could not be established due to the observed cytotoxicity. The flavonoid **8** showed significant action in both carrageenan and TPA models, with a weak NO inhibition effect, indicating that the highest component of its activity could be associated, here again, with the prostaglandin synthesis. The agarofuran derivative **9** exhibited modest effects in the anti-inflammatory tests, suppressed the NO production but exerted cytotoxic action towards Mϕ. On the other hand, compound **10** showed low activity in TPA, no activity in the carrageenan model, and its NO inhibition, with no cytotoxicity, was similar to the one observed for aminoguanidine indicating that its activity could be related to an action on the iNOS and is probably not related to prostaglandin synthesis. The latter, also suggests a different mechanism of action in **9** and **10** due to their different substitution pattern.

Acknowledgements

We are indebted to Ma. de los Angeles Peña, Isabel Chávez, Héctor Ríos, Beatríz Quiroz, Rubén Gaviño, Rocío Patiño, Javier Pérez, Luis Velasco, and Gabriela Salcedo for technical assistance. Dr. Guillermo Avila is also gratefully acknowledged for the collection of the plant.

Ahmad V. U. and Atta-ur-Rahman (1994), Handbook of Natural Products Data, Vol. 2. Elsevier, Amsterdam, London, New York, Tokyo, p. 627.

Betancor C., Freire R., Gonzalez A. G., Salazar J. A., Pascard C., and Prange T. (1980), Three triterpenes and other terpenoids from *Catha cassinoides*. Phytochemistry **19**, 1989–1993.

Di Rosa M. (1972), Biological properties of carrageenan. J. Pharm. Pharmac. **24**, 89–102.

Dirsch V., Stuppner H., and Vollmar A. (1998), The Griess assay: Suitable for a bio-guided fractionation of anti-inflammatory plant extracts? Planta Med. **64**, 423–426.

Fernández M. A., de las Heras B., García M. D., Sáens M. T., and Villar A. (2001), New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J. Pharm. Pharmac. **53**, 1533–1539.

Fushiya S., Kishi Y., Hattori K., Batkhuu J., Takano F., Singab A., and Okuyama T. (1999), Flavonoids from *Cleome droserifolia* suppress NO production in activated macrophages *in vitro*. Planta Med. **65**, 404–407.

González A. G., Núñez M. P., Bazzocchi I. L., Ravelo A. G., and Jiménez I. A. (1993), Sesquiterpenes from *Maytenus magellanica* (Celastraceae). Nat. Prod. Lett. **2**, 163–170.

Govindachari T. R., Viswanathan N., Pai B. R., Rao U. R., and Srinivasan M. (1967), Triterpenes of *Calophyllum inophyllum* Linn. Tetrahedron **23**, 1901–1910.

Hara H., Sukamoto T., Ohtaka H., Abe K., Tatumi Y., Saito Y., Suzuki A., and Tsukamoto G. (1992), Effects of baicalein and α-tocopherol on lipid peroxidation, free radical scavenging activity and 12-*O*-tetradecanoylphorbol acetate-induced ear edema. Eur. J. Pharmac. **221**, 193–198.

Huguet A. I., Recio M. C., Máñez S., Giner R. M., and Ríos J. L. (2000), Effect of triterpenoids on the inflammation induced by protein kinase C activators, neuronally acting irritants and other agents. Eur. J. Pharmac. **410**, 69–81.

Mahato S. B., Nandy A. K., and Roy G. (1992), Triterpenoids. Phytochemistry **31**, 2199–2249.

Martínez V. M., Muñoz Corona M., Sánchez Vélez C., and Rodríguez-Hahn L. (1988), Terpenoids from *Mortonia diffusa*. J. Nat. Prod. **51**, 793–796.

Martínez-Vázquez M., Ramírez Apan T. O., Lastra A. L., and Bye R. (1997), A comparative study of the analgesic and anti-inflammatory activities of pectolarin isolated from *Cirsium subcoriaceum* and linarin isolated from *Buddleia cordata*. Planta Med. **64**, 134–137.

Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immun. Meth. **65**, 55–63.

Mukherjee A., Hale V. G., Borga O., and Stein R. (1996), Predictability of the clinical potency of NSAIDs from the preclinical pharmacodynamics in rats. Inflamm. Res. **45**, 531–540.

Nicollier G., Tabacchi R., Gavin J., Breton J. L., and González A. G. (1979), Triterpènes de la mousse de chêne (*Evernia prunasti* (L.) Arch.). Helv. Chim. Acta **62**, 807–810.

Omote K., Hazama K., Kawamata T., Kawamata M., Nakayaka Y., Toriyabe M., and Namiki A. (2001), Peripheral nitric oxide in carrageenan-induced inflammation. Brain Res. **912**, 171–175.

Pérez-Castorena A. L, Arciniegas A., Ramírez Apan M. T., Villaseñor J. L., and Romo de Vivar A. (2001), Evaluation of the anti-inflammatory and antioxidant activities of the plastoquinone derivatives isolated from *Roldana barba-johannis*. Planta Med. **68**, 645–647.

Rao T. S., Currie J. L., Shaffer A. F., and Isakon P. C. (1993), Comparative evaluation of arachidonic acid (AA)- and tetradecanoylphorbol acetate (TPA)-induced dermal inflammation. Inflamm. **17**, 723–741.

Recio M. C., Giner R. M., Máñez S., and Ríos J. L. (1995), Structural requirements for the anti-inflammatory activity of natural triterpenoid. Planta Med. **61**, 182–185.

Rioja I., Ubeda A., Terencio A. C., Guillén I., Riguera R., Quinela J. M., Peinador C., González L. M., and Alcaraz M. J. (2000), An anti-inflammatory ditriazine inhibiting leukocyte functions and expression of inducible nitric oxide synthase and cyclo-oxygenase-2. Eur. J. Pharm. **397**, 207–217.

Rodríguez-Hahn L., Martínez M., Jiménez M., and Díaz E. (1988), Constituyentes sesquiterpenicos de plantas del género *Mortonia* (Celastraceae). Rev. Latinoam. Quím. **19**, 52–55.

Rózsa Z. and Pelczer I. (1989), New sesquiterpene esters from *Euonymus europaeus* and *E. latifolius*. J. Chem. Soc. Perkin Trans. I, 1089–1095.

Safayhi H. and Sailer E. R. (1997), Anti-inflammatory actions of pentacyclic triterpenes. Planta Med. **63**, 487–493.

Sánchez A., Martínez M., and Rodríguez-Hahn L. (1984), Photochemical rearrangement of mortonin C. Tetrahedron **40**, 1005–1008.

Sautebin L., Ialenti A., Ianaro A., and Di Rosa M. (1995), Modulation by nitric oxide of prostaglandin biosynthesis in the rat. Brit. J. Pharm. **114**, 323–328.

Suh N., Honda T., Finlay J., Barchowsky A., Williams C., Benoit N. E., Xie Q., Nathan C., Grible W., and Sporn M. B. (1998), Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. Cancer Res. **58**, 717–723.

Tinto W. F., Blair L. C., Alli A., Reynolds W. F., and McLean S. (1992), Lupane triterpenoids of *Salacia cordata*. J. Nat. Prod. **55**, 395–398.

Vidari G., Finzi P. V., and de Bernardi M. (1971), Flavonols and quinones in stems of *Aframomun giganteum*. Phytochemistry **10**, 3335–3339.