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Anti-inflammatory activity of erycristagallin, a pterocarpene from *Erythrina mildbraedii*☆

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Abstract

Erycristagallin, a pterocarpene isolated from *Erythrina mildbraedii*, was tested in vitro for its antioxidant properties on the stable 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) free radical and on the arachidonic acid metabolism. In addition, erycristagallin was tested on different experimental models of inflammation, such as the acute and chronic inflammation induced by the application of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on mice and the phospholipase A_2-induced mouse paw oedema test. In the carrageenan-induced mouse paw oedema test, the ethyl acetate extract obtained from *E. mildbraedii* showed anti-inflammatory activity, and erycristagallin was isolated as the active principle. In vivo, erycristagallin significantly inhibited the phospholipase A_2-induced mouse paw oedema as well as the mouse ear oedema induced by TPA ($ID_{50} < 10$ μg/ear). Moreover, it significantly reduced the chronic inflammation and leukocyte infiltration induced by repeated application of TPA. In vitro, erycristagallin inhibited the arachidonic acid metabolism via the 5-lipoxygenase pathway in rat polymorphonuclear leukocytes ($IC_{50} = 23.4$ μM), but had no effect on cyclooxygenase-1 metabolism in human platelets, while showing antioxidant activity in the DPPH test. As with other phenolics, the anti-inflammatory activity of erycristagallin may be based on its capacity to inhibit the arachidonic acid metabolism via the 5-lipoxygenase pathway.

Keywords: Erycristagallin; (*Erythrina mildbraedii*); Arachidonic acid metabolism; 5-Lipoxygenase inhibition; Anti-inflammatory activity

1. Introduction

The genus *Erythrina* contains numerous species distributed in tropical and subtropical regions (Morton, 1981; Oliver-Bever, 1986). During the last three decades, over 50 flavonoids have been obtained from 15 *Erythrina* species (Kamat et al., 1981), with prenylated flavanones, isoflavones and pterocarpans being the major nonalkaloid secondary metabolites isolated thus far (Fomum et al., 1983, 1986, 1988; Wandji et al., 1994). These compounds are of biological importance as they exhibit various pharmacological activities, including depression of the central nervous system, muscle contracting and antihypertensive effects, inhibition of β_1-adrenergic receptors and antibacterial properties (Fomum et al., 1986, 1988).

Whereas the antibacterial effect of the hot ethanol extract obtained from the roots of *Erythrina mildbraedii* has been previously reported (Mitscher et al., 1987), no studies on the anti-inflammatory activity of this species have been published. One of the secondary metabolites produced by *E. mildbraedii*, namely erycristagallin, has been studied as to its antimicrobial effects, which have

☆ Part 41 in the series *Erythrina* studies.

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been found to include activity against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Streptococcus mutans*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (Mitscher et al., 1984, 1987, 1988a,b; Iinuma et al., 1994). Again, no studies concerning this compound's anti-inflammatory effects have been reported to date.

In order to contribute to the ongoing research on the isolation and pharmacological characterisation of bioactive secondary metabolites from plants of the *Erythrina* genus, we have studied the root bark of *E. mildbraedii*, one of the 10 *Erythrina* species indigenous to Cameroon, where potions made from this plant are popularly used in village communities for the traditional treatment of dysentery, stomach pains, venereal diseases, asthma, female sterility, ulcers, boils and various types of inflammations (Oliver-Bever, 1986). A preliminary screening of crude extracts of *E. mildbraedii* in our laboratory indicated that the ethyl acetate extract of the root bark has anti-inflammatory properties. A bioassay-guided study of this crude extract led to the isolation and identification of the pterocarpene erycristagallin as the constituent endowed with a potent anti-inflammatory activity, which we describe in this article.

2. Materials and methods

2.1. Extraction

The root bark of *E. mildbraedii* Harms was collected in July 1997 in Buea, Southwest Province of Cameroon. Identification and authentication of the plant material was done at the Cameroon National Herbarium, Yaounde, where a voucher specimen is on deposit (No. 50452/HNC). Air-dried and pulverised root bark of the collected sample (5 kg) was extracted at room temperature with ethyl acetate for 2 days. After evaporation of the solvent under reduced pressure, 105 g of residue was obtained. This extract was chromatographed over silica gel packed in *n*-hexane. Gradient elution was effected with *n*-hexane, ethyl acetate and methanol. Fractions were tested on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear oedema and active fractions were selected to purify compounds. Repeated column chromatography yielded eight compounds. The structures have been successfully elucidated by using homo- and heteronuclear two-dimensional nuclear magnetic resonance (NMR) techniques such as ^{1}H-NMR, ^{13}C-NMR, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, and mass spectrum. Erycristagallin ($C_{25}H_{26}O_4$) (Fig. 1) was obtained as violet crystals (100 mg) after concentration of the fractions eluted with *n*-hexane/ethyl acetate (95:5). The ^{1}H- and ^{13}C-NMR spectra data obtained for erycristagallin in $CDCl_3$ were compared to those published by Mitscher et al. (1984). The other compounds obtained were *n*-hexatetracontanol, *n*-hexacosanol, *n*-octacosanol, and their ferulates, β-sitosterol and β-sigmasterol.

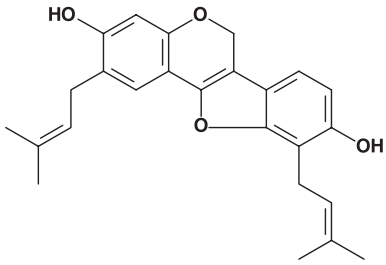

Fig. 1. The chemical structure of erycristagallin.

2.2. Animals

Female Wistar rats weighing 180–200 g and groups of six Swiss female mice weighing 25–30 g were used. All animals were fed on a standard diet ad libitum. Housing conditions and all in vivo experiments were approved by the institutional Ethical Committee of the Faculty of Pharmacy, University of Valencia (Spain), according to the guidelines established by the European Union on Animal Care (CEE Council 86/609).

2.3. Chemicals

Chemical and biochemical reagents were purchased from Sigma (St. Louis, MO), except acetone and methanol of high-performance liquid chromatography (HPLC) grade, which were from Baker (Deventer, Holland); ethanol 96°, *n*-hexane, ethanol, methanol and ethyl acetate, all of analytical grade, were from Panreac (Barcelona, Spain).

2.4. Carrageenan-induced paw oedema in mouse

Oedema was induced on the right hind paw by subplantar injection of carrageenan (3% w/v in saline, 25 μl) (Sugishita et al., 1981). The ethyl acetate extract of *E. mildbraedii*, dissolved in Tween 80/ethanol/saline (1:1:10) was orally administered at a dose of 200 mg/kg (0.2 ml), 1 h before carrageenan injection. A group received the reference drug indomethacin (10 mg/kg, p.o.). Details of the method have been described earlier (Giner et al., 2000).

2.5. Phospholipase A_2-induced paw oedema in mouse

The method was described by Neves et al. (1993). Phospholipase A_2 from *Naja mossambica* (2 units in 25 μl of sterile saline) was injected s.c. into the right hind mouse paw. The left paw received the same volume of vehicle. The test compounds (5 mg/kg) were injected i.p. 30 min before induction of inflammation with phospholipase A_2. Both the reference drug cyproheptadine and the test products were dissolved in Tween 80/ethanol/saline (1:1:10). Details of the

method have been described earlier (Giner-Larza et al., 2002).

2.6. 12-O-Tetradecanoylphorbol 13-acetate (TPA)-induced acute mouse ear oedema

Oedema was induced by topical application of 2.5 μg per ear of TPA. Erycristagallin (0.25 mg/ear) and indomethacin (0.5 mg/ear) were applied topically, simultaneously with TPA (Giner et al., 2000). Test products were dissolved in acetone. Details of the method have been described earlier (Giner et al., 2000). The 50% inhibitory dose (ID_{50}) was performed by applying erycristagallin at five different doses ranging from 10 to 100 μg/ear and applying indomethacin from 4.6 to 186 μg/ear for the control group.

2.7. Mouse ear inflammation induced by multiple topical applications of TPA

Inflammation was induced by topical application on alternate days (five applications) of 2 μg of TPA (20 μl) in each ear (Stanley et al., 1991). Erycristagallin (0.1 mg/ear) and dexamethasone (0.05 mg/ear) were applied topically twice daily for 4 days. On the last day, the compounds were applied only in the morning. The mice were killed by cervical dislocation, and two ear punches from each animal were taken ($n = 5$ animals). Details of the method have been described earlier (Giner et al., 2000).

2.8. Histology

Ear samples were fixed in 4% neutral-buffered formalin. Each sample was longitudinally cut into equal halves. Half of each was embedded in paraffin, cut into 3- to 4-μm sections and stained with haematoxylin–eosin. Epithelium thickness was evaluated using an objective $\times 100$ and expressed as the mean ± S.D. of the number of epidermal layers from the basal to the granulous stratum, inclusive (Giner et al., 2000).

2.9. Myeloperoxidase assay

According to the methods used by De Young et al. (1989), each ear sample was placed in an Eppendorf tube with sodium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide. Enzyme activity was colorimetrically determined using a Labsystems Multiskan MCC/340 plate reader set to measure absorbance at 620 nm. Details of the methods have been described earlier (Giner-Larza et al., 2001).

2.10. Cytotoxicity assay

Cytotoxicity was measured by the colorimetric assay of Mosmann (1983). Neutrophils were exposed to the product (100 μM) in a microplate for 30 min, and then 100 μl per well of a 5 mg/ml solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil-tetrazolium bromide (MTT) were added and incubated at 37 °C until blue deposits were visible. The colored metabolite was dissolved in dimethylsulfoxide (100 μl per well). This reaction was performed in triplicate. Absorbance was measured at 490 nm using a Labsystems Multiskan MCC/340 plate reader. Results are expressed in absolute absorbance readings; a decrease indicated a reduction in cell viability.

2.11. Inhibition of leukotriene B_4 production from rat polymorphonuclear leukocytes

Rat peritoneal leukocytes (95% viability) were prepared according to Safayhi et al. (1995). For 5-lipoxygenase product formation from endogenous arachidonic acid, leukocytes were stimulated at 37 °C for 5 min with the calcium ionophore A23187 (1.9 μM) and Ca^{2+} (1.8 mM). The cells were incubated in the presence of erycristagallin at a final concentration of 100 μM. All incubations including controls were carried out in the presence of 0.5% dimethylsulfoxide. Separation of arachidonic acid products was performed by high-performance liquid chromatography (HPLC) followed by diode array detection. A reversed-phase C_{18} column was employed and eluted with mobile phase [A = methanol with 0.007% (v/v) trifluoracetic acid; B = water with 0.007% trifluoracetic acid]; solvent gradient, 50% A to 74% A linear in 27 min, 50% A isocratic for 12 min; flow 1.0 ml/min. The results obtained from peak areas were normalised to prostaglandin B_2 (17 μg/ml) internal standard and expressed as a percentage of leukotriene B_4 production. Experiments were performed in triplicate. IC_{50} value was calculated by means of the linear regression plotted from the inhibition percentages obtained at four different concentrations.

2.12. Assay of cyclooxygenase-1 activity from human platelets

Blood platelets were obtained from healthy human donors and were separated by sequential centrifugation. The platelets were incubated in the presence of the test compounds at 100 μM. Stimulation was performed according to Safayhi et al. (1995) and Laufer et al. (1995) with 2.5 μM Ca^{2+} and 1.9 μM calcium ionophore 23187. Separation of 12-hydroxyheptadecatrienoic acid (12-HHTrE) was achieved by HPLC coupled to diode array detection. A reversed-phase C_{18} column was used and eluted with methanol/water (74:26) containing 0.007% (v/v) trifluoroacetic acid. The results obtained were expressed as percentage of 12-HHTrE production.

2.13. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Reduction of the stable free radical was determined according to Cavin et al. (1998), with some modifications. 2,2-Diphenyl-1-picrylhydrazyl (1.5 ml) radical in methanol

(20 mg/l) were added to 0.75 ml of a solution of test compounds in methanol (100 μM). Absorbance at 517 nm was spectrophotometrically determined after 5 min and the scavenging activity was calculated as percentage of the radical reduction. Quercetin was used as reference compounds.

2.14. Statistical analysis

Inhibition percentages were calculated from the differences between drug-treated animals and control animals treated only with the inflammatory agent. One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons of unpaired data was used for statistical evaluation.

3. Results

3.1. Effects on carrageenan-induced paw oedema in mouse

After the oral administration of the ethyl acetate extract obtained from *E. mildbraedii*, the carrageenan-induced oedema to the hind paws of mice was significantly inhibited. The extract produced a 33% and 35% inhibition of the oedema at 1 and 3 h, respectively, whereas at 5 h the effect was not significant (Fig. 1). In a bioassay-guided process, we isolated and identified the pterocarpene erycristagallin as the constituent endowed with anti-inflammatory activity (Fig. 2).

3.2. Effects on phospholipase A_2-induced acute paw oedema in mouse

When erycristagallin was assayed against the phospholipase A_2-induced oedema in mouse paws, oedema formation

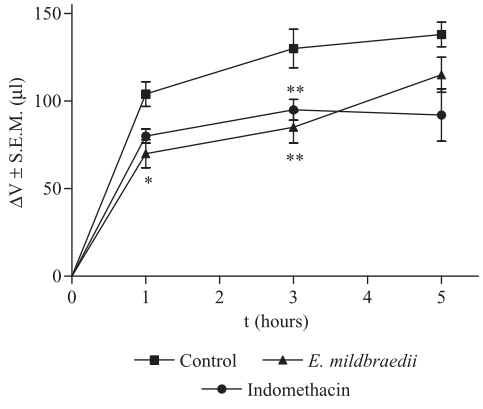

Fig. 2. Effect of *E. mildbraedii* extract on carrageenan-induced paw oedema. Footpad oedema was induced 1 h later by injection of carrageenan (3% w/v in saline). Footpad volume was measured 1, 3 and 5 h after irritant injection. Each point represents the mean from six increases in footpad volume and the vertical lines indicate the S.E.M. Statistically significant difference with respect to the control is expressed as $^{**}P<0.01$, $^{*}P<0.05$ (Dunnett's *t*-test).

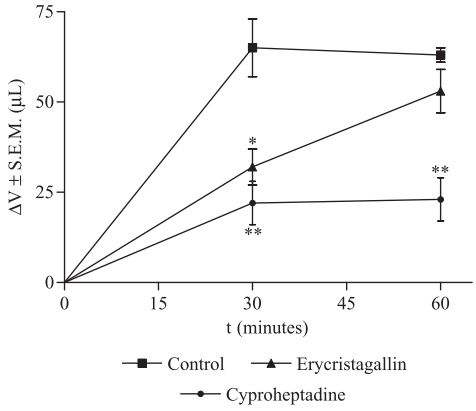

Fig. 3. Anti-inflammatory effect of erycristagallin and cyproheptadine (5 mg/kg, i.p.) on phospholipase A_2-induced paw oedema 30, 60 and 90 min after irritant injection. Each point represents the mean from six increases in footpad and the vertical lines indicate the S.E.M. $^{**}P<0.01$, $^{*}P<0.05$ (Dunnett's *t*-test).

was inhibited by 51% after 30 min at a dose of 5 mg/kg (i.p.); however, the effect was clearly reduced at 60 min. At the same dose, the reference drug cyproheptadine was effective at both 30 and 60 min (Fig. 3).

3.3. Effects on TPA-induced acute ear oedema in mouse

The ethyl acetate extract from *E. mildbraedii* inhibited oedema by 89% at a dose of 1 mg/ear (data not shown). Erycristagallin, the compound isolated from the active extract, exhibited a strong effect when assayed in the TPA acute ear oedema test, inhibiting the oedema by 94% at a dose of 0.25 mg/ear, while the reference drug indomethacin produced an 82% inhibition at 0.5 mg/ear. Both drugs influenced leukocyte infiltration, measured as the decrease of the myeloperoxidase activity, lowering it by 42% and 36%, respectively (Fig. 4). Analysis of the dose–response relationship confirmed the higher potency of erycristagallin, with an ID_{50} lower than 10 μg/ear (< 25 nmol/ear), whereas the value for indomethacin under the same experimental conditions was 103 μg/ear (288 nmol/ear) (Table 1). The histological analysis of the ears treated only with TPA (control group) showed a focal inflammatory lesion affecting both the dermis and the epidermis, with infiltrating cells (neutrophils) in the dermis. In addition, neither lymphocytes nor macrophages were observed. The presence of papillomatosis, acanthosis and hyperkeratosis was characteristic in the epidermis of the control group and the epithelium thickness was 3.1 ± 0.2 cells (Fig. 5A). Treatment with erycristagallin reduced both the leukocyte infiltration and the hyperkeratosis, and the epithelium thickness was clearly lower than that in the control and reference groups (2.3 ± 0.2 cells). However, administration of the compound had no effect on the formation of oedema, papillomatosis or acanthosis (Fig. 5B). The ears treated with indomethacin showed neither inflammatory lesions nor cell infiltration, and the epithelium thickness was 2.6 ± 0.2 cells (Fig. 5C).

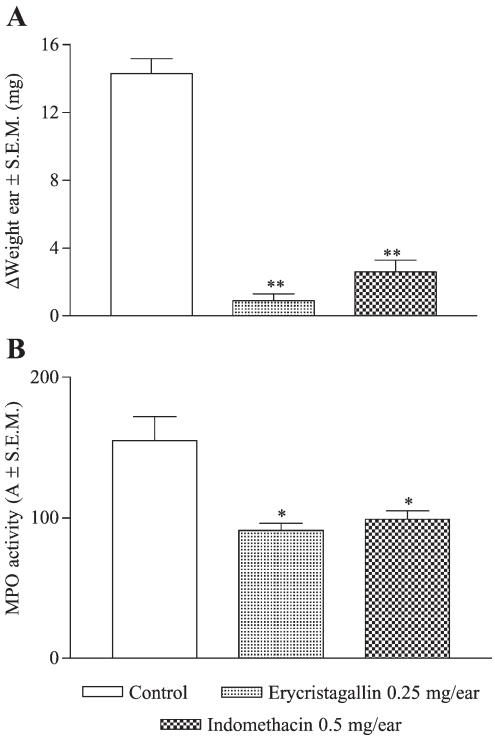

Fig. 4. Effect of erycristagallin and indomethacin on acute TPA-induced ear oedema. (A) Ear oedema expressed as the mean of the difference between ear thickness before and after challenge (mg ± S.E.M.). $n = 6$. Inhibition ratio percentage with respect to the control group treated only with TPA. (B) Neutrophil accumulation assessed from myeloperoxidase activity. $n = 8$. Inhibition ratio percentage with respect to the control group treated only with TPA. $^{**}P < 0.01$, $^{*}P < 0.05$ as compared to the control group (Dunnett's *t*-test).

3.4. Effects on mouse ear inflammation induced by multiple topical applications of TPA

In the model of chronic inflammation induced by multiple applications of TPA, erycristagallin showed a moderately significant effect at 0.1 mg/ear (seven applications), inhibiting swelling by 34% and producing 59% inhibition of neutrophil infiltration, measured as myeloperoxidase activity (Fig. 6). The inhibition of swelling and myeloperoxidase activity observed for dexamethasone was 85% and 67%, respectively. The histological study of ears treated only with repeated doses of TPA (Fig. 7A) showed an extensive and diffuse inflammatory lesion affecting both the dermis and the epidermis. The main infiltrating cells in the dermis were neutrophils, lymphocytes and macrophages; hypertrophy and hyperplasia of fibroblasts and

Table 1
Inhibitory dose (50%) of erycristagallin on acute TPA-induced ear oedema

	ΔT (μm ± S.E.M.)[a]	I.R.[b]	ID_{50} (μg/ear)[c]
Control	232.5 ± 19.8	–	–
Erycristagallin (0.01 mg/ear)	29.5 ± 3.8[d]	87	< 10 (100–10)
Indomethacin (0.5 mg/ear)	40.0 ± 4.3d	83	103 (4.6–186)

[a] Ear oedema expressed as the mean of the difference between ear thickness before and after challenge (μm/ear ± S.E.M.). $n = 6$.

[b] Inhibition ratio percentage of erycristagallin at 0.1 mg/ear and indomethacin at 0.5 mg/ear with respect to the control group treated only with TPA.

[c] 50% inhibitory dose.

[d] $P < 0.01$ as compared to the control group (Dunnett's *t*-test).

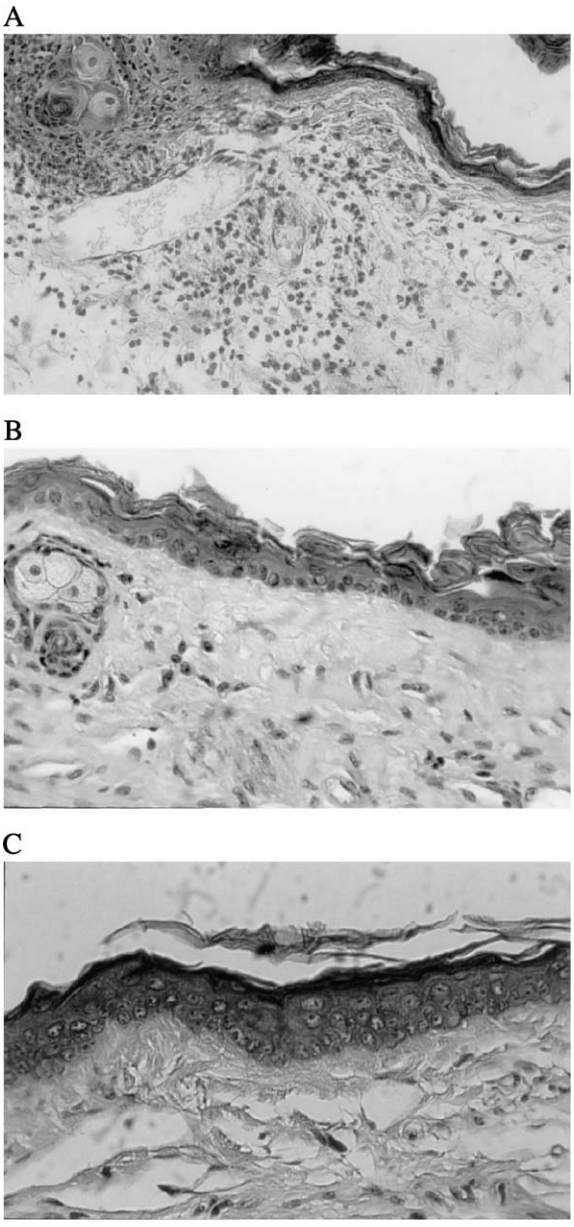

Fig. 5. Haematoxylin–eosin-stained sections of mouse ears after topical application of TPA. (A) Control treated only with TPA × 40: inflammatory lesion with oedema, focal infiltrate by polymorphonuclear leukocytes, epithelial reaction with papillomatosis, acanthosis and hyperkeratosis areas. (B) Ears treated with erycristagallin × 40: reduction of oedema and macrophage infiltration, dermal fibrosis with increase in fibroblasts. (C) Ears treated with indomethacin × 40: no oedema, leukocyte infiltration nor fibrosis, no epidermis modification.

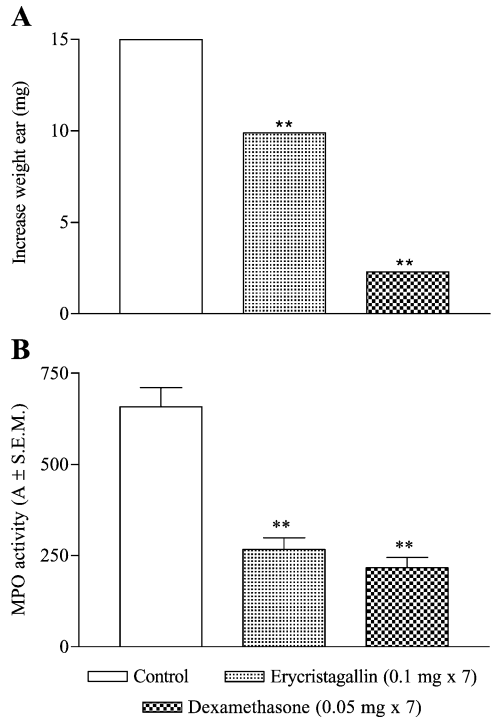

Fig. 6. Effect of erycristagallin (repeatedly 0.1 mg/ear) and dexamethasone (repeatedly 0.05 mg/ear) on chronic inflammation induced by TPA in mice (2 μg/ear). $n=5$ animals. (A) Increase in ear weight in milligrams (mean ± S.E.M.). Inhibition ratio percentage with respect to the control group treated only with TPA. (B) Neutrophil accumulation, assessed as myeloperoxidase activity. Each column with a vertical bar represents the mean for eight ear samples with S.E.M. Statistical significance of difference from the control. $^{**}P<0.01$, $^{*}P<0.05$ by Dunnett's multiple comparison test.

papillar fibrosis were also present. The epidermis in this assay was characterised by the presence of papillomatosis and acanthosis, and the epithelium was 6.7 ± 0.1-cells thick. Repeated treatment with 0.1 mg of erycristagallin (Fig. 7B) reduced the oedema and leukocyte infiltration as well as the hyperkeratosis, papillomatosis and acanthosis, and the epithelium thickness was clearly lower than that of the control group (5.0 ± 0.1 cells). As in the control group, however, hypertrophy and hyperplasia of fibroblasts were present in the ear treated with erycristagallin, but to a minor degree. In contrast, the ears treated with dexamethasone (Fig. 7C) showed neither inflammatory lesions nor cell infiltration, and the epithelium was 4.8 ± 0.2-cells thick.

3.5. Effects on arachidonic acid metabolism

Erycristagallin showed no toxic activity against rat polymorphonuclear leukocytes, giving a viability higher than 95%. When we assayed the effect of erycristagallin on leukotriene B_4 production by polymorphonuclear cells from the rat peritoneal cavity, 100% inhibition was obtained at 100 μM, but no effect was observed on cyclooxygenase-1 from human platelets at the same dose (data not shown). Testing showed a clear dose–effect dependence for erycristagallin, the IC_{50} value of which was thus determined to be 23.4 μM (Fig. 8).

3.6. DPPH scavenging activity

Erycristagallin had scavenging properties, inhibiting the stable DPPH free radical by 96% at a concentration of 50

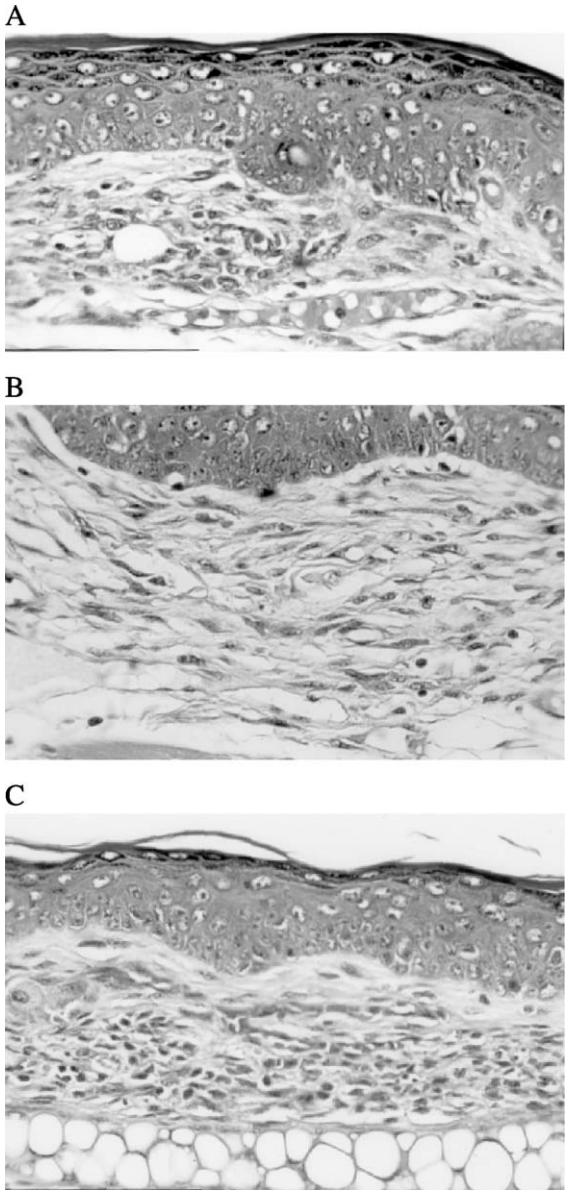

Fig. 7. Haematoxylin–eosin-stained sections of ears (× 10 and × 40) after application of TPA. (A) Control treated only with TPA: inflammatory lesion with intraepithelial microabscesses, diffuse inflammation of the conjunctive tissue by polymorphonuclear leukocytes and macrophages, epithelial reaction with hyperplasia and hypertrophy of the dermis, hyperkeratosis areas. (B) Ears treated with erycristagallin: no inflammation, dermal fibrosis with increase in fibroblasts. (C) Ears treated with dexamethasone: diffuse lesion in the dermis with increase in fibroblasts and lymphocyte infiltration and few polymorphonuclear leukocytes.

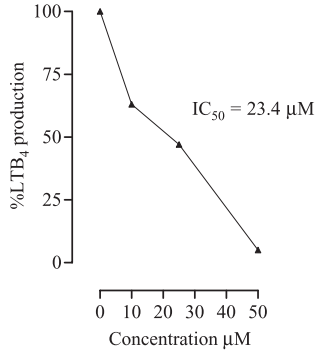

Fig. 8. Inhibition concentration-50 (IC-50) of erycristagallin on leukotriene B_4 production from endogenous arachidonic acid in Ca^{2+} ionophore-stimulated rat peritoneal leukocytes. Concentrations assayed ranged from 5 to 50 μM. Data are expressed as means ± S.E.M. percentages of metabolite formation with respect to controls: $n=4-6$. $r^2=0.9585$. $P=0.0210$ significant.

μM, similar to obtained for the reference drug at the same concentration.

4. Discussion

Studies of the antimicrobial effects of various species of the genus *Erythrina* as well as those of their isolated principles have appeared in the literature. Indeed, several species and their related compounds have been studied as anti-inflammatory agents, the most pertinent example being Pillay et al.'s (2001) description of the inhibition of cyclooxygenase activity by different *Erythrina* extracts. The present paper, however, represents the first report on the anti-inflammatory activity of both *E. mildbraedii* and its isolated principle erycristagallin.

There are few references with respect to the anti-inflammatory activity of the pterocarpans isolated from *Erythrina* species. In a previous work, Ghosh and Anandakumar (1983) studied the anti-inflammatory activity of gangetin, a pterocarpan structurally related to erycristagallin. This compound administered per os at 50 mg/kg inhibited the carrageenan-induced oedema in the hind paws of rats, but its effect was significantly lower when assayed on the cotton pellet granuloma test, again in rats. Applied at 50 mg/kg, it exhibited only 14% inhibition, while at 100 mg/kg, no effect was observed. At the latter dose, however, it did reduce the inflammatory exudate of the granuloma pouch in rats by 61%, and it additionally inhibited formaldehyde-induced arthritis in albino rats by 22%. Apart from this study, the literature also contains reports of the anti-inflammatory activity of a pterocarpene isolated from the bark of different species of *Erythrina* such as *E. variegata*, *E. caffra* and *E. lysistemon*. The anti-inflammatory mechanism of this compound, 3,9-dihydroxy-2,10-diprenyl pterocarp-6*a*-ene, seems to be related to the inhibition of cyclooxygenase.

Our work on the pterocarpan erycristagallin found that 30 min after administering the compound at 5 mg/kg, the phospholipase A_2-induced oedema decreased by 51%. Curiously, the effect disappeared 60 min after application. The mechanism underlying this effect may be either an indirect inhibition of the enzyme or a blockage of the mastocyte degranulation, which in turn leads to release of serotonin and other vasoactive amines (Cirino et al., 1989). Direct inhibition of the enzyme seems unlikely as an in vitro assay on arachidonic acid metabolism showed that erycristagallin had no effect on the production of the cyclooxygenase metabolites 5-HETE or 12-HHTrE on human platelets. On rat peritoneal leukocytes, however, application of erycristagallin inhibited the production of leukotriene B_4 (5-lipoxygenase metabolite).

This effect of erycristagallin on the arachidonic acid metabolism via the 5-lipoxygenase pathway suggests that the compound acts through a mechanism somehow related to the inhibition of the enzyme. This action may either be directly inhibitory or such that it interferes with some of the mechanisms that participate in the complex activation of this enzyme. In our experiment, erycristagallin showed scavenging properties, inhibiting the stable DPPH free radical by 96% at a concentration of 50 μM, while the reference drug quercetin produced an inhibition of 92% at the same dose. Previous studies have demonstrated that different antioxidant and free radical scavenger agents (e.g. nordihydroguaiaretic acid, phenidone, gnaphaliin and silibinin) can reduce 5-lipoxygenase activity by a mechanism that interferes with divalent ions implicated in the catabolism of arachidonic acid (Alcaraz and Ferrándiz, 1987; Dehmlow et al., 1996; De la Puerta et al., 1999). Thus, it is likely that the scavenging properties of erycristagallin are implicated in the compound's effect on 5-lipoxygenase activity.

This would explain why erycristagallin inhibited both the acute ear oedema and the chronic inflammation induced by application of TPA. Although glucocorticoids are the most active drugs against the chronic inflammation caused by application of this acetate, other pharmacological agents have been found to be effective, among them the 5-lipoxygenase inhibitors (Stanley et al., 1991). We thus propose that the observed effects of erycristagallin are due to its ability to inhibit the leukotriene B_4 formation in polymorphonuclear leukocytes.

Histological studies of ears treated with TPA indicated that erycristagallin is an efficient inhibitor of neutrophil affluence into the skin in both acute and chronic inflammations. This property would constitute a basis for the compound's cutaneous anti-inflammatory effect. It should, however, be noted that although erycristagallin was as effective as the reference drugs at reducing leukocyte infiltration, its anti-inflammatory activity in terms of swelling was weaker than that of dexamethasone in the chronic model of inflammation.

In conclusion, erycristagallin has been found to be an efficient and potent anti-inflammatory agent in experimental models of acute inflammation. Its anti-inflammatory effects

are most likely related with the inhibition of the activity of 5-lipoxygenase.

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