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ARTICLE *in* EUROPEAN JOURNAL OF PHARMACOLOGY · FEBRUARY 2003

Impact Factor: 2.53 · DOI: 10.1016/S0014-2999(02)02954-0 · Source: PubMed

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European Journal of Pharmacology 460 (2003) 219–226
www.elsevier.com/locate/ejphar

A new dual inhibitor of arachidonate metabolism isolated from *Helichrysum italicum*

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Received 8 August 2002; received in revised form 16 December 2002; accepted 20 December 2002

Abstract

Six acetophenones (**1**–**6**) and one γ-pyrone (**7**), previously isolated from *Helichrysum italicum*, were tested for their ability to inhibit enzymatic and non-enzymatic lipid peroxidation, the stable 1,1-diphenyl-2-pycryl-hydrazyl free radical, superoxide scavenging and arachidonic acid metabolism. In addition, they were studied in different experimental models such as the chronic inflammation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), the phospholipase A_2-induced mouse paw oedema test, the carrageenan-induced mouse paw oedema test, and the writhing induced by acetic acid in the mouse. Of the assayed compounds, only **1** inhibited enzymatic lipid peroxidation but had no effect on non-enzymatic lipid peroxidation. None of them scavenged the superoxide radical. Study of the inhibition of arachidonic acid metabolism demonstrated that **1** was an inhibitor of both cyclooxygenase and 5-lipoxygenase, whereas **2** was a selective inhibitor of 5-lipoxygenase. In the assay of phospholipase A_2-induced mouse paw oedema, the γ-pyrone derivative inhibited oedema formation, showing a similar profile to that obtained with cyproheptadine. The acetophenones were effective at 30 and 60 min. In the carrageenan test, acetophenone **1** gave the best results and had analgesic effects in the acetic acid writhing test. In conclusion acetophenone **1** (4-hydroxy-3-(3-methyl-2-butenyl)acetophenone) is a new dual inhibitor of arachidonate metabolism, and could be a useful tool for obtaining anti-inflammatory and analgesic drugs.

Keywords: Acetophenones; Maltol-glucoside; Arachidonic acid metabolism; Anti-inflammatory compound; Analgesic compound

1. Introduction

Helichrysum italicum is a perennial herb used in folk medicine for its anti-inflammatory properties. In previous studies (Schinella et al., 2002; Sala et al., 2002), we reported the antioxidant and anti-inflammatory activities of the aerial parts of this plant in various in vitro and in vivo experimental models. We have reported the isolation, identification and topical anti-inflammatory activity of six acetophenones and one γ-pyrone (Fig. 1) against 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear oedema (Sala et al., 2001).

Acetophenones are a group of phenolic metabolites present in various taxonomic groups in the plant kingdom and possess different pharmacological activities, such as antioxidant and anti-inflammatory effects. Several acetophenones were tested in vitro for their effects on superoxide anion production in human polymorphonuclear neutrophils. Among them, apocynin (4-hydroxy-3-methoxy-acetophenone) and 4-hydroxyethyloxy-3-methoxy-acetophenone were shown to be the most active inhibitors of the neutrophil oxidative burst (Stuppner et al., 1995). Apocynin inhibited superoxide formation by preventing the assembly of the superoxide-generating enzyme NADPH-oxidase (Stolk et al., 1994) and also strongly inhibited the formation of peroxynitrite by murine macrophages (Muijsers et al., 2000). Lapperre et al. (1999) suggested that the antioxidant properties of apocynin could be related to the increase in glutathione synthesis through activation of transcription factors such as activator protein-1 (AP-1).

There are some interesting studies in vivo of the anti-inflammatory and anti-asthmatic effects of diverse natural

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doi:10.1016/S0014-2999(02)02954-0

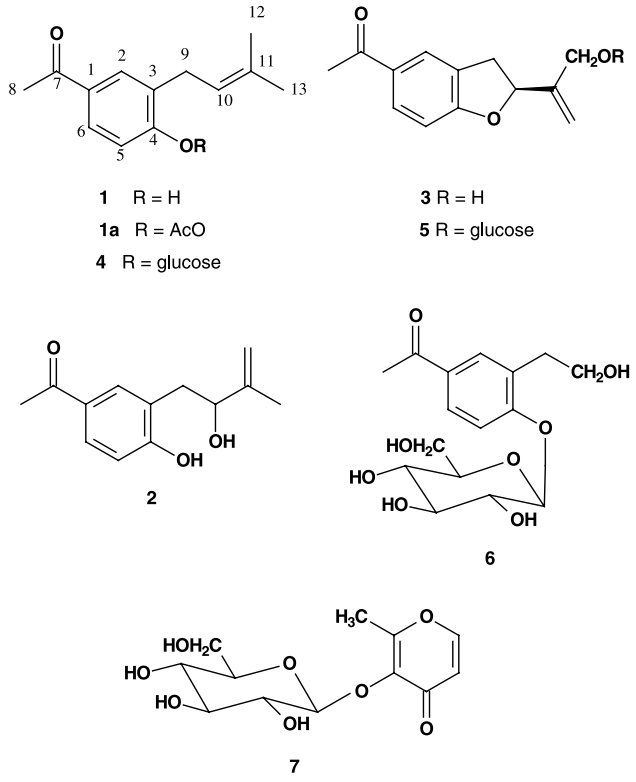

Fig. 1. Chemical structures of compounds isolated from *H. italicum*: (**1**), (**1a**), (**2**), (**3**), (**4**), (**5**), (**6**) and (**7**).

and synthetic acetophenone derivatives (Müller et al., 1999; Favier et al., 1998; Dorsch et al., 1994; Ohmi et al., 1994). Apocynin proved to be very active in rat models of arthritis or in ulcerative skin lesions in rats ('t Hart et al., 1990, 1992). More recently, Lafeber et al. (1999) described its effect on inflammation-induced cartilage destruction in rheumatoid arthritis in a human in vitro experimental model, accompanied by a decrease in interleukin-1 and tumour necrosis factor-α production by mononuclear cells.

Our study examined the antioxidant and anti-inflammatory activities of the isolated acetophenones on lipid peroxidation, free radical generation, and arachidonic acid metabolism and in different in vivo experimental models of acute and chronic inflammation and peripheral analgesia. The aim of this paper is to determine the mechanism of action of the active acetophenones and to establish their potential as new anti-inflammatory agents.

2. Materials and methods

2.1. Test compounds and chemicals

4-Hydroxy-3-(3-methyl-2-butenyl)acetophenone (**1**), 4-hydroxy-3-(2-hydroxy-3-isopentenyl) acetophenone (**2**), 12-hydroxytremetone (bitalin A) (**3**), 3-(3-methyl-2-butenyl)acetophenone-4-*O*-β-D-glucopyranose (**4**), 12-hydroxytremetone-12-*O*-β-D-glucopyranoside or bitalin A-12-*O*-β-D-glucopyranoside (**5**), 3-(2-hydroxyethyl) acetophenone-4-*O*-β-D-glucopyranoside (**6**) and maltol β-D-*O*-glucopyranoside (**7**) were isolated from the anti-inflammatory extracts of *H. italicum* (Sala et al., 2001). All of them have a purity higher than 99.9%, according to their mass spectra and nuclear magnetic resonance data. Acetic acid, allopurinol, ascorbic acid, butylated hydroxytoluene, calcium ionophore A23187, carrageenan, cyproheptadine hydrochloride, dexamethasone, dimethylsulpoxide, 1,1-diphenyl-2-pycryl-hydrazyl ethylenediaminetetraacetic acid, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, glycogen, hexadecyltrimethylammonium bromide, hydrogen peroxide, hypoxanthine, indomethacin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil-tetrazolium bromide (MTT), nitro blue tetrazolium, *N*,*N*-dimethylformamide, phosphate-buffered saline, phospholipase A_2 from *Naja mossambica* venom, prostaglandin B_2, pyrogallol, TPA, tetramethylbenzidine, 2-thiobarbituric acid, trichloroacetic acid, Trypan blue, xanthine and xanthine oxidase were purchased from Sigma (St. Louis, MO, USA); Tween 80 was from Fluka Chemika–Biochemika (Buchs, Switzerland); acetone analytical grade, and methanol of high-performance liquid chromatography gradient grade were from Baker (Deventer, Holland); ethanol 96°, methanol, sodium acetate and trifluoroacetic acid, all of them of analytical grade, were from Panreac (Barcelona, Spain).

2.2. Acetylation of acetophenone *1*

Acetylation of acetophenone **1** (50 mg) with acetic anhydride in pyridine afforded, after usual work-up, 48 mg of 4-acetoxy-3-(3-methyl-2-butenyl)acetophenone (**1a**). Acetylated compound was purified by gel filtration on a Sephadex LH-20 column with methanol as eluant.

2.3. Animals

Groups of six Swiss female mice weighing 25–30 g and female Wistar rats weighing 180–200 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 Ethics Committee of the Faculty of Pharmacy according to the guidelines established by the European Union on Animal Care (CEE Council 86/609).

2.4. Lipid peroxidation

Rat liver microsomes were prepared using a standard differential centrifugation technique (Schinella et al., 2000). Protein contents were quantified by Bradford's method using bovine serum albumin as the standard (Bradford, 1976). The antioxidant activity of the products dissolved in dimethylsulfoxide was tested at a final concentration of 100 μM.

Non-enzymatic peroxidation was induced by addition of $FeSO_4$ (5 μM) and ascorbate (500 μM) (Schinella et al.,

2000). The products of lipid peroxidation were detected by measuring the absorbance at 535 nm, using the thiobarbituric acid method. Butylated hydroxytoluene was used as a positive control. In enzymatic lipid peroxidation, the reaction mixture contained microsomal protein and an NADPH-generating system. Peroxidation was started by the addition of 10 μl CCl_4 1:4 (v/v) in dimethylsulfoxide. After a 15-min incubation at 37 °C, thiobarbituric acid-reactive species were determined as above.

2.5. Free radical generation

Superoxide radical was generated by enzymatic oxidation of hypoxanthine with xanthine oxidase grade I and was detected by nitroblue tetrazolium reduction, monitored spectrophotometrically at 560 nm (Schinella et al., 2000). Pyrogallol was used as a positive control. The influence on enzyme activity was evaluated by uric acid formation from xanthine, and absorbance was measured at 295 nm. Allopurinol was used as a positive control. A solution of 1,1-diphenyl-2-picrylhydrazyl radical in methanol was added to the compound solutions and activity was determined by spectrophotometry at 517 nm after 10 min (Schinella et al., 2000). Butylated hydroxytoluene was used as a reference compound.

2.6. Cytotoxicity assay

Cytotoxicity of the acetophenones and maltol-glucoside was measured by the colorimetric assay of Mosmann (1983). Neutrophils were exposed to the products (100 μM) in a microplate for 30 min, and then 100 μl per well of a 5 mg/ml solution of MTT was added and incubated at 37 °C until blue deposits were visible. The coloured 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 decrease in cell viability.

2.7. Inhibition of leukotriene B_4 production by 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 the test compounds 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 followed by diode array detection. A reverse-phase (RP-18) column was used 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 linear regression plotted from the inhibition percentages obtained with four different concentrations.

2.8. Assay of cyclooxygenase-1 activity in 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 **1** and **2** at 100 μM. Stimulation was performed according to Safayhi et al. (1995) and Laufer et al. (1995) with 2.5 nM Ca^{2+} and 1.9 μM calcium ionophore 23187. Separation of 12-hydroxyheptadecatrienoic acid (12-HHTrE) was achieved by high-performance liquid chromatography coupled to diode array detection. A reverse-phase C_{18} column was used and eluted with methanol/water (74:26) containing 0.007% (v/v) trifluoroacetic acid. The results obtained are expressed as percentages of 12-HHTrE production.

2.9. 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) to each ear (Stanley et al., 1991). Compounds **1** and **3** (0.5 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-Larza et al., 2001).

2.10. Myeloperoxidase assay

According to the method used by De Young et al. (1989), each ear sample was placed in an Eppendorf tube with 0.75 ml of 80 mM sodium phosphate buffer (pH = 5.4) containing 0.5% hexadecyltrimethylammonium bromide. Enzyme activity was determined colorimetrically using a Labsystems Multiskan MCC/340 plate reader set to measure absorbance at 620 nm. Details of the method have been described earlier (Giner-Larza et al., 2001).

2.11. Histology

Ear samples were fixed in 4% neutral-buffered formalin. Each sample was cut longitudinally 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 × 100 and

expressed as the mean ± S.D. of the number of epidermal layers from the basal to the granulous stratum, inclusive (Giner-Larza et al., 2001).

2.12. Phospholipase A_2-induced mouse paw oedema

The method was that described by Neves et al. (1993). Phospholipase A_2 from *N. 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 (80 mg/kg) were injected i.p. 30 min before phospholipase A_2, and the reference drug cyproheptadine (10 mg/kg) was administered p.o. 60 min prior to inflammation induction. Both the products and the reference drug were dissolved in Tween 80/ethanol/saline (1:1:10). Oedema was measured with a plethysmometer (Ugo Basile) 30, 60 and 90 min after challenge and is expressed as the difference between the right and left paw volumes. The control group was treated only with phospholipase A_2. Inhibition is expressed as a percentage of control.

2.13. Carrageenan-induced mouse paw oedema

Oedema was induced in the right hind paw by subplantar injection of carrageenan (3% w/v in saline, 25 μl) (Sugishita et al., 1981). Compounds **1** and **1a**, dissolved in Tween 80/ethanol/saline (1:1:10) were administered orally at a dose of 150 mg/kg (0.2 ml), 1 h before carrageenan injection. A group received the reference drug indomethacin (10 mg/kg, p.o.). Right and left paw volumes were measured with a plethysmometer (Ugo Basile) 1, 3 and 5 h after inflammation induction. Oedema is expressed as the difference between right and left paw volumes, and oedema inhibition is expressed as the percentage of volume reduction relative to the control.

2.14. Writhing test

The experiment was performed according to the method described by Atta-ur-Rahman et al. (2001) with minor modifications. Acetophenones **1** and **1a**, and aspirin, a reference peripheral analgesic drug, at 100 mg/kg were administered p.o. and i.p. 1 h or 30 min, respectively, before the i.p. administration of 3% acetic acid solution (0.25 ml). Control animals received vehicle under the same experimental conditions. Immediately after acetic acid injection, each animal was isolated in an individual box to be observed for 20 min. The number of writhings and stretchings was recorded. Analgesic activity is expressed as the percentage of writhing reduction relative to the control.

2.15. 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 lipid peroxidation and free radical generation

Acetophenones **1** and **2** (Fig. 1) were the only active compounds against enzymatic lipid peroxidation in rat liver microsomes (51% and 25% inhibition, respectively), while none showed activity in the non-enzymatic lipid peroxidation test. All the compounds, except acetophenone **4** (29% inhibition), were inactive against the stable 1,1-diphenyl-2-pycrylhydrazyl free radical. Finally, no compound exhibited superoxide scavenging properties in the hypoxanthine/xanthine oxidase system or in human leukocytes stimulated with TPA.

3.2. Effect on leukotriene B_4 production by rat polymorphonuclear leukocytes

None of the tested compounds had cytotoxicity in the MTT test. Fig. 2 shows the effect on the production of leukotriene B_4 by rat peritoneal leukocytes, which was clearly reduced by acetophenones **1** and **2** at 100 μM (95% and 44%, respectively). This effect was concentration dependent, and IC_{50} values were therefore determined. The IC_{50} for acetophenone **1** was nearly four times lower than that for acetophenone **2**. The rest of compounds had no activity at 100 μM.

3.3. Effect on cyclooxygenase-1 activity in human platelets

When the compounds were tested for their inhibitory effect on cyclooxygenase-1 at 100 μM, acetophenone **1**

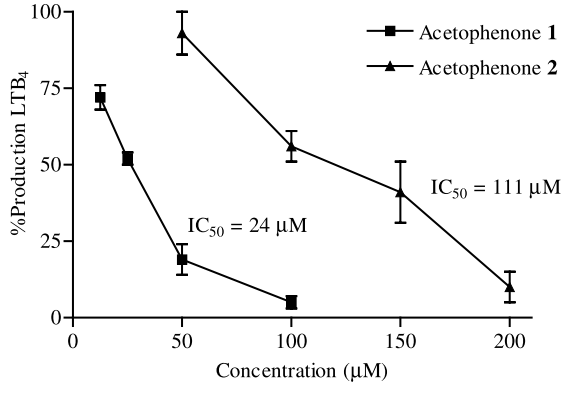

Fig. 2. Inhibition concentration-50 (IC_{50}) of **1** and **2** on leukotriene B_4 production from endogenous arachidonic acid in Ca^{2+} ionophore-stimulated rat peritoneal polymorphonuclear leukocytes. Concentrations assayed ranging from 12.5 to 200 μM. Data are expressed as means ± S.E.M. percentages of metabolite formation with respect to controls: $n = 4–6$.

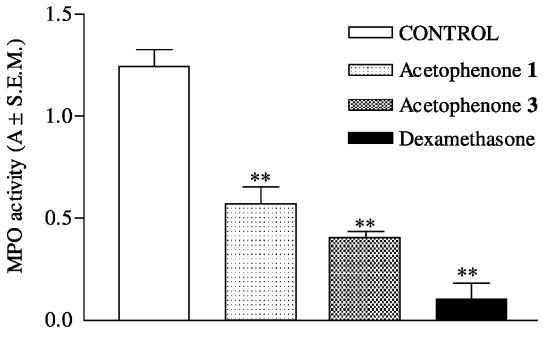

Fig. 3. Effect of acetophenones **1** and **3** (0.5 mg/ear × 7) on myeloperoxidase activity following ear inflammation induced by repeated application of TPA. ** $P<0.01$ (Dunnett's *t*-test). Dexamethasone 0.05 mg/ear × 7.

was the only one to reduce the production of 12-HHTrE by 59%.

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

Acetophenones **1** and **3** were studied in a model of chronic inflammation induced by TPA. Neither inhibited oedema formation (data not shown), but they did significantly inhibit myeloperoxidase activity by 57% and 71%, respectively (Fig. 3). The histological study demonstrated that none of the compounds affected the inflammatory process (data not shown).

3.5. Effect on phospholipase A_2-induced mouse paw oedema

All the substances, except acetophenone **2**, which was isolated in a small quantity, were studied in vivo against the phospholipase A_2-induced mouse paw oedema (Fig. 4). One hour after phospholipase A_2 injection, the most active compound was acetophenone **5** (65% oedema reduction) followed by compounds **6** and **7** (57% and 52% of oedema inhibition, respectively). These compounds were still active 90 min after phospholipase A_2 subplantar injection.

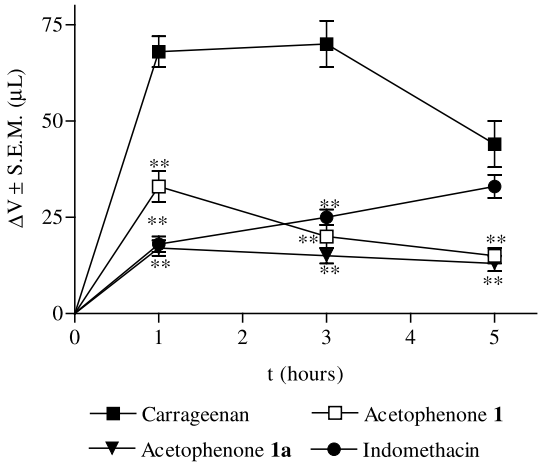

Fig. 5. Effect of **1** and its acetylated derivative **1a** (150 mg/kg, p.o.) on carrageenan-induced mouse paw oedema. Footpad oedema was induced 1 h later by injection of carrageenan (3% w/v in saline). Footpad volume was measured 1, 3 and 5 h after irritant injection. Each point represents the mean from five to six increases in footpaw volume and the vertical lines indicate the S.E.M. Statistically significant difference with respect to the control is expressed as ** $P<0.01$ (Dunnett's *t*-test).

3.6. Effect on carrageenan-induced mouse paw oedema

Acetophenone **1** was the only compound active against the cyclooxygenase pathway in isolated human platelets. This compound and its acetylated derivative (**1a**) were assayed in the carrageenan (Fig. 5) and the peripheral analgesia (Fig. 6) tests. Both compounds exhibited significant anti-inflammatory activity, inhibiting paw oedema by 51% and 75%, respectively, after the first hour. Later measurements gave similar results for both compounds, with 71% and 79% inhibition at 3 h, and 66% and 70% inhibition at 5 h, respectively.

3.7. Analgesic effect

Finally, acetophenones **1** and **1a** were tested in the acetic acid writhing test and both compounds showed analgesic

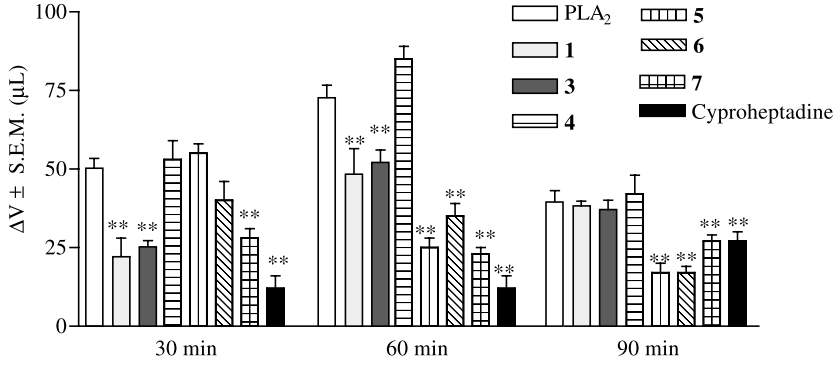

Fig. 4. Anti-inflammatory effect of acetophenones (**1**–**6**) and compound **7** (80 mg/kg, i.p.) and cyproheptadine (10 mg/kg, p.o.) on phospholipase A_2-induced paw oedema 30, 60 and 90 min after irritant injection. PLA_2: Phospholipase A_2. Each point represents the mean for five to six animals and the vertical lines indicate the S.E.M. **$P<0.01$ (Dunnett's *t*-test).

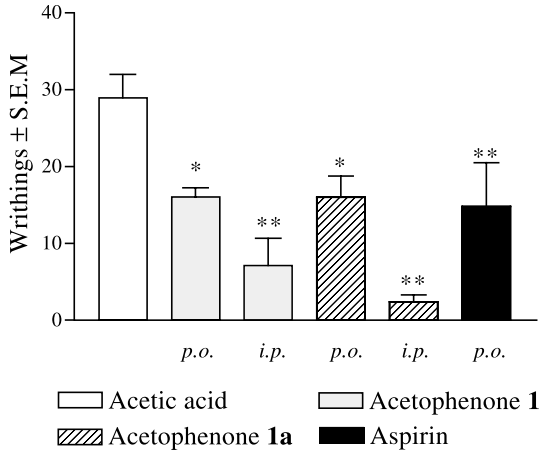

Fig. 6. Analgesic effects of **1** and its derivative **1a** in the writhing test. Compounds were administered p.o. and i.p. at 100 mg/kg. Aspirin was administered at the same dose only p.o. ** $P<0.01$; * $P<0.05$ (Dunnett's *t*-test). $n=5$ animals.

properties after oral and intraperitoneal administration. However, although similar behaviour was observed p.o., different responses were noted after intraperitoneal administration, with inhibition values of 75% and 92%, respectively.

4. Discussion

In a previous paper, Sala et al. (2001) reported the anti-inflammatory activity of some of the acetophenones isolated from *H. italicum* against TPA-induced ear oedema. In this study, we demonstrate the effects of these compounds in different experimental models in order to establish their potential as anti-inflammatory agents.

Some acetophenones have previously been reported to be inhibitors of reactive oxygen species production, and a relationship between their chemical structure and pharmacological activity has been established (Dorsch et al., 1994; Stuppner et al., 1995; Van den Worn et al., 2001). Apocynin and its derivatives are the best known of the agents with this type of structure. It is a potent inhibitor of the NADPH oxidase involved in superoxide anion generation in stimulated human neutrophils, has low toxicity and does not interfere with other neutrophil functions. Our data demonstrate that the acetophenones isolated from *H. italicum* have no antioxidant properties and only compound **1** inhibited enzymatic lipid peroxidation, while it had no effect on non-enzymatic peroxidation. The most relevant difference between apocynin and acetophenone **1** is the presence of a methoxy group at C-3 in the former and a butenyl group in the latter. Various studies of structure–activity relationships of acetophenones have highlighted the relevance of methoxylation in C-5 and its interference with the signal transduction that mediates neutrophil activation. Our findings, together with the results obtained with apocynin derivatives, suggest that the antioxidant properties of our acetophenones are not involved in their mechanism of anti-inflammatory activity.

Hydroxyacetophenone derivatives have been described as inhibitors of chemotaxis of polymorphonuclear granulocytes (Müller et al., 1999; Ohmi et al., 1994). Methyl or methoxy group substitution in C-3 resulted in pronounced inhibitory effects (Müller et al., 1999). In our studies, histology of the ear sample obtained following the chronic inflammation induced by repeated application of TPA demonstrated that acetophenones **1** and **3** had no effect on the inflammation or on the neutrophil infiltration (data not shown). These results suggest that the myeloperoxidase activity inhibition observed in the chronic model of inflammation is due to direct inhibition of the enzyme. In a previous paper, Van den Worn et al. (2001) studied the inhibition of this enzyme by different structurally related compounds, and only two inhibited the release of myeloperoxidase and/or its activity, but in both cases, the compounds were acid derivatives. There is no direct chemical relationship between these active compounds and our acetophenones.

Six of the compounds isolated from *H. italicum* were assayed in the phospholipase A_2 test, and the results demonstrated a net effect against this irritant. Of these, compound **7**, a γ-pyrone glucoside, was the only one to be effective throughout the inflammatory process. The mechanism responsible for this effect may be a direct inhibition of the enzyme, a blockage of mast cell degranulation, or an antagonism of the liberated vasoamines (Cirino et al., 1989). The behaviour of compound **7** was similar to that of cyproheptadine, the reference drug used, which is a serotonin and histamine antagonist. A structural relationship can be established between chemical composition and pharmacological response. In general, glycosylation had no effect at 30 min but the response at 90 min was greater than that of the corresponding aglycones. However, the free forms responded quickly against the oedema induced by the irritant agent. Only acetophenone **4** was ineffective at all the different times investigated, but its corresponding aglycone was active at 30 and 60 min after challenge.

Arachidonic acid metabolism was inhibited only by acetophenones **1** and **2**. At 100 μM, acetophenone **1** totally inhibited leukotriene B_4 production by 5-lipoxygenase in rat neutrophils stimulated with calcium ionophore, while its effect on the production of 12-HHTrE by cyclooxygenase-1 in human platelets, also stimulated with the same agent, was more moderate. Acetophenone **2** was a more specific inhibitor of 5-lipoxygenase than of cyclooxygenase-1, although its potency was about five times lower than that of acetophenone **1**.

The latter compound was tested in the carrageenan mouse paw oedema model in order to explore its anti-inflammatory effect. This test is a good tool for investigating the in vivo effects of cyclooxygenase inhibitors, principally its constitutive form or cyclooxygenase-1, which is the

biological source of prostaglandin E_2 production during the inflammatory process (Smith et al., 1998). Some acetophenones, such as tremetone, exhibited anti-inflammatory activity in the acute inflammatory process induced by carrageenan in the paw mouse (Favier et al., 1998). The effects of acetophenone **1** 3 h after the subcutaneous injection of carrageenan were similar to those observed for indomethacin, although we noted that the effects of acetophenone **1** were longer lasting, reducing the inflammation in the later phase (5 h). These results confirm those previously reported by Malaviya et al. (2000) for related acetophenones in a study of their effects on the production of prostaglandin E_2 in vitro as well as in vivo in the carrageenan air pouch test.

It had been demonstrated that the writhing response to intraperitoneal injection of acetic acid in mice is produced by the release of prostacyclin synthesised by cyclooxygenase-1 in the abdominal cavity (Berkenkopf and Weichman, 1988). The analgesic potency of some non-steroid anti-inflammatory drugs correlates closely with increasing selectivity for cyclooxygenase-1 rather than for cyclooxygenase-2 (Warner et al., 1999; Ballou et al., 2000). In a parallel assay, we tested the potential effect of acetophenone **1** as an analgesic agent and obtained encouraging results when it was assayed in the writhing test. Acetophenone **1** was effective after oral and intraperitoneal administration, with an effect similar to that obtained with the reference drug aspirin, at the same dose.

To complete this study, we synthesised an acetylated derivative of acetophenone **1** (**1a**) and assayed it in the same experimental protocols used for acetophenone **1**. In this case, the effects were similar to those obtained for acetophenone **1** in the paw oedema test, but the activity of acetophenone **1a** was higher at 1 h. Both compounds showed similar properties in the writhing test after oral or parenteral administration, showing a more marked effect in the latter case, in particular the acetylated derivative (**1a**). The behaviour of both compounds in this assay was similar to that of aspirin, when administered orally at the same dose as the reference drug.

These results confirm the potential of *H. italicum* as a medicinal plant to be used in different inflammatory and allergic diseases, and confirm the potential of its isolated compound, **1**, as a new dual anti-inflammatory and analgesic drug. This compound could be a useful tool for obtaining new analgesic and anti-inflammatory agents, following minor structural modifications.

Acknowledgements

The authors wish to thank the Spanish government for its financial support (DGESIC, PM98-0206). We are indebted to the Centre de Transfusions de la Comunitat Valenciana (Valencia, Spain) for its generous supply of human blood. This study is a part of the CYTED project IV.11. GS is member of CIC Provincia de Buenos Aires, Argentina.

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