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Effects of marine 2-polyprenyl-1,4-hydroquinones on phospholipase A_2 activity and some inflammatory responses

Blanca Gil [a], M. Jesús Sanz [a], M. Carmen Terencio [a], Alfonso De Giulio [b], Salvatore De Rosa [b], M. José Alcaraz [a], Miguel Payá [a,*]

[a] *Department of Pharmacology, University of Valencia, Faculty of Pharmacy, Avenida Vicent Andrés Estellés s / n, 46100 Burjassot, Valencia, Spain*
[b] *Istituto per la Chimica di Molecole di Interesse Biologico, C.N.R., 80072 Arco Felice, Naples, Italy*

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Abstract

Three 2-polyprenyl-1,4-hydroquinone derivatives (2-heptaprenyl-1,4-hydroquinone: IS1, 2-octaprenyl-1,4-hydroquinone: IS2 and 2-[24-hydroxy]-octaprenyl-1,4-hydroquinone: IS3) isolated from the Mediterranean sponge *Ircinia spinosula*, were evaluated for effects on phospholipase A_2 activity of different origin (*Naja naja* venom, human recombinant synovial fluid and bee venom), as well as on human neutrophil function and mouse ear oedema induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA). IS1 interacted minimally with these responses. In contrast, IS2 and IS3 inhibited human recombinant synovial phospholipase A_2 in a concentration-dependent manner, with minor effects on the rest of the enzymes. Both compounds slightly affected superoxide generation and degranulation in human neutrophils, whereas they decreased thromboxane B_2 and leukotriene B_4 synthesis and release in a mixed suspension of human platelets and neutrophils stimulated by ionophore A23187, with IC_{50} values in the μM range. IS3 was the most effective inhibitor of the synthesis of thromboxane B_2 by human platelet microsomes and of leukotriene B_4 by high speed supernatants from human neutrophils. IS2 and IS3 showed topical anti-inflammatory activity against the TPA-induced ear inflammation in mice, with similar effects on oedema and a higher inhibition of IS3 on leukocyte migration, estimated as myeloperoxidase activity in supernatants of ear homogenates. Some structure-activity relationships were established since differences in the prenylated chain attached to the hydroquinone moiety result in important modifications of these inflammatory responses.

Keywords: 2-Polyprenyl-1,4-hydroquinone; Marine product; Phospholipase A_2; Neutrophil, human; Anti-inflammatory drug

1. Introduction

One area of current interest concerns the study of selective inhibitors of phospholipase A_2 and their ability to control inflammatory processes, as possible alternatives to non-steroidal anti-inflammatory drugs (Miyake et al., 1993; Bomalaski and Clark, 1993; Mukherjee et al., 1994). In addition, such inhibitors may be tools for elucidation of the mechanisms of arachidonate mobilization and cell activation.

In recent years, a number of marine sponge metabolites, including manoalide, have proven effective to inhibit phospholipase A_2 activity from different sources, in vitro. This property could be responsible, at least in part, for the anti-inflammatory effects shown in animals, mainly against the ear oedema induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in mice (Bennett et al., 1987; Jacobson et al., 1990; Potts et al., 1992; De Carvalho and Jacobs, 1991; Marshall et al., 1994).

Phospholipases A_2 specifically release fatty acids from the *sn*-2 position of membrane phospholipids for production of proinflammatory mediators such as platelet activating factor and eicosanoids (Chang et al., 1987; Mukherjee et al., 1994). Secretory phospholipases A_2 are low molecular weight enzymes and can be divided into groups I (e.g. *Elapidae* venoms), II (e.g. synovial fluids) and III (bee venom) (Glaser et al., 1993; Mukherjee et al., 1994). The enzyme present in mammalian synovial fluids during inflammatory dis-

* Corresponding author. Tel. 34-6-3864292, 34-6-3864942, fax 34-6-3864292, 34-6-3864943.

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eases induces or potentiates inflammatory responses either in vitro (Miyake et al., 1994; Mukherjee et al., 1994) or in vivo when injected into animals (Vadas and Pruzanski, 1986; Vishwanath et al., 1988). Recently, cytosolic enzymes of high molecular weight and lacking significant sequence similarity with secretory pospholipases A_2 have also been isolated from different cell types (Glaser et al., 1993; Kudo et al., 1993; Mukherjee et al., 1994).

We have previously reported the anti-inflammatory activity of two natural products of marine origin, avarol and avarone, isolated from the sponge *Dysidea avara* (Ferrándiz et al., 1994). They are diterpenoids possessing a hydroquinone (avarol) or quinone (avarone) moiety. Other sponge metabolites contain a hydroquinone moiety attached to a terpene residue, like three 2-polyprenyl-1,4-hydroquinones isolated from the Mediterranean sponge *Ircinia spinosula* which have shown analgesic activity in mice with a low toxicity (20% mortality at 1 g/kg i.p., a dose 100–200 times higher than those exerting significant analgesic effects) (De Pasquale et al., 1991). These compounds also showed moderate antimicrobial effects in vitro (De Rosa et al., 1995).

In the present work we studied the effects of this series of 2-polyprenyl-1,4-hydroquinones (2-heptaprenyl-1,4-hydroquinone: IS1, 2-octaprenyl-1,4-hydroquinone: IS2 and 2-[24-hydroxy]-octaprenyl-1,4-hydroquinone: IS3) (Fig. 1) on extracellular phospholipase A_2 (groups I, II and III). In addition, we evaluated their topical anti-inflammatory activity by use of TPA-induced ear oedema in mice. In order to investigate the possible mode of action of these compounds, we also studied their ability to modify some responses related to the inflammatory process.

2. Materials and methods

2.1. Methods

Preparation of human neutrophils and platelets

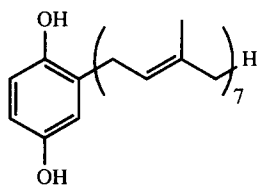
The anticoagulated blood of healthy volunteers was centrifuged at $200 \times g$ for 15 min at 4°C and the platelet-rich plasma was removed. In the residual blood, erythrocytes were sedimented in 2.0% (w/v) dextran (molecular weight 526000) in 0.9% NaCl at room temperature and the supernatant was centrifuged at 1200 $\times g$ for 10 min at 4°C. After hypotonic lysis of remaining erythrocytes the pellet was resuspended in phosphate-buffered saline solution (PBS), and neutrophils were purified by Ficoll-Hypaque sedimentation. The cells (viability greater than 95% by trypan blue test) were resuspended in PBS containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+}.

Cytotoxicity studies

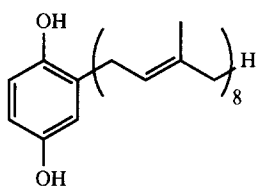
Lactate dehydrogenase was determined by the rate of oxidation of NADH (Bergmeyer and Bernt, 1974). Tubes containing Triton X-100 were used for measurement of total cellular content of enzyme.

Superoxide generation by human neutrophils

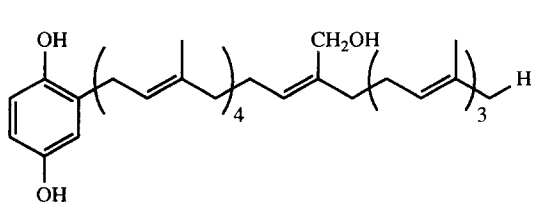
Aliquots of 0.5 ml neutrophils (2.5×10^6 cells/ml) were preincubated for 5 min at 37°C with test compounds or the vehicle (methanol, 5 μl) and nitroblue tetrazolium (100 μM). Cells were stimulated with TPA (1 μM) for 10 min. Tubes were centrifuged at $1200 \times g$ for 10 min at 4°C and the pellets were treated with 500 μl of dimethyl sulfoxide/HCl (95:5) and sonicated. Aliquots (250 μl) were transferred to a well of a flat-bottomed microtitre plate and absorbance was measured at 540 nm (Rice-Evans et al., 1991).



IS_1



IS_2



IS_3

Fig. 1. Chemical structure of IS1, IS2 and IS3.

Scavenging of superoxide

Superoxide was generated by the hypoxanthine/xanthine oxidase system and detected by the reduction of nitroblue tetrazolium. A direct inhibitory effect on xanthine oxidase activity was tested for by measuring uric acid formation from xanthine by following the rate of absorbance change at 295 nm (Payá et al., 1992).

Elastase release by human neutrophils

The cells (2.5×10^6/ml), in a volume of 500 μl, were preincubated with test compound or vehicle for 5 min at 37°C and then treated with cytochalasin B (10 μM) for 5 min followed by addition of *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (10 μM) and incubated for 10 min at 37°C. After centrifugation at 1200 $\times g$ for 10 min at 4°C, 250 μl aliquots of supernatants were transferred to a well of a flat-bottomed microtitre plate and incubated with *N*-tert-butoxy-carbonyl-L-alanine *p*-nitrophenyl ester (200 μM) for 10 min at 37°C. Absorbances were determined at 414 nm (Barrett, 1981).

Partial purification of elastase from human neutrophils

Human neutrophils (10^8/ml) were completely lysed by sonication and the mixture was then centrifuged at 100000 $\times g$ for 1 h at 4°C (Barrett, 1981). The supernatant was diluted in PBS at a protein concentration of 0.21 mg/ml. Aliquots of the diluted supernatant (250 μl) were used as the enzyme source for the above reaction.

Synthesis and release of eicosanoids by human neutrophils and platelets

A mixed suspension of neutrophils (5×10^6/ml) and platelets (5×10^6/ml) in PBS was preincubated with test compound or vehicle (5 μl) for 10 min at 37°C. The calcium ionophore, A23187 (final concentration 1 μM), was added in a volume of 5 μl and the mixture was incubated for 10 min. After centrifugation at 1200 $\times g$ for 10 min at 4°C, the supernatants were frozen at −70°C until the radioimmunoassays for thromboxane B_2 and leukotriene B_4 were performed as described previously (Moroney et al., 1988).

Synthesis of thromboxane B_2 by human platelet microsomes

Human platelet microsomes were prepared and incubated with 10 μM arachidonic acid as previously described (Brownlie et al., 1993). The samples were then boiled for 5 min and thromboxane B_2 levels were determined by radioimmunoassay as above.

Synthesis of leukotriene B_4 by high speed supernatants from human neutrophils

High speed (100000 $\times g$) supernatants from human neutrophils were obtained and incubated with 10 μM arachidonic acid using the procedure described by Tateson et al. (1988). Leukotriene B_4 levels were measured by radioimmunoassay as above.

Phospholipase A_2 assay

Phospholipase A_2 was assayed by using [^{3}H]oleate labelled membranes of *Escherichia coli*, following a modification of the method of Franson et al. (1974) described previously (Ferrándiz et al., 1994). Test compounds were preincubated with the enzyme for 5 min and after addition of substrate the incubation was allowed to proceed for 15 min. Human recombinant synovial phospholipase A_2 (0.03 μg), bee venom phospholipase A_2 (0.05 units) and *Naja naja* venom enzyme (0.1 units) were diluted in 100 μM Tris-HCl, 1 μM $CaCl_2$ buffer, pH 7.5.

Mouse ear oedema

Test compounds were applied topically in acetone before TPA administration (2.5 μg) to the right ear of Swiss mice weighing 20–25 g. The left ear (control) received only acetone. After 4 h the animals were killed by cervical dislocation and equal sections of both ears were punched out and weighed (Carlson et al., 1985). The ear sections were homogenized in 750 μl saline and after centrifugation at 10000 $\times g$ for 15 min at 4°C, myeloperoxidase activity was measured in supernatants (Suzuki et al., 1983; De Young et al., 1989). The reaction mixture contained 50 μl supernatant, 150 μl PBS, 15 μl 0.22 M NaH_2PO_4 (pH = 5.4), 20 μl 0.034% H_2O_2 and 20 μl 18 mM tetramethylbenzidine in 8% dimethyl formamide. After 3 min reaction at 37°C, 30 μl of 1.46 M sodium acetate buffer, pH 3.0, was added and absorbance at 630 nm was read using a microtitre plate reader.

2.2. Drugs

IS1, IS2 and IS3 were isolated from the marine sponge *Ircinia spinosula* (Dictyoceratida) collected in the bay of Naples (Italy), as previously described (Cimino et al., 1972). Other materials were purchased from Sigma Chemical Co. (St. Louis, MO, USA): TPA, dextran, histopaque, mepacrine, *p*-bromophenacyl bromide, indomethacin, hypoxanthine, xanthine, xanthine oxidase, ionophore A23187, cytochalasin B, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine, *N*-tert-butoxy-carbonyl-L-alanine *p*-nitrophenyl ester, sodium pyruvate, tetramethylbenzidine, Triton X-100, NADH, superoxide dismutase from bovine erythrocytes, nitroblue tetrazolium, *Naja naja* venom phospholipase A_2, bee venom phospholipase A_2, thromboxane B_2, leukotriene B_4 and the antibody against thromboxane B_2. [9,10-^{3}H]oleic acid was purchased from Du Pont (Itisa, Madrid, Spain). [5,6,8,9,11,12,14,15(n) −^{3}H]thromboxane B_2 and [5,6,8,9,11,12,14,15(n)-^{3}H]leukotriene

B_4 were from Amersham Iberica (Madrid, Spain). Human recombinant synovial phospholipase A_2, antibody against leukotriene B_4 and 6-([3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-2*H*-pyran-4-yl) phenoxy] methyl)-ethyl-2-quinolone (ZM 230,487) were a gift from Dr. R.M. McMillan, Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK. *Escherichia coli* strain CECT 101 was provided by Prof. F. Uruburu, Department of Microbiology, University of Valencia, Spain.

2.3. Statistics

The results are presented as means ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. The 50% inhibitory concentration (IC_{50}) and the 50% inhibitory dose (ID_{50}) were calculated by regression analysis.

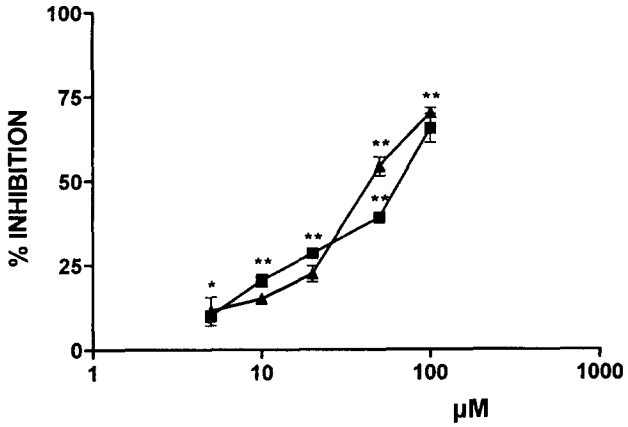


Fig. 2. Concentration-effect relationship for the inhibition by IS2 and IS3 of human recombinant synovial phospholipase A_2. (■) IS2, (▲) IS3. Points with vertical lines represent the means and S.E.M. of $n = 6$. $^*P < 0.05$, $^{**}P < 0.01$.

3. Results

3.1. Phospholipase A_2 activity

The experiments presented in Table 1 were performed to measure the activity of phospholipase A_2 exposed to 2-polyprenyl-1,4-hydroquinones. As indicated in this table, different amounts of [^{3}H]oleic acid were released from *E. coli* membranes by the direct action of phospholipase A_2 activity belonging to groups I (*Naja naja* venom), II (human recombinant synovial fluid) or III (bee venom). IS1, IS2 and IS3 interacted slightly with the phospholipid hydrolysis catalyzed by *Naja naja* venom or bee venom phospholipase A_2. Nevertheless the inhibitory effect was clearly greater on the human recombinant synovial enzyme, which was inhibited by IS2 and IS3 in a concentration-dependent manner and with the same potency (Fig. 2). Mepacrine and *p*-bromophenacyl bromide, two unspecific inhibitors of phospholipase A_2, needed higher concentrations to achieve significant enzyme inhibition.

3.2. Superoxide generation and elastase release

Table 2 summarizes the effects of 2-polyprenyl-1,4-hydroquinones on superoxide generation and elastase release by human neutrophils. The three compounds had minimal effects on both responses and failed to inhibit elastase activity present in 100 000 × *g* supernatants of sonicated human neutrophils (data not shown). IS3 was more effective than the rest of the 2-polyprenyl-1,4-hydroquinones. All the compounds failed to scavenge the superoxide anion generated by

Table 1
Effect of 2-polyprenyl-1,4-hydroquinones on three secretory phospholipase A_2 enzymes

	Naja naja venom phospholipase A_2		Human recombinant synovial phospholipase A_2		Bee venom phospholipase A_2	
	% Inhibition	IC_{50}	% Inhibition	IC_{50}	% Inhibition	IC_{50}
IS1 (100 μM)	32.8 ± 0.8 [b]	N.D.	41.1 ± 1.0 [b]	N.D.	15.8 ± 3.5 [b]	N.D.
IS2 (100 μM)	30.7 ± 2.1 [b]	N.D.	65.7 ± 4.3 [b]	48.7 μM	18.5 ± 2.9 [b]	N.D.
IS3 (100 μM)	25.5 ± 1.7 [b]	N.D.	70.2 ± 1.5 [b]	48.0 μM	21.5 ± 0.8 [b]	N.D.
Mepacrine (1 mM)	67.3 ± 4.3 [b]	0.15 mM	48.9 ± 2.6 [b]	N.D.	58.5 ± 2.4 [b]	0.5 mM
p-Bromophenacyl bromide (1 mM)	82.4 ± 1.5 [b]	0.29 mM	50.4 ± 2.9 [b]	5.4 mM	24.5 ± 3.2 [a]	3.2 mM

Results are expressed as percent inhibition (means ± S.E.M. from $n = 3$–5) and IC_{50}. N.D. = not determined. [a] $P < 0.05$, [b] $P < 0.01$. Control activity was 274.6 ± 3.2 pmol oleic acid/mg protein (*Naja naja* venom phospholipase A_2), 214.0 ± 1.2 pmol oleic acid/mg protein (human recombinant synovial phospholipase A_2) and 379.0 ± 2.0 pmol oleic acid/mg protein (bee venom phospholipase A_2).

Table 2
Effect of 2-polyprenyl-1,4-hydroquinones on superoxide anion generation and elastase release in human neutrophils

	Superoxide generation % Inhibition (10 μM)	Elastase release % Inhibition (100 μM)
IS1	11.1±5.4	23.7±4.5 [b]
IS2	23.1±1.7	24.2±4.2 [b]
IS3	27.7±6.2 [b]	40.7±4.2 [b]
Indomethacin	–	31.9±4.9 [a]
Superoxide dismutase (100 U/l)	93.4±2.3 [b]	–

Results are expressed as percent inhibition (means±S.E.M. from $n=6$). [a] $P<0.05$, [b] $P<0.01$. The highest concentration tested for inhibition of superoxide generation was 10 μM since over it 2-polyprenyl-1,4-hydroquinones interfered with the detecting molecule, nitroblue tetrazolium.

the hypoxanthine/xanthine oxidase system (data not shown).

3.3. Synthesis and release of eicosanoids by human neutrophils and platelets

In a mixed suspension of human neutrophils and platelets, reference compounds inhibited potently eicosanoid generation. The cyclo-oxygenase inhibitor, indomethacin, showed an IC_{50} value for inhibition of thromboxane B_2 synthesis and release of 3.7 nM and the 5-lipoxygenase inhibitor ZM 230,487 inhibited leukotriene B_4 synthesis and release with an IC_{50} of 59.6 nM.

Fig. 3 illustrates the concentration dependence of IS2 and IS3 on thromboxane B_2 synthesis and release. Both compounds exhibited the same potency since the calculated IC_{50} values were 3.9 μM and 3.4 μM, respectively. In contrast, IS1 did not inhibit this response in a significant way at concentrations up to 100 μM (data not shown). The three compounds inhibited the A23187-stimulated leukotriene B_4 production in human neutrophils, with marked differences in efficacy and potency (Fig. 4). IS3 was able to abolish the release of this eicosanoid at the higher concentration (100 μM) and was a potent inhibitor ($IC_{50}=7.4$ μM), followed by IS2 ($IC_{50}=23.1$ μM) and IS1 ($IC_{50}=91.1$ μM). Concentrations of 2-polyprenyl-1,4-hydroquinones that inhibited these responses did not result in cellular toxicity as determined by lactic dehydrogenase release (data not shown).

3.4. Synthesis of thromboxane B_2 by human platelet microsomes

IS1 did not inhibit thromboxane B_2 synthesis by human platelet microsomes (data not shown). In contrast, IS2 reduced this response ($IC_{50}=62.2$ μM), whereas IS3 showed higher inhibitory effects with an IC_{50} value of 29.0 μM. Indomethacin potently inhibited cyclo-oxygenase activity with an IC_{50} value of 19.4 nM.

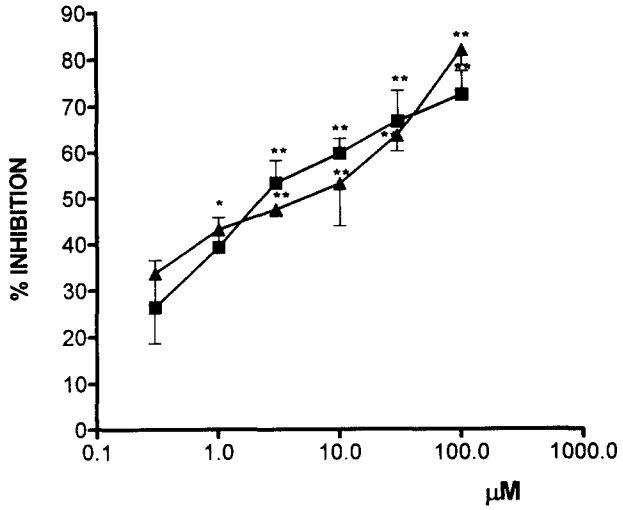


Fig. 3. Effect of IS2 and IS3 on thromboxane B_2 synthesis and release in A23187-stimulated mixed suspension of human platelets and neutrophils. (■) IS2, (▲) IS3. Points with vertical lines represent the means and S.E.M. of $n=6$. $^*P<0.05$, $^{**}P<0.01$. Non-stimulated preparations released 2.6±0.3 ng/5×10^6 platelets/ml thromboxane B_2 (means±S.E.M., $n=30$). After stimulation with ionophore A23187, control incubations released 39.2±1.9 ng/5×10^6 platelets/ml thromboxane B_2 (means±S.E.M., $n=30$).

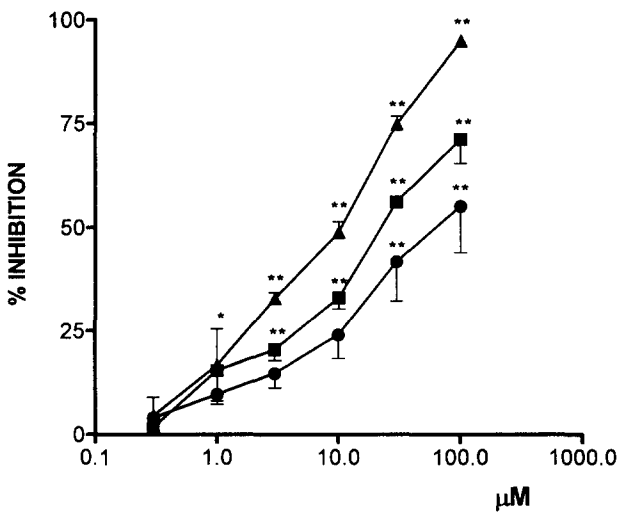


Fig. 4. Effect of IS1, IS2 and IS3 on leukotriene B_4 synthesis and release in A23187-stimulated mixed suspension of human platelets and neutrophils. (●) IS1, (■) IS2, (▲) IS3. Points with vertical lines represent the means and S.E.M. of $n=6$. $^*P<0.05$, $^{**}P<0.01$. Non-stimulated preparations released 0.47±0.01 ng/5×10^6 neutrophils/ml leukotriene B_4 (means±S.E.M., $n=30$). After stimulation with ionophore A23187, control incubations released 49.8±2.8 ng/5×10^6 neutrophils/ml leukotriene B_4 (means±S.E.M., $n=30$).

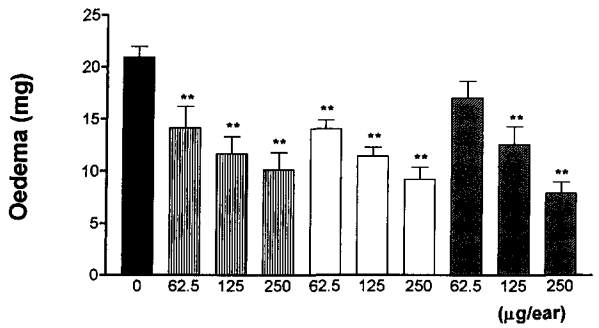


Fig. 5. Effect of IS2, IS3 and indomethacin on TPA-induced ear oedema in mice. Compounds were administered topically before the application of TPA at 2.5 μg/ear. Values are expressed as the means ± S.E.M. for 6–12 mice. $^{**}P < 0.01$. Black column: control group, vertically hatched columns: IS2, open columns: IS3, diagonally hatched columns: indomethacin.

3.5. Synthesis of leukotriene B_4 by high speed supernatants from human neutrophils

In this system, IS1 failed to modify leukotriene B_4 synthesis (data not shown). The rest of the compounds behaved as inhibitors with some differences. The effect of IS2 was weak, with percent inhibition of 27.0 ± 5.2 ($P < 0.05$) at 100 μM . IS3 and the reference inhibitor, ZM 230,487, dose-dependently reduced leukotriene B_4 synthesis with IC_{50} values of 29.4 and 6.9 μM, respectively.

3.6. Mouse ear oedema

The topical anti-inflammatory effects of IS2 and IS3 on the TPA-induced ear oedema are illustrated in Figs. 5 and 6. Both compounds dose dependently decreased the extent of swelling with a potency in the range of that of indomethacin since the approximated ID_{50} values were 212.9, 173.5 and 171.2 μg/ear for IS2, IS3 and indomethacin, respectively. IS2 and IS3 also were more effective than this reference anti-inflammatory drug at the lower doses used (62.5 and 125 μg/ear). Nevertheless IS2 only inhibited myeloperoxidase activity (an index of leukocyte migration into the inflammed ears) at the highest dose tested (250 μg/ear), whereas IS3 and indomethacin effectively inhibited this parameter at 125 and 250 μg/ear. IS1 only decreased ear oedema by 39.8 ± 6.4% ($n = 6$, $P < 0.01$), at 250 μg/ear, without any significant effect on myeloperoxidase activity.

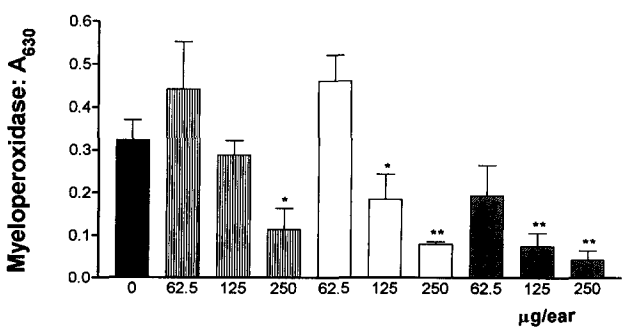


Fig. 6. Effect of IS2, IS3 and indomethacin on myeloperoxidase activity (absorbance at 630 nm) in supernatants of homogenates from TPA-treated ears. Absorbance of non-treated ears was previously subtracted from all values. Compounds were applied topically before the application of TPA at 2.5 μg/ear. Values are expressed as the means ± S.E.M. for 6–12 mice. $^{*}P < 0.05$, $^{**}P < 0.01$. Black column: control group, vertically hatched columns: IS2, open columns: IS3, diagonally hatched columns: indomethacin.

4. Discussion

Of the compounds tested, IS2 and IS3 were found to be effective inhibitors of phospholipase A_2, with selectivity for a group II secretory phospholipase A_2. This enzyme is released by different cell types, such as human synovial cells following activation by pro-inflammatory mediators. Thus, IS2 and IS3 inhibit an enzyme activity possessing a potential role in the pathogenesis of inflammatory processes (Vadas and Pruzanski, 1986; Pfeilschifter et al., 1993; Angel et al., 1993). Since there are few agents available which are known to be effective and selective phospholipase A_2 inhibitors, these marine compounds may prove useful in studies of phospholipase A_2 in other systems.

Many cells are known to generate arachidonic acid metabolites following stimulation by a variety of agents which activate phospholipase A_2 and other enzymes, via an increase in cytoplasmatic calcium. Within this scheme, phospholipase A_2 activation represents the point of regulatory control for arachidonic acid release and the subsequent eicosanoid synthesis (Walsh et al., 1983; Chang et al., 1987). In our experiments, IS2 and IS3 potently reduced eicosanoid generation by intact cells. In addition, we have confirmed the inhibitory activity of both compounds on cyclo-oxygenae and 5-lipoxygenase, showing a lower potency in these systems. Although the data presented here do not establish phospholipase A_2 as the site at which IS2 and IS3 inhibit inflammatory responses, it is evident that a decrease in the availability of arachidonic acid may participate in the observed reduction in eicosanoid synthesis and release, in addition to their inhibitory effects on the enzymes, cyclo-oxygenase and 5-lipoxygenase.

Treatment of human neutrophils with 2-polyprenyl-1,4-hydroquinones did not block the release of superoxide anion and only reduced the secretion of lysosomal elastase in human neutrophils at a high concentration (100 μM). Nevertheless the three compounds in-

fluenced the synthesis and release of leukotriene B_4 by intact human neutrophils in a concentration-dependent manner. Thus, IS3 and to a lesser extent the rest of compounds, have a specific influence on cellular responses and it seems unlikely that they act by inhibiting some step in the metabolic pathway of human neutrophils common to all the stimuli used.

TPA-stimulated superoxide anion generation in neutrophils is considered to be a protein kinase C-mediated event and phospholipase A_2 activity is not required for this cellular response (Rosenthal et al., 1992). In the system used, our results indicate that the compounds tested are not acting as classic inhibitors of protein kinase C.

IS2 and IS3 exhibited topical anti-inflammatory activity in the TPA-induced ear oedema in mice. It is interesting to note that the compounds able to inhibit group II secretory phospholipase A_2 activity also exerted anti-inflammatory effects on mouse ear oedema, an experimental model likely related with phospholipase A_2 activation (Henderson et al., 1989) and dependent on different inflammatory mediators like prostaglandin E_2 (Carlson et al., 1985) and leukotriene B_4, which would determine the cellular influx (Rao et al., 1993). In fact, the influence of these 2-polyprenyl-1,4-hydroquinones on neutrophil migration in this experimental model of inflammation is consistent with the effect shown on leukotriene B_4 synthesis and release by human neutrophils, and their topical anti-inflammatory activity on the response induced by TPA may depend on inhibition of phospholipase A_2 and the generation of mediators derived from 5-lipoxygenase and cyclo-oxygenase pathways.

On the other hand, some structural features present in these sponge metabolites can modify their pharmacological activity. Thus, inhibition of leukotriene B_4 synthesis in the presence of either high speed supernatants from human neutrophils or intact cells was enhanced by increasing the length of the prenylated chain (IS1 < IS2) as well as by the introduction of an alcoholic group in such a chain (IS2 < IS3). Similar behaviour was observed for inhibition of cyclo-oxygenase activity, while only the first feature favours the inhibition of thromboxane B_2 synthesis and release by platelets as well as the inhibition of synovial phospholipase A_2 (IS1 < IS2 = IS3). The data further indicate that the substitution of a sesquiterpenoid moiety present in avarol (Ferrándiz et al., 1994) by a prenylated chain with a length of 8 isoprene units at the C-2 of hydroquinone, adds methylene groups as well as yields a flexible structure that could facilitate the interactions with secretory phospholipase A_2.

Our results also suggest that the design of synthetic analogs related structurally to 2-polyprenyl-1,4-hydroquinones may represent a useful approach for the development of new phospholipase A_2 inhibitors.

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