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ANALOGS OF THE MARINE NATURAL PRODUCT SCALARADIAL LACKING THE α,β-UNSATURATED ALDEHYDE: EFFECTS ON HUMAN SYNOVIAL FLUID PHOSPHOLIPASE A_2 AND MACROPHAGE LIPID MEDIATOR PRODUCTION

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Abstract: The role of the α,β-unsaturated aldehyde in the inhibition of PLA_2 by scalaradial was investigated by reduction to the triol and oxidation to the diacid. The results demonstrate that the reactivity of the α,β-unsaturated aldehyde of scalaradial is not absolutely essential to its ability to inhibit PLA_2 and lipid mediator production in the intact cell but adds significantly to its inhibitory potency.

INTRODUCTION

Scalaradial (SLD) is a novel marine natural product inhibitor of extracellular phospholipase A_2 (PLA_2) described by deCarvalho and Jacobs [1,2]. The structure of scalaradial is interesting as it resembles that of a steroid but lacks the cyclopentyl D ring and has instead a cyclohexyl ring which contains an α,β-unsaturated aldehyde [3]. The α,β-unsaturated aldehyde is similar to the structure found in the open ring form of manoalide (MLD), another marine natural product inhibitor of extracellular PLA_2 [4]. MLD and SLD irreversibly inactivate bee venom PLA_2 by mechanisms which involve the α,β-unsaturated aldehyde reacting with nucleophilic groups (eg lysine residues) near the active site of the PLA_2 [5].

Inhibtion of PLA_2 by MLD and SLD results in potent antiinflammatory activities in animal models of acute inflammation [6]. SLD is a potent inhibitor of phorbol myristate acetate (PMA) induced ear edema when applied topically to the mouse ear. As predicted with other inhibitors of PLA_2, SLD does not inhibit arachidonic acid (AA) induced ear edema, suggesting that SLD is acting *in vivo* at the level of AA release. SLD has also

been demonstrated to directly inhibit AA release in the macrophage and to inhibit macrophage PLA_2 activity in cell-free homogenates [1].

The present study was undertaken to evaluate the potential intrinsic activtiy of the SLD molecule as an inhibitor of PLA_2 through modification of the α,β–unsaturated aldehyde. These studies demonstrate the SLD molecule to be a novel steroid-like structure where reduction of the α,β-unsaturated aldehyde results in loss of potency against PLA_2 and lipid mediator release but not total loss in activity as would be expected if the α,β-usaturated aldehyde was totally responsible for PLA_2 inhibitory activity.

METHODS

Materials: Scalaradial was isolated from the sponge Hyrtios erecta by Dr Valerie Paul, Univesity of Guam (Mangiloa, Guam), and was subsequently purified in our laboratories as follows : Crude scalaradial (mp 164-208°C) was first chromatographed on a Silica Prep Pak 500 HPLC column, eluting with a hexane-ethyl acetate gradient. Recrystalization was from ethanol to afford white crystals, mp 172-175°C .[7]

Preparation of Unsaturated Triol (2) and Diacid (3) of Scalaradial[8]: The known [9] triol **2** was prepared from **1** by lithium aluminum hydride reduction in tetrahydrofuran. The diacid **3** was prepared from **1** by potassium permanganate oxidation in 1:1:1 water/sulfuric acid/acetic acid.

Human Synovial Fluid Phospholipase A_2 (HSF-PLA_2) Assay: Phospholipase A_2 activity was assayed by monitoring the hydrolysis of autoclaved [^{3}H]-arachidonic acid labeled *E. coli* as previously described [10].

Eicosanoid Biosynthesis in the Murine Resident Peritoneal Macrophage: Murine resident peritoneal macrophages were obtained from CD-1 male mice as previously described and stimulated with zymosan (100 μg/ml) for 2 hr [11]. PGE_2 and LTC_4 production was quantitated using specific EIA (Cayman Chemical). Cell viability was determined by release of lactate dehydrogenase, and unless shown, was less than 10% of total LDH activity under most conditions.

Platelet-Activating Factor (PAF) Biosynthesis in the Human PMN: PAF biosynthesis was measured in purified human PMN [12]. Test compounds were preincubated with PMN for 10 min prior to stimulation with calcium ionophore A23187 (3 μM) for 15 min at 30°. [^{3}H] Acetate-labeled PAF was quantitated using a Berthold automated TLC linear analyzer or measured by radioimmunoassay (NEN).

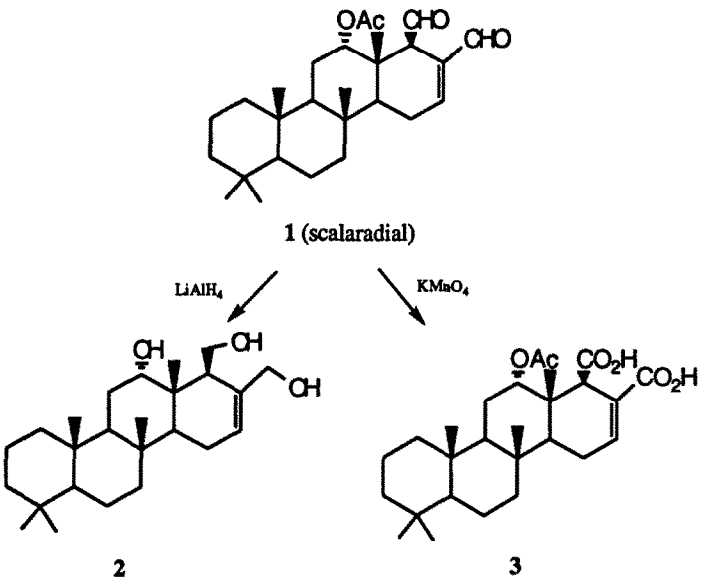

Figure 1. Synthesis of unsaturated triol (**2**) and diacid (**3**) from scalaradial (**1**) starting material.

RESULTS AND DISCUSSION

Recent SAR studies [4] and adduct NMR structural studies [5] indicate the formation of Schiff base between the aldehyde carbon of the α,β-unsaturated aldehyde and lysine residues on PLA_2 as the primary mechanism involved in irreversible inactivation of PLA_2 by the marine natural products manoalide and scalaradial. To evaluate the role of the α,β-unsaturated aldehyde in this mechanism, chemical modification was performed on scalaradial (**1**) to reduce the aldehydes to less reactive groups such as alcohols (**2**) and acids (**3**) (Fig. 1). Scalaradial is a potent inhibitor of HSF-PLA_2 (IC_{50} = 0.015 μM) (Table 1). However, reduction of the aldehyde to the unsaturated alcohol (**2**) or carboxylic acid (**3**) results in significant loss in potency (Table 1). The unsaturated triol of scalaradial (**2**) was completely inactive against HSF-PLA_2 whereas the unsaturated diacid of scalaradial (**3**) was much less active but retained a 4.8 μM IC_{50} against HSF-PLA_2 (Table 1). These observations demonstrate that the α,β-unsaturated aldehyde in the structure of scalaradial is not absolutely essential for this inhibitory activity. It is

apparent that as the aldehydes are converted to less reactive functionalities there is a concomitant decrease in activity (potency: aldehyde>carboxylic acid>alcohol).

Table 1: *In Vitro* Pharmacological Profile of Scalaradial and Analogs

Assay	IC_{50} (μM)[a,b] or % Inhibition (μM) 1	2	3
HSF-PLA_2 (hydrolysis AA-labeled *E. Coli*)	0.015[a]	21% (50)	4.8[a]
Murine Macrophage (PGE_2 production)	0.04[b]	0.3[b]	0.2[b]
Human PMN			
PAF production	1.0[b]	N.A.[c]	60% (10)
LTB_4 production	1.0[b]	N.A.[c]	42% (10)

[a] IC_{50} values determined by non-linear regression analysis of the log dose-response curve.
[b] IC_{50} values graphically determined from log dose-response curves.
[c] N.A. - not active at 50 μM.

Scalaradial is a potent inhibitor of lipid mediator generation in murine macrophages [1] and human neutrophils [13] (Table 1). The analogs of scalaradial were evaluated in both of these models. In the murine resident peritoneal macrophage both the unsaturated alcohol (**2**) and unsaturated diacid (**3**) of scalaradial retained activity against PGE_2 production in response to zymosan stimulation (Fig. 2). In this system scalaradial (IC_{50} = 0.04 μM) was five to eight fold more potent than the unsaturated diacid (**3**) or alcohol (**2**) analogs (IC_{50} = 0.2 and 0.3 μM, respectively) (Table 1 and Fig. 2). It is of interest that in this cell system the alcohol analog of scalaradial retains activity. In the human PMN, scalaradial (**1**) inhibits both LTB_4 and PAF production with an IC_{50} of 1 μM. At a concentration of 10 μM the unsaturated diacid anaolg of scalaradial (**3**) retained some (approximately 50%) activity whereas the unsaturated alcohol analog (**2**) was inactive (Table 1). These results more closely resemble the data obtained with HSF-PLA_2.

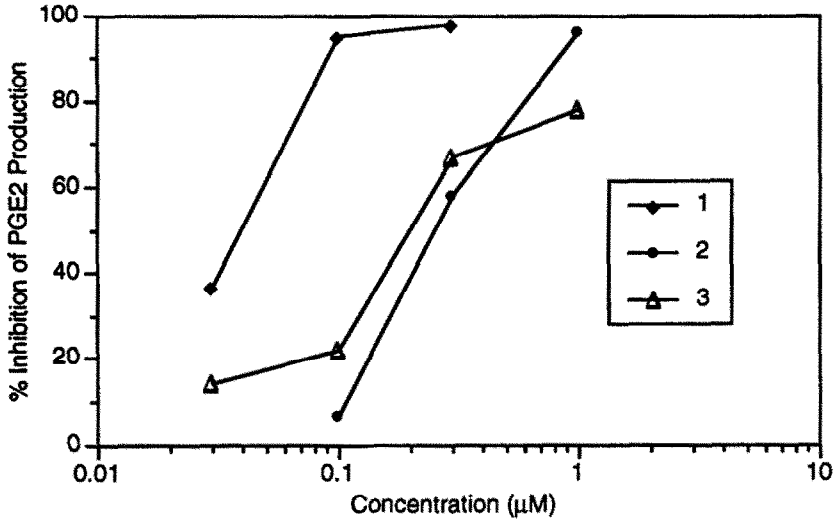

Figure 2. Inhibition of zymosan stimulated PGE_2 production in murine resident peritoneal macrophages by scalaradial (♦), the unsaturated triol (•) and diacid (Δ).

These results demonstrate that the α,β-unsaturated aldehyde in scalaradial is not an absolutely essential moiety for inhibition of PLA_2. The PLA_2 inhibitory activity of the diacid (**3**) may be due to its ability to form a Michael adduct; however, the activity of the triol (**2**) in the mouse macrophage needs further investigation. The effect(s) of the structural changes described in this report on the irreversible nature of inhibition is a key study to be performed when quantities of this novel and interesting marine natural product inhibitor of PLA_2 become available for analog synthesis. Synthetic modifications to remove the olefin from these analogs is another approach to elucidate the mechanism of scalaradial inhibition of PLA_2.

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