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Inhibition of bacterial phospholipase by methoxylated fatty acid isolated from the brown seaweed *Ishige okamurae*

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Introduction

Inflammatory processes are complex biochemical phenomena that are characterized physiologically in tissues by edema, erythema, pain, hyperthermia, and loss of function. As a biochemical manifestation in the inflammatory reaction, an elicitation of arachidonic acid metabolism involving phospholipase A_2(PLA_2), cyclooxygenase (COX), and lipoxygenase (LOX) cascades is an important factor. PLA_2 is an ubiquitous lipolytic enzyme found in all cell types including bacteria. In this work, we have isolated a methoxylated fatty acid of 7-methoxy-9-methylhexadeca-4,8-dienoic acid from the brown seaweed *Ishige okamurae* as a potent inhibitor against bacterial PLA_2 and in vivo inflammation.

Materials and methods

Isolation procedure

I. okamurae powder (1 kg) was extracted with 50 L of methanol three times, and the three extracts combined. The extract was then successively fractioned into different classes according to polarity as described in Harborne (1998). The fraction that was acidified to pH 2 with sulfuric acid and extracted three times with chloroform, contained the main PLA_2 inhibitory activity. The active substance was loaded on a silica gel column (22 g; 230-400 mesh), and eluted with each 50 mL of n-hexane. The active fraction of 7th (faint yellow; 32 mg) just following the main greenish yellow mass was dried and dissolved in 0.64 mL acetonitrile for reverse-phase HPLC.

Anti-inflammatory bioassays

BALB/c mice (810 weeks old; 2530 g body weight) were used for anti-inflammatory assays. Phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, USA) was topically applied to the inner side of the mouse ear at 0.2 mg in 10 mL acetone with an equal volume of test compounds in ethanol. Edema value was expressed as (S10S0)/S0,

where S10 is ear thickness 10 h after PMA application and S0 is ear thickness at 0 h. Erythema value was expressed as (R10R0)/R0, where R10 is ear redness 10 h after PMA application and R0 is ear redness at 0 h.

Results and discussion

Isolated compound was also tested for inhibition of mouse ear erythema induced by the same PMA application. Erythema value of PMA at 10 h reached 0.27 ± 0.01. PMA was treated with various concentrations of the isolated compound. From the dose-response curve, the IC_{50} and MIC were determined to be 4.6 and 9.1 mg mL-1, respectively (Table 1). From the Lineweaver-Burk plot, at the given concentration of inhibitor, enzyme reaction velocity decreased, i.e. 1/V increased (Figure 2)

Table 1. Inhibitory concentrations of the isolated compound and rutin as a reference against bacterial PLA_2, mouse ear edema and erythema. The inhibitory concentration was determined from the dose-response curve against inhibitor concentrations. Data of edema and erythema were derived from the mean of at least 7 mice for each concentration.

	PLA_2		Edema		Erythema	
	IC_{50} (μg mL^{-1})	MIC (μg mL^{-1})	IC_{50} (mg mL^{-1})	MIC (mg mL^{-1})	IC_{50} (mg mL^{-1})	MIC (mg mL^{-1})
Isolated compound	1.9	4.0	3.6	5.2	4.6	9.1
Rutin	32.5	66.4	11.3	76.0	8.2	32.7

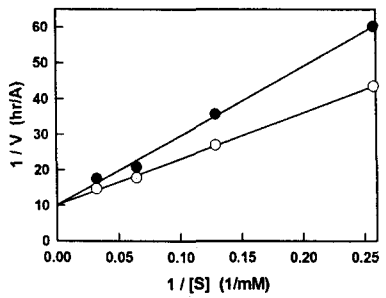

Figure 1. Lineweaver-Burk plot of competitive inhibition showing lines for no inhibitor (○) and the isolated compound (●). The compound was added to the enzyme reaction mixture with 2 mg $mL^{-1}$and reacted at 37 ℃ for 4 h.

References

Harborne J.B. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed., Chapman & Hall, London, UK, p. 302.

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