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Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 15 (1): 29 - 40

ISSN 0717 7917

www.blacpma.usach.cl

Artículo Original | Original Article

Pedalitin from *Isodon japonica*, an inactivation of soybean lipoxygenase-1

[Pedalitin de *Isodon japonica*, un inactivador de la lipo oxigenasa-1 de poroto de soya]Tae Joung Ha¹, Takahiko Isobe², Enrique Werner³, Julio Alarcon³, Isao Kubo¹ & Carlos L. Cespedes³¹Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3112, USA²Department of Chemistry, Hyogo College of Medicine, Nishinomiya-shi, Hyogo 663-8501, Japan³Biochemistry and Phytochemical-Ecology Laboratory, Department of Basic Sciences, Universidad del Bio Bio, Chillan, ChileContactos | Contacts: Isao KUBO - E-mail address: ikubo@berkeley.eduContactos | Contacts: Carlos L. CESPEDES - E-mail address: cespedes.leonardo@gmail.com

Abstract: Pedalitin, isolated from the aerial part of *Rabdosia japonica* (Labiatae), inhibited soybean lipoxygenase-1 (EC 1.13.11.12, Type I) with an IC₅₀ of 152.5 μM. The progress curves for an enzyme reaction, pedalitin inactivate the lipoxygenase-1 in a time dependent, irreversible manner, exhibiting kinetics with a kinact/KI of 59.6 ± 10 mM⁻¹min⁻¹. In the pseudoperoxidase activity, pedalitin is very slowly oxidized by the soybean lipoxygenase-1 catalyzed decomposition of lipid hydroperoxides.

Keywords: *Rabdosia japonica*, Pedalitin, Lipoxygenases inhibitor.

Resumen: Pedalitina, aislada de las partes aéreas de *Rabdosia japonica* inhibió a la lipooxigenasa-1 (EC 1.13.11.12 tipo I) con un IC₅₀ de 152.5 uM. La curva de progreso para una acción enzimática, pedalitina inactivó a la lipooxigenasa-1 de una manera dependiente del tiempo, de una manera irreversible, exhibiendo una cinética con una kinact/KI de 59.6 ± mM⁻¹min⁻¹. En la actividad pseudoperoxidasa, pedalitina es oxidada lentamente por la descomposición de la lípido hidroperóxido de la lipooxigenasa-1 de poroto de soya

Palabras clave: *Rabdosia japonica*, Pedalitina, inhibidor de Lipooxigenasas.

Recibido | Received: March 23, 2015

Aceptado | Accepted: August 27, 2015

Aceptado en versión corregida | Accepted in revised form: October 30, 2015

Publicado en línea | Published online: January 31, 2016

Declaración de intereses | Declaration of interests: This work was support in part by CONICYT-Chile Fondecyt Grant 1130242 and UC Berkeley-CONICYT-Chile. Seed funds program to IK and CLC.

Este artículo puede ser citado como / This article must be cited as: TJ Ha, T Isobe, E Werner, J Alarcon, I Kubo, CL Cespedes. 2016. Pedalitin from *Isodon japonica*, an inactivation of soybean lipoxygenase-1. *Bol Latinoam Caribe Plant Med Aromat* 15 (1): 29 – 40.

INTRODUCTION

Lipoxygenase (EC 1.13.11.12) is a non-heme ferric iron-containing enzyme, suggested to be involved in the early event of atherosclerosis by inducing plasma low-density lipoprotein (LDL) oxidation (Cornicelli & Trivedi, 1999; Kris-Etherton & Keen, 2002). Lipoxygenases are a class of enzymes that are widely distributed in both animals and plants. They catalyze the dioxygenation of unsaturated fatty acids containing at least one 1,4-*cis*, *cis*-pentadiene unit (Zheng & Brash 2010). An example of this is the peroxidation of linoleic acid by soybean lipoxygenase, to produce 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) (Scheller *et al.*, 1995). The lipoxygenase products mediate a number of biological processes not only to human implicated in a inflammatory diseases (Samuelsson *et al.*, 1987; Sigal, 1991) and cancer growth regulation (Rioux, 1998; Nie *et al.*, 1998) but also in plant involved in immunology, growth regulating, and germination (Suzuki & Matsukura, 1997; Kolomiets *et al.*, 2001). Hence, lipoxygenase inhibitors should have broad applications (Richard-Forget *et al.*, 1995).

Members of the genus *Rabdosia* grow on lands of eastern Asia, but only in China and Japan have been investigated chemically. In Japan, the leaves are used as a common household medicine for gastrointestinal disorders. In China are used as antitumor and antiphlogistic agents. The genus of *Rabdosia* (formerly known as *Isodon*) belonging to *Labiatae* (Hara, 1985) is rich source of diterpenoids, especially highly oxygenated *ent*-kaurenoids (Fujita & Node, 1984; Takeda & Otsuka, 1995). For example, a number of diterpenoids, glaucocalyxin A, B, C, D, and E, rabdosin A, and B, rabdophyllin G, acetylexidonin, and rabdosinate, were isolated from the aerial parts of *R. japonica* (Chen *et al.*, 1989; Wang *et al.*, 1990; Kim *et al.*, 1992). Previous studies on *R. japonica* led to the isolation of more than 16 diterpenoids (Li & Tian, 2001). This study report about minor diterpenoid constituents of this species was aimed at finding substances with even higher biological activities. Li and Tian, 2001, have isolated two new enmein type diterpenoids, taibaijaponicains A and B, together with six known diterpenoids, norhendosin A, rabdophyllin G, dihydroenmein, nodosin, enmein, rabdosin A, and two other known compounds, β -sitosterol and oleanolic acid (Li & Tian, 2001).

In addition to the diterpenoids, together with monoterpane phenolic acids (Gawlik-Dziki *et al.*,

2014), various phenolic compounds, quercetin (**1**), rutin (**2**), pedalitin (**3**), ursolic acid (**4**), and rosmarinic acid (**5**) were also characterized from the same source. In the previous reports, the study was emphasized on diterpenoids, and phenolic compounds were generally overlooked, especially their biological activity (Yoshimoto *et al.*, 1983; Abdel-Mageed *et al.*, 2014; Gawlik-Dziki *et al.*, 2014). It should be noted, however, the phenolic compounds isolated from *R. japonica* can be expected to possess various biological activities. For example, quercetin is known as a highly efficient hydroxyl radical scavenger, and inhibits the development of stomach, colon, and oral cancer. Therefore, the aim of this study was to reexamine various *R. japonica* biological activities which were not previously tested. Flavonoids demonstrate various pharmacological activities in vitro and in vivo including anticancer, anti-inflammatory, and antiallergic activities.

Our effort is focuses in the general effect of several compound obtained from plants in experimental models of cell growth, we are working in dissect the mechanism of lipoxygenase inhibition in which this effect is associated to mammalian lipoxygenase and tumor models. Additionally, we are looking for to define whether this model is comparative or have a similar mechanism for several lipoxygenases.

MATERIALS AND METHODS

Chemicals

Pedalitin, quercetin, rutin and ursolic acid were available as well as from extraction of *Isodon japonica* as from our previous work on *Heterotheca inuloides* (Kubo *et al.*, 1994). Rosmarinic acid was purchased from Cayman Chemical Co. (Ann Arbor, MI). Nordihydroguaiaretic acid (NDGA) and trifluoroacetic acid (TFA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Dimethyl sulfoxide (DMSO), soybean lipoxygenase-1 (EC 1.13.11.12, Type I) and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Tris was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Ethanol was purchased from Quantum Chemical Co. (Tuscola, IL). 13-Hydroperoxyoctadecadienoic acid (13-HPOD: $\lambda_{\max} = 234 \text{ nm}$, $\epsilon = 25 \text{ mM}^{-1}\text{cm}^{-1}$) was prepared enzymatically by described procedure (Gibian & Galaway, 1976) and stored in ethanol at -18°C .

Inhibition Experiments on lipoxygenase-1

These experiments were performed by measurement of the initial rate of soybean lipoxygenase-1 with a Clark type oxygen electrode (YSI 53, Yellow Springs Instrument Co., Yellow Springs, OH) at 25° C. The reaction mixture (3.0 mL) contained 80 μM linoleic acid, inhibitor and 0.1 M Tris-HCl (pH8.0). After 2 min pre-incubation, the reaction was started by addition of lipoxygenase-1 (4.35 nM). In spectrophotometric experiment, the oxygenase activity of the soybean lipoxygenase was monitored at 234 nm by Spectra MAX plus spectrophotometer (Molecular device, Sunnyvale, CA) under conditions similar to those used to follow O₂ uptake except for the concentrations of linoleic acid and lipoxygenase. A plot of the natural log of the residual enzyme activity versus pre-incubation time gave a straight line with a slope of $-k_{\text{obs}}$. The values of KI and kinact were calculated from the double reciprocal plot of the k_{obs} versus concentration of inhibitors according to the method of Kitz-Wilson (Kitz & Wilson, 1962; Parsons & Gates, 2013).

Pre-incubation Experiments

Most experiments were performed that lipoxygenase-1 (4.35 nM) and 0.5 to 10 μM for pedalitin were incubated with 0.1 M Tris-HCl (pH = 8.0) in the oxygraphic measuring chamber at 25° C. At timed intervals, reactions were started by addition of 80 μM linoleic acid. Control samples were incubated under identical conditions except for the absence of inhibitor. The oxygen consumption was monitored for the first 5min of the reaction immediately.

Measurement of Pseudoperoxidase Activity

The pseudoperoxidase activity was determined from the inhibitor dependent consumption of 13-HPOD catalyzed by the soybean lipoxygenase-1 using the variation in A_{234} as previously described (Falgueyret *et al.*, 1993). The assay mixture contained 15 μM 13-HPOD, various concentrations of inhibitors, and soybean lipoxygenase-1 (320 nM) in 0.1 M Tris-HCl, pH = 8.0.

HPLC Analysis

HPLC analysis was performed on LPG-1000 with an UV-7000 detector (Tokyo Rikakikai, Tokyo, Japan) and a 4.6mm × 250 mm i.d., 5 μm, Capcell Pack C-18 column (Shiseido, Tokyo, Japan) as previously reported with some modifications (Ha *et al.*, 2012).

The operating conditions were as follows: solvent; 30% MeCN/H₂O containing 0.1% TFA, flow rate; 0.5 mL/min, detection; UV at 254nm, injected amount; 20 μL from 3 mL assay system containing 100 μM pedalitin (1) at the same conditions of pseudoperoxidase measurement. Sampling time was chosen at 0, 30, 60, 90, and 120 min, respectively.

Data Analysis and Curve Fitting

The assay were conducted in triplicate of separate experiments. The data analysis was performed by using Sigma Plot 2000 (SPSS Inc, Chicago, IL). The inhibitory concentration leading to 50% activity loss (IC₅₀) was obtained by fitting experimental data to the logistic curve by the equation as follows (Copeland, 2000):

$$\text{Activity (\%)} = 100[1/(1-([I]/IC_{50}))]$$

The time course of inactivation of soybean lipoxygenase-1 by pedalitin was analyzed by fitting the residual activity (v/v_0) at a given pre-incubation time (t) to eq 1, where k_{obs} is the observed rate of inactivation. The rate of inactivation of the enzyme were fit to eq 2, where k_{inact} is the first-order rate constant for the conversion of the reversibly formed enzyme-inhibitor complex to irreversibly inactivated enzyme, K_1 is the dissociation constant for the complex, and $[I]$ is the concentration of pedalitin.

$$v/v_0 = \exp(-k_{\text{obs}}t) \quad (1)$$

$$1/k_{\text{obs}} = [(K_1/k_{\text{inact}})(1/[I])] + 1/k_{\text{inact}} \quad (2)$$

In the time-dependent inhibition of soybean lipoxygenase-1 by different concentrations of quercetin were fitted by nonlinear regression analysis to the integrated rate eq 3 for slow-binding inhibitors as described previously (Copeland, 2000).

$$P = v_s t + (v_i - v_s)(1 - \exp(-k_{\text{obs}}t))/k_{\text{obs}} \quad (3)$$

$$k_{\text{obs}} = k_4[I]/K_i^{\text{app}} + k_4 \quad (4)$$

Where P is the oxygen consumption concentrations at time t , v_i and v_s are the initial and final steady-state velocity, respectively, k_{obs} is the apparent first-order rate constant.

RESULTS AND DISCUSSION

Inhibition activity of soybean lipoxygenase-1

In previous work we isolated flavonoids and terpenoids from aerial parts of *R. japonica* and tested their antioxidant properties and inhibitory melanogenesis in tumor cells (Masuoka *et al.*, 2006; Satooka *et al.*, 2012).

In 2006, Sadik *et al.*, reported the inhibition of mammalian lipoxygenases by flavonoids; in our preliminary assay, the methanol extract of the aerial parts of *R. japonica* was found to inhibit the oxidation of linoleic acid by soybean lipoxygenase (EC 1.13.11.12, Type I) at 800 $\mu\text{g/mL}$; when we tested the major diterpenoids, represented by *ent*-kaurenes isolated from the same source, did not show any inhibitory activity. However the flavonoids quercetin (**1**), rutin (**2**) and pedalitin (**3**) were active. In addition, an ursane type triterpene, ursolic acid (**4**) isolated from the same plant in quantity and rosmarinic acid (**5**) (**Figure 1**), was also assayed since it was previously reported to inhibit 15-lipoxygenase (Najid *et al.* 1992). Additionally, cirsiol together pedalitin obtained synthetically showed a high selectivity inhibition activity of arachidonate 5-lipoxygenase a mammalian lipoxygenase (Yoshimoto *et al.*, 1983), also a ethanolic extract of *Simmondsia chinensis* (Jojoba) leaves showed similar effect (Abdel-Mageed *et al.*, 2014), and in similar form green coffee beans rich in 5-caffeoylquinic, 4-caffeoylquinic, 3-feruoylquinic and 5-feruoylquinic acids were potent inhibitors of lipoxygenase (Gawlik-Dziki *et al.*, 2014).

The inhibition activity of soybean lipoxygenase-1 was measured by two methods for comparison, because this enzyme seems to be sensitive to assay conditions. Soybean lipoxygenase-1 is known to catalyze the reaction of oxygen with unsaturated fatty acids containing 1,4-*cis,cis*-diene units to yield 1,3-*cis-trans*-diene-5-hydroperoxides. Hence, the enzyme assay was usually performed using a Clark type oxygen electrode at 25° C. And also this enzyme is catalyze the dioxygenation of (*Z*, *Z*)-diene moiety of linoleic acid. In plants, the primary dioxygenation product is 13*S* linoleic acid hydroperoxide (13-HPOD) (Grechkin, 1998). Hence, UV spectrophotometer to detect the increase at 234 nm associated with the (*Z*, *E*)-conjugated double bonds newly formed in the product but not the substrate. As a result, in the inhibition of soybean lipoxygenase-1 by pedalitin (**3**), the formation of conjugated diene was recorded spectrophotometrically at 234 nm, and oxygraphic measurement of oxygen consumption yielded similar curves as shown in **Figure 2**. This inhibition pattern suggests that pedalitin (**3**) inactivates soybean lipoxygenase-1 by a slow binding mechanism and irreversibly. The effect of pedalitin on the rate of enzyme inactivation can be readily observed at low concentrations (e.g. 10 μM) where a 78% decrease in product accumulation. At higher pedalitin concentrations, the rate of the reaction was also inhibited, together with the increase in enzyme inactivation (**Figure 2A**).

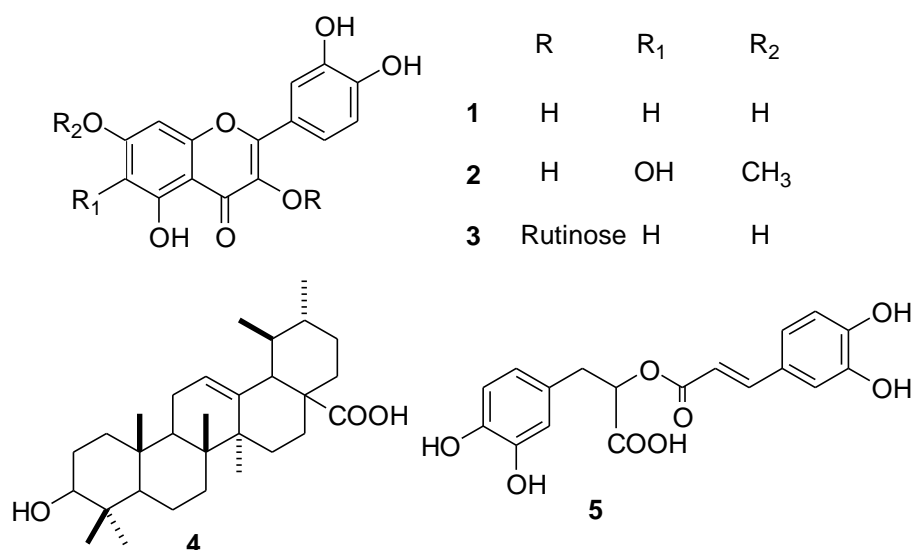


Figure 1

Chemical structures of compounds **1-5** isolated from *R. japonica*.

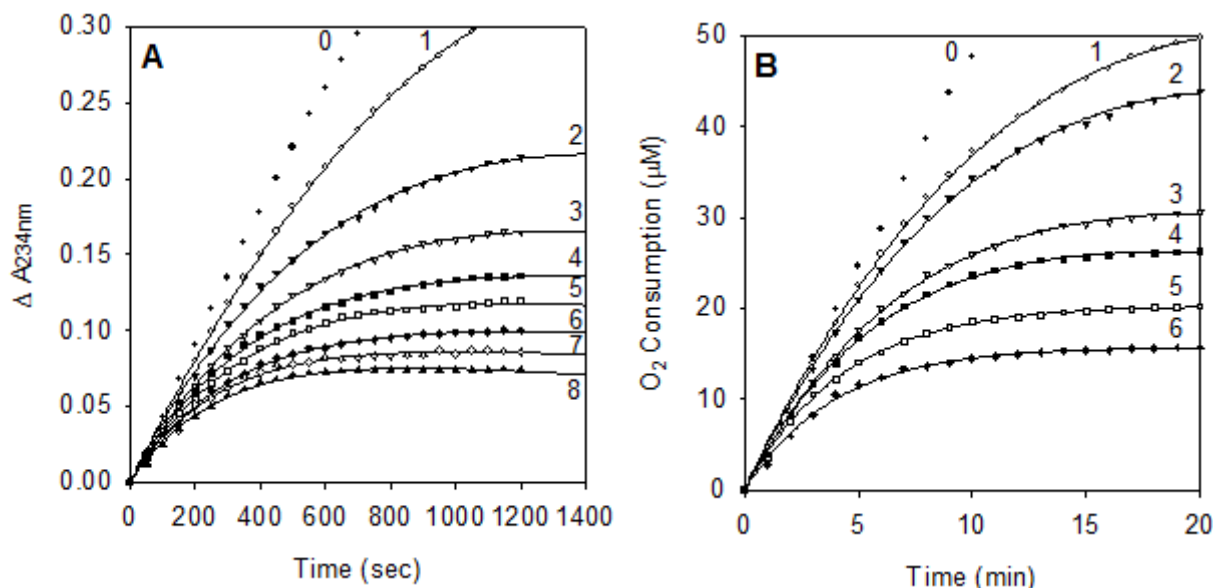


Figure 2

Progress curves for the inactivation of soybean lipoxygenase-1 by pedalitin by using a spectrophotometer (A) and a Clark-type oxygen electrode (B). A: 40 μM linoleic acid, 1.3 nM SLO-1 and concentrations of pedalitin for curves 0-8 were 0, 5, 10, 15, 20, 25, 30, 35, and 40 μM . B: 80 μM linoleic acid, 6.54 nM SLO-1 and concentrations of pedalitin for curves 0-6 were 0, 5, 10, 20, 40, 60, and 80 μM .

Pedalitin showed a dose-dependent oxygen consumption inhibitory effect on this oxidation as shown in **Figure 3**. As pedalitin increase, oxygen consumption was rapidly decreased. The inhibitory concentration leading to 50% activity lost (IC_{50}) was estimated to be 152.5 μM . And also the IC_{50} value of quercetin, rutin, ursolic acid, rosmarinic acid, and NDGA obtained are listed in **Table 1**. The enzyme pre-incubation for 5 minutes with pedalitin, resulted in about a 40-fold decrease in IC_{50} (3.7 μM).

Possible mechanism of lipoxygenase inhibition by pedalitin

Subsequently, pedalitin inhibited soybean lipoxygenase-1 in a time-dependent inactivation fashion as shown in **Figure 4A** to suggest that this inhibit the enzyme in an irreversible manner, the kinetic parameters for the irreversible inhibitions, K_i and k_{inact} were estimated from the respective double reciprocal plot $1/k_{\text{obs}}$ as a function of $1/[\text{pedalitin}]$ according to the method of Kitz and Wilson (Kitz & Wilson, 1962; Parsons & Gates, 2013) for kinetic analysis of covalent enzyme inactivation, as exemplified by **Figure 4B**, and are listed in **Table 2**. These values were found to be equal to 5.37 μM and 0.32 min^{-1} , respectively.

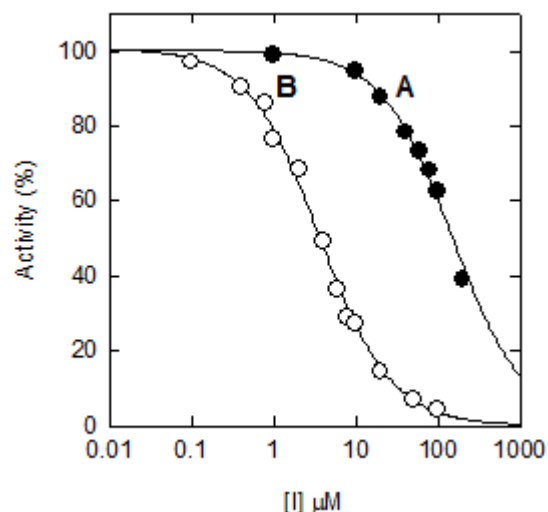


Figure 3

Effects of pedalitin on the activity of soybean lipoxygenase-1 for the catalysis of linoleic acid at 25° C. A: pedalitin was mixed with 80 μM linoleic acid in 0.1m tris-hcl buffer (ph8.0) at 25° C and the reactions were started by addition of soybean lipoxygenase-1 (6.54 nM). B: pedalitin was pre-incubated for 5 min with enzyme and the reactions were started by addition of 80 μM linoleic acid at same conditions

Compound	IC ₅₀ (μM)
1	4.8 ± 1.9
2	152 ± 37
3	379 ± 94
4	> 100
5	746 ± 140
NDGA	1.5 ± 0.3

On the other hand, progress curves of quercetin (**1**) display in **Figure 5**, show soybean lipoxygenase-1 inhibition by reversible slow-binding mechanism. The k_{obs} values for the quercetin inhibition of lipoxygenase-1 at different concentrations of quercetin were determined by fitting data to the slow-binding equation (eq 3). The k_{obs} values were plotted as a function of quercetin concentration. The results indicated that quercetin inhibits soybean lipoxygenase-1 by simple reversible slow binding. This was evidenced by the observation that the k_{obs} values exhibited a linear dependence on the inhibitor concentration as shown in **Figure 5** inset. Thus, analysis of data according to eq 3 and 4 yielded the following values: $k_3 = 0.0325 \pm 0.01 \mu\text{M}^{-1} \text{min}^{-1}$, $k_4 = 0.0345 \pm 0.02 \text{min}^{-1}$, $K_1^{app} = 1.06 \pm 0.2 \mu\text{M}$.

Effect on pseudoperoxidase activity of soybean lipoxygenase

Many of lipoxygenase inhibitors such as flavonoids, catechols, benzofuranol, and aminopyrazolines have been found (Yoshimoto *et al.*, 1983; Summer *et al.*,

1987; Jackson *et al.*, 1988) and there were act by a chelating the iron of the active site of enzyme (Clapp *et al.*, 1985; Nelson, 1988) and/or by reducing the ferric form of the enzyme to an inactive ferrous form (Kemal *et al.*, 1987; Mansuy *et al.*, 1988). Indirect evidence that N-alkylhydroxylamines, benzo-furanol and NDGA (norhydroguarectic acid) inhibitors also reduce the catalytically active ferric enzyme to the catalytically inactive ferrous enzyme lipoxygenase has been obtained from the observations that the inhibitors stimulate the lipoxygenase-catalyzed degradation of hydroperoxides (pseudoperoxidase activity). Figure 6 demonstrates a fast decrease in A234 with the NDGA addition in this pseudoperoxidase assay. Soybean lipoxygenase-1 also exhibited a pseudoperoxidase activity when is incubated with 15 μM 13-HPOD and 10 μM of quercetin (**1**) also showing a time-dependent decrease in the hydroperoxide levels. Pedalitin (**3**), however, did not cause any significant stimulation of the pseudoperoxidase activity, because observed changes in A234 represent less than 5% of that observed with NDGA.

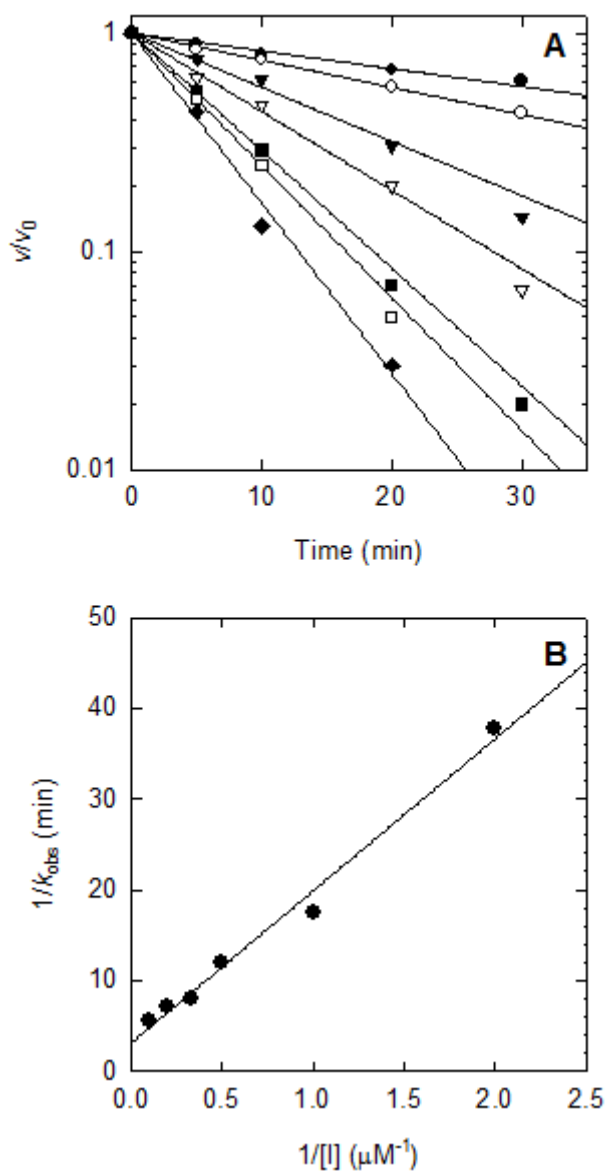


Figure 4

Time course of the inactivation of soybean lipoxygenase-1 by pedalitin. The enzyme was preincubated at 25°C with pedalitin in 0.1 M Tris-HCl buffer (pH = 8.0) and the reactions were started by addition of 80 μM linoleic acid. A: Concentrations of pedalitin were as follows: (\bullet) 0 μM ; (\circ) 0.5 μM ; (\blacktriangledown) 1 μM ; (∇) 2 μM ; (\blacksquare) 3 μM ; (\square) 5 μM ; (\blacklozenge) 10 μM . B: Double reciprocal plot of the apparent rate of inactivation as a function of pedalitin concentration.

Table 2
Kinetic parameters of soybean lipoxygenase-1 by quercetin (1) and pedalitin (2)

Compds	k_3 ($\mu\text{M}^{-1}\text{min}^{-1}$)	k_4 (min^{-1})	K_i^{app} (μM)	k_{inact} (min^{-1})	K_I (μM)	k_{inact}/K_I ($\text{mM}^{-1}\text{min}^{-1}$)
1	0.0325 \pm 0.01	0.0345 \pm 0.02	1.06 \pm 0.2			
2				0.32 \pm 0.07	5.37 \pm 0.5	59.6 \pm 10

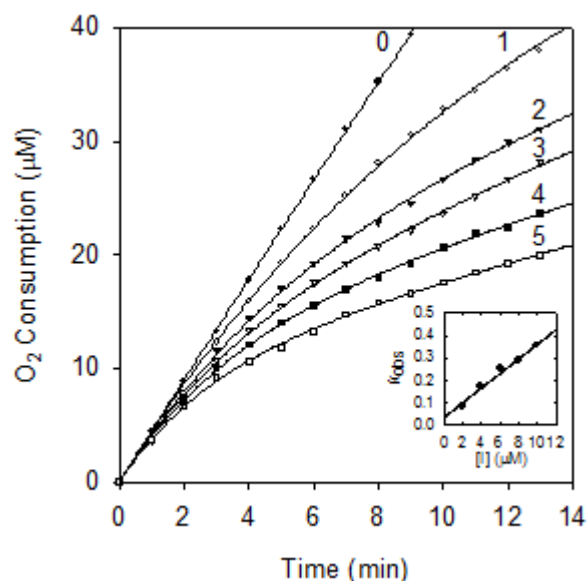


Figure 5

Time-dependent inhibition of soybean lipoxygenase-1 in the presence of quercetin. Conditions were as follows: 80 μM linoleic acid, concentrations of quercetin for curves 0-5 were 0, 2, 4, 6, 8, and 10 μM . (Inset) Dependence of the values for k_{obs} on the concentration of quercetin.

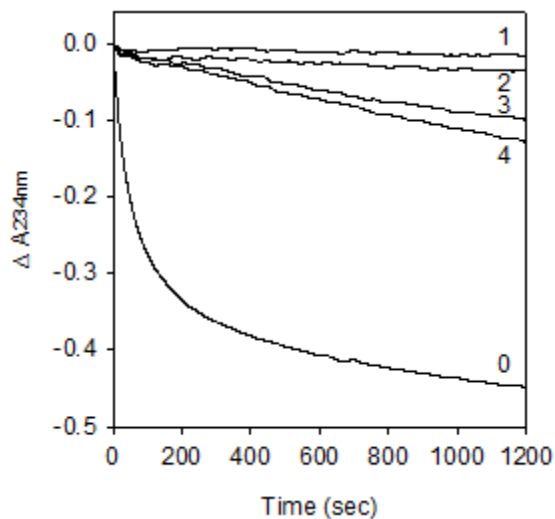


Figure 6

Effects of pedalitin, quercetin and NDGA on soybean lipoxygenase-1 catalyzed consumption of 13-HPOD. The conditions of incubation were as described for pseudoperoxidase assay using 10 μM pedalitin (1), 30 μM pedalitin (2), 10 μM quercetin (3), 30 μM quercetin (4), and 10 μM NDGA (0) in the presence of enzyme (320 nM).

Inhibitor modification (Pedalitin structure changes) during time course inhibition

Oxidation of quercetin (**1**) and pedalitin (**3**) during pseudoperoxidase activity were studied by repetitive UV-visible spectrum during 2 hrs. The UV-Vis spectra showed the formation of a new product ($\lambda_{\text{max}} = 321 \text{ nm}$) indicating the formation of an intermolecular complex between LOX and quercetin. To explain the results obtained, Pinto and Macias (2005) concluded that in the presence of hydroperoxylinoleic acid, lipoxygenase produces a quinoid product as a result of the enzymatic cooxidation (in the presence of linoleic acid) of quercetin. Ha *et al.* (2010) indicate that quercetin might first be oxidized to the corresponding o-quinone and then isomerized to p-quinone. The

pedalitin (**3**) is very slowly oxidation by soybean lipoxygenase-1 in the presence 13-HPOD, as followed by observing slowly changes in the UV-visible spectrum (**Figure 7**). Maximal spectral changes in the oxidation medium were observed at 280 and 359 nm (decreases in absorbance) and at 310 and 450 nm (increases in absorbance), and the presence of three isosbestic points at 288, 330 and 415 nm, respectively. In the contrast to the pedalitin, the quercetin (**1**) was quickly oxidized by soybean lipoxygenase-1 in the presence 13-HPOD (**Figure 7** inset). The decrease in the absorbance of the band centered at 270 and 385 nm, the concomitant increase in the absorbance at 330 nm, and the presence of two isosbestic points at 286 and 357 nm, respectively.

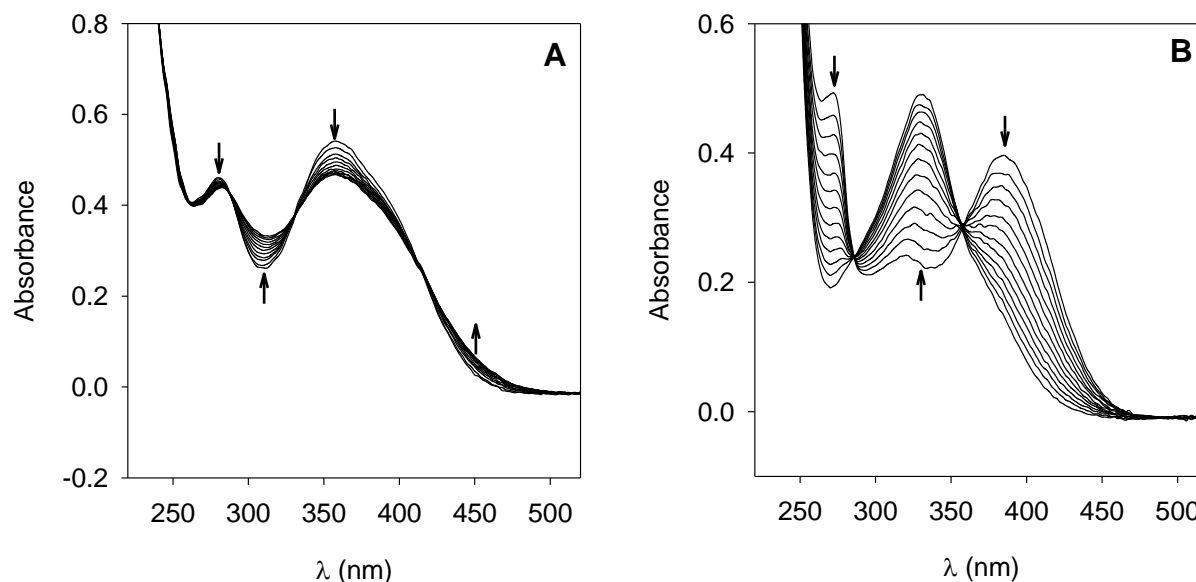


Figure 7

Repetitive scans of 30 μM pedalitin (A) and 30 μM quercetin (B) by soybean lipoxygenase-1 in the presence of 13-HPOD. The reaction medium contained 0.1M Tris-HCl buffer, pH = 8.0, 15 μM 13-HPOD, and lipoxygenase-1 (320 nM). A: The elapsed time between scans was 10 min during 2 hrs. B: The elapsed time between scans was 4 min during 48 min.

Reverse phase high pressure liquid chromatography (RP- HPLC) analysis was performed using reaction mixture with pedalitin (**3**) (100 μM) and lipoxygenase-1 under the same conditions of pseudoperoxidase experiments (**Figure 8**). It shows that peak **3** (12.2 min) which came from pedalitin, was slowly decreased and peak **a** (6.4 min) was

mainly increased.

A wide number of biological activities of flavonoids have been reported, which overall are believed to be benefic to human health like the antioxidant capacity and the inhibitory effects on prooxidant enzymes (Sadik *et al.*, 2003). Our previous paper reported that the catechol moiety is

one of the essential head portions to elicit the lipoxygenase inhibitory activity (Ha *et al.*, 2004). For example, luteolin (7), a common flavone bearing a catechol moiety in B-ring, is known to have this specific activity like a slow binding inhibitor, not oxidized despite the addition of hydroperoxides and remained without any change (Ha *et al.*, 2012). However, in our previous pseudoperoxidase assay, quercetin was slowly oxidized by hydroperoxides to a rather stable intermediate, 2-(3,4-dihydroxybenzoyl)-

2,4,6-trihydroxybenzofuran-3(2H)-one, and this oxidized intermediate still inhibited the enzymatic oxidation (Ha *et al.*, 2010).

Concluding remarks

The results obtained allow us to infer that pedalitin is very slowly oxidized by the soybean lipoxygenase-1 during a time dependent reaction inactivating the lipoxygenase-1 under irreversible way.

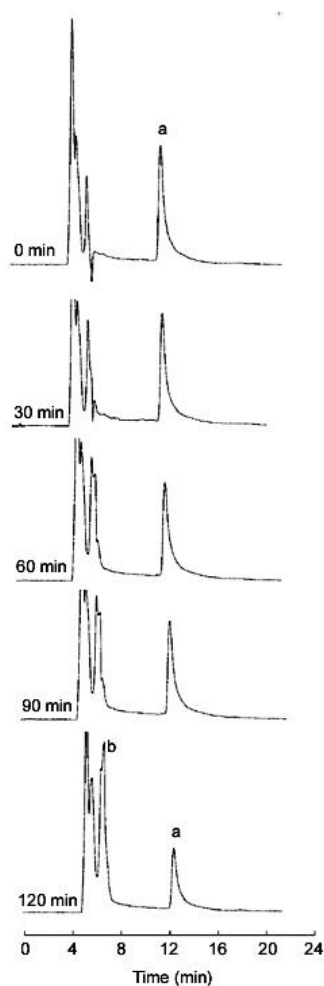


Figure 8

HPLC analysis of the reaction medium with 100 μ M of pedalitin and soybean lipoxygenase-1. Sampling time was chosen at 0, 30, 60, 90, and 120 min, respectively. The HPLC operating conditions was as follow: column; Capcell Pak C-18, solvent; 30% MeCN/H₂O containing 0.1% TFA, flow rate; 0.5 mL/min, detection; UV at 254 nm, injected amount; 20 μ L from 3 mL assay system.

ACKNOWLEDGEMENTS

This work was supported in part by CONICYT-Chile Fondecyt Grant 1130242 and UC Berkeley-CONICYT-Chile Seed funds program to IK and CLC.

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