Evaluation of Lipoxygenase Inhibitory Activity of Anacardic Acids

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6-Alkylsalicylic acids inhibit the linoleic acid peroxidation catalyzed by soybean lipoxygenase-1 (EC 1.13.11.12, type 1) competitively and without pro-oxidant effects. This activity is largely dependent on the nature of their alkyl side chains. Inhibitory activities of anacardic acids, *viz*. 6-pentadec(en)ylsalicylic acids, isolated from the cashew *Anacardium occidentale*, were initially used for comparison because their aromatic head portions are the same. Consequently, the data should be interpreted to mean that changes in the hydrophobic side chain tail portions of the molecules evaluated correlate with the specific activity determined.

Key words: Lipoxygenase, Anacardic Acids, Inhibitory Activity, Hydrophobicity

Introduction

Lipid peroxidation catalyzed by lipoxygenases (EC 1.13.11.12) is known to be one of the major factors causing deterioration of foods during their processing and storage since it leads to the development of unpleasant rancid or off-flavours as well as potentially toxic end-products (Grechkin, 1998). Octyl gallate (**1**) has previously been reported to inhibit the peroxidation of linoleic acid catalyzed by soybean lipoxygenase-1 in a cell-free experiment (Ha *et al*., 2004). As far as food protection is concerned, octyl gallate can be considered as a superior lipoxygenase inhibitor since this gallate is one of the only three alkyl gallates currently permitted for use as antioxidant additives in food. In addition, lipoxygenases have been postulated to be involved in the early stages of atherosclerosis by inducing plasma low-density lipoprotein (LDL) oxidation (Kris-Etherton and Keen, 2002). Lipoxygenase inhibitors have also been suggested to be potential cancer chemopreventives (Steele *et al*., 1999). However, after consumption, alkyl gallates are hydrolyzed to gallic acid and the corresponding alcohols, neither of which inhibit lipoxygenases. From this it is thus clear that alkyl gallates are unlikely to act as lipoxygenase inhibitors in human living systems. However, there is a positive consequence to this finding, *viz*. the discovery obtained about the inter-relationship between the head and tail moieties of alkyl gallates and their activity is useful not only to strive for activity optimization through planned synthetic strategies but also to then be able to select appropriate natural products for further evaluation. Thus 6-[8(*Z*),11(*Z*),14-pentadecatrienyl]salicylic acid (**2**), 6-[8(*Z*),11(*Z*)-pentadecadienyl]salicylic acid (**3**), and 6-[8(*Z*)-pentadecenyl]salicylic acid (**4**), isolated from the cashew *Anacardium occidentale* L. (Anacardiaceae) (Kubo *et al*., 1986) are a case in point. These salicylic acid derivatives having a non-isoprenoid C_{15}-alkenyl side chain at C-6 of the salicylic acid ring will be referred to as anacardic acid ($C_{15:3}$), anacardic acid ($C_{15:2}$) and anacardic acid ($C_{15:1}$) for simplicity, respectively (reviewed by Tyman, 1979). In addition, **4** and 6-pentadecanylsalicylic acid, referred to as **5**, were previously characterized as prostaglandin synthetase inhibitors and were isolated in acceptable quantities from the African medicinal plant *Ozoroa mucronata* (Anacardiaceae) (Kubo *et al*., 1987). The anacardic acids **4** and **5** isolated from another African *Ozoroa* species, *O. insignis*, were also reported to be cytotoxic principles against six human cancer cell lines (Rea *et al*., 2003). Among these natural lipoxygenase inhibitors, anacardic acids **4** and **5** were found to be the most appropriate lipoxygenase inhibitors without being oxidized.

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Materials and Methods

Chemicals

Anacardic acids **2**, **3**, **4**, **5** and cardanol ($C_{15:1}$, $C_{15:0}$) (Kubo *et al.*, 1986), and anacardic acid ($C_{15:1}$, *E*-isomer) (**10**) (Green and Tocoli, 2002a) as well as the four unnatural anacardic acids 6-[2′-(2″,5″-dihydroxyphenyl)ethyl]salicylic acid (**13**), 6-[2′-(3″,4″-dihydroxyphenyl)ethyl]salicylic acid (**14**), 6-[2′-(2″,4″-dihydroxyphenyl)ethyl]salicylic acid (**15**) and 6-[2′-(2″,4″,5″-trihydroxyphenyl)ethyl]salicylic acid (**16**) (Green and Tocoli, 2002a, 2002b) were available from our previous work. Soybean lipoxygenase-1 (EC 1.13.11.12, type 1), dimethyl sulfoxide (DMSO), salicylic acid, Tween-20 and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (13*S*)-Hydroperoxy(9*Z*,11*E*)-octadecadienoic acid (13-HPOD, **6**; λ_{max} = 234 nm, ε = 25 mM^{-1} cm^{-1}) was prepared enzymatically by a described procedure (Gibian and Galaway, 1976) and stored in ethanol at −18 °C.

Inhibition experiments on lipoxygenase-1

The experiments were performed by measurement of the initial rate of soybean lipoxygenase-1 with a Spectra MAX plus spectrophotometer (Molecular Device, Sunnyvale, CA, USA) at 25 °C. The enzyme assay was performed as previously reported (Rickert and Klinman, 1999) with slight modifications. In general, the reaction mixture consisted of 2.97 mL of 0.1 M sodium borate buffer (pH 9.0), 15 μL of 3 mM stock solution of linoleic acid and 5 μL of an ethanolic inhibitor solution. Then, 10 μL of 0.1 M sodium borate buffer solution (pH 9.0) of lipoxygenase (0.52 μM) were added. The resultant solution was mixed well and the linear increase of the absorbance at 234 nm for 5 min, which expresses the formation of conjugated diene hydroperoxide (13-HPOD, ε = 25000 M^{-1} cm^{-1}), was measured continuously. The lag period shown on lipoxygenase reaction (Ruddat *et al.*, 2003) was excluded for the determination of initial rates. The stock solution of linoleic acid was prepared with Tween-20 and sodium borate buffer at pH 9.0, and then the total Tween-20 content in the final assay was adjusted below 0.01%. For determining the reversible inhibition manner, the enzyme concentration was changed to 0.094, 0.141, 0.188, 0.235, and 0.282 μg/mL with a constant substrate concentration (30 μM). Three concentrations (10, 15 and 30 μM) of linoleic acid were selected for Dixon plots.

Pre-incubation experiments

Pre-incubation experiments of lipoxygenase-1 (1.74 nM) with 2–10 μM anacardic acid ($C_{15:0}$) were performed in 0.1 M sodium borate buffer (pH 9.0) at 25 °C. At timed intervals, reactions were started by addition of 30 μM linoleic acid. Control samples were incubated under identical conditions except for the absence of inhibitor. The increase in the absorbance at 234 nm was monitored for 5 min.

Data analysis and curve fitting

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

$$\text{activity (\%)} = 100/\{1 + ([I]/IC_{50})\}.$$

DPPH radical scavenging assay

First, 1 mL of 100 mM acetate buffer (pH 5.5), 1.87 mL of ethanol and 0.1 mL of ethanolic solution of 3 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) were put into a test tube. Then, 0.03 mL of the sample solution (dissolved in DMSO) was added to the tube and incubated at 25 °C for 20 min. The absorbance at 517 nm (DPPH, ε = 8320 M^{-1} cm^{-1}) was recorded. As control, 0.03 mL of DMSO was added to the tube. From the decrease of the absorbance, the scavenging activity was calculated and expressed as scavenged DPPH molecules per a test molecule.

ESI mass spectrometry

Electrospray ionization (ESI) mass spectra were obtained on a Finnegan LCQ-Deca XP (Thermo Fischer Scientific, Inc, Waltham, MA, USA) mass spectrometer. The mass spectrometer was equipped with an ESI needle, and the ion spray voltage was set at 3 kV with nitrogen as the sheath gas. Mass spectra were acquired at a mass range of *m/z* 50–2000 in the negative ion mode to detect the deprotonated molecule. The complex solution was directly infused into the mass spectrometer at a flow rate of 3 μL/min. For all ESI measurements of the hydrophobic complex ion, a non-aqueous solution was prepared: 2-propanol/hexane/ethyl acetate in the ratio of 2:1:1 with 0.05% acetic acid. A hexane solution of anacardic acid and an ethyl

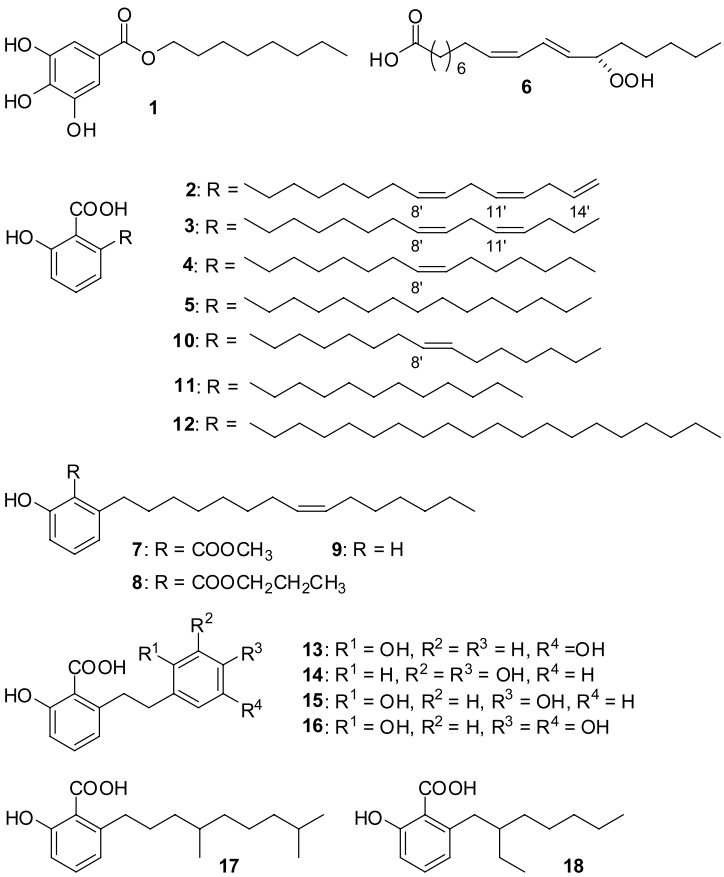

Fig. 1. Chemical structures of anacardic acids and related compounds.

acetate solution of ferric acetyl acetonate in a ratio of 1:2, respectively, were added to the solution and adjusted to a final concentration of 10 pmol/μL.

Results

Soybean lipoxygenase-1 (EC 1.13.11.12, type 1) is known to catalyze the dioxygenation of the (1*Z*,4*Z*)-diene moiety of linoleic acid. In plants, the primary dioxygenation product is (13*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid (13-HPOD, **6**) (Fig. 1) (Grechkin, 1998). The inhibition activity of soybean lipoxygenase-1 was measured by using a UV spectrophotometer to detect the increase in the absorption at 234 nm associated with the (1*Z*,3*E*)-conjugated double bonds newly formed in the product but absent in the substrate. In many previous reports, the data were obtained at pH 9 because soybean lipoxygenase-1 is reported to have its optimum activity at this pH value (Axelrod *et al.*, 1981).

Recently, **4** was described to inhibit the soybean lipoxygenase-1-catalyzed peroxidation of linoleic acid with an IC_{50} value of 6.8 μM without being oxidized (Ha and Kubo, 2005). The inhibitory activity did not increase when the enzyme was preincubated for 10 min with **4** before being added to linoleic acid. The inhibition is a slow and reversible reaction without residual enzyme activity, and the kinetics indicate that **4** is a competitive inhibitor with the inhibition constant K_I = 2.8 μM (Ha and Kubo, 2005). Pure salicylic acid did not show this inhibitory activity up to 200 μM. In addition, neither the methyl ester **7** nor the propyl ester **8** of **4** noticeably inhibited this oxidation up to 200 μM indicating that both the an alk(en)yl side chain tail as well as the salicylic acid head moiety are an essential combination to elicit the inhibitory activ-

ity supporting the previous report on the tyrosinase inhibitory activity (Nomura and Fujihara, 1994). Additionally it was also noted that anacardic acids demonstrated a high selectivity toward transition metal ions, especially iron and copper (Nagabhushana *et al.*, 1995). It therefore appears that, on the one hand, anacardic acids can be expected to chelate iron in the lipoxygenase while, on the other hand however, the pentadecenyl side chain alone is not able to elicit the activity since cardanol ($C_{15:1}$) (**9**), which possesses the same side chain as **4**, acted neither as a substrate nor as an inhibitor. It has also been suggested that the hydrophobic C_{15}-alkenyl side chain may act as an anchor in the low dielectric interior of proteins, while the hydrophilic head portion of anacardic acids first chelates the iron in the active site like a "hook" in attaching itself to this portion of the enzyme molecule after, which the hydrophobic tail portion slowly begins interacting with the *C*-terminal domain where the iron is located (Prigge *et al.*, 2003). Subsequently, based on the inhibition kinetic study, the two congeners, *viz.* **2** and **3**, were found to act as substrates at lower concentrations (<40 μM), because both possess a (1*Z*,4*Z*)-pentadiene system in their C_{15}-alkenyl side chain (Ha and Kubo, 2005). If these anacardic acids, represented as RH, are oxidized as substrates to the corresponding conjugated hydroperoxides (ROO·), these in turn would create the potential regeneration pathway, *viz.* ROO· + RH → ROOH + R· which could be followed by R· + O_2 → ROO·. Due to their free radical nature, anacardic acid hydroperoxides can be quite active by themselves. Hence, the (1*Z*,4*Z*)-pentadiene moiety should be avoided in the hydrophobic tail portion.

Subsequently, **5** was also found to inhibit the linoleic acid peroxidation catalyzed by soybean lipoxygenase-1. Under the conditions employed in the present investigation, this oxidation of linoleic acid follows Michaelis-Menten kinetics, and the kinetic parameters for this oxidase, as obtained from a Dixon plot, shows that K_m is equal to 13.1 μM and V_m is equal to 4.7 μM/min. Anacardic acid **5** demonstrated a dose-dependent inhibitory effect on this oxidation as shown in Fig. 2. The inhibition was not affected by the preincubation experiment, *vide infra*, for 10 min, in the presence of **5** but in the absence of linoleic acid. The IC_{50} value in this case was estimated to be 14.3 μM, which is about 2-fold less potent compared to that of **4**. As the concentration of **5** increased, the enzyme activity

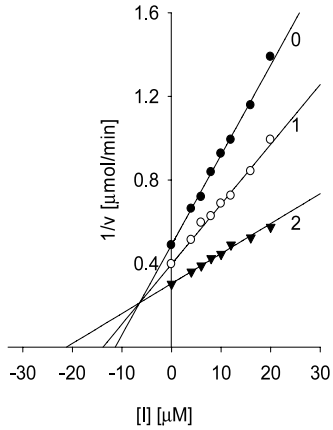

Fig. 2. Dixon plots of 13-HPOD generation by soybean lipoxygenase-1 in the presence of anacardic acid ($C_{15:0}$) in borate buffer (pH 9.0) at 25 °C. Concentrations of substrates for curves 0, 1 and 2 were 10, 15, and 30 μM, respectively.

Table I. Kinetics and inhibition constants of anacardic acids.

	Anacardic acids tested		
	$C_{15:0}$	$C_{15:1}$	$C_{12:0}$
IC_{50} [μM]	14.3	6.8	20.7
K_m [μM]	13.1	11.7	13.2
V_m [μmol/min]	4.7	4.8	4.9
Inhibition	reversible	reversible	reversible
Inhibition type	competitive	competitive	competitive
K_I [μM]	6.4	2.8	9.6

rapidly decreased but was not completely suppressed. The kinetic and inhibition constants obtained are listed in Table I where the similarities in inhibition constants of **5** and **4** should be noted. The inhibition kinetics as analyzed by Dixon plots demonstrated that **5** is a competitive inhibitor since increasing substrate concentrations resulted in a family of lines with a common intercept above the [I] axis but with different slopes. The equilibrium constant for inhibitor binding, K_I = 6.4 μM, was obtained from the −[I] value at the intersection of the three lines. The inhibition was shown again to be a slow and reversible process in which the substrate was not oxidized. It thus appears that the double bond in the side chain is not essential in eliciting the specific activity but is most certainly associated with increasing it. The *E*-isomer of anacardic acid ($C_{15:1}$) (**10**) was previously synthesized,

by the *trans* elimination of a hydroxy phenylphosphine oxide precursor with sodium hydride (Green and Tocoli, 2002a), and was also tested for comparison. The IC_{50} value estimated was nearly identical with that found for the *Z*-isomer, indicating that the stereochemistry of the C-8 double bond is not directly associated with this particular activity.

Among the naturally occurring anacardic acids, **2** was reported to exhibit the most potent antibacterial activity against *S. aureus* (Muroi and Kubo, 1994). Their antibacterial activity was found to correlate well with their partition coefficient (log *P*) as calculated by Chem Draw Pro version 4. 5. In case of their lipoxygenase inhibitory activity, anacardic acids possessing similar log *P* values may exhibit similar activity. For example, based on similarities of the log *P* values of 6.62 for **2** and 6.23 for anacardic acid ($C_{12:0}$) (**11**), it was expected that both would show similar activities in this study. Hence, the effect of **11** on the lipoxygenase-catalyzed peroxidation of linoleic acid was examined for comparison and indeed the linoleic acid peroxidation was catalyzed by soybean lipoxygenase-1 in a dose-dependent manner (Fig. 3). As the concentration of **11** increased, the enzyme activity rapidly decreased but was not completely suppressed. The IC_{50} value was estimated to be 20.7 μM, which is slightly less potent compared to that of **5**. The kinetic and inhibition constants obtained are also listed in Table I. The inhibition kinetics analyzed by Dixon plots confirmed that **11** is a competitive inhibitor. The lipoxygenase inhibitory activity seems to increase in proportion to the length of the side chain. It should be noted, however, that both **4** and **5** were previously reported to inhibit potato lipoxygenase (Grazzini *et al.*, 1991), but that the IC_{50} valueof anacardic acid ($C_{20:0}$) (**12**) against soybean lipoxygenase-1 was not obtained accurately because of the solubility limitation in the water-based test solution. Thus since the aromatic head moieties in this series are the same, data are interpreted to support the hypothesis that changes in the hydrophobic side chain tail moieties correlate to the specific activity. With the proviso that anacardic acid is taken as the head portion of the molecules studied, lipoxygenase inhibitory activity can be reasonably designed by selecting the appropriate partition coefficient (log *P*) as a standard. However, it should be noted that other factors of the hydrophobic side chain are also associated with the specific activity. For example, introduction of branching or unsaturation into the hydrophobic moiety is known to increase the solubility of the molecules in water and thus is closely associated with the activity (Rosen, 1989). The head and tail structural moieties of the anacardic acids thus further suggest that optimization of the lipoxygenase inhibitory activity is achievable through a designed synthetic approach. We thus extended the scope of our evaluations to additionally determine the effects of lipoxygenase inhibitors based on the anacardic acid scaffold (Green *et al.*, 2007).

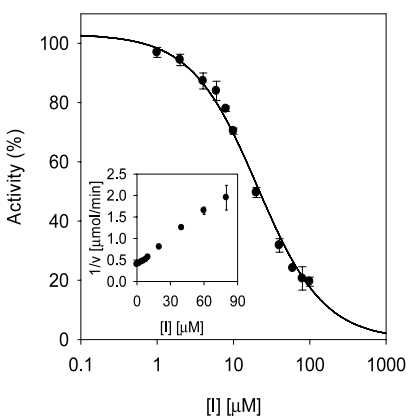

Fig. 3. Dose-response curve for the inhibition of soybean lipoxygenase-1 by anacardic acid ($C_{12:0}$) (**11**) at 25 °C. Inset replots of data as $1/v$ versus [I].

Lipoxygenase is a non-heme iron-containing enzyme, and lipoxygenase inhibitors are known to act mainly in two different ways: a) by chelating the iron of the active site of an enzyme (Nelson *et al.*, 1988) and/or b) by reducing the ferric form of the enzyme to an inactive ferrous form (Mansuy, 1988). In previous reports, anacardic acids were described to form complexes with both the divalent ferrous ion (Nagabhushana *et al.*, 1995) and trivalent ferric ion (Tsujimoto *et al.*, 2007). In the latter case, determination of the metal-complex composition by ESI mass spectrometry was effectively employed. Formation of the anacardic acids-ferric ion complex was detected in the ratio of 2:1 as the base peak in the negative ion ESI mass spectrum. Hence, anacardic acids inhibit both E_{ox} and E_{red} forms (Fig. 4). These phenomena were also observed in the case of salicylic acid (Hayashi *et al.*, 2007) but this did not inhibit the linolenic acid peroxidation catalyzed by soybean lipoxygenase-1. In addition, EDTA (ethylene diaminetetraacetic acid), a well-known chelator, did not inhibit the linolenic acid peroxidation catalyzed by soy-

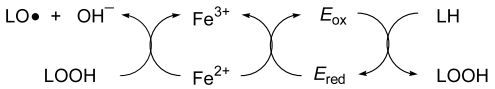

Fig. 4. Commercial lipoxygenase contains a non-heme ferrous ion (E_{red}) that must be oxidized to yield the catalytically active ferric enzyme (E_{ox}) and therefore a catalytic amount of LOOH (13-HPOD) is usually added as a cofactor to LH (linoleic acid, a substrate). Anacardic acids chelate both ferric and ferrous ions.

bean lipoxygenase-1, indicating that the chelation ability alone is not enough to inhibit the activity of the enzyme.

Anacardic acids in general act as antioxidants in a variety of ways including inhibition of various pro-oxidant enzymes involved in the production of the reactive oxygen species and additionally chelate transition metal ions, but do not quench reactive oxygen species. However, once the appropriate head portion is selected, scavenging activity can be introduced. Thus, the head portion seems to be the determining feature. For example, the four anacardic acids 6-[2′-(2″,5″-dihydroxyphenyl)ethyl]salicylic acid (**13**), 6-[2′-(3″,4″-dihydroxyphenyl)ethyl]salicylic acid (**14**), 6-[2′-(2″,4″-dihydroxyphenyl)ethyl]-salicylic acid (**15**) and 6-[2′-(2″,4″,5″-trihydroxyphenyl)ethyl]salicylic acid (**16**), previously synthesized by a *trans* elimination of a hydroxyphenylphosphine oxide precursor with sodium hydride (Green and Tocoli, 2002a), can scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. In addition, all four compounds inhibited the lipoxygenase-catalyzed oxidation of linoleic acid, but to a lesser extent compared to **5**, indicating that the tail portion is also vital for this specific activity. For example, the IC_{50} value of **13** is estimated as 400 μM. This inferior activity is likely caused by lack of the appropriate hydrophobic tail portion since the interaction of iron-chelating molecules with the iron centre of lipoxygenase is known to be influenced by the lipid solubility (Abeysinghe *et al.*, 1996). Both anacardic acids **13** and **14** were previously described to show moderate inhibitory activity on potato lipoxygenase (Nagabhushana *et al.*, 2002). On the other hand, 6-(4′,8′-dimethylnonyl)salicylic acid (**17**) and 6-(2′-ethylheptyl)salicylic acid (**18**) were found to be potent lipoxygenase inhibitors with IC_{50} values of 10.5 μM and 18.8 μM, respectively. However, both act similarly to **4** and **5** neither of which scavenged the DPPH radical.

Last but not least, the current experiment demonstrates that lipoxygenases catalyze the oxygenation of polyenoic compounds containing a (1*Z*,4*Z*)-pentadiene system such as anacardic acids and cardols into their 1-hydroperoxy-(2*E*,4*Z*)-pentadiene products. Anacardic acids are present in cashew apple which is widely consumed not only as fresh fruit but also as various processed products, especially as juice. Cardols were isolated from many edible plants such as pistachio (*Pistacia vera*), macadamia (*Macademia ternifolia*), and mango (*Mangifera indica*) (Cojocaru *et al.*, 1986). Similar resorcinolic lipids were also characterized in grain plants such as wheat (*Triticum aestivum*) and rye (*Secale cereale*) (Kubus *et al.*, 1983). Since safety is a primary and paramount consideration for food, their safety needs to be examined.

Discussion

Anacardic acids inhibit the soybean lipoxygenase-1-catalyzed peroxidation of linoleic acid, and this activity is associated to a large extent with their pentadeca(en)yl side chain, similar to the case found for their antibacterial activity (Kubo *et al.*, 1987, 2003; Muroi and Kubo, 1994). It is important to note that the olefinic bond in the side chain is not essential to elicit the specific activity but rather to increase it. Furthermore the stereochemistry (*E/Z*) of the olefinic bond is apparently not related to the activity. However, the shape and volume of the side chain seems to play a vital role in the activity, and hence additional biological activity may be optimized by the choice of different side chains, which in turn suggest very strongly that greater specific activity is indeed achievable through a systematic synthetic approach. Trends observed in results obtained in the current investigations are to a large extent similar to those observed for their antibacterial activity against Gram-positive bacteria (Kubo *et al.*, 1987, 2003). It would appear that the enzymes have a common base in that they possess a relatively non-specific and hydrophobic domain in their molecular structures, and that the anacardic acids most likely interact with this domain. This dynamic concept can be more broadly considered in that the hydrophobic alkyl side chain should allows for conformational flexibility and hence allows for interaction with many sites in the hydrophobic domain. There is no doubt that a better understanding of this interaction at the molecular level is necessary for the

design or selection of more effective lipoxygenase inhibitors.

Safety is a primary and paramount consideration to be made before incorporating antioxidants in food products. Antioxidant activity of anacardic acids is not that high due to their radical scavenging abilities but more at a preventative measure. Anacardic acids may be considered to be advantageous in the suppression of the formation of free radicals and active oxygen species as the first line of defense. Generally radical-scavenging antioxidants trap an active radical to form an antioxidant-derived radical. Evidence is accumulating of several cell types other than phagocytes which also produce extracellular free radicals *in vivo*. For example, lipids are oxidized by lipoxygenases and cyclooxygenases to generate peroxide intermediates. Typically, lipoxygenases catalyze the oxygenation of polyenoic fatty acids containing a (1*Z*,4*Z*)-pentadiene system, such as linoleic acid and arachidonic acid, into their 1-hydroperoxy-(2*E*,4*Z*)-pentadiene products. Hence, the fate of this newly formed radical is important in determining the total potency of the antioxidant. If free radicals are produced during the normal cellular metabolism in sufficient quantities to overcome the normally efficient protective mechanisms, metabolic and cellular disturbances will consequently occur in a variety of ways. The data obtained thus far indicates the advantage of anacardic acids as preventive antioxidants since they inhibit the enzymes involved in the production of reactive oxygen species, but yet do not quench reactive oxygen species as such. Since anacardic acids are found in edible plants which have been consumed for many years, this fact should be noted to be an important indicator for their physiological activity. Additionally, the metal chelation capacity of anacardic acids must provide them with a further advantage, *viz*. to reduce the need of a high concentration of the catalyzing transition metal in lipid peroxidation. It is known that chelating agents, which form bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential and thereby stabilize the oxidized form of the metal ion (Fraga and Oteiza, 2002).

Anacardic acids now appear to combine both lipoxygenase inhibitory activity and metal chelation properties in one agent without being oxidized. Inhibitory effects of anacardic acids without scavenging activity on this pro-oxidant enzyme may be relevant to health benefits through a general reduction in oxidative stress *in vivo* (Rioux and Castonguay, 1998). It is for these reasons that anacardic acids were recently suggested to be utilized in functional food formulations (Trevisan *et al*., 2006). Despite these clear advantages, the full biological significance of anacardic acids as lipoxygenase inhibitors in living systems is still largely unknown. Thus, it is not absolutely clear that, when ingested, anacardic acids are absorbed into the system through the intestinal tract and delivered to the places where antioxidants are needed. The relevance of *in vitro* experiments in simplified systems to *in vivo* protection from oxidative damage should be carefully considered. Further intensive evaluation will be required in order to identify and formulate a holistic and dynamic perspective.

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