Inhibition of Soybean Lipoxygenase-1 by Anacardic Acids, Cardols, and Cardanols

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INHIBITION OF SOYBEAN LIPOXYGENASE-1 BY ANACARDIC ACIDS, CARDOLS, AND CARDANOLS

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ABSTRACT.—The inhibitory activity of the title compounds against 15-lipoxygenase (soybean lipoxygenase-1) was examined. Anacardic acid 8′*Z*-monoene [**3**] exhibited the highest potency (IC_{50}=50 μM), followed by cardol 8′*Z*,11′*Z*,14′-triene [**5**] (IC_{50}=60 μM). Cardol [**8**] and cardanol [**12**] were ineffective, while the other unsaturated phenols were of intermediate potency.

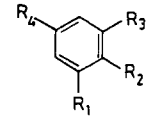
Cashewnut shells (*Anacardium occidentale* L.; Anacardiaceae) are a rich source of long-chain alkyl substituted salicylic acid and resorcinol derivatives called anacardic acids [**1–4**] and cardols [**5–8**]. Together, they form about 30% by weight of the shells, with anacardic acids forming 70% of the cashewnut shell liquid and cardols forming about 25%. The remainder consists of small quantities of 2-methylcardols and cardanols [**9–12**] (1). All the compounds are characterized by the presence of a linear 15-carbon chain with varying degrees of unsaturation, namely, 8′*Z*-monenes, 8′*Z*,11′*Z*-dienes and 8′*Z*, 11′*Z*,14′-trienes. The bioactivities of these compounds have attracted considerable attention in recent years. These include molluscicidal (2), antitumor (3), antibiotic (4), and antiacne (5) properties.

We have reported earlier that anacardic acid 8′*Z*,11′*Z*-diene [**2**] is a substrate for soybean lipoxygenase-1, with the product of the reaction being 2-hydroxy-6-[12′-hydroperoxy-(8′*Z*,10′*E*)-pentadecadienyl]-benzoic acid [**13**] identified by spectroscopic studies on the reduction product, **14** (6). Around the time this work was completed, Grazzini *et al.* (7) reported that anacardic acid [**4**] and anacardic acid 10′*Z*-monoene [**15**] from zonal geranium (*Pelargonium* x *hortorum*) were good inhibitors of 5-lipoxygenase and ovine prostaglandin endoperoxide synthase. They suggested that the natural resistance of the geranium to many arthropods and insect pests may be related to its ability to synthesize anacardic acids. They have also discussed the possibility that anacardic acid-mediated arthropod resistance could be brought about by inhibition of prostaglandin synthase and lipoxygenase enzymes in the insect leading to reproductive failure or physiological dysfunction. In view of the ecological significance of the above hypothesis, we undertook a detailed study of the effects of the various anacardic acids, cardols, and cardanols of cashewnut shell liquid on soybean lipoxygenase-1 activity.

Among the compounds tested, anacardic acid 8′-monoene [**3**] was the most potent, showing an IC_{50} value of 50 μM (Table 1). In view of the fact that certain products of lipoxygenase action on arachidonic acid (for example, 15-hydroperoxyeicosatetraenoic acid, 15-HPETE, and 12-hydroxyeicosatetraenoic acid, 12-HETE) inhibit 5- and 12-lipoxygenases (8), **13** and **14** were also tested for their inhibitory activity against lipoxygenase-1 and were found ineffective. Cardol 8′*Z*,11′*Z*,14-triene [**5**] was nearly as potent as **3** with an IC_{50} value of 60 μM. Saturated cardol [**8**] and cardanol [**12**] were ineffective, while all others were of intermediate potency. In the case of cardols and cardanols, potency of inhibition increased with an increase in unsaturation in the alkyl side-chain.

These findings, coupled with our earlier observation of the rapid accumulation of lipophilic phenols and phenolic acids during the early period of growth of

TABLE 1. Structures of Cashewnut Shell Liquid Phenols and Their Inhibitory Activity Against Lipoxygenase-1.



Compound	R_1	R_2	R_3	R_4	IC_{50}[a]
1	8′Z,11′Z,14′-Pentadecatrienyl	COOH	OH	H	>250
2	8′Z,11′Z-Pentadecadienyl	COOH	OH	H	—
3	8′Z-Pentadecenyl	COOH	OH	H	50
4	Pentadecyl	COOH	OH	H	85
5	8′Z,11′Z,14′-Pentadecatrienyl	H	OH	OH	60
6	8′Z,11′Z-Pentadecadienyl	H	OH	OH	167
7	8′Z-Pentadecenyl	H	OH	OH	250
8	Pentadecyl	H	OH	OH	—
9	8′Z,11′Z,14′-Pentadecatrienyl	H	OH	H	100
10	8′Z,11′Z-Pentadecadienyl	H	OH	H	125
11	8′Z-Pentadecenyl	H	OH	H	>250
12	Pentadecyl	H	OH	H	—
13	12′-Hydroperoxy-(8′Z,10′E)-pentadecadienyl	COOH	OH	H	—
14	12′-Hydroxy-(8′Z,10′E)-pentadecadienyl	COOH	OH	H	—
15	10′Z-Pentadecenyl	COOH	OH	H	6[b]

[a]Concentration in μM; for assay conditions, see Experimental.
[b]On 5-lipoxygenase, from Grazzini *et al.* (7).

the cashewnut (9), suggest a significant ecophysiological role for these compounds, possibly in the protection of the kernel.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Lipoxygenase-1 was isolated from soybean seeds and purified as described previously (10). The enzyme, containing 12 mg/ml protein and with a specific activity of 45 units/mg protein was used in the work. Enzyme assays were carried out on a Shimadzu 2100 spectrophotometer. Linoleic acid was purchased from Nuchek Prep, Inc., Elysian, MN. All the compounds tested were taken in EtOH as 10 mM solutions. Compounds **1–12** were obtained by extraction of cashewnut shells with petroleum ether and separation of the extract by normal-phase column lc followed by reversed-phase prep. hplc (1). Compounds **13** and **14** were prepared by reacting **2** with lipoxygenase-1 as described (6). The purity of all compounds, as determined by hplc (1), was >98%.

ASSAY PROCEDURE.—All the reactions were carried out at 20° in 0.2 M borate buffer (pH 9.0) containing 0.1 mM linoleic acid dispersed in Tween 20. For inhibition studies, 2.5 μl of an EtOH solution of the test compound was added to 100 μl of suitably diluted (1:200) enzyme and incubated for 5 min at 4–5°. As a control experiment, 2.5 μl of EtOH was added to 100 μl of the enzyme solution and incubated for 5 min at 4–5°. From these preincubation mixtures, 10 μl was used for each assay; the total volume of the assay mixture was 1 ml. The reaction was monitored by the rate of increase in absorbance at 234 nm (10). The concentrations (μM) of test compound required for 50% decrease in velocity are given as IC_{50} values in Table 1. Each value is the mean of three experiments.

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