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A PRENYLFLAVONE, ARTONIN E, AS ARACHIDONATE 5-LIPOXYGENASE INHIBITOR

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Abstract—Several naturally occurring prenylflavones were tested for their inhibitory actions on arachidonate 5-lipoxygenase purified from porcine leukocytes. Of the compounds tested, artonin E (5′-hydroxymorusin) exhibited the most potent inhibition on arachidonate 5-lipoxygenase (IC_{50} = 0.36 μM). Arachidonate 12-lipoxygenases purified from porcine leukocytes and human platelets, 15-lipoxygenase from rabbit reticulocytes and fatty acid cyclooxygenase from bovine vesicular glands were inhibited by the compound only at higher concentrations (IC_{50} = 2.3, 11, 5.2 and 2.5 μM, respectively).

Due to the proposed pathophysiological roles of leukotrienes in inflammation and immediate hypersensitivity [1], the development of selective inhibitors of arachidonate 5-lipoxygenase has been a subject of medicinal chemical interest. The enzyme oxygenates the position-5 of arachidonic acid, and produces 5*S*-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE), which is a precursor of various leukotrienes [1]. Molecular and catalytic properties of the enzyme have recently been clarified by extensive investigations [2]. In our previous study we screened various flavonoids, and found that cirsiliol (3′,4′,5-trihydroxy-6,7-dimethoxyflavone) potently inhibited 5-lipoxygenase [3]. Its structure–activity relation was also investigated [3], and more potent derivatives were prepared [4, 5]. Inhibition of 5-lipoxygenase by various flavonoids has been reported from other laboratories [6–12]. Since modification of the position 3 of cirsiliol with an alkyl group enhanced 5-lipoxygenase inhibition [5], we were interested in the inhibitory effects of eight natural 3-isoprenylated flavones [13–15] on the 5-lipoxygenase activity.

MATERIALS AND METHODS

Materials. [1-^{14}C]Arachidonic acid (58.3 mCi/mmol) was purchased from Amersham International (Amersham, U.K.), arachidonic acid from Nu-Chek-Prep (Elysian, MN, U.S.A.), and precoated silica gel 60 F-254 glass plates for thin-layer chromatography from Merck (Darmstadt, F.R.G.). Artonin E (compound **1** in Fig. 1) and cycloartobiloxanthone (**2**) were isolated from the bark of *Artocarpus communis* Forst. [13], heterophyllin (**4**), cycloheterophyllin (**5**), artonin A (**6**), and artonin B (**7**) from the root bark of *Artocarpus heterophyllus* Lamk. [14], and morusin (**8**) from the root bark of *Morus alba* L. [15]. Isolation of artobiloxanthone (**3**) from the bark of *Artocarpus rigida* Bl. will be described elsewhere. Cirsiliol was donated from Prof. T. Horie of Tokushima University Faculty of Engineering.

Preparation and assay of enzymes. Arachidonate 5-lipoxygenase was highly purified from the cytosol fraction of porcine leukocytes by immunoaffinity chromatography using a monoclonal anti-5-lipoxygenase antibody [16]. 12-Lipoxygenases from the cytosol fraction of porcine leukocytes [17] and human platelets [18] were purified by immunoaffinity chromatography. 15-Lipoxygenase of the cytosol fraction of rabbit reticulocytes was partially purified by ammonium sulfate fractionation [19]. Cyclooxygenase was purified from the microsomal fraction of bovine vesicular glands by immunoaffinity chromatography utilizing a monoclonal anti-cyclooxygenase antibody prepared previously [20].

5-Lipoxygenase (5 μg protein) was allowed to react with 25 μM [1-^{14}C]arachidonic acid (50,000 cpm) at 24° for 3 min in a 200 μL reaction mixture containing 2 mM ATP, 2 mM $CaCl_2$ and 50 mM Tris–HCl buffer (pH 7.4). The porcine leukocyte 12-lipoxygenase (2 μg protein) was assayed by incubation at 24° for 3 min in a 200-μL reaction mixture containing 25 μM [1-^{14}C]arachidonic acid (50,000 cpm) and 50 mM Tris–HCl buffer (pH 7.4). Human platelet 12-lipoxygenase (2 μg protein) was incubated at 30° for 10 min. Cyclooxygenase (14 μg protein) was assayed at 24° for 2 min in a 100-μL reaction mixture containing 50 μM [1-^{14}C]arachidonic acid (25,000 cpm), 2 μM hematin, 5 mM L-tryptophan, and 0.1 M Tris–HCl buffer (pH 8.0). The assay condition of reticulocyte 15-lipoxygenase (16.5 μg protein) was the same as that of porcine leukocyte 12-lipoxygenase assay. Termination of the enzyme reaction, ethereal extraction of reaction products, their separation by thin-layer chromatography, and determination of radioactivity were performed as described earlier [16–18, 20]. Methanol solution (4 μL) of each flavone compound at varying concentrations was

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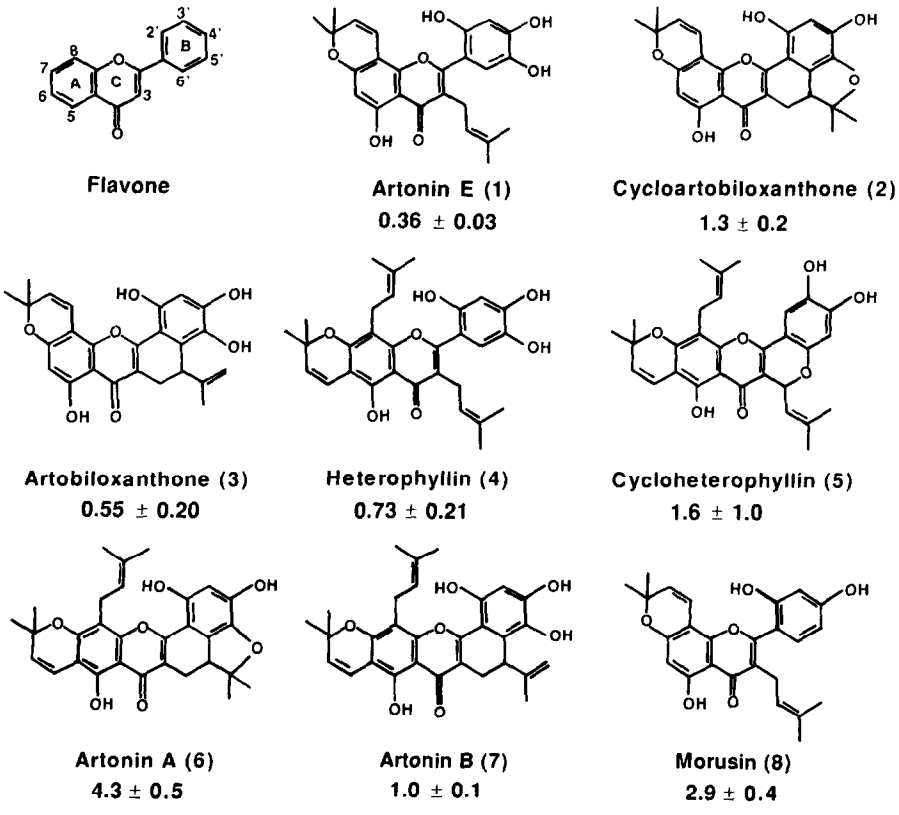

Fig. 1. Structures of prenylflavones and their inhibitory effects on arachidonate 5-lipoxygenase. Concentrations (μM) required to cause 50% inhibition (IC_{50}) were examined with the 5-lipoxygenase purified from porcine leukocytes (mean ± SD, N = 3).

preincubated with enzyme for 3 min (2 min only with cyclooxygenase) at room temperature. Protein concentration of enzyme solution was determined by the method of Lowry *et al.* [21].

RESULTS

When the activity of the purified 5-lipoxygenase from porcine leukocytes was measured in the presence of each of compounds **1–8**, all the compounds inhibited the enzyme reaction in a dose-dependent manner. As shown in Fig. 1, the IC_{50} values varied depending on the structural modification of the compound. Compounds with 4′,5′-vicinal diol in the B ring of the flavone skeleton (compounds **1**, **3**, **4** and **7**) showed lower IC_{50} values. As shown in Fig. 2, 5-lipoxygenase was inhibited depending on the concentration of artonin E (compound **1**), which gave the lowest IC_{50} (0.36 μM) of all the eight compounds. On the other hand, morusin (compound **8**), which lacked the 5′-hydroxyl group of artonin E, was a less potent 5-lipoxygenase inhibitor. The IC_{50} value of artonin E was lower than that of cirsiliol (IC_{50} = 1.3 μM). On the basis of the IC_{50} values, we selected artonin E as a representative compound out of the eight compounds.

Inhibitory actions of artonin E and morusin on other mammalian arachidonate oxygenases were examined (Table 1). Artonin E inhibited two 12-lipoxygenases from porcine leukocytes and human

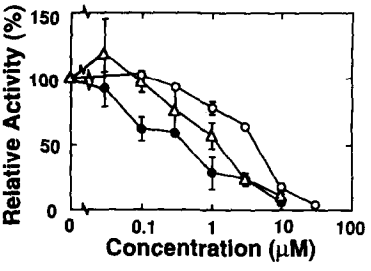

Fig. 2. Dose-dependent inhibition of 5-lipoxygenase by artonin E, morusin and cirsiliol. The 5-lipoxygenase activity was assayed in the presence of various concentrations of artonin E (●), morusin (○) and cirsiliol (△) (mean ± SD, N = 3).

platelets, 15-lipoxygenase from rabbit reticulocytes, and fatty acid cyclooxygenase from bovine vesicular glands. However, IC_{50} values for these oxygenases were higher by one order of magnitude than that for 5-lipoxygenase. These results indicated that artonin E was a relatively specific inhibitor for 5-lipoxygenase. Morusin also inhibited these enzymes (except for human platelet 12-lipoxygenase) with IC_{50} values of micromolar order. Thus, the selectivity for 5-lipoxygenase was not observed with morusin.

DISCUSSION

Flavonoids are a group of naturally occurring

Table 1. Inhibitory effects of artonin E and morusin on mammalian arachidonate oxygenases

Enzymes	IC_{50} (μM) (mean ± SD) Artonin E	Morusin
5-Lipoxygenase	0.36 ± 0.03	2.9 ± 0.4
12-Lipoxygenase (leukocytes)	2.3 ± 1.2	3.4 ± 0.2
12-Lipoxygenase (platelets)	11 ± 2.0	>30
15-Lipoxygenase	5.2 ± 0.2	3.3 ± 0.4
Cyclooxygenase	2.5 ± 0.3	1.6 ± 0.2

IC_{50} values of artonin E and morusin were determined for 5-lipoxygenase, 12-lipoxygenases of porcine leukocytes and human platelets, 15-lipoxygenase, and cyclooxygenase under the standard assay conditions (N = 3).

substances widely distributed in plants, and about 4000 flavonoids have been described so far [22]. Several flavonoids were identified as the promising drugs for free radical pathologies such as ischaemia, anaemia, arthritis and asbestosis [23]. Flavonoids possessing anti-inflammatory and gastroprotective properties were screened recently [24]. Furthermore, inhibition by a number of natural flavonoids of lipoxygenases or cyclooxygenase or both was found [3–12, 25], and the structure–activity relationship was discussed [3, 10].

In the present study we showed the inhibitory activity of prenylflavones on 5-lipoxygenase. The compounds having three hydroxyl groups at positions 2′, 4′ and 5′ on the B ring (compounds **1**, **3**, **4** and **7**) were more potent inhibitors. Thus, the vicinal diol was important for 5-lipoxygenase inhibition, and this finding was in agreement with our previous report [3]. As examined under the same assay condition using the purified porcine leukocyte 5-lipoxygenase, artonin E (compound **1**) was significantly more potent than cirsiliol (Fig. 2), which was reported as a 5-lipoxygenase inhibitor [3]. This finding was consistent with our recent report that the inhibitory activity of cirsiliol with 5-lipoxygenase was enhanced by introducing a lipophilic alkyl group at position 3 of the C ring [5].

Artonin E inhibited other mammalian arachidonate lipoxygenases; 12-lipoxygenases, 15-lipoxygenase and cyclooxygenase. However, their IC_{50} values were higher than that for 5-lipoxygenase. In contrast, morusin (compound **8**) was a non-specific lipoxygenase inhibitor, inhibiting 5-lipoxygenase with a higher IC_{50} value than that of artonin E. Significant differences of IC_{50} values of artonin E and morusin between the porcine leukocyte 12-lipoxygenase and the human platelet 12-lipoxygenase should be noted since the leukocyte and platelet 12-lipoxygenases were distinct both catalytically and immunologically [18].

Previously, it was reported that morusin inhibited the formation of thromboxane B_2 and 12-hydroxy-5,8,10-heptadecatrienoic acid (cyclooxygenase metabolites) more strongly than the formation of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (a 12-lipoxygenase metabolite) as tested on rat platelet homogenate [25]. This finding was confirmed by our present study with the purified enzymes.

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