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NEW ISOFLAVONOIDS AS INHIBITORS OF PORCINE 5-LIPOXYGENASE

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Abstract—The inhibitory activity of new isoflavonoids on 5-lipoxygenase of porcine leukocytes was investigated. Isoflavans (I) proved to be stronger inhibitors than isoflavones (II). The isoflavans containing *ortho*-hydroxy groups in ring A showed the lowest K_i values (0.8–50 μM). In comparison, isoflavans with *meta*-dihydroxy groups exhibited K_i values higher than 150 μM. The effect of commercial antioxidants was tested also on porcine 5-lipoxygenase. Butylated hydroxyanisole (K_i: 25 μM) and butylated hydroxytoluene (K_i: 55 μM) revealed moderate inhibitory activity, whereas L-ascorbic acid, L-ascorbyl palmitate, *dl*-α-tocopherol and *n*-propyl gallate showed weak inhibitory activities (K_i: 100–260 μM).

Isoflavonoids represent an important subclass of the flavonoids. The structure of isoflavonoids is based on a 3-phenylchroman skeleton, which biogenetically is derived by aryl migration from the 2-phenylchroman skeleton of the flavonoids [1]. While flavonoids are ubiquitous compounds, the isoflavonoids show a limited distribution in the plant kingdom. They are found mainly in the subfamily *Papilionoidae* of the Leguminosae (for review see Ref. 2).

Isoflavonoids are known to exhibit various biological properties, e.g. the insecticidal activity of rotenoids [2] and isoflavans [3], the anti-microbial, especially the anti-fungal, activity of the phytoalexins (pterocarpans, isoflavans and some isoflavones [2, 4]), an hypocholesterolemic effect and a triglyceride-lowering activity [5–7]. The isoflavonoids have many properties in common with the flavonoids, e.g. anti-cataract [8, 9] anti-inflammatory and anti-allergic activity [10–14]. Some biological activities are explained by special biochemical mechanisms. Thus, the anti-inflammatory and anti-allergic activity of flavonoids is in part due to inhibition of the enzymes involved in the arachidonic cascade [10–14].

One of the enzymes of the arachidonic acid cascade, the 5-lipoxygenase (5-LOX†) is the first enzyme in the biosynthetic pathway leading to LT. LTs are potent mediators, involved in immunoregulation and in various diseases, including inflammation, asthma and diverse allergic reactions. In neutrophils, stimulated with the Ionophore A 23187, the main products of arachidonic acid are 5-HETE and LTB_4 [15]. The same pattern of 5-LOX products is obtained with neutrophils derived from various species stimulated under the same conditions [16]. Previously, the HETEs were considered to be predominantly inactivation products of HPETEs without biological importance. New investigations have shown that HETEs modulate basic biological functions such as enzyme regulation, hormone secretion, ion transport and immune mechanisms. They are involved in pathological processes including various inflammatory diseases, arteriosclerosis and ischemia (for review see Ref. 17).

Due to the participation of LT and HETEs in various diseases, we evaluate in this study the inhibitory effect of different new synthetic isoflavonoids on porcine 5-LOX *in vitro*. We also compare the effect of isoflavonoids on porcine 5-LOX with that of commercial food antioxidants.

MATERIALS AND METHODS

Materials

All isoflavonoids have been synthesized at the Institute for Physiological Chemistry of the University of Bonn [18]. The antioxidants *dl*-α-tocopherol, BHT, BHA and L-ascorbic acid were purchased from Merck (Darmstadt, Germany), *n*-propyl gallate from Sigma (Munich, Germany) and ascorbyl palmitate from Serva Feinbiochemica (Heidelberg, Germany). Dextran T-500 for cell sedimentation was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Arachidonic acid (Merck) was purified by silicic acid column chromatography prior to use. The Ionophore A 23187 and PGB_2, which served as internal standard, were obtained from Sigma. ETYA was supplied by Hoffmann-La-Roche (Basle, Switzerland). All salts, organic solvents, thin layer silicic acid plates and Trypan blue were obtained from Merck. All chemicals used were of reagent grade. The solvents for HPLC were dried, distilled and filtered.

5-LOX assay

Preparation of leukocyte suspension. Porcine peripheral blood leukocytes were prepared according to the method of Kuhl *et al.* [10]. Porcine blood (1.5 L) was decoagulated with 100 mL Hank's buffer solution containing 3.8% sodium citrate, 6 U of heparin/mL of blood and passed through a Dextran

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† Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; LOX, lipoxygenase; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LT, leukotriene; PG, prostaglandin; ETYA, 5,8,11,14-eicosatetraynoic acid.

(6%) gradient. After sedimentation at 4° for 60 min, the supernatant was centrifuged at 500 *g* for 12 min. The pellet was resuspended in Hank's buffer containing 0.38% sodium citrate. The centrifugation was repeated. Residual erythrocytes were lysed after 5 min incubation at 25° with Tris buffer (17 mM, pH 7.2) containing 0.17% ammonium chloride. After centrifugation at 400 *g* for 6 min and resuspension of the cells in Hank's buffer containing 0.38% sodium citrate, the solution was centrifuged again at 400 *g* for 6 min. The cell pellet was resuspended in phosphate-buffered (25 mM, pH 7.4) isotonic saline at 4×10^7 cells/mL. The viability of the cells (higher than 90%) was checked by Trypan blue exclusion.

Incubation conditions for porcine leukocytes. Leukocyte suspension (10 mL) was preincubated for 3 min at room temperature in the presence of different concentrations (0.5–200 μM) of isoflavonoids or usual food antioxidants dissolved in ethanol or dimethyl sulfoxide. An equivalent suspension with solvent (ethanol or dimethyl sulfoxide) but lacking test substance served as control. The solvent content did not exceed 1%, to avoid an influence on 5-LOX activity [19]. During the assay for 5-LOX activity, nearly complete inhibition of 12-LOX is achieved by addition of 10 μM ETYA [11] to the reaction mixture, as the latter enzyme is the predominant LOX of porcine leukocytes [20].

The 5-LOX reaction was started by adding the following substances to the leukocyte suspension: $CaCl_2$ (2 mM), Ionophore A 23187 (10 μM), ETYA (10 μM) and the substrate arachidonic acid (100 μM). After incubating the cells for 5 min at 37°, the enzymatic reactions were stopped by adding 1.5 mL of formic acid (1%). After addition of PGB_2 (2 μg) as an internal standard, the samples were extracted immediately with chloroform/methanol (1:1, v/v; 2 × 15 mL), evaporated and stored under nitrogen at −18° prior to HPLC analysis.

HPLC analysis. Analytical HPLC was performed according to Kuhl *et al.* [10] with slight modifications. A prepacked column (Hibar RT, 250 × 4 mm, Lichrosorb 60, 7 μm, Merck) and a precolumn (RCSS Silica T 61031) from Waters, Millipore (Eschborn, Germany) were employed (instrument: S 101, Siemens; pump: DMR-AE-10,4, Orlita; Injectorsystem: U6K, Waters).

The compounds were eluted using first *n*-hexane/2-propanol/methanol/acetic acid (972/18/9/1 by vol.) containing 0.06% water. After 9.5 min the gradient elution was started. The 2-propanol content was raised during a linear gradient up to 3 vol.% in 12 min (972/30/9/1). The flow rate was 3.5 mL/min at 22°. The elution was monitored spectrophotometrically at 235 nm (0–9.5 min) and at 280 nm (9.5–24 min). 5-HETE and LTB_4 were quantified by comparing their peak areas with that of PGB_2 (internal standard). The extinction coefficients used for 5-HETE, LTB_4 and PGB_2 were $\varepsilon = 30{,}500$, 39,500 and 26,800 L mol^{-1} mm^{-1}, respectively.

RESULTS

Inhibition of porcine 5-LOX by isoflavonoids

Arachidonic acid incubated with porcine leukocytes in the presence of Ionophore A 23187, $CaCl_2$ and ETYA is converted predominantly to 5-HETE and LTB_4. Figure 1A shows a typical HPLC chromatogram of an incubation performed under the conditions described in Materials and Methods. The effect of various concentrations of 7,8-dihydroxy-4′-methoxyisoflavan (6) on 5-LOX-activity is shown in Fig. 1B and C. The formation of the 5-LOX products 5-HETE and LTB_4 was suppressed by 7,8-dihydroxy-4′-methoxyisoflavan (6) in a dose-dependent manner. The inhibition of 5-LOX by isoflavonoids is expressed as the percentage of activity related to the control value measured without inhibitor. Plotting of 1/(% activity) vs [I] was carried out to evaluate the inhibition constant K_i. The substitution patterns of isoflavans (Table 1) and isoflavones (Table 2) are listed with their corresponding K_i values. K_i values of most isoflavonoids tested ranged from 0.1–100 μM.

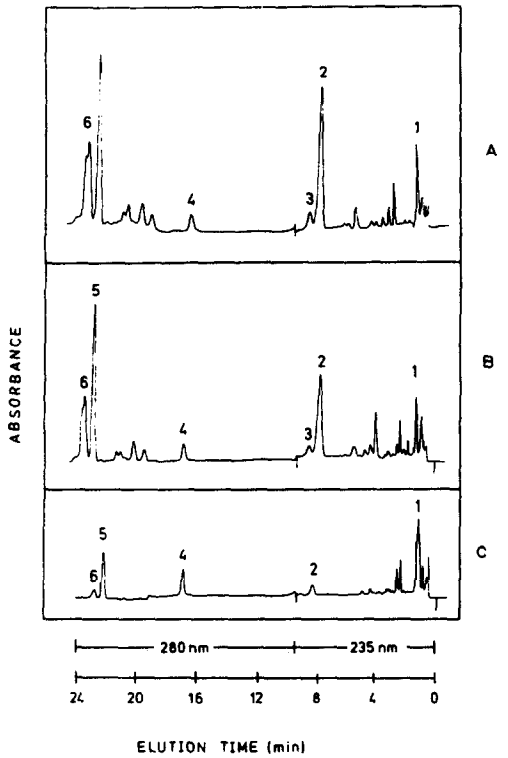
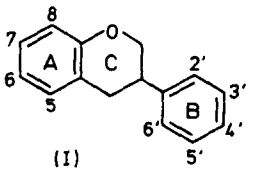


Fig. 1. Inhibition of porcine 5-LOX by 7,8-dihydroxy-4′-methoxyisoflavan (6). HPLC chromatograms of the products formed during a 5-min incubation of porcine peripheral blood leukocytes (10 mL/4 × 10^7 cells/mL) with arachidonic acid (100 μM), $CaCl_2$ (2 mM), Ionophore A 23187 (10 μM) and ETYA (10 μM). (A) Control (without test substance), (B) with 10 μM 7,8-dihydroxy-4′-methoxyisoflavan (6), (C) with 50 μM 7,8-dihydroxy-4′-methoxyisoflavan (6). Signal attenuation was three times higher at 235 than at 280 nm. Peaks: 1, arachidonic acid; 2, 5-HETE; 3, 5-HPETE; 4, PGB_2; 5, LTB_4; 6, stereoisomers of LTB_4.

Structure–activity studies showed that the isoflavans inhibited porcine 5-LOX more effectively than the corresponding isoflavones [see compounds (2) and (3), (13) and (14), (18) and (17)]. Looking

Table 1. K_i values of porcine 5-LOX inhibition by various isoflavans

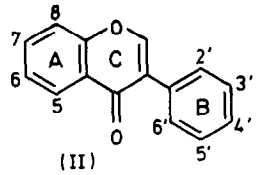


(I)

Compound	Substitution 5	6	7	8	3'	4'	K_i (μM)
(1)	H	OH	OH	H	H	OH	37
(2)	H	H	OH	OH	H	OH	0.8
(4)	H	O-Ace	O-Ace	H	H	OCH_3	15.5
(5)	H	OH	OH	H	H	OCH_3	2.5
(6)	H	H	OH	OH	H	OCH_3	21
(7)	H	OH	OH	H	H	CH_3	53
(8)	H	H	OH	OH	H	CH_3	7.5
(9)	OH	H	OH	H	H	CH_3	218
(10)	H	OH	OH	H	OCH_3	OCH_3	7
(11)	H	H	OH	OH	OCH_3	OCH_3	24
(12)	H	H	OH	CH_3	OCH_3	OCH_3	70
(13)	H	OH	OH	H	O-CH_2-O		28
(18)	H	O-CH_2-O		H	H	OCH_3	0.6
(22)	H	H	OH	OH	H	Cl	9.5
(23)	H	H	OH	CH_3	H	Cl	36
(25)*	OH	H	OH	H	H	OH	168

* See Ref. 11.

Table 2. K_i values of porcine 5-LOX inhibition by various isoflavones



(II)

Compound	Substitution 5	6	7	8	3'	4'	K_i (μM)
(3)	H	H	OH	OH	H	OH	19.5
(14)	H	OH	OH	H	O-CH_2-O		103
(15)	H	H	OH	OH	O-CH_2-O		23.5
(16)	H	O-CH_2-O		H	OCH_3	OCH_3	250
(17)	H	O-CH_2-O		H	H	OCH_3	60
(19)	H	OH	OH	H	F	H	16.5
(20)	H	H	OH	OH	F	H	21
(21)	H	H	OH	OH	CF_3	H	16
(24)	H	H	OH	OH	H	NO_2	91

for an influence of the substituents in ring B, it is obvious that there is no specific relation to 5-LOX inhibition. Among the tested isoflavans and isoflavones neither size, position nor different charge of the substituents was decisive for inhibition strength (see compounds (3), (6), (8), (11), (20), (21), (22)].

Comparing the effects of 6,7-dihydroxyisoflavonoids and of the 7,8-dihydroxyisoflavonoid isomers on porcine 5-LOX, there was no structure–activity relationship [see compounds (1) and (2), (5) and (6), (10) and (11), (19) and (20)]. In contrast, structure–activity relationships are obvious comparing *ortho*-hydroxy- and *meta*-hydroxy-substituted compounds in ring A. Among the 4'-methylisoflavans, the *ortho*-hydroxy isoflavans (7) and (8) are significantly stronger inhibitors of porcine 5-

Table 3. Inhibition of porcine 5-LOX by commercial food antioxidants (K_i values)

Compound	K_i (μM)
BHA	25
BHT	55
n-Propyl gallate	124
dl-α-Tocopherol	259
L-Ascorbic acid	100
L-Ascorbyl palmitate	239

LOX than the respective *meta*-hydroxy isoflavan (9). A similar result was obtained in the corresponding series of 4'-hydroxyisoflavans [see compounds (1), (2) and (25)]. Comparing 7,8-dihydroxyisoflavans with the 7-hydroxy-8-methylisoflavans, the *ortho*-hydroxy substituted compounds are as expected more effective 5-LOX inhibitors [see compounds (11) and (12), (22) and (23)]. Surprisingly some isoflavans, which lack free *ortho*-dihydroxy-substituents in ring A show a marked 5-LOX inhibition. These compounds are 6,7-methylenedioxy-4'-methoxyisoflavan [(18), K_i: 0.6 μM] and 6,7-diacetyl-4'-methoxyisoflavan [(4), K_i: 15.5 μM].

Inhibition of porcine 5-LOX by food antioxidants

In Table 3 the K_i values for inhibition of porcine 5-LOX by commercially available food antioxidants are summarized. Whereas BHA (K_i: 25 μM) and BHT (K_i: 55 μM) are moderate inhibitors, L-ascorbic acid, L-ascorbyl palmitate, α-tocopherol and *n*-propyl gallate are less effective inhibitors (K_i: 100–260 μM) than most isoflavans.

DISCUSSION

Inhibition of porcine 5-LOX by isoflavonoids and food antioxidants

The different isoflavonoids tested in this study inhibited the porcine polymorphonuclear leukocytes 5-LOX in a concentration range of 0.1–100 μM (K_i and IC_{50} values). The same concentration range was obtained with most flavonoids containing also hydroxy- and methoxy-substituents [21–25]. The absolute inhibition values found for various compounds in the literature, however, differ depending on the type of LOX, the enzyme source, method of enzyme isolation and the assay conditions. This fact is illustrated in Table 4 for quercetin, which may be designated as reference flavonoid. Therefore comparison of different studies should be undertaken with certain reservations.

Other compounds tested as inhibitors of porcine 5-LOX are: (*E*/*Z*)-Ajoene (IC_{50}: 1.6 μM), a garlic constituent [30]; nordihydroguairetic acid (IC_{50}: 1.5 μM); caffeic acid (IC_{50}: 46 μM); *p*-coumaric acid (IC_{50}: 2.5 μM) and wedelolactone (IC_{50}: 2.5 μM), a coumestane derivative [19]. These compounds inhibited the 5-LOX also to the same extent as the isoflavonoids tested in this study.

The structure–activity relationship of the isoflavonoids concerning 5-LOX-inhibition (Tables 1 and 2) revealed the following conclusions: (i) isoflavans were found to be more effective inhibitors than their corresponding isoflavones. An explanation may be the change in conformation of ring C after hydration of the isoflavones to isoflavans and the interruption of the fully conjugated system. (ii) *ortho*-Dihydroxy-substituted isoflavans (1), (2), (7) and (8) inhibited the 5-LOX at lower concentrations than the respective *meta*-dihydroxy-substituted isoflavans (9) and (33). The 7,8-dihydroxyisoflavans also proved to be stronger 5-LOX inhibitors than the 7-hydroxy-8-methyl-isoflavans (12) and (23).

Other authors [31] found that phenolic *ortho*-dihydroxy-compounds, including caffeic acid and the flavonoids taxifolin (3,5,7,3',4'-pentahydroxyflavanon), luteolin (5,7,3',4'-tetrahydroxyflavon) and quercitin (3,5,7,3',4'-pentahydroxyflavon), clearly had strong radical-scavenging activities, whereas monohydroxylated and *para*-dihydroxylated compounds proved to be only moderate to weak radical scavengers. Compounds lacking a free hydroxy-group in the molecule scarcely influenced radical scavenging. Some isoflavonoids examined in our study may also trap radicals. During this reaction isoflavonoids are oxidized to *ortho*- and/or *para*-benzoquinones as shown here for 6,7- and 7,8-dihydroxyisoflavans in Fig 2a and b.

Oxygen radicals, which are probably involved in inflammatory and cancerogenic processes, are generated as by-products during arachidonic acid metabolism via the cyclo-oxygenase and LOX

Table 4. Inhibition (IC_{50} values) of various LOXs by quercitin

	Enzyme source	IC_{50} (μM)	Literature
5-LOX	RBL-1	0.2	[26]
5-LOX	RBL-1	2.1	[22]
5-LOX	RBL-1	0.1–1	[23]
5-LOX	RBL-1	>1	[21]
5-LOX	Porcine leukocytes	0.8	[11]
5-LOX	Human leukocytes	≈125	[27]
12-LOX	Human thrombocytes	4–5	[26]
13-LOX	Soybeans	2–3	[28]
15-LOX	Soybeans	>10	[29]

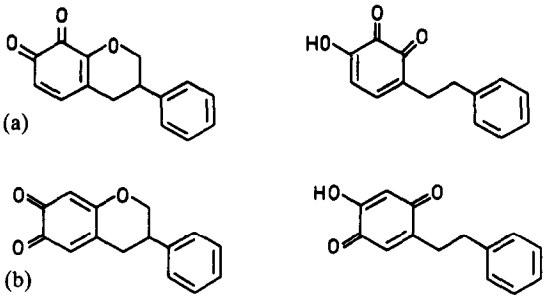


Fig. 2. (a) *ortho*-Benzoquinones of 7,8-dihydroxyisoflavan. (b) *ortho*- and *para*-Benzoquinones of 6,7-dihydroxyisoflavan.

pathway. It is known that fatty acid hydroperoxides formed in LOX reactions are necessary for LOX activity [32–35]. Therefore, trapping of these radicals by isoflavonoids may reduce the 5-LOX activity. But not all compounds with radical-scavenging properties are effective antioxidants.

LOXs are non-heme iron-containing enzymes [36, 37] existing in two different forms: a Fe^{3+} (ferric) and a Fe^{2+} (ferrous) form [38, 39]. Kemal *et al.* [34] showed that catechols reduced the catalytically active ferric of soybean LOX to the inactive ferrous form. The reduction of the Fe^{3+} LOX to the inactive Fe^{2+} form also may be performed by *ortho*-dihydroxyisoflavonoids, according to the redox cycle for LOX activation and inactivation proposed by Hatzelmann *et al.* [35]. Isoflavonoids may act as the H-atom donor and/or the radical scavenger in this cycle.

6,7-Diacetyl-4′-methoxyisoflavan (4) and 6,7-methylenedioxy-4′-methoxyisoflavan (18) which both lack the free hydroxy- group showed a strong inhibition of 5-LOX. The diacetyl- and the methylenedioxy- groups may be split off by cellular hydrolases to yield free hydroxy- groups.

The antioxidative activity of the isoflavonoids studied was examined in our laboratories [6, 7, 40]. Isoflavans, especially those containing *ortho*-dihydroxy- groups in ring A, inhibited strongly the autoxidation of vitamin E-free lard (this is for the most part in accordance with the structure–relationship found for 5-LOX inhibition). 6,7-Dihydroxyisoflavonoids from fermented soy oil were 10–20 times stronger antioxidants than vitamin E [41–43].

Comparing the effect of *dl*-α-tocopherol and the *ortho*-dihydroxyisoflavans on porcine 5-LOX, the isoflavonoids proved to be on average 150 times more active than the tocopherol. K_i values for *n*-propyl gallate, L-ascorbic acid and ascorbyl palmitate were also higher than those for the isoflavans. The inhibitory potency of BHA and BHT is comparable with the inhibition caused by isoflavonoids. However, the use of BHA and BHT as food additives is controversial [44, 45].

During the 5-LOX assay viability of the leukocytes (Trypan blue exclusion) in the presence of isoflavonoids was 80–105% of the control. In the presence of BHT the viability decreased to 37% of the control value. Some isoflavonoids were tested on P 388 leukemia in mice and were found, up to 240 mg/kg test animal, not to exert any cytotoxic effect [46]. The bioavailability of a physiologically active compound tested *in vitro* is one of the factors determinative for a possible therapeutic use of such compounds. Few data are available on the metabolism and pharmacokinetics of isoflavonoids [9, 47] (for review see Ref. 48). These studies concerned distribution, storage and elimination of isoflavonoids. We have investigated the absorption of some isoflavonoids on isolated intestinal segments of the rat (unpublished data).

REFERENCES

1. Hagmann M and Griesebach H, Enzymatic rearrangement of flavone to isoflavone. *FEBS Lett* **175**: 199–202, 1984.
2. Dewick PM, Isoflavonoids. In: *The Flavonoids. Advances in Research since 1980* (Ed. Harborne JB), pp. 125–209. Chapman and Hall, London, 1988.
3. Kumar RJ, Krupadanam GLD and Srimannarayana G, Isoflavans from milettia racemosa. *Phytochemistry* **28**: 913–916, 1989.
4. Harborne JB, Flavonoids in the environment: structure–activity relationship. In: *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties* (Eds. Cody V, Middleton E and Harborne JB), pp. 17–28. Alan R. Liss, New York, 1988.
5. Sharma RD, Isoflavones and hypercholesterolemia in rats. *Lipids* **14**: 535–540, 1979.
6. Bergmann Ch, Vergleichende Untersuchung natürlicher und synthetischer Isoflavonoide bezüglich ihrer antioxydativen, antihämolytischen und hypolipidämischen Eigenschaften. Dissertation, University Bonn, 1981.
7. Bulut M, Synthese sowie antioxidative, hypolipidämische Aktivitäten von natürlichen Isoflavonoiden, insbesondere von Isoflavonen, Isoflavanonen, Isoflavanen und deren Derivaten. Dissertation, University Bonn, 1982.
8. Varma S, Inhibition of aldose reduction by flavonoids. In: *Plant Flavonoids in Biology and Medicine. Progress in Clinical and Biological Research, Vol. 213* (Eds. Cody V, Middleton E and Harborne JB), pp. 343–358. Alan R. Liss, New York, 1986.
9. Pfaar U, Versuche zur Enantiomerentrennung von Isoflavonoiden und Wirkung natürlicher und synthetischer Isoflavonoide auf Katarakt induzierende Systeme. Dissertation, University Bonn, 1988.
10. Kuhl P, Shiloh R, Jha H, Murawski U and Zilliken F, 6,7,4′-Trihydroxyisoflavan: a potent and selective inhibitor of porcine 5-lipoxygenase in human and peripheral blood leukocytes. *Prostaglandins* **28**: 783–804, 1984.
11. Weissen N, Inhibition des Arachidonsäuremetabolismus durch Isoflavonoide. Dissertation, University Bonn, 1985.
12. Moroney MA, Alcaraz MJ, Forder RA, Carey F and Hoult JRS, Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J Pharm Pharmacol* **40**: 787–792, 1988.
13. Gábor M, Szent-György and the bioflavonoids: new results and perspectives of pharmacological research into benzopyrone derivates. In: *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties* (Eds. Cody V, Middleton E and Harborne JB), pp. 187–200. Alan R. Liss, New York, 1988.

14. Goda Y, Katayama M, Ichikawa K, Shibuya M, Kinchi F and Sankawa U, Inhibitors of prostaglandin biosynthesis from *Dalbergia odoifera*. *Chem Pharm Bull* **33**: 5606–5609, 1985.
15. Borgeat P, Naden M, Salari H, Poubelle A and Fruteau de Laclos B, Leukotrienes: biosynthesis, metabolism and analysis. *Adv Lipid Res* **21**: 47–77, 1985.
16. Borgeat P, Biochemistry of the lipoxygenase pathway in neutrophils. *Can J Physiol Pharmacol* **67**: 936–942, 1989.
17. Spector AA, Gordon JA and Moore SA, Hydroxyeicosatetraenoic acids (HETEs). *Prog Lipid Res* **27**: 271–323, 1988.
18. Albert AI and Zilliken FW, USP, no. 4,814,346, 1989.
19. Wagner H and Fessler B, *In vitro* 5-Lipoxygenasehemmung durch *Eclipta alba* Extrakte und das Coumestanderivat Wedelolacton. *Planta Medica* **52**: 374–377, 1986.
20. Kaneko S, Ueda N, Tonai T, Maruyama T, Yoshimoto T and Yamamoto S, Arachidonic 5-lipoxygenase of porcine leukocytes studied by enzyme immunoassay using monoclonal antibodies. *J Biol Chem* **262**: 6741–6745, 1987.
21. Yoshimoto T, Furukawa M, Yamamoto S, Horie I and Watanabe-Kohno S, Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochem Biophys Res Commun* **116**: 612–618, 1983.
22. Furukawa M, Yoshimoto T, Ochi K and Yamamoto S, Studies on arachidonate 5-lipoxygenase of rat basophilic leukemia cells. *Biochim Biophys Acta* **795**: 458–465, 1984.
23. Welton AF, Tobias LD, Fiedler-Nagy C, Anderson W, Hope W, Meyers K and Coffey JW, Effect of flavonoids on arachidonic acid metabolism. In: *Plant Flavonoids in Biology and Medicine. Progress in Clinical and Biological Research, Vol. 213* (Eds. Cody V, Middleton E and Harborne JB), pp. 231–244. Alan R. Liss, New York, 1986.
24. Kimura Y, Okuda H and Arichi S, Studies on scutellariae radix effects of various flavonoids on arachidonic acid metabolism in leukocytes. *Planta Medica* **51**: 132–136, 1985.
25. Koshihara Y, Neichi T, Murota S-I, Fujimoto Y and Tatsuno T, Selective inhibition of 5-lipoxygenase by natural compounds isolated from chinese plants, *Artemisa rubipes nakai*. *FEBS Lett* **158**: 41–44, 1983.
26. Hope WC, Welton AF, Fiedler-Nagy C, Batula-Bernardo C and Coffrey JW, *In vitro* inhibition of the biosynthesis of slow reacting substances of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. *Biochem Pharmacol* **32**: 367–371, 1983.
27. Corvazier E and Maclouf J, Interference of some flavonoids and non-steroidal anti-inflammatory drugs with oxidative metabolism of arachidonic acid by human platelets and neutrophils. *Biochim Biophys Acta* **835**: 315–321, 1985.
28. Takahama U, Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanisms of antioxidative function. *Phytochemistry* **24**: 1443–1446, 1985.
29. Alcaraz MJ, Alcaraz ML and Ferrandiz Villar A, Flavonoid inhibition of soybean lipoxygenase. *Pharmazie* **41**: 299–300, 1986.
30. Wagner H, Wierer M and Bauer R, *In vitro* Hemmung der Prostaglandin-Biosynthese durch ätherische Öle und phenolische Verbindungen. *Planta Medica* **53**: 184–187, 1987.
31. Baumann J, v. Bruchhausen F and Wurm G, Hemmung der Prostaglandinsynthese durch Flavonoide und Phenolderivate im Vergleich mit deren O_2-Radikalfängereigenschaften. *Arch Pharm* **313**: 330–337, 1980.
32. Maclauf J, Laclos BF and Borgeat P, Stimulation of leukotriene biosynthesis in human blood leukocytes by platelet derived 12-hydroperoxyeicosatetraenoic acid. *Proc Natl Acad Sci USA* **79**: 6042–6046, 1982.
33. Rouzer CA and Samuelsson B, The importance of hydroperoxide activation for the detection and assay of mammalian 5-lipoxygenase. *FEBS Lett* **204**: 293–296, 1986.
34. Kemal C, Louis-Flamberg P, Krupinski-Olsen R and Shorter AL, Reductive inactivation of soybean lipoxygenase 1 by catechols: A possible mechanism for regulation of lipoxygenase activity. *Biochemistry* **26**: 7064–7072, 1987.
35. Hatzelmann A, Schatz M and Ullrich V, Involvement of glutathione peroxidase activity in the stimulation of 5-lipoxygenase activity by glutathione depleting agents in human polymorphonuclear leukocytes. *Eur J Biochem* **180**: 527–533, 1989.
36. Siegel MI, McConnell RT, Porter NA and Cuatrecasas P, Arachidonate metabolism via lipoxygenase and 12-L-hydroperoxy-5,8,10,14-eicosatetraenoic acid and peroxidase sensitive to anti-inflammatory drugs. *Proc Natl Acad Sci USA* **77**: 308–312, 1980.
37. Thody VE, Buckle DR and Foster KA, Studies on the antioxidant activity 5-lipoxygenase inhibitors. *Biochem Soc Trans* **15**: 1987–1988, 1987.
38. Galpin JR, Tielens LGM, Veldink GA, Vliegenhart JFG and Boldingh J, On the interaction of some catechol derivates with the iron atom of soybean lipoxygenase. *FEBS Lett* **69**: 179–182, 1976.
39. de Groot JJMC, Veldink GA, Vliegenhart JFG, Boldingh J, Wever R and van Gelder BF, Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. *Biochim Biophys Acta* **377**: 71–79, 1975.
40. Moustakidis G, Synthesen und antioxidative Wirkung neuer Isoflavonoide. Dissertation, University Bonn, 1988.
41. Zilliken FW, USP, no. 4,157,984, 1979.
42. Zilliken FW, USP, no. 4,264,509, 1981.
43. Zilliken FW, USP, no. 4,368,264, 1983.
44. Haigh R, Safety and necessity of antioxidants: EEC approach. *Fd Chem Toxicol* **24**: 1031–1034, 1986.
45. Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T and Tatematsu M, Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. *Fd Chem Toxicol* **24**: 1071–1082, 1986.
46. Sepulveda-Boza S, unpublished data.
47. Bannwart C, Adlercreutz H, Wahala K, Kotiaho T, Hesso A, Brunow G and Hase T, Identification of the phyto-estrogen 3',7-dihydroxyisoflavan, an isomer of equol, in human urine and cow's milk. *Biomed Environ Mass Spectrom* **17**: 1–5, 1988.
48. Griffiths LA, Mammalian metabolism of flavonoids. In: *The Flavonoids: Advances in Research* (Eds. Harborne JB and Mabry TJ), pp. 681–718. Chapman and Hall, London, 1982.