Biochemical Pharmacology, Vol. 55, pp. 1369–1375, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00644-8

Structure-Related Inhibition of Human Hepatic Caffeine N3-Demethylation by Naturally Occurring Flavonoids

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ABSTRACT. The effects of flavonoids on caffeine N3-demethylation, a marker activity of CYP1A2, in human liver microsomes were investigated to elucidate the inhibition mechanism and the structure–activity relationship. Caffeine *N*3-demethylase activity was inhibited by the presence of various flavonoids, whose structures seem to be closely related to the degree of inhibition. Among twenty-one compounds tested, the most active was chrysin with an IC_{50} value of 0.2 μM. Others had IC_{50} values ranging from 1 to more than 500 μM. Kinetic analysis revealed that the mechanism of inhibition varied among the flavonoids. The inhibitory effect was postulated to be governed by factors such as the number of hydroxyl groups and glycosylation of these free hydroxyl groups. An increase in the number of free hydroxyl groups reduced the inhibitory effect on P450 activity. Analysis of the quantitative structure–activity relationship (QSAR) showed that the volume to surface area ratio was the most effective factor on the inhibition of caffeine N3-demethylation, and the electron densities on the C3 and C4′ atoms exercised significant influence on the inhibitory effect. The calculated inhibitory effect of flavonoids on CYP1A2 activity was highly correlated with the antimutagenicity of flavonoids on 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ)-induced *umu* response. BIOCHEM PHARMACOL **55**;9: 1369–1375, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. flavonoids; caffeine N3-demethylation; inhibition; QSAR

P450¶ is a major enzyme family involved in various types of monooxygenation reactions. More than 450 isozymes were found in bacteria, plants, insects, and animals. The major role of mammalian P450 is hydroxylation of xenobiotics, resulting in either detoxification of xenobiotics or an increase in toxicity through the production of more reactive chemical species. CYP1A2 has been known to play a critical role in the metabolism of aromatic amines, estradiol, and other drugs [1, 2]. Induction of CYP1A2 by aromatic hydrocarbons and heterocyclic amines has been observed in rats and presumably in humans [1]. CYP1A2 has also been reported to be induced by cigarette smoking and charcoal-broiled meat [1] and to be affected by the intake of flavonoids, which can collectively result in the interindividual variation of the enzyme activity.

Flavonoids occur widely in the natural diet in such items as vegetables, teas, and fruits. The biological effects of flavonoids include the reduction of cardiovascular disease risk, the inhibition of hepatocytic autophagy, antiviral activity, inhibition of platelet aggregation, an anticlastogenic effect, an anti-inflammatory analgesic effect, and an antiischemic effect [3–9]. Adverse effects of flavonoids, such as mutagenicity by quercetin and lipid peroxidation by kaempferol, are reported [10, 11]. Modulation of P450 activities by flavonoids has drawn great concern due to interindividual differences in susceptibility to chemical carcinogens. It is generally known that flavonoids with free hydroxyl groups inhibit P450-dependent reactions, whereas flavonoids without hydroxyl groups stimulate P450 activity [12]. The basic structure of flavonoids is a 2-phenyl-benzo(*a*)pyrene or a flavan nucleus with two benzene rings linked through a heterocyclic pyrone C ring (Fig. 1). Li *et al.* [13] reported the effect of flavonoid structure on acetaminophen oxidation activity in rat and human microsomes and in human CYP1A2 protein expressed in HepG2 cells. The presence of a free phenolic group inhibited CYP3A-dependent acetaminophen oxidation, and the addition of a methoxy group increased the activity by 2–3 times. Most of the flavonoids used showed inhibition of CYP1A2-dependent acetaminophen oxidation. The structure of flavonoids is also important in antimutagenicity toward aflatoxin B_1. The existence

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¶ *Abbreviations:* P450, cytochrome P450; QSAR, quantitative structure–activity relationship; EGC, (−)epigallocatechin; EGCG, (−)epigallocatechin gallate; and MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline.

Received 17 April 1997; accepted 16 October 1997.

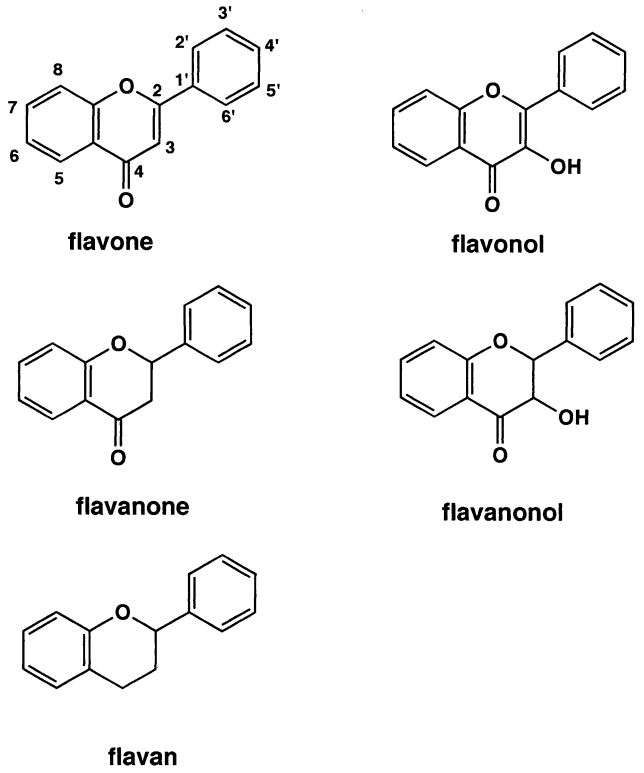

FIG. 1. Structure and numbering of flavonoids.

of a free 5,7-hydroxyl group is essential in the antimutagenicity of flavonoids [14].

In the present investigation, we have systemically investigated the relationship between flavonoids and their inhibitory effects on CYP1A2 activity by measuring the inhibitory activity of flavonoids on caffeine N3-demethylation by human liver microsomes and by computer-assisted structure–activity analysis.

MATERIALS AND METHODS

Chemicals

Chrysin, biochanin A, myricetin, and neohesperidin were purchased from the Sigma Chemical Co. EGC and EGCG were purchased from Kurida. Other flavonoids were extracted and purified according to a published procedure and confirmed by IR, MS, UV, and NMR spectroscopy [15, 16]. The purity of all flavonoids used was above 95% with the exception of myricetin (85%). Glucose-6-phosphate, $NADP^+$, glucose-6-phosphate dehydrogenase, 1,7-dimethylxanthine, *o*-nitrophenyl β-D-galactopyranoside (ONPG), sodium dodecylsulfate, and β-mercaptoethanol were purchased from Sigma. Caffeine was supplied by Fluka, and 7-ethyltheophylline was prepared as described by others [17]. Bactotryptone and bacto yeast extract were purchased from Difco, and MeIQ was purchased from Toronto Research Chemicals Inc.

Preparation of Microsomes

A human liver sample (HL136) was donated by Dr. F. P. Guengerich (Vanderbilt University), and microsomes were prepared according to previously described methods [18]. A microsomal pellet was resuspended in 0.1 M of phosphate buffer (pH 7.4) containing 20% glycerol and 1 mM of EDTA, and stored at −80° until used. Protein concentration was determined by the method of Lowry *et al.* [19], using bovine serum albumin as a standard.

Caffeine N3-Demethylation Assay

The caffeine N3-demethylation assay was carried out using the method of Grant *et al.* [20]. Caffeine and most flavonoids were dissolved in methanol. Populnin and hesperidin were dissolved in dimethyl sulfoxide. The solvent was evaporated under a stream of nitrogen gas, and 0.2 mg of microsomal protein in 0.2 mL of 0.1 M of potassium phosphate buffer (pH 7.4) was added. The final concentration of dimethyl sulfoxide did not exceed 1%. After preincubation at 37° for 5 min, the reaction was initiated by adding the NADPH-generating system (10 mM of glucose-6-phosphate, 0.67 mM of $NADP^+$ and 1 unit of glucose-6-phosphate dehydrogenase). The reaction was stopped by the addition of 50 μL of ice-cold 20% trichloroacetic acid (TCA) solution. Twenty microliters of 0.1 μM of 7-ethyltheophylline in 0.05 N of NaOH was added as an internal standard. The reaction mixture was extracted twice with 400 μL of a chloroform:isopropanol (85:15, v/v) mixture. The combined organic layer was dried *in vacuo*, and the residue was dissolved in HPLC solvents. Then the extracts were analyzed on a reverse phase C18 column (Resolve, 5 mm, 3.9 × 150 mm, Waters) using Waters HPLC. A mixture of 20 mM potassium phosphate (pH 6.8) and acetonitrile (91:1) was used as the mobile phase. The eluent was monitored at 272 nm at a flow rate of 1.2 mL/min. K_m, V_{max}, and K_i values were calculated by using the Pharmacologic Calculation system (PHARM/PCS version 4).

QSAR

INSIGHT II (version 2.3.5) was used for molecular model design. The molecular descriptors used in the statistical analysis were: the electron densities at the C3, C5, and C4′ site, the torsion angle between the C2 atom and the B ring (Phi), the length of the C3 side chain (dL in the A ring), the volume difference between substrate and flavonoid (dV), the dipole moment of the flavonoid molecule (μ), the volume to surface area ratio of the flavonoid (V/S), and the Hammett coefficient of the B ring (σ). The V/S value was calculated using a Cray supercomputer, and the other descriptors were calculated by the Mopac program. The calculated descriptors were processed for SAS analysis (SAS Institute Inc.), and the structure–activity relationships were obtained quantitatively.

SOS Chromotest

The SOS chromotest was done by the method of Quillardet and Hofnung [21]. A frozen tester strain of *Escherichia coli*

TABLE 1. IC_{50} Values for naturally occurring flavonoids on caffeine N3-demethylation activity by human hepatic microsomes

	Flavonoids	Structure	IC_{50} (M)
Flavone	Chrysin	5,7-Dihydroxyflavone	2.0×10^{-7}
	Apigenin	4′,5,7-Trihydroxyflavone	1.35×10^{-6}
	Luteolin	3′,4′,5,7-Tetrahydroflavone	1.34×10^{-5}
	Biochanin A	5,7-Dihydroxy-4′-methoxyflavone	ND*
Flavonol	Galangin	3,5,7-Trihydroxyflavone	3.06×10^{-6}
	Quercetin	3′,4′,3,5,7-Pentahydroxyflavone	1.69×10^{-4}
	Guaiyaverin	Quercetin 3-*O*-arabinopyranose	3.68×10^{-4}
	Avicularin	Quercetin 3-*O*-arabinofuranose	3.77×10^{-4}
	Quercitrin	Quercetin 3-*O*-Rha	2.24×10^{-4}
	Myricetin	3′,4′,5′,3,5,7-Hexahydroxyflavone	1.85×10^{-4}
	Fisetin	3′,4′,3,7-Tetrahydroxyflavone	2.37×10^{-4}
	Morin	2′,4′,3,5,7-Pentahydroxyflavone	9.46×10^{-6}
	Kaempferol	4′3,5,7-Tetrahydroxyflavone	7.34×10^{-5}
	Panasenoside	Kaempferol 3-*O*-Gal-Glu	3.68×10^{-4}
	Populnin	Kaempferol 7-*O*-Rha	3.27×10^{-4}
Flavanone	Hesperetin	3′,5,7-Trihydroxyflavanone	2.72×10^{-4}
	Neohesperidin	Hesperetin 7-*O*-neohesperidoside	5.05×10^{-4}
	Prunin	Naringenin 7-*O*-Glu	2.73×10^{-4}
	Hesperetin 5-glucoside	Hesperetin 5-*O*-Glu	$>6 \times 10^{-4}$
	Hesperidin	Hesperetin 7-*O*-rutinose	4.89×10^{-4}
	Naringenin	4′,5,7-Trihydroxyflavanone	1.82×10^{-4}
Flavan	(−)Epigallocatechin (EGC)	3′,4′,5′,5,7-Hexahydroxyflavan	1.05×10^{-4}
	(−)Epigallocatechin gallate (EGCG)	(−)Epigallocatechin 3-*O*-gallate	2.05×10^{-4}

*ND = not determined.

PQ37 was inoculated in Luria Broth (LB) supplemented with 20 μg/mL of ampicillin and incubated for 20 hr at 37°. Culture was transferred to fresh LB medium and incubated until $O.D._{600}$ = 0.06 to 0.08. Tester strain solution was added to each test tube containing solvent, MeIQ, and flavonoid. An SOS reaction was induced by the addition of microsomal mixture and NADPH-generating system (final volume: 1 mL); 0.3 mL of solution was transferred to a fresh tube for β-galactosidase and alkaline phosphatase assays after 2 hr of incubation at 37°. Induction of *umu* expression was represented as β-galactosidase activity divided by alkaline phosphatase activity.

RESULTS

Inhibitory Activity

The IC_{50} values of flavonoids on caffeine N3-demethylation are presented in Table 1, along with the structure of each flavonoid. The remaining activity over the negative control varied from 3 (chrysin) to 152% (populnin). Inhibition of CYP1A2 activity by flavonoids was dependent on the basic structure and concentration of the flavonoids. Flavones showed higher inhibitory activity than flavonols (apigenin > kaempferol, chrysin > galangin, luteolin > quercetin). The degree of inhibition by naringenin (a flavanone) was between those of apigenin and kaempferol. The inhibitory activities of flavonoids are postulated to be controlled by several factors. Among the flavones, chrysin, with two hydroxyl groups, showed higher inhibitory activity than other flavones, e.g. apigenin (3) and luteolin (4).* Flavonols showed a similar tendency with an increasing degree of hydroxylation. The order of inhibitory activities among flavonols was: galangin (3), kaempferol (4) > fisetin (4) > quercetin (5) > myricetin (6).* Morin, containing five hydroxyl groups, showed exceptionally high inhibitory activity, indicating that the site of hydroxylation and the number of hydroxyl groups play a role in the inhibition of CYP1A2 activity.

Kinetic Analysis

The mode of inhibition was studied further, using four flavonoids. To generate the Lineweaver–Burk plot, IC_{20}, IC_{50}, and IC_{80} were used as concentrations of the flavonoids, and caffeine concentrations of 0.1 to 4 μM were used. The results are shown in Table 2 and Fig. 2. Apigenin was a competitive inhibitor at a low concentration. By increasing the apigenin concentration above 1.5 μM, the mode of inhibition was changed from competitive to mixed. EGCG showed competitive inhibition. Noncompetitive inhibition was observed with kaempferol and naringenin. The K_i values of kaempferol and naringenin determined from this analysis were lower than the IC_{50} values.

* The numbers in parentheses are the number of hydroxyl groups in the flavonoids.

TABLE 2. Kinetic parameters associated with flavonoids in the inhibition of caffeine N3-demethylation of cytochrome P450

Flavonoids	Inhibition type	K_m (mM)	V_{max} (nmol/mg/min)	K_i (μM)
Control		0.69	291.09	
Apigenin, 0.1 μM	Competitive	0.66	274.0	4.87
Apigenin, 1.5 μM	Mixed	1.12	191.2	1.49
Apigenin, 6 μM	Mixed	0.78	95.35	2.11
EGCG, 30 μM	Competitive	0.63	236.98	171.98
EGCG, 100 μM	Competitive	0.44	126.24	108.26
EGCG, 200 μM	Competitive	0.76	136.42	160.76
Kaempferol, 5 μM	Non-competitive	0.55	177.73	12.03
Kaempferol, 20 μM	Non-competitive	0.61	194.68	16.65
Kaempferol, 80 μM	Non-competitive	1.02	164.60	28.55
Naringenin, 2 μM	Non-competitive	0.58	150.82	2.56
Naringenin, 10 μM	Non-competitive	0.81	142.20	8.11
Naringenin, 30 μM	Non-competitive	0.77	120.81	19.34

Computer-Assisted QSAR

The molecular descriptors used to calculate the structure–activity relationships represent physicochemical characteristics including hydrophobicity, electronic property, size, and shape [22]. The equation determined from SAS statistical analysis is as follows:

$$-\log IC = 52.0 - 31.5[V/S] - 3.43 \times 10^{-3}[Phi] + 6.12[\sigma] + 2.40 \times 10^{-4}[dV] + 0.52[dL] + 0.63[\mu] + 6.57[C4'] - 2.42[C3] + 1.03[C5].r = 0.775$$

The statistical data are summarized in Table 3, and the relationship between calculated and observed IC_{50} values is shown in Fig. 3. V/S (volume to surface area ratio) had the greatest effect on the inhibitory activity. The sigma factor and electron densities at C4′ and C3 atoms also had significant influence on the inhibitory activity. Planar molecules with small V/S values turned out to possess high inhibitory activity.

Antimutagenicity of Flavonoids on MeIQ

The inhibitory effect of flavonoids on the MeIQ induction of an SOS gene in *E. coli* PQ37 was determined. Eight

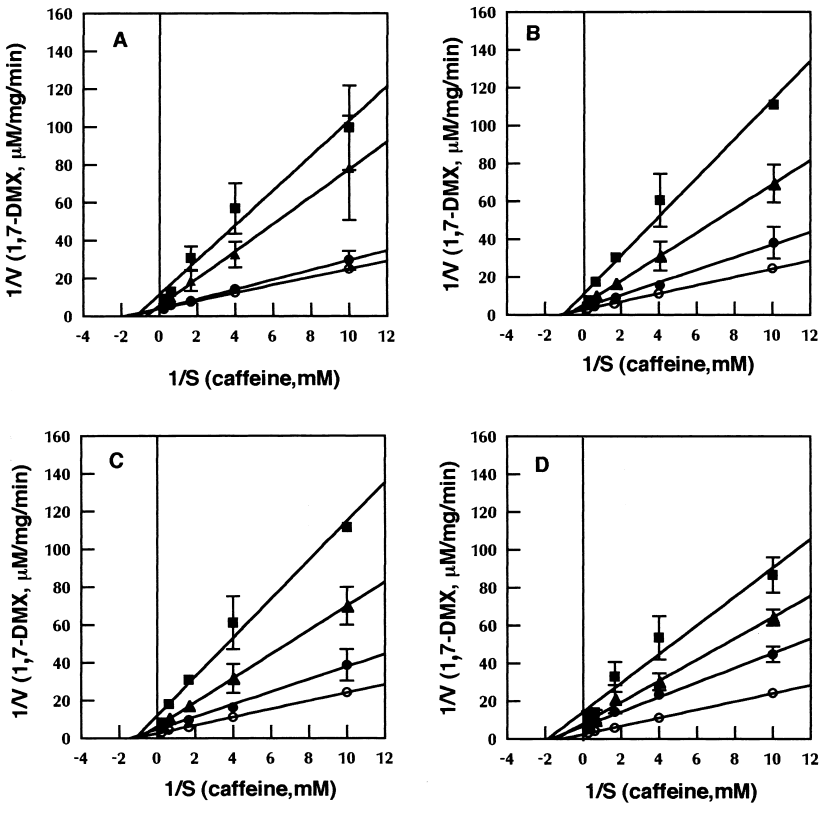

FIG. 2. Lineweaver–Burk plots of CYP1A2 inhibition by flavonoids. The incubation mixture contained microsomal protein (HL136, 0.2 mg protein), NADPH-generating system, and caffeine (0.1 to 4 mM). The reaction was incubated at 37° for 2 hr. 1,7-DMX = 1,7-dimethylxanthine. (A) Apigenin concentrations: 0 (○), 0.1 (●), 1.5 (▲), and 6 (■) μM. (B) Kaempferol concentrations: 0 (○), 5 (●), 20 (▲), and 80 (■) μM. (C) Naringenin concentrations: 0 (○), 2 (●), 10 (▲), and 30 (■) μM. (D) EGCG concentrations: 0 (○), 30 (●), 100 (▲), and 300 (■) μM. Values are means ± SEM (N = 3).

TABLE 3. Molecular descriptors of flavonoids and their inhibitory activity (IC_{50}) on CYP1A2*

Flavonoid	V/S	Phi	σ	dV	dL	μ	C3	C5	C4′	−log IC
Chrysin	1.56	0.00	0.00	316.9	1.00	4.162	−0.100	0.030	−.010	6.70
Apigenin	1.55	61.87	−0.37	355.3	1.00	3.660	−0.100	0.030	0.139	5.87
Galangin	1.55	65.45	0.00	405.4	1.00	3.151	−0.041	0.248	−0.100	5.51
Morin	1.58	86.30	−0.25	417.9	1.74	3.574	−0.051	0.245	0.170	5.02
Luteolin	1.56	63.88	−0.25	378.5	1.00	3.645	−0.200	0.241	0.110	4.87
Naringenin	1.56	43.47	−0.37	373.8	1.00	2.980	−0.073	0.248	0.127	4.74
Kaempferol	1.55	109.75	−0.37	401.4	1.74	3.195	−0.045	0.247	0.129	4.13
Myricetin	1.57	58.56	−0.13	419.8	1.74	4.030	−0.020	−0.030	−0.216	3.73
EGC	1.59	36.22	−0.13	427.5	1.74	3.186	0.050	0.030	0.030	3.69
Quercetin	1.70	110.89	−0.25	719.5	5.45	5.290	0.137	0.030	0.112	3.28
Fisetin	1.55	0.00	−0.25	460.4	1.00	3.216	−0.100	−0.100	0.030	3.63
Hesperetin	1.58	43.46	−0.15	285.8	1.00	1.650	−0.200	0.030	0.028	3.57
Populnin	1.65	62.88	−0.37	1497.8	1.74	5.400	−0.042	0.243	0.129	3.49
Panasenoside	1.73	105.54	−0.37	1076.1	12.27	2.930	0.137	0.030	0.030	3.43
Avicularin	1.70	80.92	−0.25	748.6	6.00	5.220	−0.010	0.241	0.030	3.42
Hesperetin 5-Glu	1.65	43.41	−0.15	762.2	1.00	3.371	−0.200	0.028	0.132	3.22

*Key: V/S, volume to surface area ratio of the flavonoid; Phi, torsion angle between the C2 atom and the B ring; σ, Hammett coefficient of the B ring, dV, volume difference between substrate and flavonoid; dL, length of the C3 side chain; μ, dipole moment of a flavonoid; and C3, C5, and C4′, electronic densities of C3, C5, and C4′ atoms.

flavonoids were selected to investigate the relationship between antimutagenicity and inhibitory activity. In the presence of MeIQ, induction of *umu* gene expression was 7-fold higher than in the control. Chrysin and galangin showed the highest antimutagenicity among the flavonoids tested. The suppression of MeIQ-induced *umu* gene expression by flavonoids was well correlated with the calculated CYP1A2 inhibitory potency of flavonoids (Fig. 4).

DISCUSSION

The ability of flavonoids to regulate P450 activity has been studied extensively [13]. In an experiment on benzo(*a*)pyrene and aflatoxin B_1 metabolism using human liver microsomes, it was determined that all the flavonoids possessing hydroxyl groups significantly inhibited the hydroxylation of benzo(*a*)pyrene [12]. On the other hand, flavonoids lacking hydroxyl groups stimulated benzo(*a*)pyrene hydroxylation and aflatoxin B_1 metabolism. In acetaminophen oxidation, CYP1A2-dependent oxidation was inhibited by all flavonoids. Since these experiments used only a limited number of flavonoids, it is necessary to use more flavonoids in order to deduce a structure–activity relationship.

In this study, similar trends were observed in flavonoid-induced inhibition of caffeine *N*3-demethylase activity. The basic structure of flavonoids and other structural factors turned out to be important in the inhibition mechanism. Flavone exhibited higher CYP1A2 inhibition than flavonol. Other factors that controlled inhibition

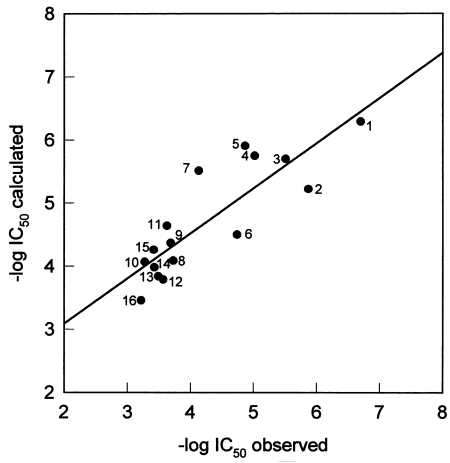

FIG. 3. Relationship between log IC_{50} calculated and log IC_{50} observed. Key: (1) chrysin, (2) apigenin, (3) galangin, (4) morin, (5) luteolin, (6) naringenin, (7) kaempferol, (8) myricetin, (9) EGC, (10) quercetin, (11) fisetin, (12) hesperetin, (13) populnin, (14) panasenoside, (15) avicularin, and (16) hesperetin 5-Glu.

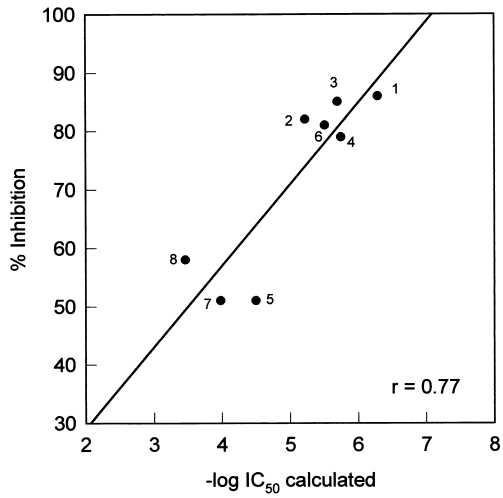

FIG. 4. Relationship between calculated −log IC_{50} on CYP1A2 activity and the inhibition of MeIQ-induced *umu* response by flavonoids. Key: (1) chrysin, (2) apigenin, (3) galangin, (4) morin, (5) naringenin, (6) kaempferol, (7) panasenoside, and (8) hesperetin 5-Glu.

were: (a) as the number of hydroxyl groups increased, the ability to inhibit decreased; (b) glycosylation of the free hydroxyl groups in flavonols decreased the ability to inhibit; the glycosylation of kaempferol abolished inhibitory activity and the glycosylated complex of quercetin showed a similar decrease; and (c) addition of a methoxy group to apigenin resulted in a drastic decrease in inhibitory activity. However, it seems that inhibitory activity cannot be ascribed only to the number of hydroxyl groups in the B ring of flavonoids. It should be noted that consideration of the structural characteristics is essential for detailed knowledge on the inhibition mechanism. Morin showed exceptionally high inhibitory activity in view of the role of the number of hydroxyl groups. The hydroxyl group at the C2′ atom of the B ring may be critical in the inhibition of CYP1A2 activity. Because morin is the only compound possessing a hydroxyl group at the C2′ atom of the B ring, it is necessary to obtain more data in order to generalize the importance of hydroxylation at the 2′ site on inhibition of CYP1A2 activity. Besides the importance of hydroxyl groups on the B ring, the orientation of the hydroxyl groups in the A ring also seems to contribute to the inhibitory activity of flavonoids. This assumption agrees well with the fact that the hydroxyl groups at C5 and C7 were crucial for inhibiting the metabolism of benzo(*a*)pyrene by rat liver microsomes [23].

The structure–activity relationship in the inhibition of P450 by coumarins was reported by Cai *et al.* [24]. The presence of a double bond in the furano or pyrano ring seems to be essential for inhibitory activity. Inhibition modes of ethoxyresorufin *O*-deethylase activity were closely related to the structure of coumarins. Coumarins bearing a C9 alkoxy group exhibited competitive inhibition, and coumarins without the C9 alkoxy group, but with a C4 alkoxy substituent showed non-competitive and mixed inhibition. Both kaempferol and naringenin underwent non-competitive modes of inhibition. It is assumed that there is no apparent relationship between the structures of flavonoids in inhibition of caffeine N3-demethylation, but it is necessary to obtain more data to generalize this assumption. Kaempferol, naringenin, and EGCG showed unaltered modes of inhibition depending on the concentration of flavonoids used, implying selective inhibition of CYP1A2 by these flavonoids.

Analysis of the relationship between structural factors of substrates and biological activity has been reported on P450 isozymes [25]. The metabolites of biphenyl and diaromatic compounds generated by CYP2D6 showed regiospecific hydroxylation. Electrophilic frontier values are a key factor in determining the site of hydroxylation. The rates of oxidative metabolism of various nitriles by CYP2E were calculated with respect to molecular polarizability and excitation energy. In the case of the CYP1A2 inhibition by flavonoids, the molecular shape and the electron densities at C3 and C4′ are the most contributing factors to biological activity. Planar flavonoids seem to have higher inhibitory activity.

There are two types of ligand–protein interactions at the active site of P450. Polar components of the ligand interact with polar moieties of the enzyme, such as the iron center or the polar functional group of the adjacent residue. Hydrophobic interactions play an important role in the binding of nonpolar components of the inhibitor to the hydrophobic residue of the protein. Hansch and Zhang [26] showed that polarizability is the most important factor in inhibition of microsomal arylhydroxylase by benzimidazoles. A simple linear relationship between polarizability and $\log(1/IC_{50})$ was obtained with a high correlation. Inhibition of aldrin epoxidation by alkylimidazoles showed a close relationship to the polarizability of the inhibitor. Unlike previous QSAR studies on microsomal P450 reactions, the most important factor in the inhibition of caffeine N3-demethylation by flavonoids was the area to volume ratio. Our results suggest that in determining inhibitory ability, the hydrophobic interaction between CYP1A2 and flavonoids seems to be the more crucial factor than the electronic property of the flavonoids.

Plant flavonoids have been demonstrated to possess a variety of biochemical and pharmacological activities. The antimutagenic effects of flavonoids and their relationship with flavonoid structure were tested using the *Salmonella* assay system [14, 26]. McGregor and Jurd [27] reported that factors such as a free hydroxyl group at C3, a double bond between C2 and C3 atoms, and a keto group at the C4 atom are essential in expressing antimutagenicity of flavonoids. On the other hand, free hydroxyl groups at the C5 and C7 atom are the controlling factors in aflatoxin B_1-derived mutation [14]. The mechanism of antimutagenicity is thought to be different depending on the mutagens. Human CYP1A1 and 1A2 are capable of activating food-derived heterocyclic aromatic amines—IQ, MeIQ, MeIQx, DiMeIQx, and PhIP. CYP1A2 is more efficient in activating heterocyclic amines to mutagens than is CYP1A1, by an order of magnitude [28]. Inhibition of CYP1A2 activity by flavonoids results in an antimutagenic effect against those amine mutagens. It is reported that the antimutagenicity of flavonoids was seen only in the mutation caused by chemicals that can be activated by the P450 enzyme [29]. The relationship between the *umu* response and the IC_{50} value of flavonoids is shown in Fig. 4. The antimutagenicity of flavonoids showed a high correlation with the IC_{50} value ($r = 0.77$), although naringin, panasenoside, kaempferol, and galangin deviated from the correlation. Therefore, the structure–activity relationship can be applied to the antimutagenicity of flavonoids on mutagens.

In summary, the inhibition of CYP1A2 activity by flavonoids showed a close relationship to the structure of the flavonoids, which was also reflected in the antimutagenicity tested by the SOS chromotest assay. The basic structure of the flavonoid, the number of free hydroxyl groups, and glycosylation were identified as being the major factors in determining inhibitory effect.

This work was supported, in part, by grants from the Korea Ministry of Science and Technology and by grants from the Korea Ministry of Health and Welfare.

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