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Inhibitory Activity of Natural Occurring Antioxidants on Thiyl Radical-Induced *trans*-Arachidonic Acid Formation

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Supporting Information

ABSTRACT: *trans*-Fatty acids in humans not only may be obtained exogenously from food intake but also could be generated endogenously in tissues. The endogenous generation of *trans*-fatty acids, especially in the cell membranes induced by radical stress, is an inevitable source for the living species. Thiyl radicals generated from thiols act as the catalyst for the *cis*−*trans* isomerization of fatty acids. Arachidonic acid (5c,8c,11c,14c-20:4) with only two of the four double bonds deriving from linoleic acid in the diet can be used to differentiate the exogenous or endogenous formation of double bonds. The aim of this study is to evaluate the effective compounds in preventing thiyl radical-induced *trans*-arachidonic acid formation during UV irradiation in vitro. The *trans*-arachidonic acids were found to be 75% after 30 min UV irradiation of all-*cis*-arachidonic acid. Myricetin, luteolin, and quercetin had the highest thiyl radical scavenging activities, whereas sesamol, gallic acid, and vitamins A, C, and E had the lowest. The structures of flavonoids with higher thiyl radical scavenging activities were a 3′,4′-*o*-dihydroxyl group in the B ring and a 2,3-double bond combined with a 4-keto group in the C ring. These effective compounds found in the present work may be used as lead compounds for the potential inhibitors in the formation of *trans*-fatty acids in vivo.

KEYWORDS: *trans*-fatty acids, arachidonic acid, thiyl radicals, antioxidants, flavonoids

INTRODUCTION

trans-Fatty acids (TFAs), which are unsaturated fatty acids with at least one double bond in the *trans*-configuration, are formed when vegetable oils are partially hydrogenated to semi-solid fats for use in margarine and shortening. For many years, TFAs in living systems have been considered as originating from exogenous dietary sources. However, recent studies showed that these exogenous dietary sources could not account for the total TFAs in the living species, and TFAs could be induced by radical stress in vivo. Three possible free radicals including thiyl radicals, sulfhydryl radicals, and nitrogen dioxide have been recognized as the free radical paths for the generation of TFA.[1−9] After a cumulative radiolysis dose of 5 kGy, all-*cis*-arachidonic acid was halved, and the geometrical isomers were found to be 75% in the 2-mercaptoethanol (2-ME)-added solution in which the $HOCH_2CH_2S^{\bullet}$ radicals were induced.[1] Biologically related thiols such as glutathione or cysteine could also induce *cis*−*trans* isomerization of unsaturated phospholipids containing oleic acid residues in either lipid solutions or lipid vesicles.[8] In addition, thiyl radicals generated from γ-irradiation of 2-ME could induce *cis*−*trans* isomerization of fatty acids in human monocytic leukemia cells.[9] The elevation of TFA induced by free radicals has also been observed in vivo. When rats were treated with carbon tetrachloride under an acute radical stress condition, 5- and 3-fold increases of the TFA were observed in erythrocyte membrane and kidney phospholipids when compared with the control group. In addition, because of continuous exposure of cellular components to increasing radicals during aging, higher amounts of TFA were accumulated in the heart and kidneys of 30 month old rats than 4 month old rats.[10]

Arachidonic acid (AA), a 20-carbon polyunsaturated fatty acid having four double bonds, is a precursor of important lipid mediators such as protaglandins and leukotrienes. Recent studies showed that the nitrogen dioxide radical ($^{\bullet}NO_2$), a potent toxic oxidant gas and a major pollutant from combustion of fossil fuels, could induce nitration of AA and other polyunsaturated fatty acids. Interestingly, nitration of AA by the nitrogen dioxide radical could also generate *trans* isomers of AA via addition/elimination of the nitrogen dioxide radical.[2] Some pathologic conditions were found to correlate with *trans*-arachidonic acids (TAAs) induced by nitro-oxidative stress. The TAA in plasma of rats increased significantly after infusion of lipopolysaccharide (LPS) from the jugular vein. This is mainly due to a LPS-induced strong response from the immune system, leading to the generation of $^{\bullet}NO$ in tissues.[3] Similarly, the ratio of TAA to AA significantly increased in serum of rats at the time points of 8, 12, and 16 weeks after streptozotocin induction.[4] The generation of TAA induced by radical stress was also found in humans. The average TAA levels in healthy humans were 68.7 nM, and in cigarette smokers, the TAA levels were 161−225% higher.[2,5,11] Moreover, TAA could induce not only a concentration- and time-dependent microvascular endothelial cell death in vitro but also retinol microvascular degeneration ex vivo and in vivo through upregulation of thromobospondin-1 (TSP-1).[6]

AA is our target fatty acid in the present study because the generation of TAA not only could correlate with radical stress but

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also be used as a marker of endogenous *trans* lipid formation. On the basis of the biosynthetic transformation of linoleic acid to AA, the double bonds of AA in positions 11 and 14 are derived from linoleic acid, and other two double bonds in positions 5 and 8 can be formed by enzymatic desaturation in vivo.[12]

In the study, we utilized thiyl radicals generated by UV irradiation of 2-ME to induce *cis*−*trans* isomerizaiton of AA, and the determination of TAA was achieved by silver ion high-performance liquid chromatography (Ag-HPLC). It has been reported that the thiyl radicals derived from photolysis of 2-ME could induce *cis*−*trans* isomerization of fatty acids such as linoleic acid and AA.[1,9,13] The mechanism of *cis*−*trans* isomerization in fatty acids occurs by the addition of thiyl radicals to the double bond to form the radical adduct, and the thermodynamically favorable *trans* isomers are formed by β-elimination of thiyl radicals.[13−15] It should be noted that positional isomers can not be formed during thiyl radical-induced isomerization of fatty acids because the mechanism does not allow a double-bond shift.

Some naturally occurring antioxidants such as vitamins and flavonoids are well-known to have free radical scavenging activities. Here, we evaluated these natural dietary antioxidants for their potential ability in preventing thiyl radical-induced TAA formation.

MATERIALS AND METHODS

Materials. 2-ME, *N*,*N*-diisopropylethylamine, 2,3,4,5,6-pentafluorobenzyl bromide (PFB-bromide), AA, sodium hydroxide, anhydrous sodium sulfate, ascorbic acid, retinyl acetate, α-tocopherol, curcumin, tetrahydrocurcumin, resveratrol, gallic acid, sesamol, all flavonids including apigenin, (+)-catechin, chrysin, daidzein, (−)-epicatechin, (−)-epigallocatechin gallate (EGCG), galangin, genistein, kaempferol, luteolin, myricetin, naringenin, quercetin, and microphotochemical reaction assembly were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, ethanol, isopropanol, acetonitrile, dichloromethane, dimethyl sulfoxide (DMSO), and *n*-hexane were purchased from Mallinckrodt Baker (Phillipsburg, NJ).

Isomerization of AA by Thiyl Radicals Induced by UV Irradiation of 2-ME. The method was adapted from Ferreri et al.[12] A solution of 1.9 mg (6.2 μmol) of AA dissolved in 800 μL of isopropanol was placed in a quartz microphotochemical reaction well. The solution was bubbled with argon for 5 min, followed by the addition of 0.25 mg of 2-ME (3.2 μmol) prior to UV irradiation. Photolysis was carried out in a microphotochemical reaction assembly equipped with a 5.5 W low-pressure UV lamp. During UV irradiation, the temperature was maintained at 22 ± 2 °C by the circulation of thermostat solution, composed of $NiSO_4 \cdot 7H_2O$ and $CoSO_4 \cdot 7H_2O$, which allows UV light pass through (250−340 nm).[16] The solution was withdrawn after UV irradiation for 10−50 min, and the solvent was removed by a stream of nitrogen gas.

Inhibitory Effects of Antioxidants on Thiyl Radical-Induced TAA Formation. The stock solution of antioxidants was prepared into 40 mM. Ascorbic acid, EGCG, sesamol, tetrahydrocurcumin, gallic acid, catechin, and epicatechin were dissolved in methanol, α-tocopherol and retinol acetate were dissolved in ethanol, and other flavonoids were dissolved in DMSO. Before the experiment, the stock solutions of antioxidants were diluted to 4 mM by isopropanol. A solution of 1.9 mg (6.2 μmol) of AA dissolved in 700 μL if *i*-PrOH was placed in a quartz microphotochemical reaction well. The solution was bubbled with argon for 5 min, followed by the addition of 0.25 mg of 2-ME (3.2 μmol) and 100 μL of antioxidant solution (0.4 μmol) prior to UV irradiation. The procedure of UV irradiation was performed as described above. The reaction time of UV irradiation was 30 min. After UV irradiation for 30 min, the sample was collected, and the solvent was removed by a stream of nitrogen gas.

Purification of AA Isomers. After UV irradiation, the residue containing AAs was partitioned between 500 μL of hexane and 0.01 M NaOH. The organic layer was collected and partitioned with 500 μL of salt water. The organic layer was collected and dried over anhydrous sodium sulfate. The solvent was removed by a stream of nitrogen gas.

Derivatization of AAs. The method was adapted from Hofmann et al.[17] The residue was dissolved in 39 μL of acetonitrile and mixed with 21 μL of PFB-bromide and 20 μL of *N*,*N*-diisopropylethylamine to prepare TAA PFB-esters. After they were incubated at 45 °C for 45 min, the solvent and excess of reagent were removed by a stream of nitrogen gas. The TAA-PFB esters were dissolved in 1 mL of hexane and subjected to purification by an open column containing silica gel (40−63 μm). The column was eluted with 15 mL of *n*-hexane to remove excess PFB bromide and unwanted byproduct. The second eluent was 15 mL of *n*-hexane:dichloromethane (7:3, v/v), which contained the desired TAA-PFB esters. After the solvent was removed by a stream of nitrogen gas, the purified TAA-PFB esters were dissolved in 200 μL of *n*-hexane, and 20 μL was injected into the HPLC.

Analysis by Ag-HPLC. The HPLC system consisted of a Hitachi L-7100 pump (Hitachi, Tokyo, Japan) equipped with a Hitachi L-7420 UV−vis detector. The separation of TAA-PFB esters was achieved by using a ChromSpher 5 Lipids analytical silver-impregnated column (250 mm × 4.6 mm, 5 μm particle size, Varian, CA). The mobile phase was 0.3% acetonitrile (v/v) in *n*-hexane and operated isocratically at a flow rate of 1.0 mL/min. The injection volume was 20 μL. The different TAA-PFB esters were detected by a UV detector at 205 nm. The inhibition percentage was calculated according to the following equation:

$$\text{inhibition } (\%) = \frac{\text{all-}cis\text{ isomer}_{\text{sample}} - \text{all-}cis\text{ isomer}_{\text{control}}}{100 - \text{all-}cis\text{ isomer}_{\text{control}}}$$

where all-*cis* isomer$_{\text{sample}}$ and all-*cis* isomer$_{\text{control}}$ represent the percentages of all-*cis* isomer in the antioxidant-treated group and control group. Data acquisition was performed on a SISC program (Taipei, Taiwan). The Supporting Information describes the identification of TAA-PFB esters isolated from Ag-HPLC by gas chromatography−electron ionization mass spectrometry (GC/EI-MS).

Statistical Analysis. All results are presented as means ± standard deviations (SDs) of at least three independent experiments. Differences between means were detected by analysis of variance, followed by multiple comparisons using Duncan's new multiple range test. Results were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Isomerization of AA by Thiyl Radicals Induced by UV Irradiation of 2-ME. Several recent studies showed that TFAs in vivo not only are originated from exogenous food sources but also are generated by free radicals such as thiyl radicals, sulfhydryl radicals, and nitrogen dioxide radicals.[1,7,18] In our current study searching for natural dietary compounds as potential inhibiors of endogenous formed TFAs, we choose AA as our target fatty acid and use thiyl radical generated by UV irradiation of 2-ME to induce *cis*−*trans* isomerizaiton of AA.

Thiyl radicals were generated by photolysis of 2-ME in isopropanol solutions. After UV irradiation, the AA and its *trans* isomers were derivatized into PFB-esters and analyzed by Ag-HPLC. The retention of fatty acids in Ag-HPLC is based on many factors such as the configuration (*cis* or *trans* form), the number (monounsaturated or polyunsaturated), and the position of double bonds in the carbon skeleton.[19] In our study, the separation of geometrical isomers of AAs was according to their number of *trans* double bonds since the *cis* configuration of

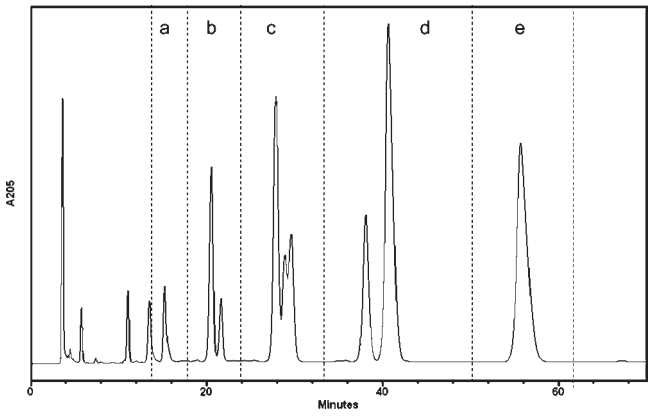

Figure 1. HPLC chromatogram of TAA-PFB esters after UV irradiation for 30 min. (a) All-*trans* isomer, (b) tri-*trans* isomers, (c) di-*trans* isomers, (d) mono-*trans* isomers, and (e) all-*cis* isomer.

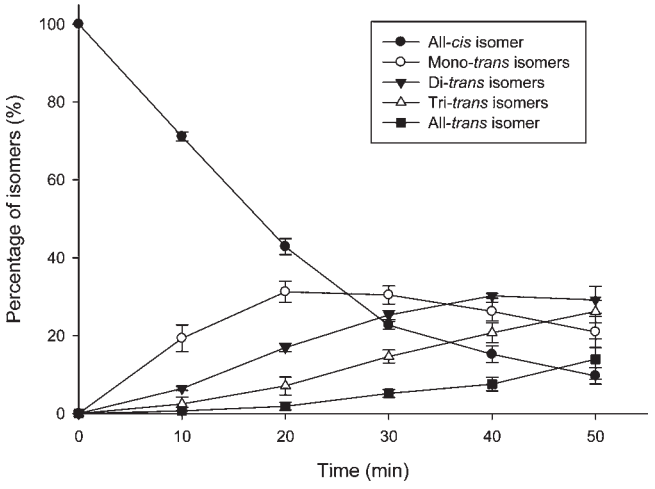

Figure 2. Time-dependent changes of all-*cis*, mono-*trans*, di-*trans*, tri-*trans*, and all-*trans* isomers of AA during UV irradiation.

unsaturated fatty acids is retained stronger than *trans* configuration.[19] The Ag-HPLC result showed that TAA-PFB esters eluted in the order of increasing number of *cis* double bonds (Figure 1). In principle, the geometrical isomers of AA contain one all-*cis* isomer, four mono-*trans* isomers, six di-*trans* isomers, four tri-*trans* isomers, and one all-*trans* isomer.[18] The detailed identification of TAA-PFB esters by GC-EI/MS is included as Supporting Information. Two peaks of mono-*trans* isomers and two peaks of tri-*trans* isomers of AA in chromatogram should each contain four isomers of AA (Figure 1b,d). Similarly, the three peaks of di-*trans* isomers should contain six isomers of AA (Figure 1c). Total isomers of AAs on the HPLC chromatography were taken as 100% and each kind of isomer interpreted as the percentage of total isomers. Time-dependent changes of all-*cis*, mono-*trans*, di-*trans*, tri-*trans*, and all-*trans* isomers of AA during UV irradiation are given in Figure 2. The dramatic decrease of the all-*cis* isomer was found during UV irradiation indicating that the *cis* double bonds of AA were quickly isomerized to *trans* configuration due to thiyl radicals. Mono-*trans* isomers increased initially and decreased after 30 min of UV irradiation because they were not only generated from the isomerizaion of the all-*cis* isomer but also were the precursors of di-*trans* isomers. Similarly, the di-*trans* isomers decreased after 40 min of UV irradiation because they were the precursors of tri-*trans* isomers. It has been reported that the isomerization of linoleic acid induced by thiyl radicals occurred as a step-by-step process that initially generated the mono-*trans* isomer (9-*trans*-linoleic acid and 12-*trans*-linoelic acid) prior to formation of the di-*trans* isomer (9-*trans*,12-*trans*-linoleic acid).[13] The tri-*trans* isomers and the all-*trans* isomer always increased during 50 min of UV irradiation. It is also worthy to know that the relative efficiency of isomerization for each *cis* double bond in a polyunsaturated fatty acid was similar and independent of their positions.[12]

Inhibitory Effects of Antixoidants on Thiyl Radical-Induced TAA Formation. Many natural occurring antioxidants are well-known to possess free radical scavenging activities. In the study, vitamins A, C, and E, and some antioxidants were studied for their potential activity in inhibiting the formation of TAA induced by thiyl radicals during UV irradiation. The results are shown in Table 1. After 30 min of UV irradiation, all-*cis* isomer was found to be 24% indicating that other 76% of AA were isomerized to mono-*trans*, di-*trans*, tri-*trans*, and all-*trans* isomers.

Retinol acetate, ascorbic acid, and α-tocophenol were selected to determine the activities of scavenging thiyl radicals in the study because it has been demonstrated that antioxidant vitamins could effectively reduce the *trans* isomerization of oleic acids, and the results showed that the ability of thiyl radical scavenging was found to increase as follows: α-tocopherol < ascorbic acid < retinol acetate.[14,20] However, our results showed that α-tocopherol, retinol acetate, and ascorbic acid had similar thiyl radical scavenging activities (24.90 ± 1.36, 24.31 ± 3.50, and 20.90 ± 5.57%, in inhibition percentage) (Table 1). The discrepancy between the literature and our result probably resulted from the low dose of UV irradiation in our system or the different experimental techniques. In addition, our study also found that sesamol and gallic acid could suppress the isomerization of AAs and their activities were similar with ascorbic acid. The antioxidant activities of sesamol and gallic acid have been found to be similar with ascorbic acid in scavenging different types of radicals such as DPPH radical, hydroxyl radical, and nitric oxide.[21,22] In other tested antioxidants, curcumin, tetrahydrocurcumin, and resveratrol had higher thiyl radical scavenging activities than vitamins A, C, and E, gallic acid, and sesamol (43.53 ± 2.38, 41.86 ± 6.87, and 40.32 ± 3.20%, in inhibition percentage). Both curcumin and its major metabolites, tetrahydrocurcumin, have chemoprevention of degenerative diseases such as Alzheimer's disease, cancer, and cardiovascular disease due to their potent antioxidant activities.[23−25] Curcumin and tetrahydrocurcumin had similar thiyl radical scavenging activities because both of them had the β-diketone moiety and two hydroxyl groups on the two aromatic rings, which are their important features to possess antioxidant activities. In addition, the result also showed that resveratrol could effectively reduce the isomerization of AAs. It has been reported that resveratrol had potent antioxidant activity, which is originated from its *para*-hydroxyl group and *meta*-hydroxyl groups.[26,27] A similar study also indicated that resveratrol could effectively inhibit the liposome oxidation induced by free radicals during γ-radiolysis or pulse radiolysis.[28] Besides, their result also indicated that resveratrol had higher free radical scavenging activities than α-tocopherol and ascorbic acid, and this trend was in accordance with our result. These results indicated that antioxidant vitamins and some polyphenol compounds, which are well-known to possess antioxidant activities, can also prevent the isomerization of fatty acids via their free radical scavenging activities.

Table 1. Inhibitory Effects of Antioxidants on Thiyl Radical-Induced TAA Formation[a]

antioxidants	all-*cis*[b]	mono-*trans*	di-*trans*	tri-*trans*	all-*trans*	inhibition (%)
control	24.43 ± 0.99 d	30.49 ± 2.35	25.31 ± 1.17	14.64 ± 1.70	5.13 ± 0.99	0.00 ± 0.00
curcumin	57.34 ± 1.42 a	24.08 ± 0.94	11.41 ± 0.50	5.46 ± 0.18	1.71 ± 0.14	43.53 ± 2.38
tetrahydrocurcumin	56.11 ± 4.71 a	25.70 ± 5.59	11.51 ± 1.65	4.55 ± 1.73	2.12 ± 0.94	41.86 ± 6.87
resveratrol	54.88 ± 3.02 a	25.97 ± 1.86	12.59 ± 1.04	5.19 ± 0.64	1.37 ± 0.29	40.32 ± 3.20
α-tocopherol	43.25 ± 1.51 bc	29.53 ± 1.53	16.56 ± 0.41	7.81 ± 0.48	2.85 ± 0.52	24.90 ± 1.36
retinol acetate	42.78 ± 3.38 bc	30.57 ± 2.67	16.34 ± 1.13	7.30 ± 0.08	3.01 ± 1.16	24.31 ± 3.50
ascorbic acid	40.23 ± 4.13 bc	30.02 ± 3.51	18.55 ± 1.73	8.21 ± 0.81	3.00 ± 1.15	20.90 ± 5.57
gallic acid	37.56 ± 4.51 c	27.81 ± 4.47	21.20 ± 1.75	10.09 ± 1.11	3.34 ± 1.80	17.31 ± 7.00
sesamol	37.03 ± 4.53 c	31.12 ± 1.72	19.56 ± 1.51	8.62 ± 1.91	3.68 ± 1.46	16.63 ± 6.56

[a] Values are expressed as means ± SDs ($n = 3$, expressed as a percentage of peak areas in the HPLC chromatogram). The time of UV irradiation is 30 min. The concentration of antioxidants is 500 μM. [b] Values with no letter in common are significantly different ($p < 0.05$).

Table 2. Inhibitory Effects of Flavonoids on Thiyl Radical-Induced TAA Formation[a]

flavonoids	all-*cis*[b]	mono-*trans*	di-*trans*	tri-*trans*	all-*trans*	inhibition (%)
control	24.43 ± 0.99 f	30.49 ± 2.35	25.31 ± 1.17	14.64 ± 1.70	5.13 ± 0.99	0.00 ± 0.00
myricetin	86.01 ± 4.40 a	10.17 ± 1.97	2.65 ± 1.79	1.00 ± 0.93	0.17 ± 0.20	81.54 ± 5.55
luteolin	84.07 ± 2.70 a	13.53 ± 2.07	1.97 ± 0.67	0.35 ± 0.05	0.09 ± 0.01	78.94 ± 3.27
quercetin	81.70 ± 5.64 ab	13.45 ± 4.40	3.22 ± 1.70	1.16 ± 1.40	0.47 ± 0.78	75.72 ± 7.73
kaempferol	76.04 ± 5.07 b	14.85 ± 1.95	5.43 ± 1.16	2.88 ± 1.29	0.80 ± 0.87	68.26 ± 6.96
apigenin	75.26 ± 4.91 b	16.31 ± 3.76	6.02 ± 1.34	2.02 ± 0.06	0.40 ± 0.12	67.20 ± 6.87
genistein	67.05 ± 4.32 c	19.45 ± 3.19	8.57 ± 1.44	3.69 ± 0.45	1.23 ± 0.54	56.35 ± 6.18
galangin	65.45 ± 3.83 c	25.52 ± 2.20	7.45 ± 1.30	1.40 ± 0.41	0.19 ± 0.06	54.24 ± 5.42
chrysin	65.07 ± 7.12 c	24.12 ± 2.83	8.31 ± 2.98	2.19 ± 1.26	0.31 ± 0.11	53.83 ± 9.00
daidzein	54.67 ± 4.61 d	24.99 ± 1.72	13.39 ± 2.37	5.47 ± 2.13	1.47 ± 0.87	40.04 ± 5.69
EGCG	54.67 ± 1.30 d	25.69 ± 1.67	11.96 ± 0.61	5.22 ± 1.11	2.46 ± 0.57	40.00 ± 1.97
epicatechin	53.99 ± 2.30 d	26.17 ± 3.66	13.32 ± 2.16	5.41 ± 2.62	1.11 ± 1.16	39.09 ± 3.44
naringenin	53.98 ± 3.64 d	25.50 ± 1.87	12.85 ± 1.92	5.52 ± 0.42	2.16 ± 0.70	39.13 ± 4.03
catechin	45.25 ± 3.97 e	32.10 ± 3.41	16.28 ± 2.03	5.46 ± 1.36	0.91 ± 0.71	27.50 ± 5.96

[a] Values are expressed as means ± SDs ($n = 3$, expressed as percentage of peak areas in the HPLC chromatogram). The time of UV irradiation is 30 min. The concentration of flavonoids is 500 μM. [b] Values with no letter in common are significantly different ($p < 0.05$).

Inhibitory Effects of Flavonoids on Thiyl Radical-Induced TAA Formation. Thirteen flavonoids were evaluated for their inhibitory effects of thiyl radical-induced *trans* arachidonic acid formation, and the results are shown in Table 2. In flavonoids, myricetin, luteolin, and quercetin had the highest thiyl radical scavenging activity, followed by kaempferol and apigenin, which had similar activities. The efficacies of the remaining flavonoids were in the following order: genistein > galangin = chrysin > daidzein = EGCG = epicatechin > naringenin > catechin.

Bors et al.[29] have indicated that three structural groups are important features for free radical scavenging activities of flavonoids: (a) the *ortho*-dihydroxy (catechol) group in the B ring, which confer a high stability to the aroxyl radical formed; (b) the 2,3-double bond combined with a 4-keto group in the C ring, which are responsible for the electron delocalization from the B ring; and (c) 3- and 5-hydroxyl groups, which allows electron delocalization from the 4-keto group to both substituents.[29] Our results show that these rules can be applied to the efficiency of flavonoid structure on inhibition of thiyl radical-induced TAA formation. The number of the B ring hydroxyl groups in flavonoids was important to thiyl radical scavenging activities. In tested flavonols, myricetin, which has a 3′,4′,5′-trihydroxy (pyrogallol) group, had the highest activity (81.54 ± 5.55%, in inhibition percentage), followed by quercetin, which has 3′,4′-*ortho*-dihydroxy (catechol) group (75.72 ± 7.73%, in inhibition percentage). Kaempferol and galangin had lower activities (68.26 ± 6.96 and 54.24 ± 5.42%, in inhibition percentage) than myricetin and quercetin since they lack the catechol group in the B ring. In flavones, luteolin, which had a catechol group in the B ring, possessed the highest thiyl radical scavenging activities (78.94 ± 3.27%, in inhibition percentage) than apigenin and chrysin (67.20 ± 6.87 and 53.83 ± 9.00%, in inhibition percentage). The trend of thiyl radical scavenging activities in flavones, which was similar with flavonols, increased with the number of the hydroxyl groups.

In flavonoids, the 2,3-double bond and 4-keto group were the other important features to contribute the higher thiyl radical scavenging activity in this study because the 2,3-double bond in conjugation with the 4-keto group can delocalize electrons from the B ring.[29] Our results showed that the loss of one or both characteristics dramatically reduced the thiyl radical scavenging activities. For flavonoids with a catechol group in the B ring (quercetin, luteolin, catechin, and epicatechin), the loss of the 2,3-double bond and 4-keto group tended to cause the reduction of thiyl radical scavenging activity. For example, catechin had much lower thiyl radical scavenging activity than quercetin (27.50 ± 5.96 vs 75.72 ± 7.73%, in inhibition percentage) because catechin lacks a 2,3-double bond and 4-keto group. 3-Hydroxyl and 5-hydroxyl groups also were proposed to be important to the antioxidant activities of flavonoids because

hydrogen bonding between the 4-keto group and the 3-hydroxyl or 5-hydroxyl groups can stabilize flavonoid radicals.[29,30] However, our result showed that the 3-hydroxyl group in the C ring might not be important to thiyl radical scavenging activities when quercetin was compared to luteolin or kaempferol was compared to apigenin. In tested flavanols (catechins), catechin and epicatechin had lower thiyl radical activities (27.50 ± 5.96 and 39.09 ± 3.44%, in inhibition percentage) than flavonols and flavones such as quercetin and luteolin because they lack the 2,3-double bond and 4-keto group in the C ring.

In summary, thiyl radicals generated from UV irradiation of 2-ME in isopropanol could effectively induce the *cis*−*trans* isomerization of AA and mono-, di-, tri-, and all-*trans* isomers of AA were produced. In tested antioxidants, curcumin, tetrahydrocurcumin, and resveratrol had higher thiyl radical scavenging activities than vitamins (A, C, and E), sesamol, and gallic acid. In 13 tested flavonoinds, myricetin, luteolin, and quercetin had higher thiyl radical scavenging activities than other flavonoids. The structural features that confer flavonoids with high thiyl radical scavenging activity were a 3′,4′-*o*-dihydroxyl group in the B ring and a 2,3-double bond combined with a 4-keto group in the C ring. The effective compounds found in this study may give a clue to find the potential inhibitors in the formation of TFAs in vivo.

■ ASSOCIATED CONTENT

Supporting Information. Identification of TAA-PFB esters by gas chromatography−electron ionization mass spectrometry. This material is available free of charge via the Internet at http://pubs.acs.org.

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