Syntheses of 5,7,8- and 5,6,7-Trioxygenated 3-Alkyl-3′,4′-dihydroxyflavones and Their Inhibitory Activities against Arachidonate 5-Lipoxygenase

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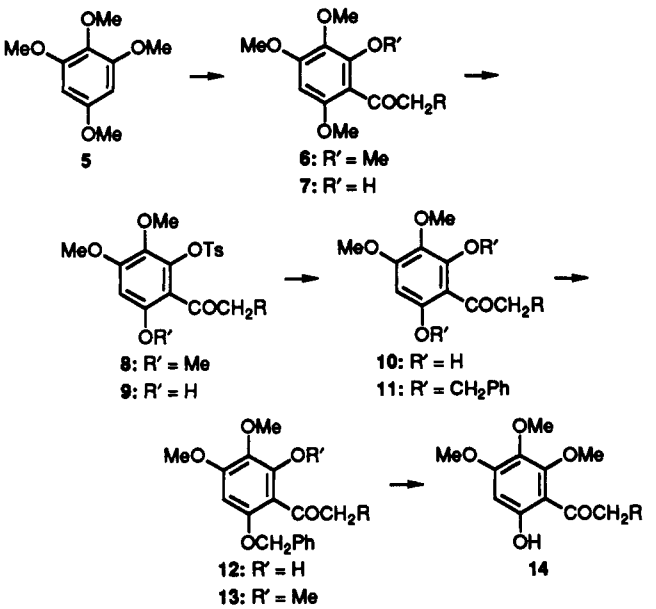
5,6,7- and 5,7,8-Trioxygenated 3′,4′-dihydroxyflavones were derivatized by introducing alkyl groups of various chain lengths at the 3-position of the flavone skeleton. These compounds were tested as inhibitors for arachidonate 5-lipoxygenase purified from porcine leukocytes. Modification of the 3-position with an alkyl group of 6–10 carbons markedly decreased the IC_{50} values. 3-Hexyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavone inhibited 5-lipoxygenase with an IC_{50} value of 58 nM. The platelet and leukocyte 12-lipoxygenases, 15-lipoxygenase of reticulocytes, and cyclooxygenase of vesicular gland were inhibited less potently (IC_{50} = 0.4, 0.4, 2.7, and 30 μM). Thus, the compound was a relatively selective inhibitor for 5-lipoxygenase.

We have been studying the selective O-alkylation and dealkylation of flavonoids to establish new convenient methods for synthesizing polyhydroxyflavones.[1] Concurrently, we have investigated the inhibitory activities of these flavones against arachidonate 5-lipoxygenase, an enzyme which initiates the biosynthesis of bioactive leukotrienes known as chemical mediators of anaphylaxis and inflammation.[2] In such a project several potent inhibitors of 5-lipoxygenase have already been found.[3-5] For example, cirsiliol (3′,4′,5-trihydroxy-6,7-dimethoxyflavone) inhibited 5-lipoxygenase,[3] and chemical modification of its 5-hydroxy group with a lipophilic alkyl group enhanced the inhibitory activity by 1 order of magnitude.[4] For in vivo application of the flavonoid inhibitor we prepared a water-soluble 5-hexyl ether derivative of cirsiliol [5-(hexyloxy)-3′,4′-dihydroxy-6,7-dimethoxyflavone 4′-(disodium phosphate)].[5] This paper reports our further attempt to develop a more potent inhibitor of 5-lipoxygenase by modification at the 3-position of the flavone skeleton.

Chemistry. The synthetic route employed for the synthesis of starting materials 2- or 6-hydroxyacylbenzenes is illustrated in Scheme I. The Friedel–Crafts reaction of 1,2,3,5-tetramethoxybenzene (**5**) with acetyl chloride in ether affords 2-hydroxy-3,4,6-trimethoxyacetophenone in favorable yield,[6] but the reaction is accompanied by 3-ethoxy-2-hydroxy-4,6-dimethoxyacetophenone.[7] The reaction of **5** with acyl chlorides also produced a large amount of the 3-ethoxy compound which was hardly removed from the desired 2-hydroxyacylbenzenes **7**.

In the reaction, however, the introduction rate of acyl group was much faster than the demethylation rate of the 2-methoxy group. Therefore, crude 2,3,4,6-tetramethoxyacylbenzenes (**6**) obtained from **5** were demethylated with anhydrous aluminum chloride in acetonitrile to give desired 2-hydroxyacylbenzenes in high yield. The direct cleavage of the 6-methoxy group in **7** is not generally achieved because of the existence of the 2-hydroxy group with the adjacent carbonyl group, and protection of the hydroxy group with sulfonyl groups is expected to solve the difficulty.[1,8] Actually, the 6-methoxy group in the tosylates (**8**) of **7** was selectively cleaved with anhydrous aluminum bromide in acetonitrile and 6-hydroxyacylbenzenes **9**, which were easily hydrolyzed into 2,6-dihydroxy-3,4-dimethoxyacylbenzenes (**10**), were obtained in quantitative yields. From our studies,[1,9-11] the selectivities of the partial dealkylation in polyalkoxyflavones is generally higher than that of partial alkylation in polyhydroxyflavones and the results suggest that 6-(benzyloxy)-2-hydroxyacylbenzenes **12** are derived more easily from **10** than **9**. Thus, acylbenzenes **12** were obtained via the corresponding dibenzyl ether **11** and converted into

Scheme I



a: R = CH_3, **b**: R = C_4H_9, **c**: R = C_6H_{13}, **d**: R = C_8H_{17}, **e**: R = $C_{10}H_{21}$, **f**: R = $C_{12}H_{25}$, **g**: R = $C_{14}H_{29}$, **h**: R = $C_{16}H_{33}$

(1) Horie, T.; Kawamura, Y.; Tsukayama, M.; Yoshizaki, S. *Chem. Pharm. Bull.* **1989**, *37*, 1216–1220 and references cited therein.
(2) Samuelsson, B. *Science* **1983**, *220*, 568–575.
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(11) Horie, T.; Kourai, H.; Fujita, N. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3773–3780.

† Department of Biochemistry.

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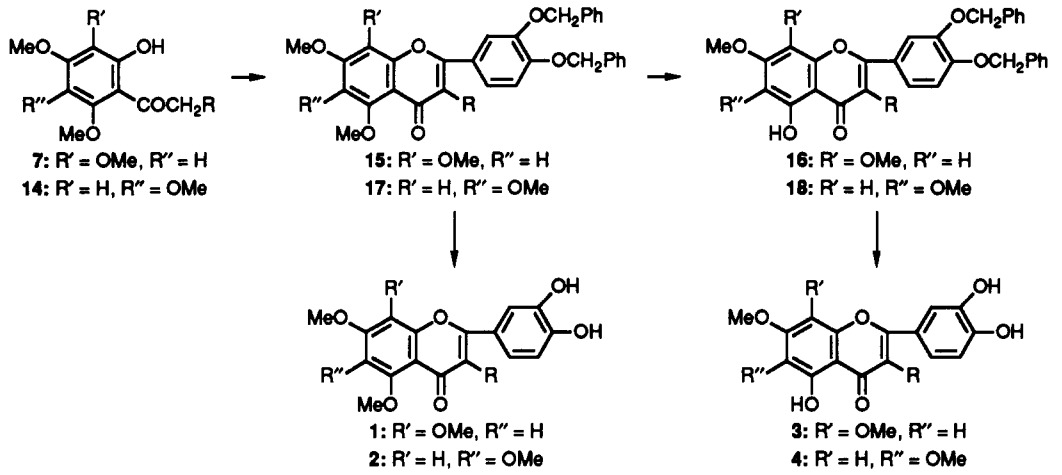
Scheme II



a: R = CH_3, b: R = C_4H_9, c: R = C_6H_{13}, d: R = C_8H_{17}, e: R = $C_{10}H_{21}$, f: R = $C_{12}H_{25}$, g: R = $C_{14}H_{29}$, h: R = $C_{16}H_{33}$

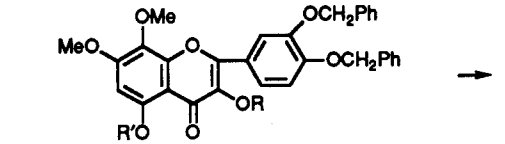
6-hydroxy-2,3,4-trimethoxyacylbenzenes (14) by the methylation and followed hydrogenolysis of the resultant methyl ether 13.

3-Alkylflavones 1–4 were synthesized from acylbenzenes 7 and 14 as shown in Scheme II. Baker–Venkataraman transformation of the 4-methoxybenzoate of **7a** with potassium hydroxide in pyridine afforded the corresponding diketone derivative, but the yield was very low because the product was unstable under the reaction conditions. Therefore, 3-alkyl-3′,4′-bis(benzyloxy)-5,7,8-trimethoxyflavones (15) were synthesized from the corresponding acylbenzenes 7 by the Allan–Robinson reaction. The 5-methoxy group in 15 was selectively cleaved with anhydrous aluminum bromide in acetonitrile to give the corresponding 5-hydroxyflavones 16. Hydrogenolysis of 16 with palladium on charcoal afforded 3-alkyl-5,3′,4′-trihydroxy-7,8-dimethoxyflavones (3) in high yields, but that of 15 formed some byproducts because of the low stability under the reduction conditions, and the yields of 3-alkyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavones (1) were 40–70%. 3-Alkyl-5,3′,4′-trihydroxy-6,7-dimethoxyflavones (4) and 3-alkyl-3′,4′-dihydroxy-5,6,7-trimethoxyflavones (2) were also synthesized from the corresponding acylbenzenes 14 by the same method (Scheme II). The physical and biological properties of the 3′,4′-dihydroxyflavones obtained here are summarized in Table I.

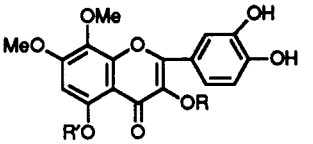
The 3-oxygenated 3′,4′-dihydroxyflavones 19–26 with a hexyloxy group were synthesized by a method as shown in Scheme III. The demethylation of 3′,4′-bis(benzyloxy)-3,5,7,8-tetramethoxyflavone (27) with anhydrous aluminum bromide in acetonitrile affords quantitatively a mixture of the corresponding 3- and 5-hydroxyflavones 28 and 29.[12] These hydroxyflavones were converted into hexyl ethers 30 and 31 by the alkylation with hexyl iodide in acetone–*N*,*N*-dimethylformamide. The demethylation of the two isomeric hexyl ethers 30 and 31 with anhydrous aluminum bromide in acetonitrile formed the corresponding 5- and 3-hydroxyflavones 32 and 33 in high yield, respectively. The 3,5,7,8-tetraoxygenated 3′,4′-dihydroxyflavones 19–22 were obtained from the corresponding 3′,4′-bis(benzyloxy)flavones 30–33 by the hydrogenolysis with palladium on charcoal, respectively.

The demethylation of 3′,4′-bis(benzyloxy)-3,5,6,7-tetramethoxyflavone (34) with anhydrous aluminum bromide affords only the corresponding 5-hydroxyflavone 35.[1] In

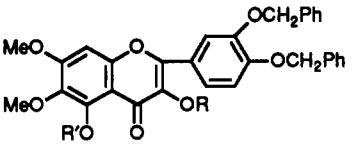
Scheme III



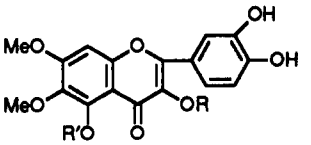
27: R = R′ =Me
28: R = H, R′ = Me
29: R = Me, R′ = H
30: R = C_6H_{13}, R′ = Me
31: R = Me, R′ = C_6H_{13}
32: R = C_6H_{13}, R′ = H
33: R = H, R′ = C_6H_{13}



19: R = C_6H_{13}, R′ = Me
20: R = Me, R′ = C_6H_{13}
21: R = C_6H_{13}, R′ = H
22: R = H, R′ = C_6H_{13}



34: R = R′ =Me
35: R = Me, R′ = H
36 :R = Me, R′ = C_6H_{13}
37: R = H, R′ =C_6H_{13},
38: R = H, R′ = Ts
39: R = C_6H_{13}, R′ = Ts
40: R = C_6H_{13}, R′ = H
41: R = C_6H_{13}, R′ = Me

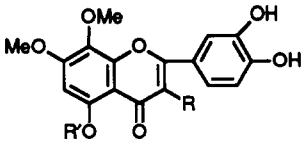


23: R = C_6H_{13}, R′ = Me
24: R = Me, R′ = C_6H_{13}
25: R = C_6H_{13}, R′ = H
26: R = H, R′ = C_6H_{13}

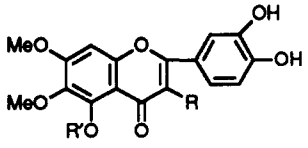
contrast to the result, the 3-methoxy group of the hexyl ether of 35 (36) was selectively cleaved with anhydrous aluminum bromide in acetonitrile to give the corresponding 3-hydroxyflavone 37. On the other hand, 3-hydroxy-5-(tosyloxy)flavone 38[1] obtained from the tosylate of 35 was easily converted into 3-(hexyloxy)-5-hydroxyflavone 40 via the corresponding hexyl ether 39. 5-Hydroxyflavone 40 was methylated to methyl ether 41. 3,5,6,7-Tetraoxygenated 3′,4′-dihydroxyflavones 23–26 were synthesized

(12) Horie, T.; Tsukayama, M.; Kawamura, Y.; Yamamoto, S. *Phytochemistry* 1988, *27*, 1491–1495.

Table I. 3-Alkyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavones (**1**), 3-Alkyl-3′,4′-dihydroxy-5,6,7-trimethoxyflavones (**2**), 3-Alkyl-3′,4′,5-trihydroxy-7,8-dimethoxyflavones (**3**), and 3-Alkyl-3′,4′,5-trihydroxy-6,7-dimethoxyflavones (**4**): Their Inhibitory Effects on Arachidonate 5-Lipoxygenase



1 and 3

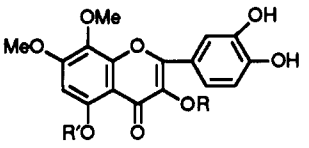


2 and 4

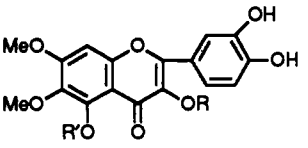
compd	R	R′	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_6- or C_8-H	formula	anal.	IC_{50},[b] nM
1a	CH_3	CH_3	280–282	MeOH–EtOAc	86	6.65[c]	$C_{19}H_{18}O_7$	C, H	800
1b	C_4H_9	CH_3	239–241	EtOAc–hexane	79	6.40	$C_{22}H_{24}O_7$	C, H	100
1c	C_6H_{13}	CH_3	188–189.5	$CHCl_3$–hexane	52	6.39	$C_{24}H_{28}O_7$	C, H	58
1d	C_8H_{17}	CH_3	158–160	Et_2O–hexane	84	6.42	$C_{26}H_{32}O_7$	C, H	62
1e	$C_{10}H_{21}$	CH_3	151–153	Et_2O	70	6.43	$C_{28}H_{36}O_7$	C, H	86
1f	$C_{12}H_{25}$	CH_3	143–144	Et_2O	76	6.43	$C_{30}H_{40}O_7$	C, H	105
1g	$C_{14}H_{29}$	CH_3	127–128.5	Et_2O	57	6.40	$C_{32}H_{44}O_7$	C, H	115
1h	$C_{16}H_{33}$	CH_3	127–129	Et_2O	56	6.40	$C_{34}H_{48}O_7$	C, H	500
2a	CH_3	CH_3	213–214	$CHCl_3$–Et_2O	56	6.99[c]	$C_{19}H_{18}O_7$	C, H	1400
2b	C_4H_9	CH_3	185–187	Et_2O–hexane	46	6.65	$C_{22}H_{24}O_7$	C, H	500
2c	C_6H_{13}	CH_3	178–180	Et_2O–hexane	66	6.65	$C_{24}H_{28}O_7$	C, H	220
2d	C_8H_{17}	CH_3	122–123	Et_2O–hexane	59	6.65	$C_{26}H_{32}O_7$	C, H	250
2e	$C_{10}H_{21}$	CH_3	100–102	Et_2O–hexane	41	6.64	$C_{28}H_{36}O_7$	C, H	190
2f	$C_{12}H_{25}$	CH_3	108–109	Et_2O–hexane	56	6.64	$C_{30}H_{40}O_7$	C, H	175
2g	$C_{14}H_{29}$	CH_3	106.5–108	Et_2O–hexane	61	6.64	$C_{32}H_{44}O_7$	C, H	240
2h	$C_{16}H_{33}$	CH_3	108–109	Et_2O–hexane	50	6.64	$C_{34}H_{48}O_7$	C, H	950
3a	CH_3	H	261–262	MeOH	95	6.57[c]	$C_{18}H_{16}O_7$	C, H	300
3b	C_4H_9	H	123–125	Et_2O–hexane	82	6.41	$C_{21}H_{22}O_7$	C, H	115
3c	C_6H_{13}	H	125–126	$CHCl_3$–hexane	93	6.41	$C_{23}H_{26}O_7$	C, H	115
3d	C_8H_{17}	H	125–127	$CHCl_3$–hexane	95	6.41	$C_{25}H_{30}O_7$	C, H	140
3e	$C_{10}H_{21}$	H	115–116	$CHCl_3$–hexane	89	6.41	$C_{27}H_{34}O_7$	C, H	135
3f	$C_{12}H_{25}$	H	104–105	EtOAc–hexane	81	6.41	$C_{29}H_{38}O_7$	C, H	340
3g	$C_{14}H_{29}$	H	109–111	$CHCl_3$–hexane	82	6.41	$C_{31}H_{42}O_7$	C, H	500
3h	$C_{16}H_{33}$	H	112–113.5	$CHCl_3$–hexane	42	6.41	$C_{33}H_{46}O_7$	C, H	1500
4a	CH_3	H	204–205	EtOAc–EtOH	64	6.77[c]	$C_{18}H_{16}O_7$	C, H	480
4b	C_4H_9	H	172–174	Et_2O–hexane	40	6.41	$C_{21}H_{22}O_7$	C, H	265
4c	C_6H_{13}	H	153–155	$CHCl_3$–hexane	90	6.41	$C_{23}H_{26}O_7$	C, H	110
4d	C_8H_{17}	H	76–78	$CHCl_3$–hexane	71	6.41	$C_{25}H_{30}O_7$	C, H	155
4e	$C_{10}H_{21}$	H	67.5–68.5	Et_2O–hexane	71	6.41	$C_{27}H_{34}O_7$	C, H	270
4f	$C_{12}H_{25}$	H	67–69	$CHCl_3$–hexane	68	6.41	$C_{29}H_{38}O_7$	C, H	210
4g	$C_{14}H_{29}$	H	68.5–70	$CHCl_3$–hexane	75	6.41	$C_{31}H_{42}O_7$	C, H	870
4h	$C_{16}H_{33}$	H	76–78	Et_2O–hexane	69	6.41	$C_{33}H_{46}O_7$	C, H	1250

[a] Measured with a Bruker 400 spectrometer in $CDCl_3$. [b] IC_{50} values for 5-lipoxygenase purified from porcine leukocytes (n = 3). [c] Measured in DMSO-d_6.

Table II. 3,5,7,8- and 3,5,6,7-Tetraoxygenated 3′,4′-Dihydroxyflavones **19–26**: Their Inhibitory Effects on Arachidonate 5-Lipoxygenase



19–22



23–26

compd	R	R′	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_6- or C_8-H	formula	anal.	IC_{50},[b] nM
19	C_6H_{13}	Me	170–171.5	MeOH–Et_2O	84	6.62	$C_{24}H_{28}O_8 \cdot {}^1/_2H_2O$	C, H	88
20	Me	C_6H_{13}	210–212	MeOH–Et_2O	70	6.59	$C_{24}H_{28}O_8 \cdot {}^1/_2H_2O$	C, H	128
21	C_6H_{13}	H	176–178	MeOH–Et_2O	72	6.55	$C_{23}H_{26}O_8$	C, H	350
22	H	C_6H_{13}	212–213	MeOH	62	6.60	$C_{23}H_{26}O_8$	C, H	770
23	C_6H_{13}	Me	126.5–128	$CHCl_3$–MeOH	56	6.73[c]	$C_{24}H_{28}O_8$	C, H	126
24	Me	C_6H_{13}	162.5–164	$CHCl_3$–MeOH	83	6.73[c]	$C_{24}H_{28}O_8$	C, H	280
25	C_6H_{13}	H	150–152	$CHCl_3$–MeOH	70	6.50[c]	$C_{23}H_{26}O_8 \cdot {}^1/_2H_2O$	C, H	480
26	H	C_6H_{13}	201–202	$CHCl_3$–MeOH	73	7.02	$C_{23}H_{26}O_8$	C, H	1350

[a] Measured in DMSO-d_6. [b] IC_{50} values for 5-lipoxygenase purified from porcine leukocytes (n = 3). [c] Measured in $CDCl_3$.

from the corresponding flavones **41**, **36**, **40**, and **37**, respectively. The physical and biological properties of the 3,5,7,8- and 3,5,6,7-tetraoxygenated 3′,4′-dihydroxyflavones are summarized in Table II.

Results and Discussion

Our previous investigation showed that the modification of cirsiliol with lipophilic alkyl groups at the 5- or 6-position enhanced the activity against arachidonate 5-lipoxygenase by 1 order of magnitude, although the activity was weakened by introduction of similar groups at the 7-position in cirsiliol.[4] In connection with this study, we were interested in the modification of the 3-position, which had not been tested, and examined the inhibitory activities of the 3,5,7,8- and 3,5,6,7-tetraoxygenated 3′,4′-dihydroxyflavones with a hexyloxy group at the 3- or 5-

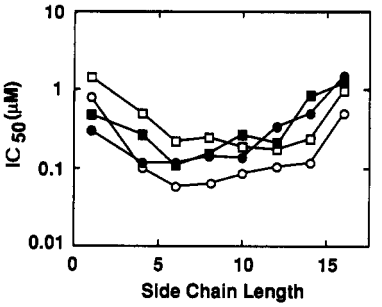


Figure 1. Effect of the alkyl chain length of 3-alkylflavones on 5-lipoxygenase inhibition. The purified 5-lipoxygenase of porcine leukocytes was assayed in the presence of each compound; compound 1 (open circle), 2 (open square), 3 (closed circle), 4 (closed square). Mean values of three experiments were plotted.

position. As shown in Table II, the 3-(hexyloxy)flavones (**19** and **23**, **21** and **25**) exhibited lower IC_{50} values than 5-hexyloxyflavones (**20** and **24**, **22** and **26**). Thus, the chemical modification of the 3-position of a flavone skeleton with lipophilic alkoxy groups increased the inhibitory activity more markedly than modification of the 5-position. In addition, the presence of a hydroxy group at the 3-position decreased the inhibitory activity, and the IC_{50} values of 3-hydroxyflavones (**22** and **26**) were higher than those of their methyl ethers (**20** and **24**). Previously, Wheeler and Berry surveyed the structure–activity relationship of flavonoids to inhibit a mouse epidermal lipoxygenase of unknown oxygenation regiospecificity, and reported that the 3-hydroxy group was important for the lipoxygenase inhibition.[13] This finding was not agreeable with our observation that the presence of the 3-hydroxy group itself decreased the activity of flavonoids to inhibit 5-lipoxygenase.

The finding led us to expect that the introduction of a lipophilic alkyl group at the 3-position in 5,7,8- and 5,6,7-trioxygenated 3′,4′-dihydroxyflavones may increase the inhibitory activity against the 5-lipoxygenase. This held true as shown in Figure 1 and Table I. The purified 5-lipoxygenase of porcine leukocytes was assayed in the presence of compounds **1**–**4** with 3-alkyl groups of various chain lengths. Each compound inhibited the 5-lipoxygenase reaction in a concentration-dependent manner, and their IC_{50} values changed depending on the alkyl chain length. As shown in Figure 1, the compounds with an alkyl chain length of C_6–C_{12} exhibited stronger inhibitions, and all of such modified compounds **1**–**4** showed stronger activities than cirsiliol 5-hexyl ether (IC_{50} = 0.29 μM). 5,7,8-Trioxygenated flavones with a 5-methoxy group (open circles in Figure 1) brought about a stronger inhibition than those with a 5-hydroxy group (closed circles in Figure 1). This was also the case of the compounds with the 3-hexyloxy group (Table II: **19** vs **21**, **23** vs **25**). As shown in Figure 1 (open circles), the 5,7,8-trimethoxyflavones with C_6–C_{10} alkyl groups at the 3-position exhibited the lowest IC_{50} values (58–86 nM). Accordingly, we selected 3-hexyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavone (**1c**) as a representative compound.

Figure 2 shows the dose–response curve of **1c** (open circles) as compared with those of cirsiliol (closed triangles) and its 5-hexyl ether (closed circles), which were studied previously.[4] Flavone **1c** gave an IC_{50} value of 58 nM and was potent by more than 1 order of magnitude than cirsiliol 5-hexyl ether (IC_{50} = 0.29 μM) and cirsiliol (IC_{50} = 0.95 μM). The above-mentioned IC_{50} values for cirsiliol 5-hexyl ether and cirsiliol were higher than those reported previously (15 nM[4] and 0.1 μM,[3] respectively). The difference was attributed to the use of different enzyme preparations: the purified porcine enzyme in the present work and the crude rat enzyme in the previous works. Actually, the IC_{50} values of the two compounds were determined at one time with the two enzymes, and high values were obtained with the purified porcine enzyme. The corresponding 3-(hexyloxy)flavone **19** showed almost the same activity as compound **1c**. These findings support that the introduction of a lipophilic alkyl group at the 3-position is more effective on 5-lipoxygenase inhibition than modification at the other positions.

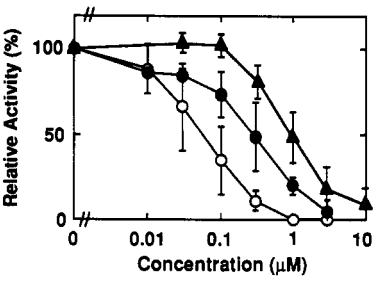


Figure 2. 5-Lipoxygenase inhibition by 3-hexyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavone (**1c**) (open circle) as compared with that of cirsiliol (closed triangle) and its 5-hexyl ether (closed circle). The purified 5-lipoxygenase of porcine leukocytes was assayed under the standard conditions in the presence of each compound at various concentrations [mean ± SD (n = 3)].

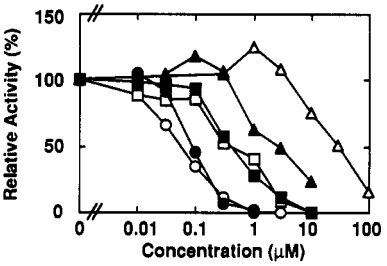


Figure 3. Inhibitory effect of 3-hexyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavone (**1c**) on various lipoxygenases: 5-lipoxygenases of rat basophilic leukemia cells (closed circle) and porcine leukocytes (open circle), 12-lipoxygenases of human platelets (closed square) and porcine leukocytes (open square), 15-lipoxygenase (closed triangle), and cyclooxygenase (open triangle). The enzymes were assayed under the standard condition in the presence of **1c** at various concentrations. Mean values of three experiments were plotted.

Flavone **1c** was also tested with other enzymes: 5-lipoxygenase of rat basophilic leukemia cells, 12-lipoxygenases of porcine leukocytes and human platelets, 15-lipoxygenase of rabbit reticulocytes, and cyclooxygenase of bovine seminal vesicle. As shown in Figure 3, a crude preparation of 5-lipoxygenase from rat basophilic leukemia cells (IC_{50} = 90 nM) was inhibited as much as the purified 5-lipoxygenase of porcine leukocytes (IC_{50} = 58 nM). On the other hand, IC_{50} values for the two 12-lipoxygenases (0.4 μM) were higher by 1 order of magnitude than those for 5-lipoxygenases. 15-Lipoxygenase and cyclooxygenase gave much higher IC_{50} values (2.7 and 30 μM). Thus, flavone **1c** was found to be a relatively specific 5-lipoxygenase inhibitor like cirsiliol and its 5-hexyl ether. Since compound **1c** is essentially insoluble in water and inconvenient for in vivo study, chemical modifications of this compound for improving the water solubility are under investigations.

Experimental Section

Chemistry. All melting points were determined in glass capillaries and were uncorrected. ^1H NMR spectra were recorded on a Hitachi R-24 B spectrometer (60 MHz), using tetra-

(13) Wheeler, E. L.; Berry, D. L. *Carcinogenesis* **1986**, *7*, 33–36.

Table III. 2-Hydroxy-3,4,6-trimethoxyacylbenzenes (7) and 6-Hydroxy-2,3,4-trimethoxyacylbenzenes (14)

compd	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_5-H	formula	anal.
7a	128–130	EtOAc–MeOH	82	5.91	$C_{12}H_{16}O_5$	C, H
7b	62.5–64	MeOH–H_2O	80	5.90	$C_{15}H_{22}O_5$	C, H
7c	49–50	MeOH–H_2O	82	5.92	$C_{17}H_{26}O_5$	C, H
7d	47.5–48.5	MeOH–H_2O	83	5.92	$C_{19}H_{30}O_5$	C, H
7e	62–63	MeOH	87	5.92	$C_{21}H_{34}O_5$	C, H
7f	66–68	MeOH	82	5.91	$C_{23}H_{38}O_5$	C, H
7g	74–75	MeOH	85	5.90	$C_{25}H_{42}O_5$	C, H
7h	72.5–74.5	MeOH	87	5.92	$C_{27}H_{46}O_5$	C, H
14a	42.5–43.5	MeOH–H_2O	89	6.16	$C_{12}H_{16}O_5$	C, H
14b	46.5–48	MeOH	87	6.15	$C_{15}H_{22}O_5$	C, H
14c	40–42	MeOH	88	6.15	$C_{17}H_{26}O_5$	C, H
14d	31–31.8	MeOH	93	6.17	$C_{19}H_{30}O_5$	C, H
14e	43.5–44.5	MeOH	91	6.18	$C_{21}H_{34}O_5$	C, H
14f	52.5–54	MeOH	90	6.16	$C_{23}H_{38}O_5$	C, H
14g	59–60.5	MeOH	91	6.17	$C_{25}H_{42}O_5$	C, H
14h	64.5–66	MeOH	92	6.18	$C_{27}H_{46}O_5$	C, H

[a] Measured in $CDCl_3$.

Table IV. 3,4,6-Trimethoxy-2-(tosyloxy)acylbenzenes (8), 6-Hydroxy-3,4-dimethoxy-2-(tosyloxy)acylbenzenes (9), 2,6-Dihydroxy-3,4-dimethoxyacylbenzenes (10), and 6-(Benzyloxy)-2-hydroxy-3,4-dimethoxyacylbenzenes (12)

compd	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_5-H	formula	anal.
8a	121–123	EtOAc	94	6.38	$C_{19}H_{22}O_7S$	C, H
8b	91.5–92.5	EtOAc–hexane	96	6.36	$C_{22}H_{28}O_7S$	C, H
8c	83–85	MeOH	93	6.37	$C_{24}H_{32}O_7S$	C, H
8d	84–85	MeOH	83	6.34	$C_{26}H_{36}O_7S$	C, H
8e	85–87	MeOH	92	6.38	$C_{28}H_{40}O_7S$	C, H
8f	58.5–60	MeOH	84	6.39	$C_{30}H_{44}O_7S$	C, H
8g	65.5–67.5	MeOH	96	6.34	$C_{32}H_{48}O_7S$	C, H
8h	67–68.5	MeOH	92	6.38	$C_{34}H_{52}O_7S$	C, H
9a	120.5–121	EtOAc	88	6.33	$C_{18}H_{20}O_7S$	C, H
9b	53–54	MeOH	80	6.36	$C_{21}H_{26}O_7S$	C, H
9c	63–64.5	MeOH–H_2O	84	6.36	$C_{23}H_{30}O_7S$	C, H
9d	59–60	MeOH–H_2O	90	6.30	$C_{25}H_{34}O_7S$	C, H
9e	55.5–56	MeOH	92	6.29	$C_{27}H_{38}O_7S$	C, H
9f	59–61	MeOH–H_2O	78	6.31	$C_{29}H_{42}O_7S$	C, H
9g	54–56	MeOH	90	6.33	$C_{31}H_{46}O_7S$	C, H
9h	58–60	MeOH	72	6.33	$C_{33}H_{50}O_7S$	C, H
10a	142–143	MeOH	82	5.99	$C_{11}H_{14}O_5$	C, H
10b	96.5–97	EtOAc–hexane	87	5.99	$C_{14}H_{20}O_5$	C, H
10c	93–94.5	MeOH	82	5.98	$C_{16}H_{24}O_5$	C, H
10d	71–72.5	MeOH–H_2O	83	6.00	$C_{18}H_{28}O_5$	C, H
10e	73–74	MeOH	85	5.97	$C_{20}H_{32}O_5$	C, H
10f	79–80	MeOH	82	5.99	$C_{22}H_{36}O_5$	C, H
10g	83.5–85	EtOAc–MeOH	89	6.00	$C_{24}H_{40}O_5$	C, H
10h	86–87	EtOAc–MeOH	76	5.99	$C_{26}H_{44}O_5$	C, H
12a	111.5–113	EtOAc–MeOH	85	5.99	$C_{18}H_{20}O_5$	C, H
12b	104.5–105.5	MeOH	81	6.00	$C_{21}H_{26}O_5$	C, H
12c	85.5–86	MeOH	84	5.97	$C_{23}H_{30}O_5$	C, H
12d	83–83.5	MeOH	80	5.97	$C_{25}H_{34}O_5$	C, H
12e	80.5–81	MeOH	82	6.01	$C_{27}H_{38}O_5$	C, H
12f	80.5–82.5	MeOH	82	6.01	$C_{29}H_{42}O_5$	C, H
12g	79–81	EtOAc–MeOH	82	5.99	$C_{31}H_{46}O_5$	C, H
12h	83–84	EtOAc–MeOH	85	6.00	$C_{33}H_{50}O_5$	C, H

[a] Measured in $CDCl_3$.

methylsilane as an internal standard, and chemical shifts are given in δ values. UV spectra were recorded on a Hitachi 124 spectrophotometer. Elemental analyses were performed with a Yanaco CHN corder Model MT-3.

2-Hydroxy-3,4,6-trimethoxyacylbenzenes (7a–h). To a solution of 1,2,3,5-tetramethoxybenzene (15 g, 0.076 mol) and anhydrous aluminum chloride (30 g, 0.22 mol) in ether (250 mL) was added acyl chloride (0.090 mol) at 0 °C, the solution was stirred at 0 °C for 30 min and then poured into ice–hydrochloric acid with stirring. The mixture was warmed at 60–70 °C for 20–30 min (ether was removed) and the separated oily material was extracted with ethyl acetate. The extract was washed with aqueous potassium carbonate and then water and dried over sodium sulfate. The solvent was evaporated to dryness to give crude **6a–h**.

Dried crude **6a–h** were dissolved into a solution of aluminum chloride (30 g, 0.22 mol) in acetonitrile (100 mL); the solution was allowed to stand at 50 °C for 1–2 h and then poured into ice–hydrochloric acid with stirring. The mixture was warmed at 60–70 °C for 15–20 min and the separated product was collected by filtration or by extraction with ethyl acetate. The product was recrystallized to give **7a–h** (Table III).

3,4,6-Trimethoxy-2-(tosyloxy)acylbenzenes (8a–h). A mixture of **7a–h** (40 mmol), *p*-toluenesulfonyl chloride (11 g, 60 mmol), and freshly powdered anhydrous potassium carbonate (35 g, 240 mmol) in acetone (150–200 mL) was refluxed with stirring until the starting material disappeared (6–14 h). The reaction mixture was cooled and potassium carbonate was filtered off. The filtrate was acidified with dilute hydrochloric acid and then concentrated under reduced pressure. The separated crystals were collected and washed with water and a small amount of hexane and then recrystallized to give **8a–h** (Table IV).

6-Hydroxy-3,4-dimethoxy-2-(tosyloxy)acylbenzenes (9a–h). Acylbenzene 8 (30 mmol) was dissolved in 25% (w/v) solution

Table V. 3-Alkyl-3′,4′-bis(benzyloxy)-5,7,8- and 5,6,7-trimethoxyflavones (15 and 17) and 3-Alkyl-3′,4′-bis(benzyloxy)-5-hydroxy-7,8- and 6,7-dimethoxyflavones (16 and 18)

compd	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_6- or C_8-H	formula	anal.
15a	137–138	MeOH	61	6.32	$C_{33}H_{30}O_7$	C, H
15b	142–143	$CHCl_3$–MeOH	42	6.33	$C_{36}H_{36}O_7$	C, H
15c	123–125	EtOAc–hexane	48	6.33	$C_{38}H_{40}O_7$	C, H
15d	124–126	$CHCl_3$–MeOH	46	6.35	$C_{40}H_{44}O_7$	C, H
15e	128–128.5	$CHCl_3$–MeOH	65	6.36	$C_{42}H_{48}O_7$	C, H
15f	124.5–125.5	$CHCl_3$–MeOH	47	6.33	$C_{44}H_{52}O_7$	C, H
15g	125.5–127	$CHCl_3$–MeOH	42	6.33	$C_{46}H_{56}O_7$	C, H
15h	113.5–115	$CHCl_3$–MeOH	53	6.34	$C_{48}H_{60}O_7$	C, H
17a	103–104.5	$CHCl_3$–hexane	44	6.50	$C_{33}H_{30}O_7$	C, H
17b	98.5–100.5	$CHCl_3$–MeOH	52	6.53	$C_{36}H_{36}O_7$	C, H
17c	82–84	MeOH–Et_2O	36	6.53	$C_{38}H_{40}O_7$	C, H
17d	95–96	EtOH	50	6.50	$C_{40}H_{44}O_7$	C, H
17e	56–57.5	Et_2O–hexane	55	6.53	$C_{42}H_{48}O_7$	C, H
17f	51–53	Et_2O–hexane	47	6.53	$C_{44}H_{52}O_7$	C, H
17g	51–53	Et_2O–hexane	52	6.54	$C_{46}H_{56}O_7$	C, H
17h	64–66	EtOH	62	6.50	$C_{48}H_{60}O_7$	C, H
16a	161–163	EtOAc–MeOH	78	6.30	$C_{32}H_{28}O_7$	C, H
16b	131–133	EtOAc–EtOH	91	6.27	$C_{35}H_{34}O_7$	C, H
16c	117–118	EtOAc–EtOH	91	6.28	$C_{37}H_{38}O_7$	C, H
16d	115–117	EtOAc–EtOH	81	6.26	$C_{39}H_{42}O_7$	C, H
16e	121–123	EtOAc–EtOH	94	6.29	$C_{41}H_{46}O_7$	C, H
16f	104–105.5	EtOAc–EtOH	87	6.32	$C_{43}H_{50}O_7$	C, H
16g	112–114	EtOAc–EtOH	87	6.32	$C_{45}H_{54}O_7$	C, H
16h	103–104	EtOAc–EtOH	82	6.30	$C_{47}H_{58}O_7$	C, H
18a	167–169	EtOAc–MeOH	74	6.29	$C_{32}H_{28}O_7$	C, H
18b	123–125	EtOH	79	6.31	$C_{35}H_{34}O_7$	C, H
18c	98–100.5	EtOH–hexane	75	6.30	$C_{37}H_{38}O_7$	C, H
18d	80.5–82	EtOH–hexane	76	6.30	$C_{39}H_{42}O_7$	C, H
18e	80–81.5	EtOH	72	6.31	$C_{41}H_{46}O_7$	C, H
18f	92–93	EtOH–hexane	65	6.32	$C_{43}H_{50}O_7$	C, H
18g	74–76	EtOH	74	6.24	$C_{45}H_{54}O_7$	C, H
18h	67–68.5	EtOH–hexane	73	6.28	$C_{47}H_{58}O_7$	C, H

[a] Measured in $CDCl_3$.

of anhydrous aluminum bromide in acetonitrile (100 mL) and allowed to stand at room temperature for 2–3 h. The mixture was poured into 2–3% hydrochloric acid (200 mL), warmed at 60–70 °C for 15–20 min and then cooled. The separated crystals were collected and recrystallized to give **9a–h** (Table IV).

2,6-Dihydroxy-3,4-dimethoxyacylbenzenes (10a–h). A mixture of acylbenzene **9** (20 mmol) and anhydrous potassium carbonate (14 g, 100 mmol) in methanol (150–200 mL) was refluxed with stirring for 1–3 h and then diluted with water. The mixture was acidified with hydrochloric acid and concentrated under reduced pressure. The separated crystals were collected and recrystallized to give **10a–h** (Table IV).

6-(Benzyloxy)-2-hydroxy-3,4-dimethoxyacylbenzenes (12a–h). To a solution of acylbenzene **10** (15 mmol) and benzyl chloride (5.5 g, 45 mmol) in *N*,*N*-dimethylformamide (150 mL), freshly powdered potassium carbonate (10 g, 75 mmol) was added and the mixture was immediately heated at 150–160 °C with vigorous stirring for 10–20 min. The reaction mixture was diluted with water and the excess benzyl chloride was removed by steam distillation under reduced pressure. The separated oily materials were extracted with ether and the extract was washed with dilute hydrochloric acid and then water. The solvent was evaporated off to give the crude product (**11a–h**) as an oil (**11a** and **11h** as crystals).

To a solution of crude **11** in acetic acid (50 mL) was added a freshly prepared mixture of concentrated hydrochloric acid (10 mL) and acetic acid (50 mL); the mixture was allowed to stand at room temperature with stirring for 2–3 h and diluted with water. The separated crystals were collected after cooling and recrystallized to give **12a–h** (Table IV).

6-Hydroxy-2,3,4-trimethoxyacylbenzenes (14a–h). A mixture of acylbenzenes **12** (12 mmol), dimethyl sulfate (3 g, 36 mmol), and anhydrous potassium carbonate (8.3 g, 60 mmol) in acetone (150 mL) was refluxed with stirring for 4–9 h. The mixture was diluted with water, then refluxed additionally for 20 min, and concentrated under reduced pressure. The separated oily materials were collected by extraction with ether to give crude **13a–h**. Crude acylbenzene **13** was hydrogenated over palladium on charcoal (10%, 0.8 g) in ethyl acetate–methanol (1:1, ca. 100 mL) until the uptake of hydrogen ceased. After the catalyst was filtered off, the filtrate was evaporated and the residue was recrystallized to give **14a–h** (Table III).

3-Alkyl-3′,4′-bis(benzyloxy)-5,7,8-trimethoxyflavones (15a–h) and 3-Alkyl-3′,4′-bis(benzyloxy)-5,6,7-trimethoxyflavones (17a–h). A mixture of acylbenzene **7** or **14** (4 mmol), 3,4-bis(benzyloxy)benzoic anhydride (6.5 g, 10 mmol), and potassium 3,4-bis(benzyloxy)benzoate (1.5 g, 4 mmol) was heated under reduced pressure at 170–180 °C for 6–8 h. The reaction mixture was cooled and dissolved in aqueous acetone (50–80 mL). The solution was gently refluxed with a solution of potassium hydroxide (KOH, 3.5 g, 60 mmol; H_2O, 10 mL; MeOH, 20 mL) for 20–30 min, concentrated under reduced pressure, and diluted with water. The separated precipitates or oily materials were collected by the filtration or by the extraction with ethyl acetate and recrystallized to give **15a–h** or **17a–h** (Table V).

3-Alkyl-3′,4′-bis(benzyloxy)-5-hydroxy-7,8-dimethoxyflavones (16a–h). Flavone **15** (1 mmol) was dissolved in 5% (w/v) solution of anhydrous aluminum bromide in acetonitrile (20 mL, 3.8 mmol) and the solution was allowed to stand at room temperature for 1–1.5 h. The solution was poured into 2–3% hydrochloric acid (ca. 20 mL) and warmed at 70–80 °C for 15–20 min. The separated yellow crystals were collected and recrystallized to give **16a–h** (Table V).

3-Alkyl-3′,4′-bis(benzyloxy)-5-hydroxy-6,7-dimethoxyflavones (18a–h). Flavone **17** (1 mmol) was dissolved in cold 5% (w/v) solution of anhydrous aluminum bromide in acetonitrile (20 mL, 3.8 mmol) and the solution was allowed to stand at 0 °C for 20–30 min. The reaction mixture was treated by the method described above to give **18a–h** (Table V).

3-Alkyl-3′,4′-dihydroxy-5,7,8-trimethoxyflavones (1a–h), 3-Alkyl-3′,4′-dihydroxy-5,6,7-trimethoxyflavones (2a–h), 3-Alkyl-3′,4′,5-trihydroxy-7,8-dimethoxyflavones (3a–h), and 3-Alkyl-3′,4′,5-trihydroxy-6,7-dimethoxyflavones (4a–h). Flavone **15**, **16**, **17**, or **18** (0.5 mmol) was hydrogenated over palladium on charcoal (10%, 50 mg) in ethyl acetate–methanol (1:1, ca. 40 mL) until two molar equiv of hydrogen was absorbed (ca. 40 min; excess absorption of hydrogen increased the amount of colored byproducts). After the catalyst was filtered off, the

Table VI. 3,5,7,8- and 3,5,6,7-Tetraoxygenated 3′,4′-Bis(benzyloxy)flavones with a Hexyloxy Group at the 3- or 5-Position

compd	mp, °C	recrystn solvent	% yield	^{1}H NMR[a] C_6- or C_8-H	formula	anal.
30	128–129.5	$CHCl_3$–MeOH	87	6.38	$C_{38}H_{40}O_8$	C, H
31	104–105	$CHCl_3$–MeOH	96	6.40	$C_{38}H_{40}O_8$	C, H
32	124–125.5	$CHCl_3$–MeOH	94	6.37	$C_{37}H_{38}O_8$	C, H
33	148.5–149.5	$CHCl_3$–MeOH	82	6.36	$C_{37}H_{38}O_8$	C, H
36	131.5–132.5	$CHCl_3$–MeOH	95	6.66	$C_{38}H_{40}O_8$	C, H
37	99–100.5	$CHCl_3$–MeOH	65	6.67	$C_{37}H_{38}O_8$	C, H
40	92–94	$CHCl_3$–MeOH	70	6.41	$C_{37}H_{38}O_8$	C, H
41	89–90.5	$CHCl_3$–MeOH	90	6.64	$C_{38}H_{40}O_8$	C, H

[a] Measured in $CDCl_3$.

filtrate was evaporated under reduced pressure and the residue was recrystallized to give the desired hydroxyflavones (Table I).

3′,4′-Bis(benzyloxy)-3-hydroxy- and 3′,4′-Bis(benzyloxy)-5-hydroxy-5,7,8-trimethoxyflavones (28 and 29). 3′,4′-Bis(benzyloxy)-3,5,7,8-tetramethoxyflavone (**27**) (1.0 g) was demethylated with a 5% (w/v) solution of anhydrous aluminum bromide in acetonitrile (25 mL) at room temperature for 1.5 h.[12] The crude demethylated product was chromatographed on a silica gel column eluting with chloroform. 5-Hydroxyflavone **29** was obtained from the first eluate: mp 136–137 °C (from chloroform–ether); yield 390 mg (44%). From the second eluate, 3-hydroxyflavone **28** was obtained: mp 164–166 °C (from chloroform–ether); yield 300 mg (34%).

3′,4′-Bis(benzyloxy)-3-(hexyloxy)-5,7,8-trimethoxyflavone (30), 3′,4′-Bis(benzyloxy)-5-(hexyloxy)-3,7,8-trimethoxyflavone (31), and 3′,4′-Bis(benzyloxy)-5-(hexyloxy)-3,6,7-trimethoxyflavone (36). A mixture of 5-hydroxyflavone **28**, **29**, or **35**[1] (0.4 g), hexyl iodide (0.8 g), and powdered anhydrous potassium carbonate (5 g) was refluxed in acetone–*N*,*N*-dimethylformamide (each 20 mL) with vigorous stirring until the starting material disappeared (4–6 h). The mixture was diluted with water and concentrated under reduced pressure. The separated precipitates were collected, washed with water and hexane, and then recrystallized to give hexyl ether **30**, **31**, or **36** (Table VI).

3′,4′-Bis(benzyloxy)-3-(hexyloxy)-5-hydroxy-6,7-dimethoxyflavone (40). A mixture of 3′,4′-Bis(benzyloxy)-3-hydroxy-6,7-dimethoxy-5-(tosyloxy)flavone (**38**)[1] synthesized from **34** (0.6 g), hexyl iodide (1.0 g), and powdered anhydrous potassium carbonate (2 g) in acetone (40 mL) was refluxed with vigorous stirring for 5 h and then the solvent was distilled off under reduced pressure. To the mixture was added methanol (30 mL); the mixture was additionally refluxed with stirring for 2 h and then diluted with water. After the solvent was concentrated under reduced pressure, the separated precipitates were collected and recrystallized to give **40** (Table VI).

3′,4′-Bis(benzyloxy)-3-(hexyloxy)-5,6,7-trimethoxyflavone (41). A mixture of **40** (0.3 g), dimethyl sulfate (0.25 mL), and anhydrous potassium carbonate (1 g) in acetone (30 mL) was refluxed with stirring for 3 h, diluted with water, and then additionally refluxed for 30 min. After the solvent was distilled off, the separated precipitates were collected and recrystallized to give **41** (Table VI).

3′,4′-Bis(benzyloxy)-3-(hexyloxy)-5-hydroxy-7,8-dimethoxyflavone (32), 3′,4′-Bis(benzyloxy)-5-(hexyloxy)-3-hydroxy-7,8-dimethoxyflavone (33), and 3′,4′-Bis(benzyloxy)-5-(hexyloxy)-3-hydroxy-6,7-dimethoxyflavone (37). To a solution of flavone **30**, **31**, or **36** (0.2 g, 0.32 mmol) in acetonitrile (4 mL) was added a 10% (w/v) solution of anhydrous aluminum bromide in acetonitrile (4 mL, 1.5 mmol); the mixture was allowed to stand at room temperature (ca. 25 °C) for 1 h and poured into 5% hydrochloric acid (30 mL). The mixture was warmed at 50–60 °C for 20 min, and the separated oily materials were extracted with chloroform. Since the extract contained a large amount of an aluminum complex of the demethylated product, the chloroform extract was stirred with 10% hydrochloric acid (20 mL) at 25–30 °C for 2–3 h and then separated from the aqueous layer. The chloroform layer was evaporated and the residue was recrystallized to give **32**, **33**, or **37** (Table VI).

3-(Hexyloxy)-5,7,8-trimethoxy- and 5-(Hexyloxy)-3,7,8-trimethoxy-3′,4′-dihydroxyflavones (19 and 20), 3-(Hexyloxy)-3′,4′,5-trihydroxy- and 5-(Hexyloxy)-3,3′,4′-trihydroxy-7,8-dimethoxyflavones (21 and 22), 3-(Hexyloxy)-5,6,7-trimethoxy- and 5-(Hexyloxy)-3,6,7-trimethoxy-3′,4′-dihydroxyflavones (23 and 24), and 3-(Hexyloxy)-3′,4′,5-trihydroxy- and 5-(Hexyloxy)-3,3′,4′-trihydroxy-6,7-dimethoxyflavones (25 and 26). Flavone **30**, **31**, **32**, **33**, **41**, **36**, **40**, or **37** (0.25 mmol) was hydrogenated over palladium on charcoal (10%, 50 mg) in ethyl acetate–methanol (1:1, ca. 40 mL) until the uptake of hydrogen ceased. The catalyst was filtered off, the filtrate was evaporated, and the residue was recrystallized to give the 3′,4′-dihydroxyflavone (Table II).

Preparation and Assay of Enzymes. Two preparations of arachidonate 5-lipoxygenases were used in this work. The porcine enzyme was highly purified from the cytosol fraction of porcine leukocytes by immunoaffinity chromatography using a monoclonal anti-5-lipoxygenase antibody.[14] The rat enzyme was the cytosol fraction of rat basophilic leukemia cells[15] which was used without further purification. Arachidonate 12-lipoxygenases were purified from the cytosol fractions of porcine leukocytes[16] and human platelets[17] by immunoaffinity chromatography. 15-Lipoxygenase was partially purified by ammonium sulfate fractionation from the cytosol fraction of rabbit reticulocytes.[18] Cyclooxygenase was purified from the microsomal fraction of bovine vesicular gland by immunoaffinity chromatography utilizing a monoclonal anti-cyclooxygenase antibody prepared as described previously.[19] 5-Lipoxygenase (porcine enzyme, 2.3 μg, or rat enzyme, 115 μg) was allowed to react with 25 μM [1-^{14}C]arachidonic acid (50 000 cpm) at 24 °C 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 activity of 12-lipoxygenase (porcine enzyme, 4.9 μg, or human enzyme, 1.7 μg) was assayed by incubation at 30 °C for 3 and 10 min, respectively, 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). The 15-lipoxygenase (83 μg) was incubated at 24 °C for 3 min in 200-μL reaction mixture containing 25 μM [1-^{14}C]-arachidonic acid (50 000 cpm) and 50 mM Tris-HCl buffer (pH 7.4). The cyclooxygenase (4.5 μg) was assayed at 24 °C for 2 min in a 200-μL reaction mixture containing 25 μM [1-^{14}C]arachidonic acid (50 000 cpm), 2 μM hematin, 5 mM L-tryptophan, and 0.1 M Tris-HCl buffer (pH 8.0). Termination of the enzyme reaction, ethereal extraction of the reaction products and their separation by thin-layer chromatography, and determination of radioactivity were performed as described previously.[14-17,19] A methanol solution (4 μL) of each flavone compound at varying concentrations was preincubated with enzyme for 3 min at room temperature. The coefficient of intraassay variation for the degree of 5-lipoxygenase inhibition by compound **1c** was 4.3% (n = 6) at 30 nM and 6.0% (n = 6) at 100 nM. The coefficient of interassay variation was

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5.8% (n = 6) at 30 nM and 10.6% (n = 6) at 100 nM. The inhibitory effect of AA861 as a reference compound was tested by the standard 5-lipoxygenase assay. An IC_{50} value of 10 μM was obtained, and the value was higher by 1 order of magnitude than the previous value (0.8 μM).[20] The difference of two IC_{50} values may be attributed to the different preparations as described in the text for cirsiliol.

Registry No. **1a**, 134081-23-5; **1b**, 134081-24-6; **1c**, 134081-25-7; **1d**, 134081-26-8; **1e**, 134081-27-9; **1f**, 134081-28-0; **1g**, 134081-29-1; **1h**, 134081-30-4; **2a**, 134081-31-5; **2b**, 134081-32-6; **2c**, 134081-33-7; **2d**, 134081-34-8; **2e**, 134081-35-9; **2f**, 134081-36-0; **2g**, 134081-37-1; **2h**, 134081-38-2; **3a**, 134081-39-3; **3b**, 134081-40-6; **3c**, 134081-41-7; **3d**, 134081-42-8; **3e**, 134081-43-9; **3f**, 134081-44-0; **3g**, 134081-45-1; **3h**, 134081-46-2; **4a**, 134081-47-3; **4b**, 134081-48-4; **4c**, 134081-49-5; **4d**, 134081-50-8; **4e**, 134081-51-9; **4f**, 134081-52-0; **4g**, 134081-53-1; **4h**, 134081-54-2; **5**, 5333-45-9; **7a**, 51379-76-1; **7b**, 134081-63-3; **7c**, 134081-64-4; **7d**, 134081-65-5; **7e**, 134081-66-6; **7f**, 134081-67-7; **7g**, 134081-68-8; **7h**, 134081-69-9; **8a**, 134081-77-9; **8b**, 134081-78-0; **8c**, 134081-79-1; **8d**, 134081-80-4; **8e**, 134081-81-5; **8f**, 134081-82-6; **8g**, 134081-83-7; **8h**, 134081-84-8; **9a**, 134081-85-9; **9b**, 134081-86-0; **9c**, 134081-87-1; **9d**, 134081-88-2; **9e**, 134081-89-3; **9f**, 134081-90-6; **9g**, 134081-91-7; **9h**, 134081-92-8; **10a**, 134081-93-9; **10b**, 134081-94-0; **10c**, 134081-95-1; **10d**, 134081-96-2; **10e**, 134081-97-3; **10f**, 134081-98-4; **10g**, 134081-99-5; **10h**, 134082-00-1; **12a**, 134082-01-2; **12b**, 134082-02-3; **12c**, 134082-03-4; **12d**, 134082-04-5; **12e**, 134082-05-6; **12f**, 134082-06-7; **12g**, 134082-07-8; **12h**, 134082-08-9; **14a**, 52099-20-4; **14b**, 134081-70-2; **14c**, 134081-71-3; **14d**, 134081-72-4; **14e**, 134081-73-5; **14f**, 134081-74-6; **14g**, 134081-75-7; **14h**, 134081-76-8; **15a**, 134082-09-0; **15b**, 134082-10-3; **15c**, 134082-11-4; **15d**, 134082-12-5; **15e**, 134109-93-6; **15f**, 134082-13-6; **15g**, 134082-14-7; **15h**, 134082-15-8; **16a**, 134082-24-9; **16b**, 134082-25-0; **16c**, 134082-26-1; **16d**, 134082-27-2; **16e**, 134082-28-3; **16f**, 134082-29-4; **16g**, 134082-30-7; **16h**, 134082-31-8; **17a**, 134082-16-9; **17b**, 134082-17-0; **17c**, 134082-18-1; **17d**, 134082-19-2; **17e**, 134082-20-5; **17f**, 134082-21-6; **17g**, 134082-22-7; **17h**, 134082-23-8; **18a**, 134082-32-9; **18b**, 134082-33-0; **18c**, 134082-34-1; **18d**, 134082-35-2; **18e**, 134082-36-3; **18f**, 134082-37-4; **18g**, 134109-94-7; **18h**, 134082-38-5; **19**, 134081-55-3; **20**, 134081-56-4; **21**, 134081-57-5; **22**, 134081-58-6; **23**, 134081-59-7; **24**, 134081-60-0; **25**, 134081-61-1; **26**, 134081-62-2; **27**, 7622-60-8; **28**, 116512-06-2; **29**, 134082-46-5; **30**, 134082-39-6; **31**, 134109-95-8; **32**, 134082-40-9; **33**, 134082-41-0; **35**, 24160-94-9; **36**, 134082-42-1; **37**, 134082-43-2; **38**, 124910-02-7; **40**, 134082-44-3; **41**, 134082-45-4; CH_3CH_2COCl, 79-03-8; $CH_3(CH_2)_4COCl$, 142-61-0; $C_6H_{13}CH_2COCl$, 111-64-8; $C_8H_{17}CH_2COCl$, 112-13-0; $C_{10}H_{21}CH_2COCl$, 112-16-3; $C_{12}H_{25}CH_2COCl$, 112-64-1; $C_{14}H_{29}CH_2COCl$, 112-67-4; $C_{16}H_{33}CH_2COCl$, 112-76-5; 3,4-bis(benzyloxy)benzoic anhydride, 1592-48-9; potassium 3,4-bis(benzyloxy)benzoate, 110193-71-0; arachidonate 5-lipoxygenase, 80619-02-9.

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(Methoxyalkyl)thiazoles: A New Series of Potent, Selective, and Orally Active 5-Lipoxygenase Inhibitors Displaying High Enantioselectivity[1]

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(Methoxyalkyl)thiazoles are novel 5-lipoxygenase (5-LPO) inhibitors that are neither redox agents nor iron chelators. Consideration of a hypothetical model of the enzyme active site led to this series which is exemplified by 1-[3-(naphth-2-ylmethoxy)phenyl]-1-(thiazol-2-yl)propyl methyl ether (**2d**, ICI211965). **2d** inhibits cell-free guinea pig 5-LPO activity, LTC_4 synthesis in plasma free mouse macrophages, and LTB_4 synthesis in rat and human blood (IC_{50}s 0.1 μM, 8 nM, 0.5 μM, and 0.4 μM, respectively) but does not inhibit the synthesis of cyclooxygenase products at concentrations up to 50 μM in macrophages and 100 μM in blood. **2d** is orally active in rat (ex vivo ED_{50} 10 mg/kg in blood taken in 1 h after dosing). SAR studies show that high in vitro potency requires methoxy, thiazolyl, and naphthyl groups and depends critically on the substitution pattern. (Methoxyalkyl)thiazoles are chiral. Resolution of 1-methoxy-6-(naphth-2-ylmethoxy)-1-(thiazol-2-yl)indan (**2j**, ICI216800) shows that (+)-**2j** is 50-150-fold more potent than (-)-**2j** in in vitro assays. Thus, (methoxyalkyl)thiazoles are a new series of orally active, selective 5-LPO inhibitors and represent the first class of inhibitors in which inhibition is mediated by specific, enantioselective interactions with the enzyme.

Introduction

Arachidonic acid is metabolized to inflammatory mediators by two major oxidative pathways. 5-Lipoxygenase (5-LPO) is the first enzyme in a cascade which produces the leukotrienes (LTs) while cyclooxygenase (CO) initiates the cyclic pathway leading to prostaglandins and thromboxanes. Inhibition of CO is a well-established clinical treatment for inflammation although this mechanism of action is associated with ulceration of the gastrointestinal tract.

The LTs are a family of important biologically active molecules. LTB_4 is a potent chemotactic agent and inflammatory mediator[2] and the peptidoleukotrienes LTC_4 and LTD_4 are powerful spasmogens in vascular and bronchial tissues.[3] Elevated levels of LTs are associated with a number of inflammatory conditions, and indeed LTs have been recovered from various pathological tissues. For these reasons it is believed that restricting LT synthesis by inhibition of 5-LPO will have therapeutic utility for the treatment of a variety of inflammatory conditions including asthma, rheumatoid arthritis, inflammatory bowel disease, and psoriasis. However, only when orally active inhibitors of 5-LPO free from CO inhibitory activity are evaluated clinically will the value of 5-LPO inhibition in the treatment of inflammatory conditions become clear.

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