

## STUDIES ON LIPOXYGENASE INHIBITORS

II. KF8940 (2-*n*-HEPTYL-4-HYDROXYQUINOLINE-*N*-OXIDE),  
A POTENT AND SELECTIVE INHIBITOR OF 5-LIPOXYGENASE,  
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(Received for publication February 12, 1986)

*Pseudomonas methanica* KY4634 was found to produce 5-lipoxygenase inhibitor designated KF8940, MY12-62a and MY12-62c. The inhibitors were purified by solvent extraction, silica gel column chromatography, reversed-phase low pressure liquid chromatography and crystallization. The chemical structures of KF8940, MY12-62a and MY12-62c were determined to be 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, 2-*n*-heptyl-4-hydroxyquinoline and 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione, respectively, on the basis of their physico-chemical properties. Among them, KF8940 was the most potent inhibitor. The compound inhibited 5-lipoxygenase of rat basophilic leukemia cells in a dose-dependent manner and the half maximal inhibitory concentration (IC<sub>50</sub>) was  $1.5 \times 10^{-7}$  M. At this concentration, KF8940 did not inhibit bovine platelet 12-lipoxygenase and cyclooxygenase, and the IC<sub>50</sub> values for these enzyme were  $3.5 \times 10^{-5}$  M and  $1.7 \times 10^{-4}$  M, respectively. The results indicated that KF8940 is a potent and selective inhibitor of 5-lipoxygenase. The IC<sub>50</sub> value of MY12-62c for 5-lipoxygenase was  $1.9 \times 10^{-5}$  M and that of MY12-62a was  $1.9 \times 10^{-5}$  M.

Much attention has been focused on 5-lipoxygenase inhibitors as anti-asthmatic and anti-inflammatory agents since SAMUELSSON and co-workers<sup>1,2)</sup> showed that slow reacting substances of anaphylaxis (SRS-A), potent mediators generated during anaphylactic reaction, were oxidative products of arachidonate 5-lipoxygenase. The enzyme is mainly distributed in leukocytes<sup>3)</sup> and catalyzes the oxygenation of arachidonic acid at C-5 position to produce 5-HPETE. The product can be either reduced enzymatically to its hydroxy derivative, 5-HETE, or transformed to the peptide LTs and LTB<sub>4</sub>. LTB<sub>4</sub> was proposed to be implicated in inflammatory reactions because of its potent chemotactic activity<sup>4)</sup>.

Many compounds have recently been reported as 5-lipoxygenase inhibitors<sup>5-9)</sup> and some of them exhibited anti-asthmatic activities<sup>10,11)</sup>. In the course of screening for lipoxygenase inhibitors from microbial origin, we found that *Pseudomonas methanica* KY4634 produced potent 5-lipoxygenase inhibitors. The compounds, designated KF8940, MY12-62a and MY12-62c, were isolated from the culture broth and their structures were determined. Among them, KF8940, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, was a potent and selective inhibitor of the enzyme. This manuscript describes the fermentation, isolation and purification, structural identification, and some biological properties of the inhibitors.

Abbreviations: 5-HPETE; 5-L-hydroperoxy-6,8,11,14-eicosatetraenoic acid, 12-HPETE; 12-L-hydroperoxy-5,8,10,14-eicosatetraenoic acid, 5-HETE; 5-L-hydroxy-6,8,11,14-eicosatetraenoic acid, 12-HETE; 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid, LT; leukotriene.

## Materials and Methods

### Materials

[1-<sup>14</sup>C]Arachidonic acid (55.4 mCi/mmol), [5,6,8,9,11,12,14,15-<sup>3</sup>H]5-HETE (60.0 mCi/mmol) and [5,6,8,9,11,12,14,15-<sup>3</sup>H]thromboxane B<sub>2</sub> (150 mCi/mmol) were obtained from New England Nuclear (Boston). 12-HETE was a generous gift from Dr. D. H. NUGTEREN (Uniliver Research Laboratorium Vlaardingen). Rat basophilic leukemia (RBL-1) cells [ATCC CRL1378] were obtained from the American Type Culture Collection (Rockville). Silica gel precoated TLC plate was obtained from E. Merck (Darmstadt). All other reagents were of analytical grade.

### Microorganism, Medium and Culture Conditions

*P. methanica* KY4634 was used in this study. The stock medium contained meat extract 1%, peptone 1%, NaCl 0.5% and agar 2%, pH 7.2. The seed medium contained glucose 2%, peptone 1%, yeast extract 1% and NaCl 0.25%, pH 7.4. The production medium contained glucose 1%, glycerol 0.25%, soybean meal 1%, peptone 0.5%, NaCl 0.3%, KCl 0.03%, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05% and CaCO<sub>3</sub> 0.3%, pH 7.8. A slant culture grown on the stock medium was inoculated into a test tube (2.5 × 19 cm) containing 10 ml of the seed medium and incubated on a test tube shaker at 30°C for 24 hours. Twenty ml of the first seed culture was transferred to two 2-liter Erlenmeyer flasks containing 450 ml each of the seed medium. The flasks were incubated on a rotary shaker at 30°C for 24 hours (220 rpm). The content of the flasks was transferred into 18 liters of the fermentation medium in a 30-liter jar fermentor. The fermentation was performed for 4 to 6 days at 28°C by stirring at 180 rpm with aeration of 18 liters per minute.

### Preparation of 5-Lipoxygenase

RBL-1 cells were cultured in Eagle's minimal essential medium containing 20% fetal calf serum and 2 mM glutamine. Cells were maintained in flasks and grown in spinner culture for large scale production<sup>12</sup>. Cells were harvested by centrifugation at 400 × *g* for 10 minutes, washed once with phosphate buffered saline without calcium and magnesium, and then resuspended in the same buffer at 4 × 10<sup>8</sup> cells/ml. When stored at -80°C, the RBL-1 cell suspension retained 5-lipoxygenase activity for several month. The stored cell suspension was lysed by freeze-thawing before use. The frozen-thawed homogenate or its 10,000 × *g* supernatant was used as the enzyme preparation of 5-lipoxygenase.

### Measurement of 5-Lipoxygenase Activity

The reaction mixture of lipoxygenase contained 0.1 M Tris-HCl (pH 7.4), 1 mM reduced glutathione, 1 mM CaCl<sub>2</sub> and 2 mM ATP<sup>13,14</sup>. The RBL-1 cell homogenate (2 × 10<sup>7</sup> cells/ml) or its 10,000 × *g* supernatant (3.6 mg protein/ml) was preincubated with test samples at 37°C for 5 minutes and then incubated with 30 μM [1-<sup>14</sup>C]arachidonic acid (1.6 μCi/ml) for 5 minutes. The reaction mixture was acidified with 0.2 M citric acid and the radioactive metabolites were extracted with EtOAc. The organic phase was evaporated under N<sub>2</sub>-gas stream. The residues were dissolved in EtOAc and applied to silica gel TLC. Petroleum ether - diethyl ether - AcOH (50:50:1) was used as developing solvent<sup>15</sup>. The radioactive product, [1-<sup>14</sup>C]5-HETE, was detected by autoradiography using [<sup>3</sup>H]5-HETE as an authentic sample, scraped off and counted in a liquid scintillation counter. In our assay system, the conversion rate from arachidonic acid to 5-HETE without inhibitors was approximately 7%.

### Measurement of 12-Lipoxygenase and Cyclooxygenase Activities

12-Lipoxygenase was prepared from bovine platelet cytosol by ammonium sulfate fraction according to the method of NUGTEREN<sup>16</sup>, and assay of the enzyme was performed as follows by the modified method of SIEGEL *et al.*<sup>17</sup>. The reaction of 12-lipoxygenase was conducted in 0.1 M Tris-HCl (pH 7.4), 2 mM reduced glutathione and 30 μM [1-<sup>14</sup>C]arachidonic acid at 30°C for 10 minutes. The radioactive product, [1-<sup>14</sup>C]12-HETE, was isolated by TLC using ligroine - diethyl ether - AcOH (50:50:1) as developing solvent.

Bovine platelet microsomes were used as cyclooxygenase by the method of YOSHIMOTO *et al.*<sup>18</sup>.

The reaction was performed in 0.1 M Tris-HCl (pH 7.4), 5 mM L-tryptophan, 2  $\mu$ M hemoglobin and 30  $\mu$ M [1-<sup>14</sup>C]arachidonic acid at 30°C for 10 minutes. The radioactive thromboxane B<sub>2</sub>, a cyclooxygenase metabolite in this system, was isolated by TLC developed with petroleum ether - diethyl ether - AcOH (15:85:0.1). The extraction, detection and quantification procedures of each radioactive product were the same as described in the 5-lipoxygenase assay.

## Results

### Production of Inhibitor by *P. methanica* KY4634

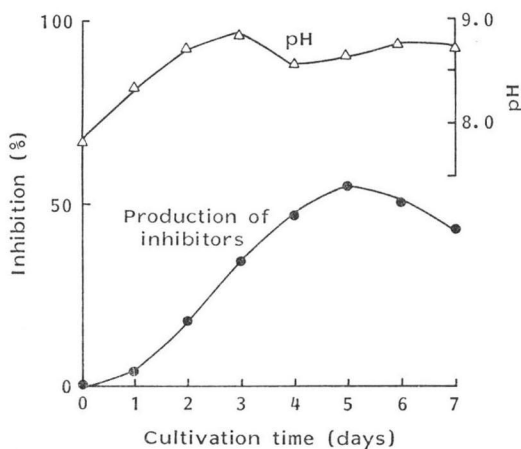
A typical time course of the production in a 30-liter jar fermentor is shown in Fig. 1. The inhibitory activity reached its maximum on the 5th day and then gradually decreased.

### Isolation and Characterization of the Inhibitors

The inhibitors were isolated from the fermentation broth by the following procedures (Fig. 2). The filtered broth (18 liters) was acidified with HCl to pH 2.0 and extracted twice with 18 liters of ethyl acetate. The extract was evaporated *in vacuo* to remove ethyl acetate. The remained aqueous phase was adsorbed to 1 liter of Diaion HP-20 and eluted with methanol. The eluate was concentrated *in vacuo* and the resulting oily residue was applied to silica gel (Wako gel C-200) column (5.0  $\times$  50 cm). Chromatography was performed with chloroform - methanol (9:1). Inhibitory activities were separated into two fractions (F-1 and F-2) and each fraction was concentrated to dryness *in vacuo*. KF8940 and MY12-62c were obtained from the F-2 fraction as follows. The F-2 was dissolved in minimal volume of 80% methanol and applied to reversed-phase low pressure liquid chromatography, Lobar RP-8 type B (Merck), using 80% methanol as a mobile phase. Activities were separated into

Fig. 1. Time course of the production of 5-lipoxygenase inhibitors by *Pseudomonas methanica* KY4634 in a 30-liter jar fermentor.

The production of inhibitors was monitored by measuring the percent inhibition of 5-lipoxygenase as described under "Materials and Methods", where the broth supernatant was added at 5% volume of the reaction mixture. The frozen-thawed homogenate of RBL-1 cells was used as 5-lipoxygenase.



two fractions. KF8940 was contained in the former fraction (F-2a) and MY12-62c was contained in the latter fraction (F-2b). Each fraction was evaporated to dryness and resuspended in a small volume of ethyl acetate. White crystallines of KF8940 and MY12-62c were obtained when each solution was kept standing at 4°C.

Purification of MY12-62a from F-1 fraction was performed by silica gel column chromatography (1.5  $\times$  30 cm) using ethyl acetate as a eluting solvent. MY12-62a was obtained as crystalline material from the active fractions. The yields of KF8940, MY12-62a and MY12-62c were 18, 25 and 15 mg, respectively.

Each crystalline material showed one spot on silica gel TLC developed with chloroform - methanol - aq ammonium hydroxide (76:20:4). The R<sub>f</sub> values of KF8940, MY12-62c and MY12-62a were 0.52, 0.52 and 0.83, respectively. KF8940 and MY12-62c were clearly separated with reversed-phase HPLC, Nucleosil C<sub>8</sub> 5  $\mu$

Fig. 2. Isolation procedures for KF8940, MY12-62c and MY12-62a.

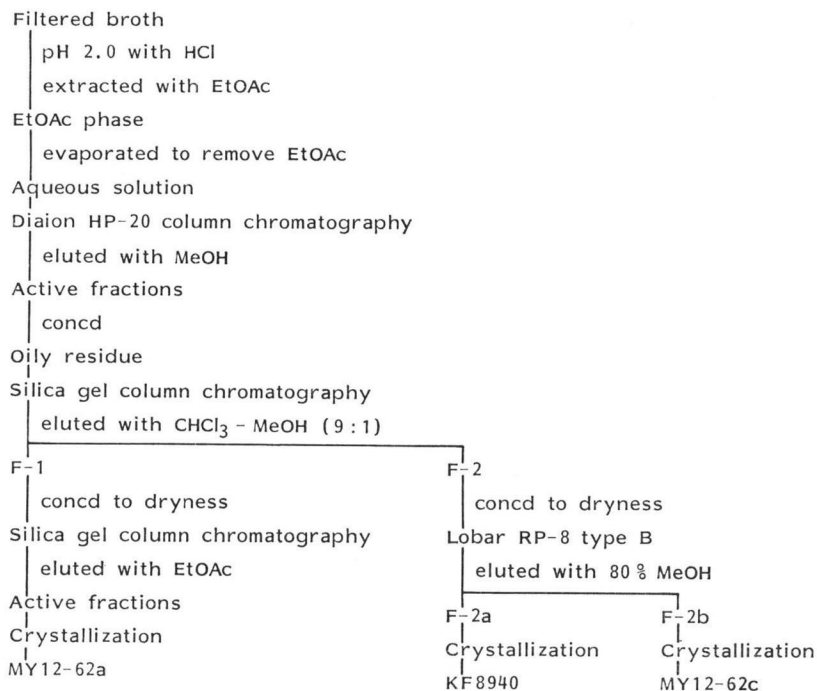
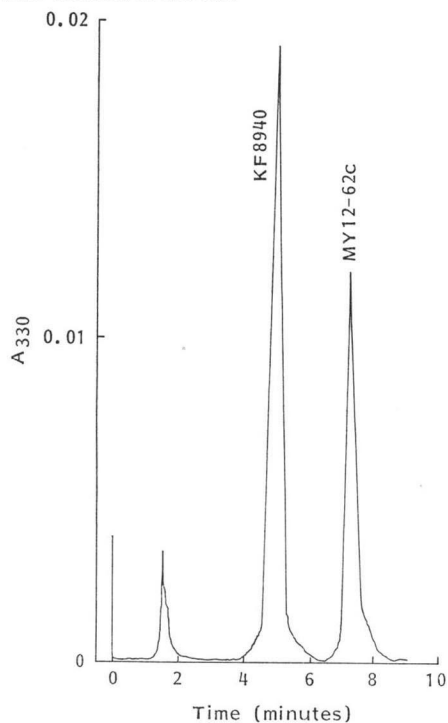


Fig. 3. Reversed-phase HPLC chromatogram of KF8940, MY12-62c.

Column; Nucleosil C<sub>8</sub> 5 μ (4.6 × 280 mm, Nagel), mobile phase; acetonitrile - H<sub>2</sub>O (60:40), peaks were detected at 330 nm.

Table 1. <sup>13</sup>C NMR (25 MHz, CDCl<sub>3</sub>) chemical shifts of KF8940, MY12-62c and MY12-62a.

	KF8940	MY12-62c	MY12-62a
C-2	155.6	155.6	173.7
C-3	105.5	108.2	82.7
C-4	170.4	178.9	195.9
C-4a	123.7	124.9	119.2
C-5	* 132.1	* 131.7	127.7
C-6	124.9	125.2	123.8
C-7	124.8	123.6	136.4
C-8	116.5	118.7	116.6
C-8a	140.6	140.8	140.6
C-1'	31.7	34.4	41.0
C-2'	27.5	* 29.2	* 31.6
C-3'	* 29.5	29.2	29.3
C-4'	29.0	29.0	28.9
C-5'	31.7	31.6	22.8
C-6'	22.6	22.6	22.5
C-7'	14.0	14.0	14.0

\* The assignments for these signals may be interchanged.

(4.6 × 280 mm, Nagel) using 60% acetonitril as a mobile phase (Fig. 3).

Structure Elucidation of KF8940, MY12-62a and MY12-62c

Table 1 shows <sup>13</sup>C NMR spectral data. <sup>1</sup>H

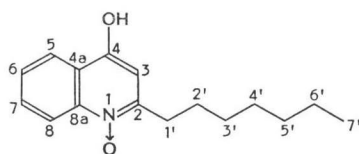
NMR spectral data are as follows:  $^1\text{H}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ).

KF8940; 0.84 (3H, t, H-7'), 1.2 (8H, br) and 1.6 (2H, br) (H-2', 3', 4', 5' and 6'), 2.78 (2H, br t, H-1'), 6.39 (1H, br s, H-3), 8.27 (1H, br d) and 8.07 (1H, br d) (H-5 and 8), 7.58 (1H, br t) and 7.36 (1H, br t) (H-6 and 7).

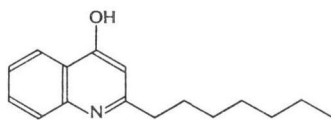
MY12-62c; 0.83 (3H, t, H-7'), 1.2 (6H) and 1.7~1.9 (4H) (H-2', 3', 4', 5' and 6'), 2.68 (2H, br t, H-1'), 6.24 (1H, br s, H-3), 7.3 (1H, m), 7.6 (2H, m) and 8.36 (1H, d) (H-5, 6, 7 and 8), 11.2 (1H, br s, 4-OH).

MY12-62a; 0.83 (3H, t, H-7'), 1.2 (10H, br, H-2', 3', 4', 5' and 6'), 1.8 (2H, m, H-1'), 3.83 (1H, br s, 3-OH), 7.07 (1H, d,  $J=7.8$  Hz, H-8), 7.18 (1H, dd,  $J=6.8$  and 7.5 Hz, H-6), 7.59 (1H, ddd,  $J=1.5$ , 6.8 and 7.8 Hz, H-7), 7.91 (1H, dd,  $J=1.5$  and 7.5 Hz, H-5), 9.30 (1H, br s, 1-NH).

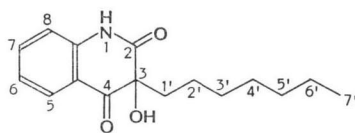
Fig. 4. Structures of KF8940, MY12-62c and MY12-62a.



KF8940



MY12-62c



MY12-62a

Fig. 5. Inhibition of 5- (○) and 12-lipoxygenase (●), and cyclooxygenase (△) versus the concentration of KF8940.

The  $10,000\times g$  supernatant of the RBL-1 homogenate was used as 5-lipoxygenase (see "Materials and Methods").

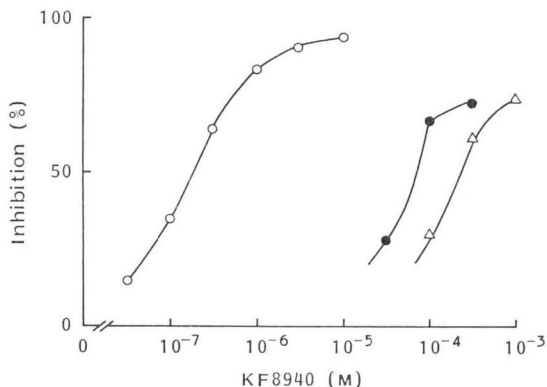


Table 2. Inhibitory activities of KF8940, MY12-62c, MY12-62a and the representative 5-lipoxygenase inhibitors.

5-LO; 5-lipoxygenase ( $10,000\times g$  supernatant of RBL-1), 12-LO; 12-lipoxygenase, CO; cyclooxygenase, AA-861; 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadynyl)-1,4-benzoquinone, U-60,257; 6,9-deepoxy-6,9-(phenylimino)- $\Delta^9,^8$ -prostaglandin  $I_1$ .

Selectivity ratio,  $\text{IC}_{50}$  for 12-LO/ that for 5-LO.

Inhibitors	$\text{IC}_{50}$ (M)			Selectivity ratio
	5-LO	12-LO	CO	
KF8940	$1.5\times 10^{-7}$	$3.5\times 10^{-5}$	$1.7\times 10^{-4}$	233
MY12-62a	$8.0\times 10^{-5}$	$1.4\times 10^{-4}$	$2.7\times 10^{-4}$	1.7
MY12-62c	$1.9\times 10^{-5}$	$5.1\times 10^{-4}$	$2.8\times 10^{-4}$	26.8
Cirsiliol <sup>(9)</sup>	$1.0\times 10^{-7}$	$1.0\times 10^{-6}$	—	10
Caffeic acid <sup>(7)</sup>	$3.7\times 10^{-6}$	$3.0\times 10^{-5}$	$2.0\times 10^{-4}$	81
AA-861 <sup>(8)</sup>	$8.0\times 10^{-7}$	$>10^{-4}$	$>10^{-4}$	$>125$
U-60,257 <sup>(9)</sup>	$2.1\times 10^{-6}$	$>10^{-4}$	—	$>48$

These data indicated that KF8940, MY12-62c and MY12-62a were 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, 2-*n*-heptyl-4-hydroxyquinoline and 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione, respectively (Fig. 4). These structures were confirmed by comparing with spectra of authentic compounds.

2-*n*-Heptyl-4-hydroxyquinoline-*N*-oxide and 2-*n*-heptyl-4-hydroxyquinoline were isolated by HAYS *et al.*<sup>10)</sup> as antibiotic substances from *Pseudomonas aeruginosa*. Afterward LIGHTBOWN and JACKSON<sup>20,21)</sup> revealed that 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide was an antagonist of dihydrostreptomycin and an inhibitor of electron transport through the cytochrome  $b\sim c_1$  segment of the respiratory chain.

3-*n*-Heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione was isolated by NEUENHAUX *et al.*<sup>22)</sup> as a metabolite of *P. aeruginosa*. However, no reports that these compounds possess lipoxygenase inhibitory activities have been described so far.

#### Biochemical Properties of KF8940

As shown in Fig. 5, KF8940 was found to inhibit 5-lipoxygenase of RBL-1 in a dose dependent manner. The concentration causing 50% inhibition ( $IC_{50}$ ) was  $1.5 \times 10^{-7}$  M. The effects of KF8940 on bovine platelet 12-lipoxygenase and bovine platelet cyclooxygenase were also studied. These enzymes were not inhibited by KF8940 at  $1 \times 10^{-5}$  M, the concentration that 5-lipoxygenase was inhibited completely. At higher concentrations, KF8940 inhibited 12-lipoxygenase and cyclooxygenase. The  $IC_{50}$  values for 12-lipoxygenase and cyclooxygenase were  $3.5 \times 10^{-5}$  M and  $1.7 \times 10^{-4}$  M, respectively (Fig. 5). These data indicated that KF8940 is a potent and selective inhibitor of 5-lipoxygenase.

#### Biochemical Properties of MY12-62c and MY12-62a

Other 5-lipoxygenase inhibitors from *P. methanica* KY4634, MY12-62c and MY12-62a, were examined on their selectivity and potency. The  $IC_{50}$  values of the compounds for 5- and 12-lipoxygenase, and cyclooxygenase are listed in Table 2. Although the inhibitory activity of MY12-62c against 5-lipoxygenase was much less potent than that of KF8940, the compound selectively inhibited 5-lipoxygenase:  $IC_{50}$  values for 5- and 12-lipoxygenase, and cyclooxygenase were  $1.9 \times 10^{-5}$ ,  $5.1 \times 10^{-4}$  and  $2.8 \times 10^{-4}$  M, respectively. The  $IC_{50}$  values of MY12-62a for 5- and 12-lipoxygenase and cyclooxygenase were  $8.0 \times 10^{-5}$ ,  $1.4 \times 10^{-4}$  and  $2.7 \times 10^{-4}$  M, respectively, indicating that the compound was a rather non-selective inhibitor for these enzymes.

#### Discussion

A number of 5-lipoxygenase inhibitors have recently been found from synthetic compounds and herb medicine components. However, no microbial metabolites have been reported to inhibit 5-lipoxygenase. Now, we have found that *P. methanica* KY4634 produced potent inhibitors of 5-lipoxygenase. From the culture broth, three 5-lipoxygenase inhibitors were isolated. Among them, KF8940, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, was a potent and selective inhibitor of 5-lipoxygenase.

Most compounds reported to inhibit 5-lipoxygenase inhibited 12-lipoxygenase or cyclooxygenase simultaneously. There have been only several 5-lipoxygenase inhibitors without cyclooxygenase and 12-lipoxygenase inhibition. The representative 5-lipoxygenase inhibitors and their inhibitory profiles together with those of KF8940 are listed in Table 2. Comparing the inhibitors so far reported, KF8940 may be one of the most potent and selective 5-lipoxygenase inhibitors;  $IC_{50}$  was  $1.5 \times 10^{-7}$  M and its selectivity ratio ( $IC_{50}$  for 12-lipoxygenase/ $IC_{50}$  for 5-lipoxygenase) was 233. As for the selectivity, it will be further required to examine the effect on other lipoxygenases, particularly mammalian 15-

lipoxygenase, and we are now investigating this subject. More precise biochemical and pharmacological properties of the compound will be reported in succeeding papers of this series.

#### References

- 1) MURPHY, R. C.; S. HAMMARSTRÖM & B. SAMUELSSON: Leukotriene C: A slow-reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 76: 4275~4279, 1980
- 2) ÖRNING, L.; S. HAMMARSTRÖM & B. SAMUELSSON: Leukotriene D: A slow reacting substance from rat basophilic leukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* 77: 2014~2017, 1980
- 3) YAMAMOTO, S.: Enzymes in the arachidonic acid cascades. *In Prostaglandins and Related Substances. Eds., C. R. PACE-ASCIAC & E. GRANSTRÖM*, pp. 171~202, Elsevier Science Publishers, Amsterdam, 1983
- 4) HANSSON, G.; C. MALMSTEN & O. RÅDMARK: The leukotrienes and other lipoxygenase products. *In Prostaglandins and Related Substances. Eds., C. R. PACE-ASCIAC & E. GRANSTRÖM*, pp. 127~169, Elsevier Science Publishers, Amsterdam, 1983
- 5) EÁGN, R. W.; A. N. TISCHLER, E. M. BAPTISTA, E. A. HAM, D. D. SODERMAN & P. H. GALE: Specific inhibition and oxidative regulation of 5-lipoxygenase. *In Advances in Prostaglandin, Thromboxane, and Leukotriene Research. Vol. 11, Ed., B. SAMUELSSON, et al.*, pp. 151~157, Raven Press, New York, 1983
- 6) YOSHIMOTO, T.; M. FURUKAWA, S. YAMAMOTO, T. HORIE & S. KOHNO: Flavonoids: Potent inhibitors of arachidonate 5-lipoxygenase. *Biochem. Biophys. Res. Commun.* 116: 612~618, 1983
- 7) KOSHIHARA, Y.; T. NEICHI, S. MUROTA, A.-N. LAO, Y. FUJIMOTO & T. TATSUNO: Caffeic acid is a selective inhibitor for leukotriene biosynthesis. *Biochim. Biophys. Acta* 792: 92~97, 1984
- 8) YOSHIMOTO, T.; C. YOKOYAMA, K. OCHI, S. YAMAMOTO, Y. MAKI, Y. ASHIDA, S. TERA0 & M. SHIRAISHI: 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadinyl)-1,4-benzoquinone (AA 861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. *Biochim. Biophys. Acta* 713: 470~473, 1982
- 9) SUN, F. F. & J. C. MCGUIRE: Inhibition of human neutrophil arachidonate 5-lipoxygenase by U-60,257. *Prostaglandins* 26: 211~221, 1983
- 10) SMITH, R. J.; F. F. SUN, B. J. BOWMAN, S. S. IDEN, H. W. SMITH & J. C. MCGUIRE: Effect of 6,9-deepoxy-6,9-(phenylimino)- $\Delta^{9,8}$ -prostaglandin I<sub>1</sub> (U-60,257), an inhibitor of leukotriene synthesis, on human neutrophil function. *Biochem. Biophys. Res. Commun.* 109: 943~949, 1982
- 11) ASHIDA, Y.; T. SAJO, H. KURIKI, H. MAKINO, S. TERA0 & Y. MAKI: Pharmacological profile of AA-861, a 5-lipoxygenase inhibitor. *Prostaglandins* 26: 955~972, 1983
- 12) KULCZYCKI, A., Jr.; C. ISERSKY & M. METZGER: The interaction of IgE with rat basophilic leukemia cells. I. Evidence for specific binding of IgE. *J. Exp. Med.* 140: 600~616, 1974
- 13) JASCHIK, B. A.; F. F. SUN, L. LEE & M. M. STEINHOFF: Calcium stimulation of a novel lipoxygenase. *Biochem. Biophys. Res. Commun.* 95: 103~110, 1980
- 14) FURUKAWA, M.; T. YOSHIMOTO, K. OCHI & S. YAMAMOTO: Studies on arachidonate 5-lipoxygenase of rat basophilic leukemia cells. *Biochim. Biophys. Acta* 795: 458~465, 1985
- 15) VANDERHOEK, J. Y.; R. W. BRYANT & J. M. BAILEY: Inhibition of leukotriene biosynthesis by leukocyte product 15-hydroxy-5,8,11,13-eicosatetraenoic acid. *J. Biol. Chem.* 225: 10064~10065, 1980
- 16) NUGTEREN, D. H.: Arachidonate lipoxygenase in blood platelets. *Biochim. Biophys. Acta* 380: 299~307, 1975
- 17) SIEGEL, M. I.; R. T. MCCONNELL & P. CUATRECASAS: Aspirin-like drugs interfere with arachidonate metabolism by inhibition of the 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid peroxidase activity of the lipoxygenase pathways. *Proc. Natl. Acad. Sci. U.S.A.* 76: 3774~3779, 1979
- 18) YOSHIMOTO, T.; S. YAMAMOTO, M. OCHI & O. HAYAISHI: Solubilization and resolution of TX synthesizing system from microsomes of bovine blood platelet. *J. Biol. Chem.* 252: 5871~5874, 1977
- 19) HAYS, E. E.; I. C. WELLS, P. A. KATZMAN, C. K. CAIN, F. A. JACOBS, S. A. THAYE & E. A. DOISY: Antibiotic substances produced by *Pseudomonas aeruginosa*. *J. Biol. Chem.* 159: 725~749, 1945
- 20) JACKSON, F. L. & J. W. LIGHTBOWN: Observations on the inhibitory and growth-promoting activities of streptomycin and dihydrostreptomycin on bacteria. *J. Gen. Microbiol.* 11 (Proc. of the Society for General Microbiology): iv~v, 1954
- 21) LIGHTBOWN, J. W. & F. L. JACKSON: Inhibition cytochrome systems of heart muscle and certain bacteria by the antagonists of dihydrostreptomycin: 2-Alkyl-4-hydroxyquinoline-N-oxides. *Biochem. J.* 63: 130~137, 1956
- 22) NEUENHAUX, W.; H. BUDZIKIEWICZ, H. KORTH & G. PULVERES: Bakterieninhyllsstoffe, III. 3-Alkyl-tetrahydrochinolinderivate *Pseudomonas*. *Z. Naturforsch* 34b: 313~315, 1979