Inhibitory Activities and Inhibition Specificities of Caffeic Acid Derivatives and Related Compounds toward 5-Lipoxygenase

Masanori SUGIURA, Youichiro NAITO, Yasunari YAMAURA, Chikara FUKAYA,* and Kazumasa YOKOYAMA

Central Research Laboratories, The Green Cross Corporation, Shodai-ohtani, Hirakata, Osaka 573, Japan. *Received August 1, 1988*

Various caffeic acid derivatives were synthesized, and their effects on 5-lipoxygenase (5-LO), 12-lipoxygenase (12-LO) and prostaglandin (PG) synthase activities were investigated. Among them, caffeic acid octyl amide (5) and 1-(3,4-dihydroxyphenyl)-1-octen-3-one (11) showed very potent inhibitory activities toward 5-LO with IC_{50} values of 4.2×10^{-8} and 3.5×10^{-8} M, respectively. They were very selective inhibitors for 5-LO. Compound 11 showed non-competitive inhibition, and the two adjacent hydroxy groups attached to the benzene ring, as well as the hydrophobic alkyl side chain, were required for its strong binding to 5-LO.

Keywords 5-lipoxygenase; caffeic acid; 1-(3,4-dihydroxyphenyl)-1-octen-3-one; caffeic acid octyl amide; inhibitory activity; inhibition specificity

Arachidonate 5-lipoxygenase (5-LO) is well known to be the key enzyme in the biosynthesis of leukotrienes, which are thought to be related to many diseases such as allergic asthma,[1-4] psoriasis,[5,6] myocardial infarction[7] and so on.[8] Therefore specific inhibitors of 5-LO are of interest as candidate drugs for the treatment of these diseases.

Since the discovery of Koshihara *et al.*[9] that caffeic acid and its methyl ester have strong and specific inhibitory activity toward 5-LO, we have synthesized various caffeic acid derivatives with the aim of finding stronger and more specific inhibitors of 5-LO.

Four types of derivatives were synthesized, mainly by the following methods. 1) Caffeic acid esters were prepared by acid catalyzed esterification. 2) Caffeic acid amides were prepared by the reaction of caffeic acid with the corresponding amines using dicyclohexylcarbodiimide as the dehydrating agent. 3) 1-(3,4-Dihydroxyphenyl)-1-alken-3-one compounds were prepared by means of the Horner–Emmons reaction of veratraldehyde with dimethyl-2-oxoalkylphosphonate followed by removal of the methyl group. 4) 1-(3,4-Dihydroxyphenyl)-1-alkene compounds were prepared by means of the Witting reaction of 3,4-bis(tetrahydro-2*H*-pyran-2-yloxy)benzaldehyde with alkyltriphenylphosphonium bromide followed by removal of the tetrahydro-2*H*-pyran group.

Several of the compounds so prepared are very potent and selective 5-LO inhibitors.

Experimental

Materials [1-^{14}C]Arachidonic acid (57.6 mCi/mmol) was obtained from Amersham Co. Caffeic acid was purchased from Nakarai Chemicals Co., Osaka. Prostaglandin E_2 (PGE_2), 6-ketoprostaglandin $F_{1\alpha}$ (6-KT-$PGF_{1\alpha}$), thromboxane B_2(TXB_2) and 12-hydroxyeicosatetraenoic acid (12-HETE) were obtained from Funakoshi Pharmaceuticals Co., Ltd., Tokyo. 5-Hydroxyeicosatetraenoic acid (5-HETE) was synthesized according to the method of Corey *et al.*[10] Adenosine triphosphate (ATP) was obtained from Wako Pure Chemicals Industries, Osaka. Precoated Silica gel 60 thin-layer chromatography (TLC) plates were obtained from E. Merck. All of the other chemicals were of reagent grade.

Analysis Melting points were determined with a Yanaco melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured on a Hitachi R-24B NMR spectrometer with tetramethylsilane (TMS) as an internal standard; chemical shifts are given on the δ (ppm) scale. Infrared (IR) spectra were obtained on a Shimadzu IR-420 spectrometer.

Caffeic Acid Butyl Ester (2) Caffeic acid (2.0 g) was added to butanol (50 ml) into which hydrogen chloride gas had been introduced beforehand for about 10 min. The resultant mixture was heated at 90—100 °C for 3 h. The reaction mixture was concentrated under reduced pressure, and the precipitated solid was purified by silica gel column chromatography (hexane: ethyl acetate = 2 : 1) and crystallized from ether and hexane to give **2** (1.4 g, 53%). mp 111.0—111.5 °C. IR (KBr) cm^{-1}: 3490, 3320, 1682, 1603. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.97 (t, 3H, J=6 Hz), 1.2—2.0 (m, 4H), 4.16 (t, 2H, J=6Hz), 6.14 (d, 1H, J=16 Hz), 6.6—7.1 (m, 3H), 7.52 (d, 1H, J=16 Hz), 8.65 (m, 2H).

Caffeic Acid Ethyl Ester (1) The procedure used for the preparation of **2** was repeated with caffeic acid (200 mg) except for the use of ethanol as the solvent to obtain **1** (100 mg, 43%). mp 142.0—143.0°C. IR (KBr) cm^{-1}: 3499, 3300, 1679, 1638, 1602, 978. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 1.30 (t, 3H, J=7 Hz), 4.20 (q, 2H, J=7 Hz), 7.12 (d, 1H, J=16 Hz), 6.65—7.1 (m, 3H), 7.50 (d, 1H, J=16 Hz), 8.70 (m, 2H).

Caffeic Acid Nonyl Ester (3) The procedure used for the preparation of **2** was repeated with caffeic acid (200 mg) except for the use of 1-nonanol as the solvent, to obtain **3** (100 mg, 34%). mp 107.0–108.0 °C. IR (KBr)cm^{-1}: 3499, 3310, 1685, 1619, 1603, 975. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.89 (t, 3H, J=7 Hz), 1.1—1.9 (m, 14H), 4.16 (t, 2H, J=7 Hz), 6.22 (d, 1H, J=16 Hz), 6.6—7.1 (m, 3H), 7.53 (d, 1H, J=16 Hz).

Caffeic Acid Hexyl Amide (4) Dicyclohexylcarbodiimide (206 mg) and hexylamine (100 mg) were successively added to a solution of caffeic acid (180 mg) in tetrahydrofuran (5 ml). The resultant mixture was stirred at 50°C for 7 h and then the reaction mixture was filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane: ethyl acetate = 1 : 1) and crystallized from ethyl acetate and hexane to give **4** (100 mg, 38%). mp 141.0—143.0 °C. IR (KBr) cm^{-1}: 3500, 1645, 1585, 970. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.92 (t, 3H, J=6 Hz), 1.0—1.7 (m, 8H), 3.1—3.4 (m, 2H), 6.40 (d, 1H, J=15 Hz), 6.8—7.1 (m, 3H), 7.40 (d, 1H, J=15 Hz).

Caffeic Acid Octyl Amide (5) The procedure used for the preparation of **4** was repeated with caffeic acid (3.0 g) and octylamine to obtain **5** (4.0 g, 82%). mp 126.0—128.5 °C. IR (KBr) cm^{-1}: 3520, 3400, 3180, 1645, 1595. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.89 (t, 3H, J=6 Hz), 1.0—1.7 (m, 12H), 3.0—3.4 (m, 2H), 6.26 (d, 1H, J=16 Hz), 6.7—7.0 (m, 3H), 7.22 (d, 1H, J=16 Hz), 7.7 (m, 1H), 8.01 (m, 1H), 8.9 (m, 1H).

Caffeic Acid Decyl Amide (6) The procedure used for the preparation of **4** was repeated with caffeic acid (180 mg) and decylamine to obtain **6** (50 mg, 16%). IR (KBr) cm^{-1}: 3500, 3330, 3180, 1645, 1595. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.88 (m, 3H), 1.1—1.6 (m, 16H), 3.0—3.4 (m, 2H), 6.28 (d, 1H, J=15 Hz), 6.7—7.0 (m, 3H), 7.22 (d, 1H, J=15 Hz), 8.0 (m, 1H), 8.85 (m, 2H).

Caffeic Acid Dodecyl Amide (7) The procedure used for the preparation of **4** was repeated with caffeic acid (360 mg) and dodecylamine to obtain **7** (70 mg, 10%). mp 124.0—125.0 °C. IR (KBr) cm^{-1}: 3500, 3380, 3150, 1650, 1595. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.89 (t, 3H, J=5 Hz), 1.1—1.7 (m, 20H), 3.0—3.4 (m, 2H), 6.36 (d, 1H, J=15 Hz), 6.7—7.0 (m, 3H), 7.29 (d, 1H, J=15 Hz), 8.01 (m, 1H).

Caffeic Acid Tetradecyl Amide (8) The procedure used for the preparation of **4** was repeated with caffeic acid (360 mg) and tetradecylamine to obtain **8** (50 mg, 7%). mp 119.0—120.0 °C. IR (KBr) cm^{-1}: 3500, 3340, 3150, 1645, 1590. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.89 (m, 3H), 1.1—1.7 (m, 24H), 3.0—3.4 (m, 2H), 6.27 (d, 1H, J=15 Hz), 6.7—7.0 (m, 3H), 7.34 (d, 1H, J=15 Hz), 7.95 (m, 1H).

1-(3,4-Dihydroxyphenyl)-1-octen-3-one (11) 1-(3,4-Dimethoxyphenyl)-

1-octen-3-one (**12**): A solution of dimethyl 2-oxoheptylphosphonate (4.0 g) in 1,2-dimethoxyethane (30 ml) was added dropwise to a suspension of sodium hydride (0.87 g, 50% in mineral oil) and 1,2-dimethoxyethane (70 ml) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was cooled to −30 °C. Then a solution of veratraldehyde (3.0 g) in 1,2-dimethoxyethane (3 ml) was added dropwise to the cooled solution. The reaction mixture was gradually warmed up to room temperature, poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine successively, and dried over magnesium sulfate. After evaporation of the solvent, **12** was purified by silica gel column chromatography (hexane : ethyl acetate = 3 : 1) (1.3 g, 27%). IR (film) cm^{-1}: 1685, 1655, 980. ^{1}H-NMR ($CDCl_3$): 0.90 (t, 3H, J = 6 Hz), 1.05—2.0 (m, 6H), 2.61 (t, 2H, J = 7 Hz), 3.90 (s, 6H), 6.59 (d, 1H, J = 16 Hz), 6.8—7.2 (m, 3H), 7.50 (d, 1H, J = 16 Hz).

1-(3,4-Dihydroxyphenyl)-1-octen-3-one (**11**): A 1 M solution of boron tribromide in dichloromethane (1.5 ml) was added to a cooled (−78 °C) solution of **12** (128 mg) in dry dichloromethane (1 ml). After the addition was completed, the reaction mixture was gradually warmed to room temperature. Then the reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water and brine successively, and dried over magnesium sulfate. After evaporation of the solvent, **11** was purified by recrystallization from water and ethanol (60 mg, 52%). mp 130.0—131.0 °C. IR (KBr) cm^{-1}: 3500, 1678, 1640, 1598, 980. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.90 (m, 3H), 1.1—1.8 (m, 6H), 2.60 (t, 2H, J = 6 Hz), 6.50 (d, 1H, J = 16 Hz), 6.7—7.1 (m, 3H), 7.43 (d, 1H, J = 16 Hz), 8.99 (s, 1H), 9.37 (s, 1H).

1-(3,4-Dihydroxyphenyl)-1-hexen-3-one (9) 1-(3,4-Dimethoxyphenyl)-1-hexen-3-one(**10**): The procedure used for the preparation of **12** was repeated with veratraldehyde (0.5 g) and dimethyl 2-oxo-pentylphosphonate (0.58 g)[11] to obtain **10** (0.5 g, 70%). IR (KBr) cm^{-1}: 1655, 1619, 1595, 977. ^{1}H-NMR ($CDCl_3$): 0.98 (t, 3H, J = 6 Hz), 1.4—2.05 (m, 2H), 2.62 (t, 2H, J = 7 Hz), 3.90 (s, 6H), 6.57 (d, 1H, J = 16 Hz), 6.7—7.3 (m, 3H), 7.48 (d, 1H, J = 16 Hz).

1-(3,4-Dihydroxyphenyl)-1-hexen-3-one (**9**): The procedure used for the preparation of **11** was repeated with **10** (188 mg) to obtain **9** (69 mg, 41%). mp 131.0—132.0 °C. IR (KBr) cm^{-1}: 3450, 3230, 1640, 1620, 1600, 978. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.94 (t, 3H, J = 6 Hz), 1.63 (tq, 2H, J = 6, 6 Hz), 2.59 (t, 2H, J = 6 Hz), 6.44 (d, 1H, J = 16 Hz), 6.65—7.1 (m, 3H), 7.46 (d, 1H, J = 16 Hz), 9.03 (s, 2H).

1-(3,4-Dihydroxyphenyl)-1-decen-3-one (13) 1-(3,4-Dimethoxyphenyl)-1-decen-3-one (**14**): The procedure used for the preparation of **12** was repeated with veratraldehyde (0.5 g) and dimethyl 2-oxo-nonylphosphonate (0.75 g)[11] to obtain **14** (0.19 g, 22%). IR (KBr) cm^{-1}: 1682, 1610, 1595, 1577, 975. ^{1}H-NMR ($CDCl_3$): 0.88 (t, 3H, J = 6 Hz), 1.05—1.9 (m, 10H), 2.63 (t, 2H, J = 6 Hz), 3.90 (s, 6H), 6.68 (d, 1H, J = 16 Hz), 6.7—7.3 (m, 3H), 7.50 (d, 1H, J = 16 Hz).

1-(3,4-Dihydroxyphenyl)-1-decen-3-one (**13**): The procedure used for the preparation of **11** was repeated with **14** (186 mg) to obtain **13** (99 mg, 59%). mp 115.0—116.5 °C. IR (KBr) cm^{-1}: 3500, 3310, 1678, 1642, 1600, 972. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): 0.89 (t, 3H, J = 6 Hz), 1.1—1.9 (m, 10H), 2.60 (t, 2H, J = 7 Hz), 6.47 (d, 1H, J = 16 Hz), 6.7—7.1 (m, 3H), 7.39 (d, 1H, J = 16 Hz), 9.04 (s, 2H).

1-(3,4-Dihydroxyphenyl)-1-butene (15) 3,4-Bis(tetrahydro-2*H*-pyran-2-yloxy)benzaldehyde (**16**): A catalytic amount of *p*-toluenesulfonic acid was added to a cooled (0 °C) solution of protocatechualdehyde (1.0 g), 3,4-dihydro-2*H*-pyran (2.45 ml) and dichloromethane (10 ml). The resultant solution was stirred at room temperature for 2 h. After the reaction was completed, the reaction mixture was poured into dichloromethane (80 ml) and saturated sodium bicarbonate solution (50 ml), and extracted with dichloromethane. The organic layer was washed with water and brine successively, and dried over magnesium sulfate. After evaporation of the solvent, **16** was purified by silica gel column chromatography (hexane : ether = 2 : 1) (500 mg, 23%). IR (film) cm^{-1}: 1690, 1598. ^{1}H-NMR ($CDCl_3$): 1.1—2.2 (m, 12H), 3.1—4.2 (m, 4H), 5.45 (s, 1H), 5.51 (s, 1H), 7.1—7.6 (m, 3H), 9.80 (s, 1H).

1-[3,4-Bis(tetrahydro-2*H*-pyran-2-yloxy)phenyl]-1-butene (**17**): A suspension of sodium hydride (80 mg, 50% in mineral oil) in dry dimethyl sulfoxide (1 ml) was stirred at 78—80 °C for 15 min. After being cooled to room temperature, a solution of propyltriphenylphosphonium bromide (640 mg) in dimethyl sulfoxide (1.5 ml) was added dropwise to the above solution. The resultant solution was stirred at room temperature for 20 min, then cooled with water, and a solution of **16** (250 mg) in dimethyl sulfoxide (1.5 ml) was added dropwise. The solution thus obtained was stirred at room temperature for 1.5 h, poured into ice-water and extracted with ether. The organic layer was washed with water and brine successively, and dried over magnesium sulfate. After evaporation of the solvent, **17** was purified by silica gel column chromatography (hexane : ether = 6 : 1) (212 mg, 77%). IR (film) cm^{-1}: 1603, 1578. ^{1}H-NMR ($CDCl_3$): 1.06 (t, 3H, J = 7 Hz), 1.4—2.1 (m, 12H), 2.1—2.5 (m, 2H), 3.4—4.3 (m, 4H), 5.45 (s, 1H), 5.47 (s, 1H), 5.4—6.5 (m, 2H), 6.75—7.2 (m, 3H).

1-(3,4-Dihydroxyphenyl)-1-butene (**15**): A catalytic amount of *p*-toluenesulfonic acid was added to a cooled (0 °C) solution of **17** (210 mg) in dry methanol (10 ml). The resultant solution was stirred at 0 °C for 2 h, and then a few drops of triethylamine were added to the solution. After evaporation of the solvent, **15** was purified by column chromatography (hexane : ether = 2 : 1) (82 mg, 79%). IR (film) cm^{-1}: 3350, 1600. ^{1}H-NMR ($CDCl_3$): 0.99 (t, 3H, J = 7 Hz), 2.21 (dq, 2H, J = 6, 7 Hz), 5.48 (dt, 1H, J = 11, 6 Hz), 6.20 (d, 1H, J = 11 Hz), 6.5—7.0 (m, 3H).

1-(3,4-Dihydroxyphenyl)-1-hexene (18) 1-[3,4-Bis(tetrahydro-2*H*-pyran-2-yloxy)phenyl]-1-hexene (**19**): The procedure used for the preparation of **17** was repeated with **16** (250 mg) and pentyltriphenylphosphonium bromide (690 mg) to obtain **19** (198 mg, 66%). IR (film) cm^{-1}: 1603, 1579. ^{1}H-NMR ($CDCl_3$): 0.95 (t, 3H, J = 6 Hz), 1.2—2.1 (m, 16H), 2.1—2.6 (m, 2H), 3.4—4.3 (m, 4H), 5.45 (s, 2H), 5.4—6.5 (m, 2H), 6.75—7.2 (m, 3H).

1-(3,4-Dihydroxyphenyl)-1-hexene (**18**): The procedure used for the preparation of **15** was repeated with **19** (198 mg) to obtain **18** (61 mg, 58%). IR (film) cm^{-1}: 3300, 1600. ^{1}H-NMR $CDCl_3$): 0.89 (m, 3H), 1.1—1.6 (m, 4H), 2.20 (dt, 2H, J = 7, 6 Hz), 5.0 (br s, 2H), 5.55 (dt, 1H, J = 11, 7 Hz), 6.16 (d, 1H, J = 11 Hz), 6.7—7.0 (m, 3H).

1-(3,4-Dihydroxyphenyl)-1-octene (20) 1-[3,4-Bis(tetrahydro-2*H*-pyran-2-yloxy)phenyl]-1-octene (**21**): The procedure used for the preparation of **17** was repeated with **16** (250 mg) and heptyltriphenylphosphonium bromide (730 mg) to obtain **21** (250 mg, 78%). IR (film) cm^{-1}: 1603, 1579. ^{1}H-NMR ($CDCl_3$): 0.97 (t, 3H, J = 5 Hz), 1.0—2.1 (m, 20H), 2.1—2.6 (m, 2H), 3.4—4.3 (m, 4H), 5.43 (s, 2H), 5.4—6.5 (m, 2H), 6.70—7.2 (m, 3H).

1-(3,4-Dihydroxyphenyl)-1-octene (**20**): The procedure used for the preparation of **15** was repeated with **21** (250 mg) to obtain **20** (76 mg, 54%). IR (film) cm^{-1}: 3350, 1600. ^{1}H-NMR ($CDCl_3$): 0.88 (t, 3H, J = 5 Hz), 1.0—1.6 (m, 8H), 2.20 (t, 2H, J = 6 Hz), 5.34 (br s, 2H), 5.52 (dt, 1H, J = 12, 6Hz), 6.23 (d, 1H, J = 12 Hz), 6.6—7.0 (m, 3H).

1-(4-Hydroxy-3-methoxyphenyl)-1-octen-3-one (22) 4-Ethoxycarboxy-3-methoxybenzaldehyde (**23**): Ethyl choloroformate (1.9 ml) was added dropwise to a cooled (0 °C) solution of vanillin (1.5 g) in 1 M NaOH solution (20 ml). The resultant mixture was stirred at 0 °C for 8 h. After the reaction was completed, the reaction mixture was extracted with ether. The ether layer was washed with 0.1 M HCl solution, water and brine successively, and dried over magnesium sulfate. After evaporation of the solvent, **23** was purified by recrystallization from ether and ethyl acetate (1.53 g, 69%). IR (KBr) cm^{-1}: 1758, 1700, 1603. ^{1}H-NMR ($CDCl_3$): 1.35 (t, 3H, J = 7 Hz), 3.86 (s, 3H), 4.29 (q, 2H, J = 7 Hz), 7.15—7.55 (m, 3H), 9.85 (s, 1H).

1-(4-Ethoxycarboxy-3-methoxyphenyl)-1-octen-3-one (**24**): The procedure used for the preparation of **12** was repeated with **23** (1.53 g) except for the use of tetrahydrofuran as the solvent to obtain **24** (1.63 g, 74%). IR (KBr) cm^{-1}: 1760, 1690, 1660, 1615, 975. ^{1}H-NMR ($CDCl_3$): 0.90 (t, 2H, J = 5 Hz), 1.37 (t, 3H, J = 5 Hz), 1.1—2.0 (m, 6H), 2.62 (t, 2H, J = 7 Hz), 3.83 (s, 3H), 4.26 (q, 2H, J = 7 Hz), 6.61 (d, 1H, J = 15 Hz), 7.0—7.2 (m, 3H), 7.46 (d, 1H, J = 15 Hz).

1-(4-Hydroxy-3-methoxyphenyl)-1-octen-3-one (**22**): A 1 M NaOH solution (5.2 ml) was added dropwise to a cooled (0 °C) solution of **24** (1.50 g) in ethanol (80 ml). The resultant solution was stirred at 0 °C for 1.5 h. The solution was brought to pH 3 with 1 M HCl solution, and was extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate. After evaporation of the solvent, **22** was purified by silica gel column chromatography (hexane : ether) (1.1 g, 93%). mp 48—49.5 °C. IR (KBr) cm^{-1}: 3450, 3320, 1635, 1592. ^{1}H-NMR ($CDCl_3$): 0.91 (t, 3H, J = 5Hz), 1.05—1.9 (m, 6H), 2.57 (t, 2H, J = 7 Hz), 3.85 (s, 3H), 5.97 (s, 1H), 6.50 (d, 1H, J = 16 Hz), 6.7—7.2 (m, 3H), 7.43 (d, 1H, J = 16 Hz).

1-(3-Hydroxy-4-methoxyphenyl)-1-octen-3-one (25) 3-Ethoxycarboxy-4-methoxybenzaldehyde (**26**): The procedure used for the preparation of **23** was repeated with isovanillin (1.5 g) to obtain **26** (2.0 g, 90%). IR (KBr) cm^{-1}: 1760, 1690, 1605. ^{1}H-NMR ($CDCl_3$): 1.35 (t, 3H, J = 7 Hz), 3.85 (s, 3H), 4.22 (q, 2H, J = 7 Hz), 6.96 (d, 1H, J = 9 Hz) 7.50—7.75 (m, 2H), 9.74 (s, 1H).

1-(3-Ethoxycarboxy-4-methoxyphenyl)-1-octen-3-one (**27**): The procedure used for the preparation of **12** was repeated with **26** (1.53 g) except for the use of tetrahydrofuran as the solvent to obtain **27** (1.89 g, 86%). IR (KBr) cm^{-1}: 1760, 1695, 1658, 1603. ^{1}H-NMR ($CDCl_3$): 0.87 (t, 3H, J = 5 Hz), 1.37 (t, 3H, J = 7 Hz). 1.1—2.0 (m, 6H), 2.57 (t, 2H, J = 6 Hz), 3.85 (s, 3H), 4.28 (q, 2H, J = 7 Hz), 6.53 (d, 1H, J = 16 Hz), 6.8—7.6 (m,

3H), 7.50 (d, 1H, $J=16$ Hz).

1-(3-Hydroxy-4-methoxyphenyl)-1-octen-3-one (**25**): The procedure used for the preparation of **22** was repeated with **27** (1.80 g) to obtain **25** (1.16 g, 83%). mp 68.5—69.0 °C. IR (KBr) cm^{-1}: 3280, 1630, 1605, 970. ^{1}H-NMR ($CDCl_3$): 0.91 (t, 3H, $J=6$ Hz), 1.1—2.0 (m, 6H), 2.62 (t, 2H, $J=7$ Hz), 3.90 (s, 3H), 5.80 (s, 1H), 6.55 (d, 1H, $J=15$ Hz), 6.85—7.25 (m, 3H), 7.44 (d, 1H, $J=15$ Hz).

8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenol (28) 3,4-Bis(ethoxycarboxy)-benzaldehyde (**29**): The procedure used for the preparation of **23** was repeated with protocatechualdehyde (10 g) except for the use of 4 eq of 1 M NaOH and ethyl chloroformate, to obtain **29** (19.3 g, 94%). IR (film) cm^{-1}: 1760, 1690, 1605. ^{1}H-NMR ($CDCl_3$): 1.38 (t, 6H, $J=7$ Hz), 4.33 (q, 4H, $J=7$ Hz), 7.44 (d, 1H, $J=8$ Hz) 7.60—7.90 (m, 2H), 9.91 (s, 1H).

1-[3,4-Bis(ethoxycarboxy)phenyl]-8-(tetrahydro-2*H*-pyran-2-yl)oxy-1-octen-3-one (**30**): The procedure used for the preparation of **12** was repeated with **29** (16.6 g) except for the use of dimethyl 2-oxo-7-(tetrahydro-2*H*-pyran-2-yl)oxyheptylphosphonate[12] to obtain **30** (22.6 g, 80%). IR (film) cm^{-1}: 1770, 1695, 1665, 1616, 1585. ^{1}H-NMR ($CDCl_3$): 1.38 (t, 6H, $J=7$ Hz), 1.2—2.0 (m, 12H), 2.65 (t, 2H, $J=6$ Hz), 3.2—4.0 (m, 4H), 4.31 (q, 4H, $J=7$ Hz), 4.55 (s, 1H), 6.62 (d, 1H, $J=16$ Hz) 7.2—7.7 (m, 4H).

8-[3,4-Bis(ethoxycarboxy)phenyl]-6-oxo-7-octenol (**31**): A solution of **31** (22.6 g) in acetic acid, water and tetrahydrofuran (3:3:1, 280 ml) was stirred at 40 °C for 4 h. After being cooled to 0 °C, the reaction mixture was diluted with ethyl acetate. The organic layer was washed with 2.5 M NaOH solution, 0.1 M HCl solution and brine successively and dried over magnesium sulfate. Removal of solvent under reduced pressure gave **31**, which was purified by silica gel column chromatography (ethyl acetate : hexane) (17.28 g, 93%). IR (film) cm^{-1}: 3400, 1765, 1690, 1660, 1610. ^{1}H-NMR ($CDCl_3$): 1.37 (t, 6H, $J=7$ Hz), 1.0—2.0 (m, 6H), 2.63 (t, 2H, $J=6$ Hz), 3.60 (t, 2H, $J=6$ Hz), 4.27 (q, 4H, $J=7$ Hz), 6.55 (d, 1H, $J=16$ Hz) 7.1—7.6 (m, 4H).

8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenol (**28**): The procedure used for the preparation of **22** was repeated with **27** (5.5 g), except for the use of 2 eq of 1 M NaOH solution to obtain **28** (2.24 g, 64%). IR (KBr) cm^{-1}: 3500, 1665, 1600. ^{1}H-NMR ($CDCl_3$, DMSO-d_6): 1.0–1.82 (m, 6H), 2.60 (t, 2H, $J=6$ Hz), 3.50 (m, 2H), 6.45 (d, 1H, $J=15$ Hz) 6.7—7.1 (m, 3H), 7.35 (d, 1H, $J=15$ Hz).

8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenoic Acid (32) 8-[3,4-Bis(ethoxycarboxy)phenyl]-6-oxo-7-octenoic acid (**33**): Jones reagent (12.6 ml) was added dropwise to a cooled (−10 °C) solution of **31** (5.0 g) in acetone (80 ml). The reaction mixture was stirred at −10 °C for 15 min, then 2-propanol (10 ml) was added dropwise. After evaporation of the solvent, the residue was dissolved by the addition of 1 M NaOH solution at 0 °C. The aqueous layer was washed with ether, acidified with 1 M HCl solution and extracted with ethyl acetate. The extract was washed with water and brine successively, and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the residue was used in the next step without further purification.

8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenoic Acid (**32**): The procedure used for the preparation of **22** was repeated with **33** (3.72 g), except for the use of 3 eq of 1 M NaOH solution, to obtain **32** (1.62 g, 48%). mp 137.5—138.5 °C. IR (KBr) cm^{-1}: 3450, 3120, 1720, 1665, 1648, 1595. ^{1}H-NMR ($CDCl_3$, DMSO-d_6): 1.5—2.0 (m, 4H), 2.38 (m, 2H), 2.62 (m, 2H), 6.40 (d, 1H, $J=15$ Hz) 6.7—7.0 (m, 3H), 7.30 (d, 1H, $J=15$ Hz).

Enzyme Preparation All of the procedures described below were performed at 0—4 °C.

Polymorphonuclear leukocytes (PMNLs) were collected and purified from peritoneal cavities of female guinea pigs (300—350 g, Hartley strain) as described by Kakinuma.[13] Suspended PMNLs ($2.0—4.0\times10^7$ cells/ml) were sonicated by an ultrasonic disruptor (UR-200P, Tomy Seiko Co., Ltd.) at a setting of 5, and then centrifuged at a speed of 10000 *g* for 10 min. The supernatant was used as an enzyme source for 5-LO or prostaglandin (PG) synthase.

Blood was collected in 77 mM ethylenediaminetetraacetic acid (EDTA), 7.5% v/v, from donors which had not taken drugs for at least 1 week. The washed platelets were prepared as described by Hamberg *et al.*[14] and suspended in 67 mM sodium phosphate (pH 8.0) containing 77 mM NaCl. Suspended platelets (5×10^8 cells/ml) were sonicated by the same procedure as described for PMNLs, and then centrifuged for 60 min at 100000 *g*. The supernatant was used as an enzyme source for 12-lipoxygenase (12-LO).

Enzyme Assays For assay of 5-LO, the mixture contained 50 mM sodium phosphate (pH 7.4), 0.1% gelatin, 2.8 mM $CaCl_2$, 1.9 mM ATP, 3.2 μM [1-^{14}C]arachidonic acid (0.1 μCi), 0.95% propylene glycol and an enzyme source (500 μl) in a final volume of 0.525 ml. The reaction was carried out at 37 °C for 20 min. The reaction was stopped by acidifing the mixture with 25 μl of 1 M HCl. The reaction mixture was treated four times each with 2.5 ml of ice-cold ethyl acetate. The combined ethyl acetate extracts were concentrated *in vacuo* and spotted on a TLC plate, which was developed in a solvent system of ethyl acetate–petroleum ether–acetic acid (50:50:1). After development, the positions corresponding to both leukotriene B_4 (LTB_4) and 5-HETE were scraped off and counted with a Packard Liquid scintillation counter (TRI-CARB 4640). The amounts of products (*i.e.* 5-HETE and LTB_4) formed in the absence of synthesized compounds were 0.079—0.12 nmol. Each experiment was done in duplicate.

For assay of PG synthase, the mixture contained 50 mM sodium phosphate (pH 7.4), 0.95% propylene glycol, 3.2 μM [1-^{14}C]arachidonic acid (0.051 μCi), 1.0 mM EDTA and an enzyme source (250 μl) in a final volume of 0.255 ml. The reaction was performed at 37 °C for 30 min and then stopped by adding 25 μl of 0.5 M HCl. The reaction products were extracted twice with 2.5 ml of ice-cold ethyl acetate. The combined extracts were evaporated *in vacuo* and spotted on a TLC plate, which was developed in a solvent system of ethyl acetate–isooctane–acetic acid–H_2O (110:50:20:100, upper layer). After development, the areas corresponding to the both authentic TXB_2 and PGE_2 were scraped off and counted with a Packard Liquid scintillation counter (TRI-CARB 4640). The amounts of products (*i.e.* PGE_2 and TXB_2) formed in the absence of synthesized compounds were 0.055—0.071 nmol. Each experiment was done in duplicate.

For assay of 12-LO, the reaction mixture contained 3.2 μM [1-^{14}C] arachidonic acid (0.032 μCi), 1.0 mM EDTA, 50 mM sodium acetate (pH 7.4) and an enzyme source (130 μl) in a final volume of 0.151 ml. The reaction was continued for 30 min at 37 °C. After termination of the reaction by adding 35.4 μl of 0.25 M HCl, the mixture was extracted twice with 2.0 ml of ice-cold ethyl acetate. The combined ethyl acetate extracts were concentrated *in vacuo* and spotted on a TLC plate, which was developed in a solvent system of ethyl acetate–Ligroin–acetic acid (50:50:1). The area corresponding to 12-HETE was scraped off and counted with a Packard Liquid scintillation counter (TRI-CARB 4640). The amounts of products (*i.e.* 12-HETE) formed in the absence of synthesized compounds were 0.020—0.039 nmol. Each experiment was done in duplicate.

Addition of Inhibitors Synthesized compounds dissolved in ethyl alcohol were added to an assay tube containing [1-^{14}C]arachidonic acid dissolved in ethyl alcohol and a mixture of propylene glycol/ethyl alcohol. After removing the ethyl alcohol by blowing N_2 gas at 37 °C, other components required for the enzyme assay were added. As a control, ethyl alcohol was added to an assay tube instead of a compound solution.

Results

Effect of Caffeic Acid Esters on 5-LO, 12-LO and PG Synthase Activities Inhibitory effects of caffeic acid esters as well as caffeic acid on 5-LO, 12-LO and PG synthase activities are listed in Table I. They are expressed as the concentrations which cause 50% inhibition (IC_{50} values) of each enzyme activity with respect to the value determined in the absence of the compounds.

Among these compounds, the butyl ester (**2**) showed the

TABLE I. IC_{50} Values of Caffeic Acid Esters for 5-LO, 12-LO and PG Synthase Activities

Compound	IC_{50} (μM)[a]		
	5-LO	12-LO	PG synthase
1	0.165 (1)	6.50 (39)	>10.0 (>61)
2	0.067 (1)	1.85 (28)	2.80 (42)
3	0.188 (1)	1.80 (10)	6.20 (33)
Caffeic acid	1.0	10.0	100.0

a) Numbers in parentheses indicates the ratios of the IC_{50} values for 5-LO, 12-LO or PG synthase to that for 5-LO.

most potent inhibitory activity toward 5-LO, its IC_{50} value being less than one-fourteenth of that of caffeic acid. As to the inhibition specificities for 5-LO of these compounds, there was a tendency for elongation of the straight alkyl chain to diminish the specificity.

Effect of Caffeic Acid Amides on 5-LO, 12-LO and PG Synthase Activities The inhibitory activities of five caffeic acid amides toward 5-LO were investigated. Table II shows that the compound having 8 or 10 carbon atoms in its alkyl chain (**5** or **6**, respectively) exerts the most potent inhibitory activity. Four of them were also examined with respect to the inhibition specificities for 5-LO (Table II). Among them, compound **5** showed the highest specificity. The IC_{50} value for 5-LO was more than 100 times lower than those for 12-LO and PG synthase. In general, the inhibition specificities of the amide derivatives were higher than those of esters.

Effect of 1-(3,4-Dihydroxyphenyl)-1-alken-3-one Compounds on 5-LO, 12-LO and PG Synthase Activities Table III shows the inhibitory activities and inhibition specificities for 5-LO of three 1-(3,4-dihydroxyphenyl)-1-alken-3-one compounds (**9**, **11** and **13**). The compound having 5 carbon atoms in its alkyl chain (**11**) showed not only the most potent inhibitory activity but also the greatest inhibition specificity for 5-LO among the three compounds. Both the inhibitory activity and the inhibition specificity of **11** are comparable to those of **5**. The dose-dependent inhibition curves of 5-LO, 12-LO and PG synthase by **11** are depicted in Fig. 1. Complete inhibition of 5-LO was observed at 10^{-6} M of **11**.

Effect of 1-(3,4-Dihydroxyphenyl)-1-alkenes on 5-LO, 12-LO and PG Synthase Activities Three 1-(3,4-dihydroxyphenyl)-alkenes (**15**, **18** and **20**) were investigated for inhibitory activities and inhibition specificities toward 5-LO. The results are listed in Table IV. Two of them showed the IC_{50} values of about 10^{-8} M for 5-LO. However, their inhibition specificities for 5-LO were not so high as those of **5** and **11**.

Inhibitory Mode of 11 toward 5-LO The inhibitory mode of **11** toward 5-LO was investigated by the method of Lineweaver and Burk (Fig. 2)[15] to find that the inhibition was of non-competitive type. The K_i value of **11** was calculated from the figure to be 0.138 μM.

Effect of 1-(4-Hydroxy-3-mthoxyphenyl)-, 1-(3-Hydroxy-4-methoxyphenyl)- and 1-(3,4-Dimethoxyphenyl)-1-octen-3-one Compounds on 5-LO Activities As mentioned above, **11** showed a very strong inhibitory activity toward 5-LO with an IC_{50} value of 3.5×10^{-8} M. We next examined how the inhibitory activities toward 5-LO change on substituting one or both hydroxy groups with methoxy group(s). Three enone compounds, 1-(4-hydroxy-3-me-

TABLE II. IC_{50} Values of Caffeic Acid Amides for 5-LO, 12-LO and PG Synthase Activities

Compound	IC_{50} (μM)[a]		
	5-LO	12-LO	PG synthase
4	0.130	n.d.	n.d.
5	0.042 (1)	4.20 (100)	>10 (>238)
6	0.045 (1)	3.20 (71)	>10 (>222)
7	0.065 (1)	3.70 (57)	>10 (>154)
8	0.155 (1)	5.20 (34)	>10 (>65)

a) Numbers in parentheses indicates the ratios of the IC_{50} values for 5-LO, 12-LO or PG synthase to that for 5-LO. n.d., not determined.

TABLE III. IC_{50} Values of Enone-Type Compounds on 5-LO, 12-LO and PG Synthase Activities

Compound	IC_{50} (μM)[a]		
	5-LO	12-LO	PG synthase
9	0.275 (1)	8.30 (30)	>10 (>36)
11	0.035 (1)	3.50 (100)	13.0 (371)
13	0.058 (1)	0.87 (15)	4.50 (78)

a) Numbers in parentheses indicates the ratios of the IC_{50} values for 5-LO, 12-LO or PG synthase to that for 5-LO.

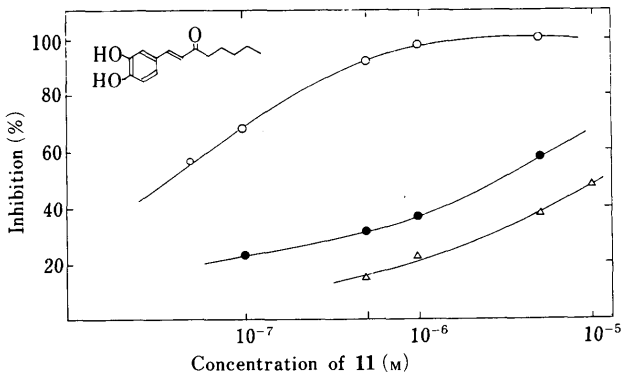

Fig. 1. Effect of **11** on 5-LO (○), 12-LO (●) and PG Synthase (△) Activities

TABLE IV. IC_{50} Values of Olefin-Type Compounds for 5-LO, 12-LO and PG Synthase Activities

Compound[b]	IC_{50} (μM)[a]		
	5-LO	12-LO	PG synthase
15	0.095 (1)	0.50 (5)	5.60 (59)
18	0.012 (1)	0.23 (19)	2.90 (242)
20	0.015 (1)	0.17 (11)	0.88 (59)

a) Numbers in parentheses indicates the ratios of the IC_{50} values for 5-LO, 12-LO or PG synthase to that for 5-LO. *b*) These derivatives are *cis-trans* mixtures, although the *cis* isomers are predominant in general.

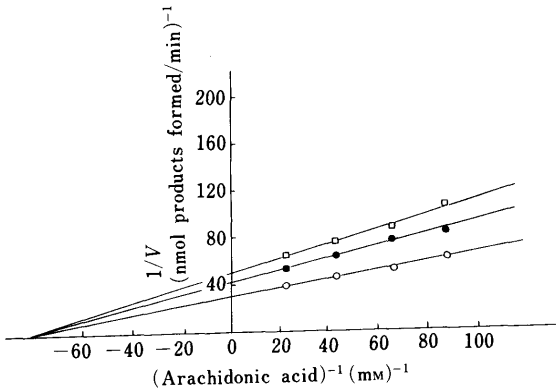

Fig. 2. Lineweaver-Burk Plots of the Formation of 5-HETE and LTB_4 with [1-^{14}C] Arachidonic Acid as a Substrate in the Presence of Various Concentrations of **11**

Concentration of **11** used: 0 M (○), 5×10^{-8} M (●), 10^{-7} M (□).

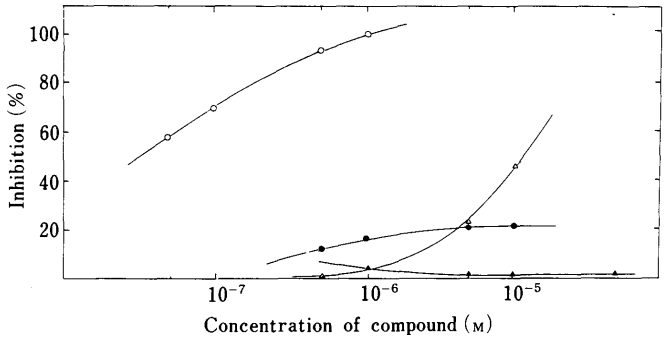

Fig. 3. Effect of **11** (○), **22** (△), **25** (●) and **12** (▲) on 5-LO Activity

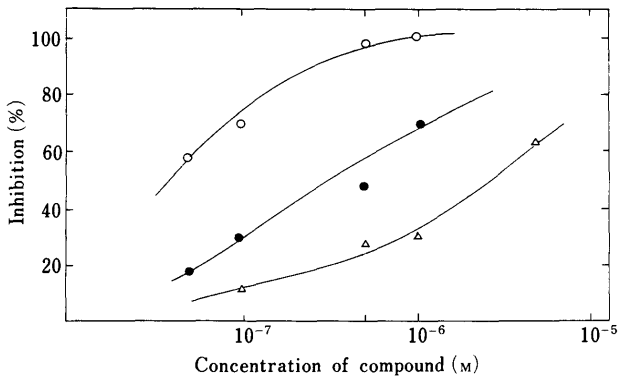

Fig. 4. Effect of **11** (○), **28** (●) and **32** (△) on 5-LO Activity

thoxyphenyl)-1-octen-3-one (**22**), 1-(3-hydroxy-4-methoxyphenyl)-1-octen-3-one (**25**) and 1-(3,4-dimethoxyphenyl)-1-octen-3-one (**12**), were synthesized, and their inhibitory activities toward 5-LO were compared to that of **11**. Figure 3 clearly shows that if one or both methoxy groups are introduced into the benzene ring inplace of the hydroxy group(s), a drastic decrease in the inhibitory activities toward 5-LO was observed. In particular, no inhibition was observed even at a high concentration of 500 μM in the case of **12**. This result suggests that the two hydroxy groups in the compound **11** are essential for the strong inhibition of 5-LO.

Effect of 8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenol and 8-(3,4-Dihydroxyphenyl)-6-oxo-7-octenoic Acid on 5-LO Activities The inhibitory effects on 5-LO of the compounds in which a hydroxy or carboxy group was introduced at the terminal of the alkyl side chain of **11** were investigated. The compounds examined were 8-(3,4-dihydroxyphenyl)-6-oxo-7-octenol (**28**) and 8-(3,4-dihydroxyphenyl)-6-oxo-7-octenoic acid (**32**). The inhibitory activities for 5-LO were in the order of **11**, **28** and **32** (Fig. 4), the IC_{50} values of **28** and **32** being about 17 and 100 times higher, respectively, than that of **11**.

Discussion

Since the report of Koshihara *et al.*[9] that caffeic acid and its methyl ester show very potent inhibitory activity toward 5-LO, we have synthesized various caffeic acid derivatives having straight alkyl chains and investigated their inhibitory activities and inhibition specificities toward 5-LO.

We found several compounds having much more potent inhibitory activities than those of caffeic acid and its methyl ester. Among them, 1-(3,4-dihydroxyphenyl)-1-octen-3-one(**11**) seems to be the best since it exhibits not only strong inhibitory activity toward 5-LO with the IC_{50} value of 3.5×10^{-8} M but also has the highest inhibition specificity for the enzyme. Caffeic acid octyl amide (**5**) is also an excellent compound comparable to **11** in inhibitory activity as well as specificity. However this compound would be decomposed to caffeic acid and octylamine when examined in tissues or in *in vivo* systems.

Compound **11** was found to show inhibition of non-competitive type, the same inhibitory pattern as circiliol[16] and, probably, TMK-777.[17] This type of inhibition contrasts to that of a potent and selective 5-LO inhibitor, AA-861, which was reported to be of competitive type.[18]

Although the precise binding site of **11** is not clear at present, it seems that there are at least three factors which are essential for its inhibitory activity toward 5-LO: an appropriate alkyl chain length, a hydrophobic binding site for the straight alkyl chain, and the two hydroxy groups attached to the benzene ring.

Compound **11** should be a useful tool to examine the roles of leukotrienes at the tissue level and in *in vivo* systems. Furthermore, it seems to be an interesting candidate drug for the treatment of diseases in which overproduction of leukotrienes is involved.

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