Inhibition of *in Vitro* Prostaglandin and Leukotriene Biosyntheses by Cinnamoyl-β-phenethylamine and *N*-Acyldopamine Derivatives

Chen-Fang TSENG,[a] Satoshi IWAKAMI,[a] Akihiro MIKAJIRI,[a] Masaaki SHIBUYA,[a] Fumio HANAOKA,[a] Yutaka EBIZUKA,[a] Kosashi PADMAWINATA[b] and Ushio SANKAWA*,[a]

Faculty of Pharmaceutical Sciences, The University of Tokyo,[a] 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan and Department of Pharmacy, Faculty of Science and Mathematics, Bandung Institute of Technology,[b] Jl. Ganesha, Bandung, Indonesia. *Received July 22, 1991*

***N-trans-* and *N-cis*-Feruloyltyramines were isolated as the inhibitors of *in vitro* prostaglandin (PG) synthesis from an Indonesian medicinal plant, *Ipomoea aquatica* (Convolvulaceae). In order to clarify structure activity relationships, cinnamoyl-β-phenethylamines with possible combinations of naturally occurring cinnamic acids and β-phenethylamines were synthesized and tested for their inhibitory activities against PG synthetase and arachidonate 5-lipoxygenase. The compounds containing catechol groups such as *N*-caffeoyl-β-phenethylamine (CaP) showed higher inhibitory effects on PG synthetase. The catechol group was found to be essential for the inhibition of arachidonate 5-lipoxygenase. The investigation of concentration dependent effects on PG biosynthesis revealed that CaP enhanced PG biosynthesis at a lower concentration range, whereas it inhibited the reaction at a higher concentration. The effects of CaP on each reaction step were investigated with purified PG endoperoxide synthase and microsomal PG synthetase. CaP inhibited the cyclooxygenase reaction, while it enhanced the hydroperoxidase reaction. *N*-Acyldopamines which contain catechol and lipophylic group were synthesized from dopamine and fatty acids to test their inhibitory effects on arachidonate 5-lipoxygenase. *N*-Linoleoyldopamine was the most active compound and its IC_{50} value was 2.3 nM in our assay system, in which an IC_{50} value of AA 861, a specific inhibitor of 5-lipoxygenase, was 8 nM.**

Keywords *Ipomoea aquatica*; Convolvulaceae; prostaglandin; arachidonate 5-lipoxygenase; structure activity relationship; *N*-feruloyltyramine; *N*-caffeoylphenethylamine; *N*-acyldopamine; *N*-linoleoyldopamine

A bioassay system that evaluates the inhibitory effect on a prostaglandin biosynthesizing enzyme (PG synthetase) has been extensively used in screening test to detect biologically active compounds in hot aqueous extracts of medicinal plants used in traditional Japanese Kampo medicine.[1,2] Following the screening test, medicinal plants that exhibited inhibitory activities were investigated to identify active compounds using PG synthetase as a monitor to detect biological activity during the fractionation and separation process. A number of phenolic compounds was isolated as the potent inhibitors of PG synthetase from *Zingiber officinale*,[1a] *Alpinia officinarum*,[1b] *Arnebia euchroma*,[1c-e,j,k] *Dalbergia odorifera*,[1f] *Allium chinense*,[1h] and *Mucuna birdwoodiana*.[1i] The phenolic inhibitors of PG synthetase have characteristic structures containing phenol as well as lipophylic group. The phenol groups are expected to inhibit radical reaction involved in the PG synthetase reaction, while the lipophylic groups assist the binding of the inhibitors to the enzyme, indicating that they are not strictly specific inhibitors of PG synthetase, but general inhibitors of lipoxygenases.[1l] The phenolic inhibitors were then found to inhibit arachidonate 5-lypoxygenase of RBL-1 cells, the first enzyme of leukotriene (LT) biosynthesis.[1l,3]

A considerable number of aqueous ethanol extracts of Indonesian medicinal plants, which have been used in traditional Jamu medicine, were accumulated during the course of academic exchange and overseas survey programmes. The extracts were subjected to a screening test on PG synthetase inhibition followed by identification of active constituents. This paper describes the results of the screening test, isolation and identification of active compounds, and also discusses structure–activity relationships of bioactive phenolic compounds.

Experimental

Mass spectra (MS) were measured on a JEOL JMS DX300. ^{1}H- and ^{13}C-Nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were measured on a JEOL JNM FX-100. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected Authentic samples of coumarins are from our collection. Authentic samples of feruloyltyramines were a kind gift of Prof. S. Sakamura of Hokkaido University. Mn-Protoporphyrin was obtained from Prof. S. Yamamoto of Tokushima University.

TABLE I. Screening Test of Indonesian Medicinal Plants with PG Synthetase[a]

Names	Family	Indonesian name	Part	Inhibition % (750 μg/ml)
Baeckea frutenscens L.	Myrtaceae	Jungrahab	Leaves	54.4
Bixa orellana L.	Bixaceae	Kasumba-kelling	Leaves	73.2
Cassytha filiformis L.	Lauraceae	Sanga-langit	Herbs	96.0
Dendrophthoe pentandra MIQ.	Loranthaceae	Api-api	Leaves	93.7
Guazma ulmifolia LAMARK	Sterculiaceae	Jati-landa	Leaves	61.8
Ipomoea aquatica FORSK.	Convolvulaceae	Kangkung	Stems	76.2
Alpinia galana L.	Zingiberaceae	Laos	Rhizomes	52.2
Melaceuca leucadendren L.	Myrtaceae	Borong-borong	Fruits	87.0
Melastoma polyanthum BL.	Melastomataceae	Senggai	Leaves	59.6
Ocimen sanctum L.	Labiatae	Lampas	Leaves	88.0

a) Medicinal plants showing more than 50% inhibition are shown.

Assay Methods Radioisotope and oxygen electrode methods of PG synthetase assay were described in a previous paper.[2] The assay of arachidonate 5-lipoxygenase was performed using RBL-1 cell lysate as an enzyme source as described in previous papers.[1f,3]

Preparation of Aqueous Ethanolic Extracts of Indonesian Medicinal Plants and Screening Test with PG Synthetase Air dried medicinal plants were extracted with hot 50% aqueous EtOH and filtrated solutions were concentrated *in vacuo*. Obtained aqueous solutions were frozen and dried to give dried extracts. Screening test with PG synthetase was performed at a test samples concentration of 750 μg/ml using the radioisotope method.[2] The results are summarized in Table I, where medicinal plants with more than 50% inhibition were listed.

Purification of PG Endoperoxide Synthase Purification of PG endoperoxide synthase from sheep seminal vesicles was carried out according to the method described for purification from bovine seminal vesicles.[4] Sheep seminal vesicle microsomes were prepared as described in a previous paper.[2] Microsomal preparation (312 mg protein) suspended in 20 mM potassium phosphate buffer (KPB) pH 7.4 (30 ml) was diluted with 20 mM KPB pH 7.4 containing 1% Tween 20 and 20% glycerol (20 ml). The solution was stirred at 0 °C for 30 min and then centrifuged at 105000 × *g* for 60 min. The solubilized enzyme solution was loaded on a diethylaminoethyl (DEAE)-cellulose column (Whatman DE-52, 3.2 × 10.5 cm) and eluted with 20 mM KPB pH 7.4 containing 0.1% Tween 20 and 20% glycerol at a flow rate of 0.9 ml/min. Active fractions (63 ml) were pooled and concentrated to 9.8 ml with a Diaflo membrane (Amicon, XM-50) to give DEAE-eluate (32.6 mg protein), which was subjected to isoelectric focusing using a LKB 8102 (110 ml) apparatus. LKB ampholine carrier ampholite (pH 3.5—10) was used to produce a pH gradient along a stepwise sucrose density gradient containing Tween-20 (7.1–0.1%), prepared according to the LKB manual. Electrofocusing was performed at 400 V for 16 h, at 800 V for 5 h and then at 900 V for 16 h. Active fractions of pH 6.4—7.2 were pooled and concentrated with a Diaflo membrane (XM-50) to give the electrofocusing fraction (0.5 ml). To remove Ampholite and sucrose, the fraction was subjected to a Sepharose 6B column (2.5 × 39 cm) which had been equillibrated with 50 mM KPB pH 7.4 containing 0.05% Tween 20 and 20% glycerol and the column was eluted with same buffer. Active fractions were pooled and concentrated by a Diaflo membrane (XM-50) to give purified endoperoxide synthase (11.3 mg protein) with specific activity of 1.62 units/mg protein, which showed a single band in sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

Preparation of [^{14}C]-PGG$_2$ and [^{14}C]-PGH$_2$ Labelled compounds were prepared with purified PG endoperoxide synthase according to the method described by Miyamoto *et al.* and Yamamoto.[4,5]

Assay of Cyclooxygenase Reaction (Arachidonic Acid (AA) to PGG$_2$) Reaction mixture (100 μl) contained Tris–HCl pH 8.0 (0.1 M), Mn-protoporphyrin (4 μM), caffeoyl-β-phenethylamine (CaP: **7**) (0 or 0.2 mM), PG endoperoxide synthase (2.4 μl, 6 μg protein) and [1-^{14}C]-AA (10 μl, 95 μM, 5.5 × 10^4 dpm). The reaction was initiated by adding [1-^{14}C]-AA solution and the reaction mixture was incubated for 1 min at 24 °C. The reaction was stopped by adding EtOAc–MeOH–1 M citric acid (30 : 4 : 1, 300 μl) which had been precooled at −70 °C. Na_2SO_4 (0.5 g) was added to the reaction mixture and an aliquot of the EtOAc layer (75 μl) was spotted on a thin layer chromatography (TLC) plate (Merck with concentration zone) precooled to 4 °C. The plate was developed with ether–petrol ether–AcOH (85 : 15 : 0.1) at −20 °C. Radioactive zone corresponding to PGG$_2$, estimated from the spot of a marker (PGB$_2$), was scraped off and subjected to radioactivity counting on a liquid scintillation counter.

Assay of Hydroperoxidase Reaction (PGG$_2$ to PGH$_2$) Reaction mixture contained Tris–HCl pH 8.0, tryptophan (10 mM), haemoglobin (1 μM), CaP (0 or 0.2 mM) and PG endoperoxide synthase (2.4 μl, 6 μg protein). [^{14}C]-PGG$_2$ (95 μM, 5.5 × 10^4 dpm) was added to the reaction mixture to initiate the reaction. The reaction mixture was incubated for 1 min at 24 °C and was worked up as in the case of the cyclooxygenase.

Assay of Endoperoxide Synthase Reaction (AA to PGH$_2$) Assay mixture (100 μl) contained Tris–HCl pH 8.0 (0.1 M), haemoglobin (1 μM), tryptophan (10 mM), glutathione (4 μM), Cap (0 or 0.2 mM), sheep seminal vesicle microsomes (10 μl, 100 μg protein) and [1-^{14}C]-AA (10 μl, 95 μM, 5.5 × 10^4 dpm). The reaction was initiated by adding radioactive AA to the solution and incubated at 24 °C for 90 s. The reaction mixture was worked up as in the case of the cyclooxygenase assay.

Assay of PGE$_2$ Synthetase (PGH$_2$ to PGE$_2$) Assay mixture contained Tris–HCl pH 8.0 (0.1 M), glutathione (4 mM), CaP (0 or 0.2 mM), sheep seminal vesicle microsomes (10 μl, 100 μg protein) and [^{14}C]-PGH$_2$ (59 μM, 3.3 × 10^4 dpm). The reaction was initiated by adding [^{14}C]-PGH$_2$ to the reaction mixture, which was incubated for 90 s at 24 °C. The reaction mixture was worked up as in the case of the cyclooxygenase.

Isolation of Coumarins and Amides Dried stems of *I. aquatica* (500 g) was extracted twice with hexane (4 l) for 4 h and then four times with acetone (4 l) for 4 h. The extraction process was repeated ten times to extract 5 kg of material and acetone was removed from the acetone solution *in vacuo* to give an acetone extract (33.5 g). The acetone extract was distributed between aqueous MeOH and hexane to give aqueous MeOH fraction (4.2 g), which showed relatively high activity against PG synthetase. The aqueous MeOH extract was repeatedly chromatographed on Sephadex LH-20 (MeOH) and silica gel (benzene–EtOAc, $CHCl_3$–MeOH) columns to afford umbelliferone (60 mg), scopoletin (70 mg), *N-trans*-feruloyltyramine (30 mg) and *N-cis*-feruloyltyramine (8 mg). They are identified by the comparison of physical data with those of authentic samples.[6]

Synthesis of *N-trans*-Feruloyltyramine (11) Acetylferulic acid (2.36 g, 0.01 mol), prepared by acetylation of ferulic acid with pyridine and acetic anhydride, was dissolved in tetrahydrofuran (THF) (30 ml) and Et_3N (1.02 g, 0.01 mol). The solution was cooled at −15 °C (NaCl-ice). Isobutyl chlorocarbonate (1.37 g, 0.01 mol) was added to the solution under vigorous shaking to give a gel-like reaction mixture, to which a solution of tyramine–HCl (1.37 g, 0.01 mol) in THF (30 ml) and Et_3N (1.02 g, 0.01 mol) was added. The reaction mixture was stirred below 0 °C for 1 h and then at r.t. over night. The solvents were removed *in vacuo* and the residue was dissolved in EtOAc (300 ml). The solution was washed with 4% $NaHCO_3$, H_2O and 2% HCl, dried over $CaCl_2$, filtered and evaporated *in vacuo*. The reaction product was hydrolyzed in 3% aqueous Na_2CO_3 and EtOH (60 mg) under reflux for 2 h. The reaction mixture was concentrated *in vacuo*, diluted with H_2O (100 ml) and neutralized with 1 N HCl. The neutralized solution was extracted with EtOAC (150 ml × 3). Thus obtained crude *N-trans*-feruloyltyramine was purified by silica-gel column chromatography to give *N-trans*-feruloyltyramine, which was crystallized from $CHCl_3$–acetone to give colourless needles (1.76 g, 54%), mp 144.5—145 °C (lit. 144—145 °C).[6]

Synthesis of Cinnamoyl-β-phenethylamine Analogues *p*-Coumaroyl-β-phenethylamine, *N-p*-coumaroyltyramine, *N-p*-coumaroyldopamine, *N*-feruloyldopamine, caffeoyl-β-phenethylamine, *N*-caffeoyltyramine and *N*-caffeoyldopamine were synthesized by the same procedure used in the synthesis of *N*-feruloyltyramine, using acetylated carboxylic acids. Cinnamoyl-β-phenethylamine, *N*-cinnamoyltyramine and *N*-cinnamoyldopamine were synthesized directly from cinnamic acid. The synthesized amides gave satisfactory ^{1}H- and ^{13}C-NMR, infrared (IR) and MS data.

Cinnamoyl-β-phenethylamine (1) mp 126—127 °C (benzene). Yield: 81%. *Anal.* Calcd for $C_{17}H_{17}NO$: C, 81.27; H, 6.77; N, 5.58. Found: C, 80.94; H, 6.85; N, 5.49.

***N*-Cinnamoyltyramine (2)** mp 194—195 °C (EtOH). Yield: 77%. *Anal.* Calcd for $C_{17}H_{17}NO_2$; C, 76.22; H, 6.37; N, 5.41. Found: C, 76.36; H, 6.46; N, 5.14.

***N*-Cinnamoyldopamine (3)** mp 165—166 °C (benzene–acetone). Yield: 71%. *Anal.* Calcd for $C_{17}H_{17}NO_3$: C, 72.09; H, 6.01; N, 4.95. Found: C, 71.96; H, 5.99; N, 5.14.

***p*-Coumaroyl-β-phenethylamine (4)** mp 148—149 °C (benzene–acetone). Yield: 56%. *Anal.* Calcd for $C_{17}H_{17}NO_2$: C, 76.22; H, 6.37; N, 5.24. Found: C, 76.12; H, 6.37; N, 5.24.

***N-p*-Coumaroyltyramine (5)** mp 260—261 °C (MeOH–H_2O). Yield: 54%. *Anal.* Calcd for $C_{17}H_{17}NO_3$: C, 72.09; H, 6.01; N, 4.95. Found: C, 71.89; H, 6.00; N, 5.17.

***N-p*-Coumaroyldopamine (6)** mp 204—206 °C ($CHCl_3$–MeOH). Yield: 37%. *Anal.* Calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.69; N, 4.68. Found: C, 67.94; H, 5.69; N, 4.74.

Caffeoyl-β-phenethylamine (7) mp 157—158 °C ($CHCl_3$–acetone). Yield: 42% *Anal.* Calcd for $C_{17}H_{17}NO_3$: C, 72.09; H, 6.01; N, 4.95. Found: C, 71.87; H, 5.99; N, 5.00.

***N*-Caffeoyltyramine (8)** mp 215—217 °C ($CHCl_3$–MeOH). Yield: 33%. *Anal.* Calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.69; N, 4.68. Found: C, 68.08; H, 5.77: N, 4.78.

***N*-Caffeoyldopamine (9)** mp 180—182 °C (MeOH–H_2O). Yield: 35%. *Anal.* Calcd for $C_{17}H_{17}NO_5$: C, 64.76; H, 5.40: N, 4.42. Found: C, 64.62; H, 5.44; N, 4.40.

***N*-Feruloyldopamine (12)** mp 144—146 °C (MeOH–H_2O). Yield 4.1%. *Anal.* Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81; N, 4.25. Found: C, 65.43; H, 5.83; N, 4.20.

Synthesis of *N*-Pelargonoyldopamine (23) Isobutyl chlorocarbonate (2.63 ml, 20 mmol) was added to a solution of pelargonic acid (3.49 ml,

20 mmol) in THF (50 ml) and Et_3N (2.79 ml, 20 mmol) under stirring. After stirring for 15 min dopamine–HCl (3.06 g, 16 mmol) in THF (50 ml) and Et_3N (2.79 ml) was added dropwise to the mixed anhydride solution and the reaction mixture was stirred for 1 h under ice cooling. Stirring was continued over night at r.t. The reaction mixture was diluted with H_2O (50 ml) and extracted with EtOAc (50 ml) twice and the EtOAc was successively washed with 4% $NaHCO_3$, H_2O, 2% HCl, H_2O and NaCl saturated H_2O. The EtOAc layer was dried over Na_2SO_4. The crude reaction product was chromatographed over silica-gel using $CHCl_3$–MeOH to give *N*-pelargonoyldopamine, which was recrystallized from benzene to give colourless needles of mp 67—69 °C (1.2 g, 21%). *Anal.* Calcd for $C_{17}H_{27}NO_3$: C, 69.59; H, 9.28; N, 4.79. Found: C, 69.40; H, 9.40; N, 4.65.

Synthesis of *N*-Acyldopamines *N*-Perfluoropelargonoyldopamine, *N*-undecyloyldopamine, *N*-stearoyldopamine, *N*-oleanoyldopamine, *N*-linoleoyldopamine and *N*-linolenoyldopamine were synthesized basically by the same method described for *N*-pelargonoyldopamine.

***N*-Perfluoropelargonoyldopamine (24)** Perfluoropelargonic acid was a kind gift of Asahi Glass Co. White powder from benzene, mp 136—137 °C. Yield: 1.9%. HR MS *m/z*: Calcd for $C_{17}H_{10}F_{17}NO_3$ 599.0384. Found 599.0274.

***N*-Undecyloyldopamine (25)** Pale brown needles from benzene–hexane, mp 75—77 °C. Yield 36.7%. *Anal.* Calcd for $C_{19}H_{31}NO_3$; C, 70.99; H, 9.64; N, 4.36. Found: C, 70.94; H, 9.81; N, 4.53.

***N*-Stearoyldopamine (26)** White needles form $CHCl_3$–MeOH (9 : 1), mp 96—98.5 °C. *Anal.* Calcd for $C_{26}H_{45}NO_3$: C, 74.42; H, 10.72; N, 3.34. Found: C, 74.38; H, 10.96; N, 3.59.

***N*-Oleoyldopamine (27)** Colourless needles from benzene–hexane, mp 50.5—52.0 °C. *Anal.* Calcd for $C_{26}H_{43}NO_3$: C, 74.78; H, 10.30; N, 3.35. Found: C, 74.72; H, 10.45; N, 3.56.

***N*-Linoleoyldopamine (28)** White powder from benzene–acetone, mp <20 °C. Yield: 46.1%. HR MS *m/z*: Calcd for $C_{26}H_{41}NO_3$ 415.3086, Found 415.3123.

***N*-Linolenoyldopamine (29)** Colourless oil. Yield: 13.5%. HR MS *m/z*: Calcd for $C_{26}H_{33}NO_3$ 413.2930, Found 413.2965.

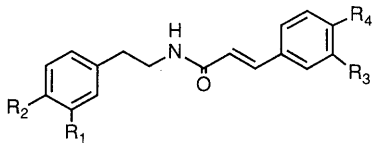
Results and Discussion

More than 40 extracts were tested for their inhibitory effects on PG synthetase as a screening test to find biologically active medicinal plants and the results are shown in Table I. Greater than 70% inhibition was observed in the following material: the extract of the leaves of *Bixaorellana* (Bixaceae), the herbs of *Cassytha filiformis* (Lauraceae), the leaves of *Dendrophthoe pantandra* (Loranthaceae), the fruits of *Melaleuca leucadendron* (Myrtaceae), the stems of *Ipomoea aquatica* (Convolvulaceae), and the leaves of *Ocimen sanctum* (Labiatae). The extract of the fruits of *M. leucadendron* showed inhibitory activities not only against PG biosynthesis but also against induced histamine relaeased from rat mast cells.[7] Two stilbenes, piceatannol and oxyresveratrol, were identified as the active compounds responsible for the inhibition of induced histamine release from mast cells.[7] They also inhibited PG synthetase (data not shown). According to the availability of enough material for phytochemical investigation the constituents of *I. aquatica* were first investigated. The acetone extract of the dried stems of *I. aquatica* inhibited *in vitro* PG biosynthesis by 90% at a concentration of 750 μg/ml. The extract was chromatographed on Sephadex LH-20 and silica-gel columns by monitoring each fraction with inhibitory activity against PG synthetase to afford four phenolic compounds, umbelliferone, scopoletin, *N-trans*-feruloyltyramine and *N-cis*-feruloyltyramine.[6] *N*-Feruloyltyramines consist of ferulic acid and tyramine, both of which exhibited no inhibitory effects on PG biosynthesis, indicating that when the two inactive components were linked by an amide bond the products became active.

Following this finding, amides with the possible combinations of naturally occurring C_6-C_3 acids and β-phenethylamines (C_6-C_2 amines) were synthesized in order to test their effects on PG synthetase and arachidonate 5-lipoxygenase and to clarify their structure–activity relationships. C_6-C_3 acids used in the synthesis were cinnamic, *p*-hydroxycinnamic, caffeic and ferulic acids, while C_6-C_2 amines were β-phenethylamine, tyramine and dopamine. The results of the bioassay test are summarized in Table II.

Caffeoyl-β-phenethylamine (CaP: **7**) and cinnamoyldopamine (CiD: **3**) were highly active in inhibiting PG synthetase as well as arachidonate 5-lipoxygenase. To investigate the mechanism of inhibition against PG biosynthesis by phenolic compounds the concentration-dependent effects of the most active CaP (**7**) was measured with an

TABLE II. Inhibition of PG Synthetase and 5-Lipoxygenase by Cinnamoyl-β-phenethylamine Derivatives



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	OH	H	H
3	OH	OH	H	H
4	H	H	H	OH
5	H	OH	H	OH
6	OH	OH	H	OH
7	H	H	OH	OH
8	H	OH	OH	OH
9	OH	OH	OH	OH
10	H	H	MeO	OH
11	H	OH	MeO	OH
12	OH	OH	MeO	OH

Compound	Abbr.	No.	IC_{50} (μM)	
			PG Syn	5-Lipo
Cinnamoyl-β-phenethylamine	CiP	**1**	180	—
N-Cinnamoyltyramine	CiT	**2**	120	—
N-Cinnamoyldopamine	CiD	**3**	90	0.20
p-Coumaroyl-β-phenethylamine	CoP	**4**	100	—
N-p-Coumaroyltyramine	CoT	**5**	280	—
N-p-Coumaroyldopamine	CoD	**6**	230	0.36
Caffeoyl-β-phenethylamine	CaP	**7**	80	0.12
N-Caffeoyltyramine	CaT	**8**	210	0.16
N-Caffeoyldopamine	CaD	**9**	270	0.23
Feruloyl-β-phenethylamine	FeP	**10**	90	21% (1 μM)
N-Feryroyltyramine	FeT	**11**	270	—
N-Feryloyldopamine	FeD	**12**	440	0.48

Abbr.: abbreviation, PG Syn: PG synthetase, 5-Lipo: arachidonate 5-lipoxygenase.

TABLE III. Concentration Dependent Effects of CaP (**7**) on Oxygen Uptake in PG Biosynthesis

Sample	0.02	0.08	0.20	0.80	2.0 mM
CaP (**7**)	105	121	127	75	23
Aspirin	90	82	75	62	43

Figures indicate reaction % when control value is taken as 100%.

TABLE IV. Activation of Hydroperoxidase and Inhibition of Cyclooxygenase in PG Biosynthesis by CaP (7)

Reaction	PG endperoxide synthetase				Microsomes			
	Cyclooxygenase		Hydroperoxidase		PGE_2 synthetase		PG synthetase	
Substrate and product	AA	PGG_2	PGG_2	PGH_2	PGH_2	PGE_2	AA	PGE_2
CaP (7) 0 μM	14.5	85.5	47.1	52.9	4.5	95.5	12.9	87.1
CaP (7) 200 μM	85.4	14.6	28.2	71.8	5.7	94.3	75.6	24.4

Figures indicate the ratios of substrates and products when their sums are taken as 100.

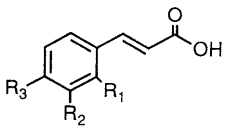
oxygen electrode using aspirin as a reference compound. At a concentration range of 0.08—0.2 mM CaP (7) enhanced reaction rate by 5—27%, while at a higher than 0.8 mM concentration the reaction was significantly inhibited (Table III).

Since aspirin inhibited the reaction in a dose dependent manner, CaP (7) should have dual effects on PG synthetase, *i.e.* the enhancing effect at a lower concentration and the inhibitory effect at a higher concentration. The enzyme system of PG synthetase used in this study contains three different reaction steps, cyclooxygenase, hydroperoxidase and PGE_2 synthetase, however the exact step in which the phenolic compounds inhibit the reaction has not been fully clarified. Tyramine and ferulic acid have been reported to enhance PG biosynthesis by acting as tryptophan like cofactors which enhanced the hydroperoxidase reaction, the conversion of PGG_2 into PGH_2.[5,8] The enhancing activity of CaP (7) at a lower concentration was suggested to owe its tryptophan like activity.[5,8] In order to clarify in which step CaP (7) acts as an inhibitor or a promoter, PG endoperoxide synthase which catalyzes two step reactions, cyclooxygenase and hydroperoxidase, was purified from sheep seminal vesicles.[4] The effects of CaP (7) were investigated in each reaction using purified endoperoxide synthetase and a microsomal preparation from sheep seminal vesicles. Cyclooxygenase reaction, the conversion of AA into PGG_2, and hydroperoxidase reaction, the conversion of PGG_2 into PGH_2, were measured with purified PG endoperoxide synthetase using ^{14}C-labelled AA and PGG_2, respectively. PGE_2 synthase reaction, the conversion of PGH_2 into PGE_2, was determined with the microsomal preparation using ^{14}C-labelled PGH_2. The results shown in Table IV clearly demonstrate that CaP (7) acts as a tryptophan like cofactor and enhances the hydroperoxidase reaction, while the cyclooxygenase reaction is inhibited by CaP (7) at a concentration of 200 mM. As a result the overall reaction of PG synthesis was inhibited by CaP (7), which can be regarded as a representative phenolic inhibitor. The result demonstrated that phenolic inhibitors of PG synthetase inhibit the cyclooxygenase reaction. Phenolic compounds are sometimes regarded as non specific inhibitors of all enzymes by their non specific binding to the enzyme proteins, however, the present results demonstrated CaP (7) inhibits specifically the cyclooxygenase reaction.

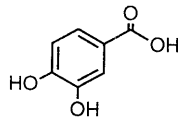
Hydroxycinnamic acids and related compounds were then tested for their tryptophan like activities with an assay system without tryptophan. The tested compounds were divided into two groups according to their modes of inhibition. Caffeic (**13**), ferulic (**14**), isoferulic (**15**) and *p*-coumaric (**16**) acids form a group which enhanced PGE_2 formation at a lower concentration by acting as tryptophan like cofactors, while they became inhibitory at a higher concentration, resulting in cancelling the enhancing effects. *o*-Coumaric (**17**), *m*-coumaric (**18**), protocatechuic (**19**) and homovanillic (**20**) acids fall in the second group which enhanced PGE_2 formation at any concentration. It is a very interesting phenomenon that a significant difference was observed among the regio-isomers of coumaric acids. Since the precise mechanism of the hydroperoxidase reaction have not been clarified, the different behavior of hydroxycinnamic acids may be helpful to clarify the effect of tryptophan like cofactors on endoperoxide synthase.

The structure–activity relationships of the amides on arachidonate 5-lipoxygenase however are more clear. The amides containing caffeic acid or dopamine exhibited higher inhibitory activities, indicating catechol structure is essential for the higher inhibition of arachidonae 5-lipoxygenase. The results are well in accord with those observed in caffeic acid,[9] flavonoids[10] and diarylheptanoids,[3] where the compounds possessing a catechol group inhibited arachidonate 5-lypoxygenase more significantly. Next we at-

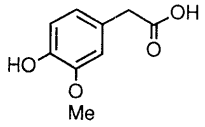
TABLE V. Effects of Hydroxycinnamic Acids and Related Compounds on PG Synthetase



	R_1	R_2	R_3
13	H	OH	OH
14	H	OMe	OH
15	H	OH	OMe
16	H	H	OH
17	OH	H	H
18	H	OH	H
21	H	H	OMe
22	H	H	H



19

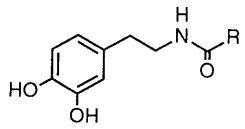


20

Sample	0.37	1.11	3.33	10.0 mM
Caffeic acid (**13**)	210.5	201.8	145.9	19.2
Ferulic acid (**14**)	191.4	191.1	157.1	98.1
Isoferulic acid (**15**)	172.4	185.9	170.4	109.6
p-Coumaric acid (**16**)	171.0	178.4	173.3	50.8
o-Coumatic acid (**17**)	171.0	173.9	172.5	153.8
m-Coumaric acid (**18**)	174.2	178.4	214.4	206.2
Protocatechuic acid (**19**)	174.2	168.4	183.3	154.5
Homovanilic acid (**20**)	146.3	174.3	177.1	151.4
p-Methoxycinnamic acid (**21**)	93.7	88.7	85.4	88.5
Cinnamic acid (**22**)	102.3	99.8	91.2	101.4

Figures indicate reaction % when control is taken as 100%.

TABLE VI. IC_{50} Values of Acyldopamines against Arachidonate 5-Lipoxygenase



Compound	R	IC_{50} (nM)
N-Pelargonoyldopamine (**23**)	n-C_8H_{17}	45
N-Perfluoropelargonoyldopamine (**24**)	n-C_8F_{17}	66
N-Undecyloyldopamine (**25**)	n-$C_{10}H_{21}$	17
N-Stearoyldopamine (**26**)	n-$C_{17}H_{35}$	16
N-Oleoyldopamine (**27**)	n-$C_{17}H_{33}$	7.5
N-Linoleoyldopamine (**28**)	n-$C_{17}H_{31}$	2.7
N-Linolenoyldopamine (**29**)	n-$C_{17}H_{29}$	3.2

tempted to synthesize the series of compounds containing catechol and lipophylic alkyl groups in their structures. The natural substrate of arachidonate 5-lipoxygenase is unsaturated fatty acids and hence the enzyme should have affinity to unsaturated alkyl group. We synthesized therefore *N*-acyldopamines which satisfied the requirements for higher activities against arachidonate 5-lipoxygenase. We investigated the effects of acyl residue from several different aspects. The first is to compare the effects of alkyl chain length among several *N*-acyldopamines synthesized from different fatty acids. The second is to clarify the contribution of double bonds to the activity. The results are remarkable. Alkyl groups of more than C_{10} seems to be necessary for high activity. Introduction of perfluoroalkyl instead of normal alkyl gave no significantly different effects as was observed between *N*-perfluoropelargonoyl and *N*-pelargonoyl dopamines (**23**,**24**). The result may indicate that a C_8 alkyl group can be accommodated as the non flexible strait chain in the enzyme. However, perfluorofatty acids of different chain lengths were not available and further investigation of the effect of the perfluoroalkyl group was not possible. Introduction of a double bond further increased inhibitory activity against arachidonate 5-lipoxygenase. This may reflect the nature of an enzyme whose natural substrate is unsaturated fatty acids. The IC_{50} value of the most active *N*-linoleoyldopamine (**28**) was higher than that of AA-861 (IC_{50} 8 nM) as far as our assay system was concerned. As we have discussed in previous papers the fundamental requirement for phenolic inhibitors of higher activities for PG and LT biosynthesis is the presence of phenol and lipophylic groups. This structural characteristic indicates that the phenolic inhibitors are not necessarily specific to the PG and LT biosynthesis enzymes, but they act as anti-oxidants to prevent the formation of radicals or to quench generated radicals in the fatty acid lipoxygenase reactions. Other potent phenolic inhibitors of PG and LT biosynthesis such as gingerols and diarylheptanoids can also be regarded as the phenolic inhibitors acting as anti-oxidants.[1a,b,l]

Acknowledgments This work forms a part of the survey studies on Indonesian Jamu medicinal drugs supported by Oversea Scientific Survey Grant No. 60041044, Ministry of Education, Science and Culture Japan. The author (U.S.) thanks Professor Kitagawa of Osaka University for providing an opportunity to join a team for the survey of Jamu medicinal drugs in Indonesia. Thanks are also due to the Japan Society for the Promotion of Science (JSPS) to supply travel grants to U. S., Y. E. and K. P. We also thank PT Eisai Indonesia for helping to collect medicinal drugs and medicinal plants and the prepation of ethanolic extracts. Thanks are also due to Prof. S. Sakamura of Hokkaido University for his kind supply of authentic samples of *N*-feruloyltyramines, Prof. S. Yamamoto for Mn-protoporphyrin and Asahi Glass Co. for perfluoropelargonic acid.

References

1) *a*) F. Kiuchi, M. Shibuya and U. Sankawa, *Chem. Pharm. Bull.*, **30**, 754 (1982); *b*) *Idem*, *ibid.*, **30**, 2279 (1982); *c*) Yao X.-S., Y. Ebizuka, H. Noguchi, F. Kiuchi, U. Sankawa and H. Seto, *Tetrahedron Lett.*, **24**, 2407 (1983); *d*) *Idem*, *ibid.*, **24**, 3247 (1983); *e*) Yao X.-S., Y. Ebizuka, H. Noguchi, F. Kiuchi, H. Seto and U. Sankawa, *ibid.*, **25**, 5541 (1984); *f*) Y. Goda, M. Katayama, K. Ichikawa, M. Shibuya, F. Kiuchi and U. Sankawa, *Chem. Pharm. Bull.*, **33**, 5606 (1985); *g*) C.-F. Tseng, A. Mikajiri, M. Shibuya, T. Goda, Y. Ebizuka, K. Padwaminata and U. Sankawa, *ibid.*, **34**, 1380 (1986), *h*) Y. Goda, M. Shibuya and U. Sankawa, *ibid.*, **35**, 2668 (1987); *i*) *Idem*, *ibid.*, **35**, 2675 (1987); *j*) Yao X.-S., Y. Ebizuka, H. Noguchi, F. Kiuchi, M. Shibuya, Y. Iitaka, H. Seto and U. Sankawa, *ibid.*, **39**, 2956 (1991); *k*) *Idem*, *ibid.*, **39**, 2962 (1991); *l*) F. Kiuchi, S. Iwakami, M. Shibuya, F. Hanaoka and U. Sankawa, *ibid.*, **40**, 387 (1992).
2) F. Kiuchi, M. Shibuya, T. Kinoshita and U. Sankawa, *Chem. Pharm. Bull.*, **31**, 3391 (1983).
3) S. Iwakami, M. Shibuya, C.-F. Tseng, F. Hanaoka and U. Sankawa, *Chem. Pharm. Bull.*, **34**, 3960 (1986).
4) R. Miyamoto, N. Ogino, S. Yamamoto and O. Hayaishi, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3645 (1974); S. Yamamoto, "Method in Enzymology," Vol. 86, ed. by W. E. M. Lands and W. L. Smith, Academic Press, New York, 1982, p. 55.
5) S. Ohki, N. Ogino, S. Yamamoto and O. Hayaishi, *J. Biol. Chem.*, **253**, 829 (1978).
6) T. Yoshihara, S. Takamatsu and S. Sakamura, *Agric. Biol. Chem.*, **42**, 623 (1978); T. Yoshihara, K. Yamaguchi, S. Takamatsu and S. Sakamura, *ibid.*, **45**, 2593 (1981); N, Fukuda, M. Yonemitsu and T. Kumura, *Chem. Pharm. Bull.*, **31**, 156 (1983).
7) T. Tsuruga, Y.-T. Chun, Y. Ebizuka and U. Sankawa, *Chem. Pharm. Bull.*, **39**, 3276 (1991).
8) J. Bauman, F. von Bruchhausen and G. Wurm, *Nunyan-Schmiedeberg's Archiev of Pharmacol.*, **307**, 73 (1979).
9) Y. Koshihara, T. Neichi, S. Murota, A.-N. Lao, Y. Fujimoto and T. Tatsuno, *FEBS Lett.*, **158**, 41 (1983).
10) T. Yoshimoto, M. Furukawa, S. Yamamoto, T. Horie and S. Watanabe-Kohno, *Biochem. Biophys. Res. Comm.*, **116**, 612 (1983).