Inhibitory Activity of Unsaturated Fatty Acids and Anacardic Acids toward Soluble Tissue Factor−Factor VIIa Complex

Dandan Wang, Thomas J. Girard, Thomas P. Kasten, Rhonda M. LaChance, Margaret A. Miller-Wideman, and Richard C. Durley*

Searle Discovery Research, Monsanto Company, 700 Chesterfield Parkway North, St. Louis, Missouri 63198

Received March 26, 1998

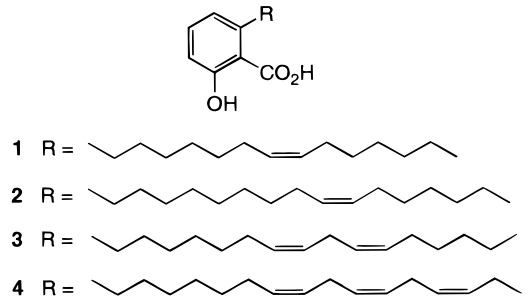
Five compounds, which inhibited the amidolytic activity of soluble tissue factor/activated factor VII complex (sTF/VIIa), were isolated from two traditional Chinese medicinal plants commonly used in the treatment of cardiovascular and cerebrovascular diseases. The active compounds were found to be linolenic, linoleic, and oleic acids from roots of *Salvia miltiorrhiza*; and two anacardic acids, 6-(8′*Z*-pentadecenyl)- and 6-(10′*Z*-heptadecenyl)-salicylic acids, from leaves of *Ginkgo biloba*. The IC_{50} values were in the range 30−80 μmol/L. Palmitic acid, isolated from roots of *Salvia miltiorrhiza*, and 2-[(3′,7′,11′,15′-tetramethyl)-2′*E*,6′*E*,-10′*E*,14′*E*-hexadecatetraenyl]-1,4-hydroquinone, isolated from the marine sponge *Adocia viola*, did not inhibit sTF/VIIa. Further expansion of the structure−activity relationship to include anacardic acids, 6-(8′*Z*,11′*Z*-heptadecadienyl)- and 6-(8′*Z*, 11′*Z*,14′*Z*-heptadecatrienyl)-salicylic acids from leaves of *Anacardium spondias*, and other fatty acids demonstrated that at least one cis double bond was essential for inhibitory activity, and that fatty acids containing two or three cis double bonds were optimal. Evidence from preincubation studies implied that these fatty acids may exert their effect by binding to VIIa and consequently preventing binding of sTF to VIIa.

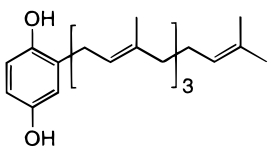
The extrinsic pathway of blood coagulation is initiated when circulating blood is exposed to subendothelium cells expressing tissue factor (TF) after vessel-wall injury.[1] TF is a membrane-bound glycoprotein, which functions as a cofactor for activated factor VII (VIIa). The TF-factor VIIa complex (TF/VIIa) proteolytically activates factors IX and X, which leads to the generation of thrombin and fibrin clots.[2] According to traditional Chinese medicinal postulation, *Salvia miltiorrhiza* Bunge (Labiatae) belongs to a class of Chinese medicinal herbs believed to "invigorate the circulation of blood" and is effective in the treatment of the cardiovascular and cerebrovascular diseases.[3] Many compounds with antibacterial,[4] antiinflammatory, antineoplastic, antiplatelet aggregation,[5] and tranquilizer activities[6,7] have been identified from this medicinal herb. Similarly, *Ginkgo biloba* L. (Ginkgoaceae) is widely used in China for protection against and treatment of cardiovascular and cerebrovascular diseases. In this study, we report three unsaturated fatty acids (linolenic, linoleic, and oleic acids) isolated from *S. miltiorrhiza* and two anacardic acids, [6-(8′*Z*-pentadecenyl)-salicylic acid (**1**) and 6-(10′*Z*-heptadecenyl)-salicylic acid (**2**)][8] isolated from *G. biloba*, as inhibitors of soluble TF/VIIa (sTF/VIIa) amidolytic activity.

The beneficial effects of unsaturated fatty acids on the cardiovascular system are well known. In human studies, unsaturated fatty acids cause a reduction in blood viscosity and blood pressure, a lowering of plasma cholesterol and triglyceride concentrations, and a decrease in platelet aggregation associated with prolonged bleeding times.[9] We therefore extended our study of inhibitors of sTF/VIIa to include other fatty acids and long-chain anacardic acids in order to explore the structure−activity relationship of these active molecules. These results are also reported herein.

Results and Discussion

Methylene chloride extracts of roots of *S. miltiorrhiza*, leaves of *G. biloba*, and leaves of *Anacardium spondias* (Anacardiceae) were found to inhibit sTF/VIIa amidolytic activity. Purification of the active principals by LC and HPLC and identification by MS and NMR yielded linolenic, linoleic, and oleic acids from *S. miltiorrhiza*, anacardic acids 6-(8′*Z*-pentadecenyl)-salicylic acid (**1**) and 6-(10′*Z*-heptadecenyl)-salicylic acid (**2**) from *G. biloba*,[8] and 6-(8′*Z*,11′*Z*-heptadecadienyl)-salicylic acid (**3**) and 6-(8′*Z*, 11′*Z*, 14′*Z*-heptadecatrienyl)-salicylic acid (**4**) from *A. spondias*.[10] Palmitic acid was also isolated from roots of *S. miltiorrhiza*, and 2-[(3′,7′,11′,15′-tetramethyl)-2′*E*,6′*E*,10′*E*,-14′*E*-hexadecatetraenyl]-1,4-hydroquinone (**5**)[11] was isolated from the marine sponge *Adocia viola* (Chalinidae), but neither was found to be active against sTF/VIIa.





5

The sTF/VIIa amidolytic inhibitory activity of these unsaturated acids, together with analogues and other compounds with lipid side chains, are summarized in Table 1. Comparison of fatty acid data revealed that at least one cis double bond is required for activity (compare 10-

* To whom correspondence should be addressed: Tel.: (314)737-6792. Fax: (314)737-7425. E-mail: richard.c.durley@monsanto.com.

10.1021/np980117p CCC: $15.00
Published on Web 09/11/1998

Table 1. TF/VIIa Inhibitory Activity of Fatty Acids, Selected Lipids, and Anacardic Acids

compound name	TF/VIIa IC_{50} (μM)[a,b,c]	log *p*
myristoleic acid (14:1)	66 ± 5[d]	NT[e]
palmitoleic acid (16:1)	120 ± 6[d]	2.73
oleic acid (18:1)	80 ± 5[f]	3.54
linoleic acid (18:2)	41 ± 3[f]	3.00
linolenic acid (18:3)	30 ± 2[f]	2.51
γ-linolenic acid (18:3)	30 ± 2[f]	2.55
cis-11,14-eicosadienoic acid (20:2)	52 ± 8[d]	2.41
cis-8,11,14-eicosatrienoic acid (20:3)	75 ± 4[d]	3.28
cis-11,14,17-eicosatrienoic acid (20:3)	100 ± 3[d]	3.30
cis-5,8,11,14-eicosatetraenoic acid (20:4)	105 ± 4[d]	2.90
cis-5,8,11,14,17-eicosapentaenoic acid (20:5)	78 ± 6[d]	2.48
6-(8′*Z*-pentadecenyl)-salicylic acid (**1**)	32 ± 3[f]	3.99
6-(10′*Z*-heptadecenyl)-salicylic acid (**2**)	68 ± 5[f]	4.04
6-(8′*Z*,11′*Z*-heptadecadienyl)-salicylic acid (**3**)	33 ± 2[f]	3.72
6-(8′*Z*,11′*Z*,14′*Z*-heptadecatrienyl)-salicylic acid (**4**)	30 ± 2[f]	3.50

[a] Values are expressed as mean ± SE. [b] All compounds had no inhibitory activity toward trypsin (IC_{50} > 200 μM). [c] The following were found to have TF/VIIa IC_{50} > 200 μM: 10-hydroxydecanoic acid, undecylenic acid, myristic acid (14:0), 16-hydroxyhexadecanoic acid, palmitic acid (16:0), stearic acid (18:0), elaidic acid (18:1$_t$) (trans double bond), linolenic acid methyl ester, 2-[(3′,7′,11′,15′-tetramethyl)-2′*E*,6′*E*,10′*E*,14′*E*-hexadecatetraenyl]-1,4-hydroquinone (**5**), prostaglandin A_1, prostaglandin B_1, prostaglandin I_2Na, prostaglandin E_1, prostaglandin E_2, prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$, vitamin E, folic acid. [d] *n* = 3. [e] NT = not tested. [f] *n* = 5.

hydroxydecanoic, undecylenic, myristic, 16-hydroxyhexadecanoic, palmitic, stearic, and elaidic acids with myristoleic, palmitoleic, and oleic acids). The highest activity was observed in fatty acids with two (linoleic and *cis*-11,-14-eicosadienoic acids) or three (linolenic, γ-linolenic, and *cis*-8,11,14-eicosatrienoic acids) double bonds. Four or more double bonds did not enhance the activity (*cis*-5,8,-11,14-eicosatetraenoic and *cis*-5,8,11,14,17-eicosapentaenoic acids). From the data given, no correlation was observed between the position of double bonds and the activity. The acid function was required for activity (compare linolenic acid and methyl linolenate). The log *p* values for the most active compounds were 2.41−3.54, which implied that such compounds would be expected to be absorbed systemically in humans.

The anacardic acids (**1**−**4**), which have lipid side chains with cis double bonds, were active to about the same extent as the unsaturated fatty acids. However, correlation of number of the double bonds to activity was not apparent. The log *p* values, 3.50−4.04, were higher in this group than in the active unsaturated fatty acids, although not out of range for good bioavailability. Other compounds with lipid-like side chains were not found to be active. These included the hydroquinone (**5**), the prostaglandin acid A_1, prostaglandin B_1, prostaglandin E_1, prostaglandin E_2, prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$, prostaglandin I_2, vitamin E, and folic acid.

Preincubation of linolenic acid with factor VIIa prior to the addition of sTF showed a dose- and time-dependent inhibition of sTF/VIIa amidolytic activity (Figure 1). Similarly, preincubation of other unsaturated fatty acids with VIIa prior to the addition of the sTF showed time-dependent inhibition (Table 2). On the other hand, preincubation with sTF did not cause amidolytic inhibition. These data are consistent with the unsaturated fatty acids binding to or otherwise affecting VIIa rather than sTF.

Similar inhibition was observed when thromboplastin (TF plus phospholipid) served as a cofactor (data not given),

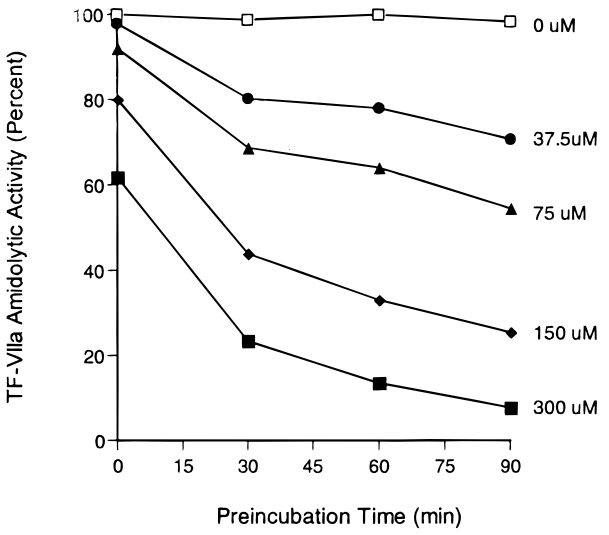


Figure 1. Preincubation of linolenic acid with factor VIIa affects the amidolytic activity of sTFVIIa. Factor VIIa (10 nM) was preincubated with the indicated concentration of linolenic acid for various times at room temperature. Following preincubation the mixtures were diluted fivefold into the amidolytic assay. Activity was measured as described in the Experimental Section. Activity at 100% represents the activity for VIIa in the absence of inhibitor and without preincubation. Each value is a mean of three experimental points.

Table 2. Effect of Preincubation Time on sTF/VIIa Inhibitory Activity

sample	no preincubation (IC_{50}, μM)	preincubation of inhibitor with (IC_{50}, μM)			
		VIIa for 15 min	VIIa for 90 min	TF for 15 min	TF for 90 min
p-aminobenzamidine	83	81	80	85	80
γ-linolenic acid	>200	30	21	>200	>200
linolenic acid	>200	27	30	>200	>200
linoleic acid	>200	30	19	>200	>200
oleic acid	>200	45	20	>200	>200
palmitic acid	>200	>200	>200	>200	>200

indicating that the unsaturated fatty acids also inhibited VIIa activated by natural TF. All the above fatty acids had no inhibitory activity toward trypsin, another serine protease, demonstrating a degree of selective inhibitory activity toward sTF/VIIa.

Some recent reports have indicated an inverse correlation between the levels of unsaturated fatty acids in serum and certain thrombotic and cardiovascular diseases. Patients with coronary heart disease or diabetes mellitus were shown to have lower serum levels of γ-linolenic, arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic acids (DHA).[12] Because AA and EPA are precursors to prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, I_2, and I_3, which are potent platelet antiaggregators and vasodilators and which have been shown to prevent thrombosis and atherosclerosis, it was proposed that the low levels of unsaturated fatty acids were causative to these diseases.[9,13] Other evidence suggests that linolenic acid inhibited thrombogenicity by a mechanism involving lipoxygenase oxidation in vessel walls to 13-hydroxyoctadecadienoic acid (13-HODE), which decreases platelet/vessel-wall interactions.[14] Some reports have implied that oleic, linoleic, and linolenic acids may influence expression of TF, because these unsaturated fatty acids were shown to be strong inhibitors of LPS- or IL1β-induced TF expression in monocytes and macrophages.[15] Saturated fatty acids such as palmitic acid were ineffective.[16] Ethyl esters of eicosapentaenoic and docosahexaenoic acid administered to healthy volunteers reduced TF activity in adherent monocytes either in the unstimulated

condition or after exposure to endotoxin, and the TF activity remained inhibited for 14 weeks after stopping treatment.[17]

As an alterative mechanism of inhibition of thrombosis by unsaturated long-chain acids, we have proposed inhibition of sTF/VIIa. Fatty acids with 18−22 carbon chains and containing two or three cis double bonds were optimal for activity. Anacardic acids with lipid side chains containing cis double bonds were equally as effective. This inhibition is likely due to the unsaturated acids themselves and not due to conversion to prostaglandins, inasmuchas the latter compounds were not inhibitory against sTF/VIIa amidolytic activity. Because inhibition was observed after preincubation with factor VIIa, binding of unsaturated fatty acids to VIIa is suggested. The position of binding to one or more of the four factor VIIa domains was not determined. If the unsaturated fatty acid inhibition is caused by binding and consequent conformational change of VIIa, this could affect TF binding, which requires all four domains,[18] or alternatively could directly affect the amidolytic active site.

Evidence has been presented herein that unsaturated fatty acids and anacardic acids are effective in the inhibition of the amidolytic activity of sTF/VIIa. In a single preliminary experiment linolenic acid did not initiate the clotting process when incubated with plasma as a source of VIIa and thromboplastin (data not given). However, other fatty acids, derivatives, or mixtures were not tested. It may be possible to design more effective inhibitors based on unsaturated fatty acids or anacardic acids, since serum levels of long-chain unsaturated acids are inversely correlated with certain thrombotic and cardiovascular diseases.

Experimental Section

General Experimental Procedures. Unless otherwise specified, all chemicals were of the highest quality available from Sigma−Aldrich Chemical Co. (St. Louis). Analytical HPLC was performed on a Thermo Separation Products P4000 pump, AS3000 injector, and Spectra Focus forward optics UV scanning detector. Columns were Rainin C_{18} (10 × 250 mm, 5 μm particle size) and Alltech Alltima C_{18} (10 × 250 mm, 5 μm particle size). The mobile phases were 0.05% trifluoroacetic acid in H_2O and 0.05% trifluoroacetic acid in MeCN, and mixtures of these phases are given as % MeCN for brevity. Flow rate was 3 mL/min on all columns. ^{1}H NMR spectra were collected on a Varian VXR-300 spectrometer. The samples were dissolved in $CDCl_3$ 100 atom % (MSD Isotopes, St. Louis) at a concentration of 0.1−0.7 wt % and placed in 3-mm NMR tubes (Wilmad Glass, Buena, NJ). MS was performed using a SCIEX (Thornhill, Ontario, Canada) API-III mass spectrometer utilizing an electrospray interface.

Plant/Animal Material and Sources of Test Compounds. Dried roots of *S. miltiorrhiza* Bunge were purchased in Chengdu, China, and St. Louis, MO. *G. biloba* L. leaves were collected in St. Louis in July and September 1996. *A. spondias* leaves were collected in Agumatsa Wildlife Sanctuary−National Park, Ghana, at the town of Wli-Agorviafe. *A. viola* was collected 500 m off the coast of Kolonia, Pohnpei, in the Federated States of Micronesia in July 1993. This sponge was identified by Dr. Michelle Kelly-Borges (Zoology Department, The Natural History Museum, London, UK). Test compounds not extracted from plants or animals were purchased from Sigma−Aldrich Chemical Co. (St. Louis).

Production of Recombinant Human Soluble Tissue Factor. The extracellular domain of human soluble tissue factor (Ala-TF $_{1-219}$) was expressed in *E. coli* inclusion bodies using conventional molecular biology techniques.[19,20] *E. coli* inclusion bodies containing sTF were dissolved in 50 mmol/L Tris-HCl, pH 8.0, containing 8 mol/L urea, and 5 mmol/L dithiothreitol and dialyzed against the same buffer. All dialysis was carried out at 4 °C. To refold the sTF, extension dialysis was performed over a 1-week period using buffer with progressively lower concentrations of urea (24 h/dialysis, urea concentration decreasing by 1mol/day). The refolded sTF was then purified by Mono Q column. Fractions containing sTF were pooled and analyzed by SDS−PAGE, and the sTF product was shown to be >95% pure.[21,22] EIMS spectral data indicated that the molecular weight was the expected 24 870 daltons. The purified sTF was crystallized, and the crystallized structure was shown to be similar to that reported.[23]

sTF/VIIa Amidolytic Inhibitory Activity Determination. The sTF/VIIa activity was measured using an amidolytic assay and chromozyme t-PA as substrate. sTF, chromozym-tPA, and inhibitors were prepared in assay buffer: 50 mmol/L Tris-HCl pH 7.5, 100 mmol/L NaCl, and 0.1% (w/v) bovine serum albumin. Factor VIIa was prepared in assay buffer plus 25 mmol/L $CaCl_2$. To each well of a 96-well plate (Falcon 3915, Lincoln Park, NJ) was added assay buffer plus or minus inhibitor, 0.01 μg of human factor VIIa (BiosPacific, Emeryville, CA), 0.35 μg of human sTF, and 23 μg of chromozym-tPA (American Peptide Co., Sunnyvale, CA) in a total reaction volume of 100 μL. Inhibitors were preincubated with factor VIIa (2 nM) for 15 min. The reaction was started with the addition of sTF (140 nM) and substrate chromozym-tPA. The plate was read immediately for absorbance at 405 nm (Biorad model 3550). After a 60-min incubation at room temperature, the plate was read a second time. The sTF/VIIa enzymatic activity was calculated as the change in absorbance at 405 nm over 60 min. The assay showed linearity over 60 min.

Preincubation Assay Determination. The assay method was as described above. Preincubation time and inhibitor concentrations were as indicated (Figure 1 and Table 2).

Trypsin Inhibitory Activity Determination. Trypsin, *N*-benzoyl-L-arginine-*p*-nitroanilide, and inhibitors were prepared in assay buffer: 100 mmol/L Tris-HCl, pH 8.2, 20 mmol/L $CaCl_2$, and 0.1% (w/v) bovine serum albumin. To each well of a 96-well plate was added assay buffer plus or minus inhibitor, 0.07 μg of trypsin (porcine pancreas), and 32 μg of *N*-benzoyl-L-arginine-*p*-nitroanilide in a total reaction volume of 100 μL. Inhibitors were preincubated with trypsin for 15 min at room temperature. The reaction was initiated with the addition of substrate, and the plate was read immediately for absorbance at 405 nm. After incubation at room temperature for 60 min, the plate was read a second time. Trypsin enzymatic activity was calculated as the change in absorbance at 405 nm over 60 min.

Extraction and Purification of Fatty Acids. *S. miltiorrhiza* roots (10 g) were extracted with CH_2Cl_2 (2 × 30 mL) overnight at room temperature. After removal of organic solvent, the extract was separated by flash column chromatography (Si gel 200 g, 230−400 mesh 60 A, Merck 9385), using mobile phase hexane−EtOAc (100:0 to 50:50). Fractions from this column were purified by HPLC on Rainin C_{18} (50 to 100% MeCN over 70 min). Active fractions corresponding to peaks eluting at 52, 60, and 69 min were collected. Residues from the 52- and 60-min fractions were subjected to further HPLC purification on Alltech Alltima C_{18} (78% MeCN for the 52-min fraction and 82% MeCN for the 60-min fraction) to give, respectively, linolenic acid and linoleic acid, identified by MS and NMR. The residue from 69-min fraction was subjected to GC−MS, to reveal the presence of oleic acid and palmitic acid identified by MS, and subsequently, after separation, by NMR.

Extraction and Purification of Anacardic Acids. *G. biloba* leaves (15 g) were extracted in CH_2Cl_2. After removal of solvent, the residue was chromatographed on flash LC as described above. Fractions from this column were purified by HPLC on Rainin C_{18} (70 to 100% CH_3CN over 30 min). Active fractions corresponding to peaks eluting at 38 and 46 min were collected. Residues from these fractions were subjected to further HPLC purification on Alltech Alltima C_{18} (96% MeCN) to give 6-(8′*Z*-pentadecenyl)-salicylic acid (**1**) and 6-(10′*Z*-heptadecenyl)-salicylic acid (**2**), respectively, identified by comparison of MS and NMR with published data.[8]

A. spondias leaves (4 g) were extracted and chromatographed by flash LC as described above. Fractions from this column were purified by HPLC on Rainin C_{18} (70 to 100% MeCN over 60 min). Active fractions corresponding to peaks eluting at 44, 54, and 63 min were collected, and mobile phase was removed. From these fractions 6-(10′*Z*-heptadecenyl)-salicylic acid (**2**), 6-(8′*Z*,11′*Z*-heptadecadienyl)-salicylic acid (**3**), and 6-(8′*Z*,11′*Z*,14′*Z*-heptadecatrienyl)-salicylic acid (**4**) were isolated, respectively, and identified by comparison of MS and NMR with published data.[10]

Extraction and Purification of 2-[(3′,7′,11′,15′-Tetramethyl)-(*2′E,6′E,10′E,14′E*)-hexadecatetraenyl]-1,4-hydroquinone (5). Whole sponge *A. viola* (2 g) was extracted and chromatographed by flash LC as described above. Fractions from this column were purified by HPLC on Rainin C_{18} (85 to 90% MeCN over 60 min). A compound eluting at 47 min was collected and, after removal of mobile phase, was determined by MS and NMR to be the title compound (**5**) by comparison with published data.[11]

Determination of Log *p* Data. Log *p* represents octanol−H_2O partition coefficients and reflects the degree to which lipids and other substances would be absorbed systemically in humans. Approximately 10 000 ppm solutions of the fatty acids were dissolved in MeOH and examined by HPLC using Biotage C_{18} (4.6 × 250 mm) and a mobile phase of MeCN−4 mmol/L phosphate buffer at pH 7.4 (10:90 to 80:20 over 20 min, and hold at 80:20 for a further 10 min) at a flow rate of 1.0 mL/min. The injection volume was 10 μL; monitoring was set at 215 nm. The system was calibrated with reference compounds for the determination of log *p*. Capacity factors were compared with the reference compounds of known log *p* in octanol−H_2O via Microsoft Excel regressions.

Acknowledgment. The authors wish to thank Dr. Ravi G. Kurumbail, Dr. Roderick A. Stegeman, Dr. Huey S. Shieh, and Prof. Paul Bajaj (St. Louis University) for helpful suggestions in the discussion of the assay results, Dr. Dutt V. Vinjamoori and Dave W. Kuneman for the log *p* determinations, Mr. James P. Doom for mass spectral analysis, the Monsanto NMR consortium personnel for NMR data and Eric E. Rhoden for his excellent assistance in the Tissue Factor/VIIa assay. Also we want to thank Michael J. Prinsen and Norma J. Bischoff, who extracted the samples.

References and Notes

(1) Broze, G. J. Jr.; Girard, T. J.; Novotny, W. F. *Biochemistry* **1990**, *29*, 7539−7549.
(2) Davie, E. W. *Thromb. Haemostasis* **1995**, *74*, 1−6.
(3) Kong, D. Y. *Chin. J. Pharm.* **1989**, *20*, 279−285.
(4) Gao, Y. G.; Song, Y. M.; Yang, Y. Y.; Tang J. X.; Liu, W. F. *Acta Pharm. Sin.* **1979**, *14*, 75−82.
(5) Wang, N.; Luo H. W.; Niwa, M.; Ji, J. *Planta Med.* **1989**, *55*, 390−391.
(6) Li, C. M.; Sham, H. L.; Wong, H. N. C. *J. Med. Chem.* **1991**, *34*, 1675−1692.
(7) Lee, C. M.; Wong, H. N. C.; Chui, K. Y.; Choang, T. F.; Hon, P. M.; Chang, H. M. *Neuroscience Lett.* **1991**, *127*, 237−241.
(8) Itokawa, H.; Totsuka, N.; Nakahara, K.; Takeya, K.; Lepoittevin, J. P.; Asakawa, Y. *Chem. Pharm. Bull.* **1987**, *35*, 3016−3020.
(9) a. Bjerve, K. S.; Brekke, O. L.; Fougner, K. J.; Midthjell, K. b. Renaud, S. c. Knapp, H.; Gregory, D.; Nolan, S. In *Dietary Omega3 and Omega6 Fatty Acids: Biological Effects and Nutritional Essentiality*; Galli, C., Simopoulos, A. P., Eds.; Plenum: New York, 1989; Series A: Life Sciences. Vol 171; pp 241−251, 263−271, 283−295.
(10) Coates, N. J.; Gilpin, M. L.; Gwynn, M. N.; Lewis, D. E.; Milner, P. H.; Sprer, S. R.; Tyler, J. W. *J. Nat. Prod.* **1994**, *57*, 654−657.
(11) Cimino, G.; Stefeno, S.; Minale, L. *Experimentia* **1972**, *28*, 1−2.
(12) Das, U. N. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1995**, *52*, 387−391.
(13) Nakahara, T.; Yokochi, T.; Kamisaka, Y.; Yamaoka, M.; Suzuki, O.; Sato, M.; Okazaki, S.; Ohshima, N. *Thromb. Res.* **1990**, *57*, 371−381.
(14) Bertomeu, M. C.; Crozier, L. C.; Haas, T. A.; Fleith, M.; Buchanan, M. R. *Thromb. Res.* **1990**, *59*, 819−830.
(15) Chu, A. J.; Moore, J. *Cell Biochem. Function.* **1991**, *9*, 231−238.
(16) Laie, A.; Herbert, J. M. *Biochem. Pharmacol.* **1994**, *48*, 429−431.
(17) Tremoli, E.; Eligini, S.; Colli, S.; Maderna, P.; Rise, P.; Pazzucconi, F.; Marangoni, F.; Sirtori, C. R.; Galli, C. *Arterioscler. Thromb.* **1994**, *14*, 1600−1608.
(18) Banner, D. W.; Arcy, A. D.; Chene, C.; Winkler, F. K.; Guha, A.; Konigsberg, W. H.; Nemerson, Y.; Kirchhofer, D. *Nature* **1996**, *380*, 41−46.
(19) Olins, P. O.; Rangwala, S. H. *Meth. Enzymol.* **1990**, *185*, 115−119.
(20) Obukowicz, M. G.; Staten, N. R.; Krivi, G. G. *Appl. Environ. Microbiol.* **1992**, *58*, 1511−1523.
(21) Laemmli, U. K. *Nature* **1970**, *227*, 680−685.
(22) Heukeshowen, J.; Denrick, R. *Electrophoresis* **1985**, *6*, 103−112.
(23) Muller, Y. A.; Ultsch, M. H.; Yos, A. M. *J. Mol. Biol.* **1996**, *156*, 144−159.

NP980117P