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Isolation and Identification of Anti-platelet Aggregation Principles from the Bark of *Fraxinus japonica* BLUME[1)]

HARUYOSHI KODAIRA, MASAYUKI ISHIKAWA,* YASUO KOMODA, and TERUMI NAKAJIMA

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2–3–10, Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan

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The methanol extract of the bark of *Fraxinus japonica* BLUME was fractionated, and by following the inhibitory activities on rat hind paw edema induced by carrageenin and on rabbit platelet aggregation induced by arachidonic acid, the following active principles were isolated and identified: 2-(3-methoxy-4-hydroxyphenyl)ethanol (I) (which also exists in animals and man as a dopamine metabolite), 2-(*p*-hydroxyphenyl)ethanol (II), 2,6-dimethoxy-*p*-benzoquinone (III), compounds IV, V, and esculetin.

Keywords——bark of *Fraxinus japonica*; anti-inflammatory activity; anti-platelet aggregating activity; 2-(3-methoxy-4-hydroxyphenyl)ethanol; 2-(*p*-hydroxyphenyl)ethanol; 2,6-dimethoxy-*p*-benzoquinone; esculetin; 2-(3,4-dihydroxyphenyl)ethanol; dopamine metabolite

The dried bark of *Fraxinus japonica* BLUME (the oriental medicine "Shinpi"), which is widely distributed in Japan, has been used as a home remedy, diuretic, antifebrile, analgesic and so on.[2)]

The methanol extract of the bark was reported to have anti-inflammatory action and to promote the excretion of uric acid.[3)] Esculin, a coumarin glycoside which is a main constituent of the extract, and its aglycone, esculetin, are known to be active principles having these pharmacological activities.[3c,f,g,4)] However, some of the authors[3c,f,g)] reported that the methanol extract showed more potent inhibitory activity than that expected from the amount of esculin contained, and suggested that the more potent effect might be associated with active compounds other than esculin. Until now, no other biologically active compounds have been found in the crude extract.

We fractionated the methanol extract as described later and subjected the fractions to bioassays of inhibitory activity on rat hind paw edema induced by carrageenin and on rabbit platelet aggregation induced by arachidonic acid (AA). We found that fraction 3 (fr. 3), which did not contain the known active compounds, esculin and esculetin, showed potent inhibitory activity in both bioassays.

In the present paper, we report the isolation and identification of the active principles (compounds I, II, and III) from fr. 3. Anti-inflammatory bioassay was performed according to the method reported by Van Arman *et al.*,[5)] and the anti-platelet aggregation bioassay was performed by the modified method of Born.[6)] The potency of these activities was used as a guide in the isolation of active principles. The behavior of esculin and esculetin in the course of the fractionation was confirmed, and their amounts were determined, by high performance liquid chromatography (HPLC).

The methanol extract of the bark was fractionated as shown in Chart 1. Table I shows the biological activities of each fraction. The water-soluble fraction (B) containing 2.7% esculin inhibited the rat hind paw edema, but not the rabbit platelet aggregation. On the other hand, the ethyl acetate-soluble fraction (C) containing 1% esculetin inhibited both the edema and

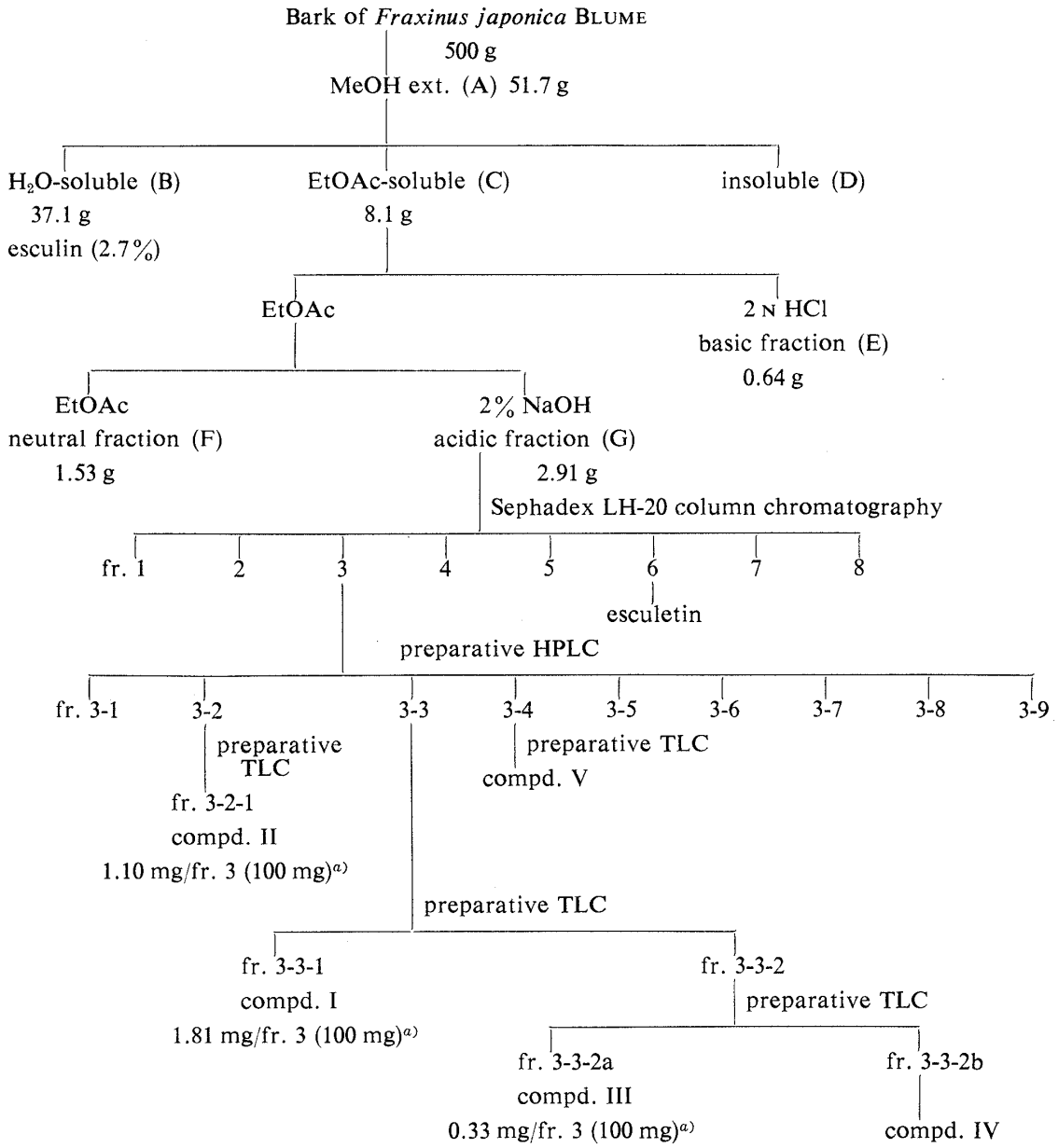

Chart 1. Isolation of Biologically Active Constituents from the Bark of *Fraxinus japonica* BLUME

a) Total yield reduced from 100 mg of fr. 3.

AA-induced aggregation. Thus, fraction C was further fractionated into basic (E), neutral (F), and acidic (G) fractions. Fraction E showed no inhibitory effects in the bioassays. Fraction F did not inhibit the edema significantly, but showed an inhibitory effect on the aggregation. In contrast, fraction G showed significant inhibitory activities on both the edema (46.7% inhibition at a dose of 300 mg/kg) and the aggregation (50—75% inhibition at 250—321 μg/ml). Fraction G contained 1.2% esculetin. Esculetin was thus tested for inhibitory activity on the rat hind paw edema and the platelet aggregation, but exhibited no significant activities at a dose (3.6 mg/kg) equivalent to 300 mg of fraction G in the former case and at a level (2.9—3.7 μg/ml) equivalent to 250—321 μg of fraction G in the latter case. From these results, it was concluded that the inhibitory activities in both bioassays shown by fraction G were not mainly associated with esculetin. Thus, other active compounds might contribute to the inhibitions.

TABLE I. Biological Activities of Fractions from Methanol Extract (A)

Fractions and compounds	Dose (mg/kg)	Route	Inhibitory activity on rat hind paw edema induced by carrageenin (1%, 0.05 ml) 4 h[a]	Amount added (μg/ml)[b]	Inhibitory activity on rabbit platelet aggregation induced by	
					AA (100 μM)[b,c]	ADP (10 μM)[b,c]
B	600	*i.p.*	16.1	250—321	±	−
C	300	*i.p.*	29.0	250—321	+	−
E	300	*i.p.*	—	250—321	−	−
F	400	*p.o.*	5.2	250—321	+	−
G	300	*i.p.*	46.7	250—321	++	−
	100	*i.p.*	40.5			
	50	*i.p.*	12.2			
Esculin	16.2	*i.p.*	—	250—321	−	−
Esculetin	3.6	*i.p.*	—	2.9—3.7	±	−
Aspirin	[d]	*i.p.*	[d]	5.82	++	−
Indomethacin	5.0	*i.p.*	62.0	0.33	++	−

a) Inhibitory % at 4 h after injection of carrageenin. −, no effect.
b) Final concentration. AA, sodium arachidonate; ADP, adenosine 5′-diphosphate.
c) Symbols indicate the degree of anti-platelet aggregating activity: −, no effect; ±, 5—25% inhibition; +, 25—50% inhibition; ++, 50—75% inhibition; +++, 75—100% inhibition.
d) Not tested.

TABLE II. Biological Activities of Fractions from Acidic Fraction (G)

Fractions and compounds	Dose (mg/kg)	Route	Inhibitory activity on rat hind paw edema induced by carrageenin (1%, 0.1 ml) 3 h[a]	Amount added (μg/ml)[b]	Inhibitory activity on rabbit platelet aggregation induced by
					AA (100 μM)[b,c]
G	400	*p.o.*	16.4	106—143	+
1	40	*p.o.*	—	106—143	−
2	202	*p.o.*	—	106—143	−
3	53	*p.o.*	13.0	106—143	+++
4	20	*p.o.*	—	106—143	±
5	19	*p.o.*	—	106—143	±
6	47	*p.o.*	5.5	106—143	+++
7	11	*p.o.*	—	106—143	−
8	11	*p.o.*	—	106—143	−
Esculetin	47	*p.o.*	7.1	106—143	+++
Aspirin	150	*p.o.*	36.5	5.82	++
Indomethacin	5.0	*p.o.*	49.0	0.33	++

a) Inhibitory % at 3 h after injection of carrageenin. −, no effect.
b) and *c*) See footnotes to Table I.

Fraction G was then subjected to Sephadex LH-20 column chromatography, and separated into eight fractions, monitored by ultraviolet (UV) absorption measurement at 260 nm. Table II shows the biological activities of each fraction. Fraction 3 showed inhibitory activity nearly equal to that of fraction G on the edema at a dose (53 mg/kg) equivalent to 400 mg of fraction G given *p.o.*, though this inhibitory effect was less than that after *i.p.* administration. In the cases of fraction 6 (fr. 6) containing mainly esculetin and esculetin itself, both showed no significant activity at the same dose (47 mg/kg, a dose equivalent to 400 mg of fraction G). On the other hand, the anti-platelet aggregating activity was observed

in frs. 3—6. In paticular, frs. 3 and 6 exhibited potent inhibitory activity. Judging from the content of esculetin in fr. 6 and its anti-platelet potency, it was considered that esculetin might contribute to the anti-aggregating action of fr. 6. Thus, fr. 3 not containing esculetin was further purified by preparative HPLC, affording fractions 3-1 to 3-9 (frs. 3-1—3-9). The elution profile was monitored by UV absorption measurement at 254 and 280 nm. The inhibitory effect of the samples obtained on the rat hind paw edema was not investigated, because large amounts of sample are needed for this bioassay. However, the anti-platelet aggregation bioassay required only a small amount of sample, and anti-inflammatory drugs, such as aspirin and indomethacin, also inhibit AA-induced platelet aggregation. Therefore, from this stage, only anti-platelet aggregation bioassay was performed for the isolation of biologically active compounds. Anti-aggregating activities of frs. 3-1 to 3-9 are shown in Table III. Fractions 3-2 and 3-3 among the fractions showed potent inhibitory activities, and fr. 3-4 showed significant activity. These fractions were further purified by repeated thin layer chromatography (TLC) on silica gel, affording active compounds, compound II from fr. 3-2, compounds I, III, and IV from fr. 3-3, and compound V from fr. 3-4.

Table III. Inhibitory Activity of Fractions from Fr. 3 on Rabbit Platelet Aggregation induced by Arachidonic Acid (100 μM)[b]

	Amount added (20—30 μg/ml each)[b]								
Fraction No.	3-1	3-2	3-3	3-4	3-5	3-6	3-7	3-8	3-9
Activity[c]	±	++	+++	+	±	−	−	−	−

b) and *c*) See footnotes to Table I.

Compound I, colorless oil, $C_9H_{12}O_3$ (M^+ *m/z* 168.0821, calcd 168.0786). The infrared (IR, ν_{max}^{KBr} 1601 cm^{-1}) and UV (λ_{max}^{MeOH} 228, 281, 287 sh nm) spectra suggested that I is an aromatic type compound. The proton nuclear magnetic resonance (^1H NMR) spectrum ($CDCl_3$) of I showed signals due to a Phe–$C\underline{H}_2$–$C\underline{H}_2$–O– group at δ 2.80 and 3.83 (2H, triplet, J=7 Hz, respectively), an aromatic methoxyl group at δ 3.89 (3H, singlet), and a 1,2,4-trisubstituted benzene ring system at δ 6.70 (1H, double doublet, J=2 and 8 Hz), 6.73 (1H, doublet, J=2 Hz) and 6.87 (1H, doublet, J=8 Hz). The above data indicated I to be 2-(3-methoxy-4-hydroxyphenyl)ethanol, which is an alkaline hydrogenation product of wood[7] and a dopamine metabolite.[8] This was confirmed by direct comparison of the IR, mass (MS), and ^1H NMR spectra with those of an authentic specimen.

Compound II, colorless needles, mp 92 °C, $C_8H_{10}O_2$ (M^+ *m/z* 138.0678, calcd 138.0678). The IR (ν_{max}^{KBr} 1603, 1596 cm^{-1}) and UV (λ_{max}^{MeOH} 224, 278, 285 sh nm) spectra suggested that II is also an aromatic type compound. The ^1H NMR spectrum (CD_3OD) showed the presence of a Phe–$C\underline{H}_2$–$C\underline{H}_2$–O– group at δ 2.71 and 3.68 (each 2H, triplet, J=7 Hz), and a *p*-substituted benzene ring system at δ 6.69 and 7.02 (each 2H, doublet, J=9 Hz). These data indicated II to be 2-(*p*-hydroxyphenyl)ethanol and it was identified by direct comparison of the mp, and IR, MS, and ^1H NMR spectra with those of an authentic sample.

Compound III, yellow needles; sublimes; $C_8H_8O_4$ (M^+ *m/z* 168.0424, calcd 168.0423); IR ν_{max}^{KBr} cm^{-1}: 1669, 1642, 1621, 1596; UV λ_{max}^{MeOH} nm: 286, 294 sh, 382. The ^1H NMR spectrum ($CDCl_3$) showed the presence of two equivalent methoxyl groups at δ 3.82 (6H, singlet) and two equivalent olefinic protons at δ 5.85 (2H, singlet). From these data, III was assumed to be 2,6-dimethoxy-*p*-benzoquinone, and this was confirmed by direct comparison of the IR and MS spectra with those of an authentic specimen.

Inhibitory potencies of compounds I, II, III, and esculetin on rabbit platelet aggregation induced by AA or collagen were examined (Table IV). Table IV includes the results obtained

TABLE IV. Inhibitory Effects on Platelet Aggregation[a)]

Inhibitors	Platelet aggregation induced by	
	Arachidonic acid (100 μM)[b,c)]	Collagen (15 μg/ml)[b)]
2-(3-Methoxy-4-hydroxyphenyl)-ethanol[d)] (I)	13.9 (10.9—17.7)	30.5 (23.0—40.6)
2-(3,4-Dihydroxyphenyl)ethanol[d)]	19.7 (13.6—28.6)	35.4 (23.9—52.6)
2-(*p*-Hydroxyphenyl)ethanol (II)	119.3 (83.9—169.6)	157.4 (111.8—221.6)
2,6-Dimethoxy-*p*-benzoquinone (III)	145.2 (96.7—217.8)	48.5 (40.5—58.1)
Esculetin	62.6 (47.8—82.0)	71.6 (57.2—89.6)
Aspirin	32.3 (24.8—42.0)	20.0 (14.5—27.6)
Indomethacin	1.01 (0.80—1.26)	0.91 (0.77—1.09)

For each assay, rabbit PRP (440 μl) was preincubated with inhibitor (2 μl in ethanol or DMSO) for 3 min at 37 °C before the addition of the aggregating agent (40 μl).

a) Figures represent IC_{50} in μM (concentration that inhibits 50% of the agonists' effects) evaluated from 2 to 3 different concentrations of the inhibitor on log probit paper. Each value is the mean and its 95% confidence limits for individual determinations in 5 to 8 different platelet preparations.
b) Final concentration.
c) Sodium arachidonate.
d) Found as a dopamine metabolite in animals and man.

for aspirin and indomethacin as positive controls, and for 2-(3,4-dihydroxyphenylethanol,[9)] which is a dopamine metabolite (like compound I) and was found to have relatively potent inhibitory effects. Compounds I, II, and 2-(3,4-dihydroxyphenyl)ethanol had no effect on adenosine 5′-diphosphate (ADP)-induced rabbit platelet aggregation, whereas compound III (ADP 10 μM final concentration: IC_{50} = 80—100 μM[10)]) inhibited it. Aspirin and indomethacin, anti-inflammatory drugs which are well known as inhibitors of prostaglandin biosynthesis,[11)] have no effect on the ADP action. Thus, the above active compounds, like aspirin and indomethacin, may be expected to have some effect on prostaglandin biosynthesis and anti-inflammatory activity. Two dopamine metabolites (compound I and 2-(3,4-dihydroxyphenyl)ethanol exhibited anti-aggregating activity and were about twice as effective as aspirin in AA-induced aggregation. An investigation of the relationship between the activities of dopamine and its metabolites on platelet function would be of interest.

Acidic hydrolysis of the water-soluble fraction (B), which showed an inhibitory effect on the rat hind paw edema, gave rise to compounds (compounds I and II) with inhibitory activity on platelet aggregation other than esculetin,[12)] suggesting that compounds I and II exist as glycosides, or as polymerized compounds such as lignin.

The structures of compounds IV and V, which were also isolated as inhibitory principles on platelet aggregation and are assumed to be structually different from compounds I, II, and III, will be reported elsewhere.

Experimental

All melting points were determined with a Yanagimoto microscope hot plate and are uncorrected. UV spectra were obtained with a Hitachi 323 spectrometer. IR spectra were taken in KBr pellets with a Hitachi 285 spectrometer. MS spectra were recorded on a JEOL JMS-300 mass spectrometer. ^1H NMR spectra were taken at 100 MHz with a JEOL JNM-MH-100 spectrometer in $CDCl_3$ or CD_3OD solution. Chemical shifts (δ) are given in ppm downfield from internal tetramethylsilane and the following abbreviations are used: s = singlet, d = doublet, and t = triplet. HPLC was performed on an ALC/GPC 204 (Waters Associates Inc.) machine equipped with a UV detector operated at 254, 280, and 365 nm. A μBondapak C_{18} column (4 mm i.d. × 30 cm, Waters) was used for all analyses. TLC was performed on Silica gel 60 F_{254} pre-coated plates (Merck). Detection was done under UV light or by exposure to iodine vapor. Column chromatography was carried out with Sephadex LH-20 (Pharmacia Fine Chemicals). Esculin was obtained from Nakarai Chemicals Ltd. Esculetin was prepared by acidic hydrolysis of esculin. 2-(3-Meth-

oxy-4-hydroxyphenyl)ethanol, 2-(*p*-hydroxyphenyl)ethanol, and 2-(3,4-dihydroxyphenyl)ethanol were prepared by reduction of the corresponding acids with $LiAlH_4$.[13] 2,6-Dimethoxy-*p*-benzoquinone was synthesized from syringic acid by oxidation.[14] Arachidonic acid (Grade I, Sigma Chemical Co.) was used as the sodium salt. Carrageenin was presented by Dr. T. Moriwaki. Other materials used in experiments were as follows: indomethacin and ADP sodium salt type I (Sigma); aspirin (Sanko Seiyaku Kogyo Co.); collagen (from equine tendon, Hormon Chemie).

Preparation of Rabbit Platelet-rich Plasma (PRP)——Blood was collected into a plastic tube containing 0.1 volume of sodium citrate solution (3.8%, v/v) from the carotid artery of a male or female rabbit (2.5—3.5 kg). The citrated blood was centrifuged at 150 g for 20 min. PRP was carefully separated from the bottom layer, and kept at room temperature in a siliconized glass tube. Platelet-poor plasma (PPP) was prepared by centrifugation of PRP at 1500 g for 10 min. The final platelet concentration in the PRP was adjusted to 3 to 3.5×10^5 platelets/μl by appropriate dilution with autologous PPP. This platelet count was determined by a Coulter counter, model Z-B (Coulter Electronic Inc.).

Assay for Anti-platelet Aggregating Activity——The aggregation experiments to assess the inhibitory effect of each fraction or isolated compound on platelet aggregation induced by aggregating agents were performed at 37 °C in a dual sample aggregation meter (SIENCO, model DP-247E) using the modified method of Born.[6] The light transmission was adjusted to 0 and 100% with PRP and PPP, respectively. PRP (440 μl) in a siliconized glass cuvette was preincubated with a solution of each test sample or vehicle (ethanol or dimethylsulfoxide (DMSO)) for 3 min, while stirring was carried out at 1200 rpm with a siliconized bar. Platelet aggregation was then initiated by the addition of an aggregating agent (40 μl) (100 μM AA, 15 μg/ml collagen, 10 μM ADP: final concentrations). The percentage of inhibition of aggregation by each test fraction was determined by measuring the aggregation response 4 min after the addition of the aggregating agent. The inhibitory potencies of isolated compounds are given as the concentration causing 50% inhibition of the platelet aggregation at 4 min.

Assay for Anti-inflammatory Activity——Measurement of activity on carrageenin-induced rat hind paw edema was performed as follows. A group of 5 male Wistar rats (120—150 g) was used at each dose level. The volume of the rat hind paw was measured according to the method reported by Van Arman *et al.*[5] The foot was immersed in a pool of mercury above the topmost callus pad. The pressure increase caused by the slight rise in mercury level was transmitted to a pressure transducer (Statham, P23BB). Recordings were made on a ink-writing oscillograph through a carrier amplifier (Nihon Koden, PMP-3002). The subplantar injection of 0.05 or 0.1 ml of 1% carrageenin in physiological saline solution was performed, then the volume of the foot was determined every 1 h for 5 h, and the increase in volume was taken as the volume of edema. The swelling of the paw reached a peak 4 h after the injection of 0.05 ml of carrageenin, or 3 h after the injection of 0.1 ml of carrageenin. The samples to be tested were administered *i.p.* or *p.o.* as a 0.25% carboxymethylcellulose (CMC) suspension 60 min before the carrageenin treatment. Results were expressed as percent inhibition of swelling at the above time (4 or 3 h), relative to the control group given the vehicle.

Extraction and Partition——The dried bark of *Fraxinus japonica* BLUME (500 g) was extracted five times with 1.5 l each of MeOH at room temperature. The mixture was filtered and the filtrate was concentrated *in vacuo* at 35—40 °C to give a methanolic extract (fraction A, 51.7 g). The isolation of biologically active constituents from fraction A is shown in Chart 1. The biological activities of each fraction are shown in Tables I, II, and III. Fraction A (51.7 g) was partitioned between EtOAc and water (total, 600 ml each). The combined H_2O layer was lyophilized to afford H_2O-soluble materials (fraction B, 37.1 g). The combined EtOAc layer was concentrated *in vacuo*, affording EtOAc-soluble materials (fraction C, 8.1 g). Fraction C was dissolved in EtOAc (300 ml) and fractionated into basic fraction (E, 0.64 g) [by extraction with 2 N hydrochloric acid (total volume: 150 ml)], neutral fraction (F, 1.53 g), and acidic fraction (G, 2.91 g) [by extraction with 2% sodium hydroxide (total volume: 150 ml)] in the usual manner.

HPLC Analysis of Esculin and Esculetin——The behavior of esculin and esculetin in the course of the fractionation was confirmed and the amounts were determined, by HPLC with monitoring of the UV absorption at 365 nm. A mixture of acetonitrile and water (15 : 85, v/v) was used as the eluting solvent for esculin. For the analysis of esculetin, a mixture of acetonitrile, acetic acid (AcOH), and water (5 : 10 : 85, v/v) was employed. These mobile phases were used at a flow rate of 1 ml/min at 40 °C. The concentration of esculin (retention volume (r.v.): 4.6 ml) or esculetin (r.v.: 7.1 ml) in each fraction was determined from calibration curves constructed by measuring the peak heights at 5—6 different concentrations.

Sephadex LH-20 Column Chromatography——The acidic portion (G, 150 mg) was chromatographed on Sephadex LH-20 (2.6 × 90 cm, 150 g) with MeOH, giving eight fractions, fr. 1 (161—336 ml), fr. 2 (336—403 ml), fr. 3 (403—480 ml), fr. 4 (480—515 ml), fr. 5 (515—543 ml), fr. 6 (543—634 ml), fr. 7 (634—760 ml), and fr. 8 (760—872 ml) based on monitoring of the UV absorption at 260 nm. This chromatography was repeated twelve times (total weight of G: 1.8 g), and each fraction was concentrated *in vacuo*. The total weight of each fraction was as follows: fr. 1 (171 mg), fr. 2 (880 ml), fr. 3 (226 mg), fr. 4 (84 mg), fr. 5 (81 mg), fr. 6 (202 mg), fr. 7 (47 mg), and fr. 8 (42 mg).

Preparative HPLC——Fr. 3 (1.4 mg) was subjected to preparative HPLC (solvent system, $CH_3CN : H_2O =$ 12 : 88; flow rate, 2 ml/min; column temperature, 40 °C), and its elution profile was monitored by measuring the UV absorption at 254 and 280 nm. Eight fractions were obtained on the basis of eight main peaks, fr. 3-1 (6—13 ml), fr. 3-

2 (13—16 ml), fr. 3-3 (16—21 ml), fr. 3-4 (21—25 ml), fr. 3-5 (25—32 ml), fr. 3-6 (32—37 ml), fr. 3-7 (37—43 ml), fr. 3-8 (43—52 ml), and at the end of the elution MeOH (0.5 ml) was injected into the HPLC machine to recover remaining materials on the column (corresponding to fr. 3-9). This preparative HPLC was performed repeatedly, and 100 mg of fr. 3 (total weight) was fractionated. Each fraction was concentrated to half the initial volume *in vacuo* at 35—40 °C and extracted with EtOAc (100 ml × 2), and the EtOAc layer was concentrated *in vacuo* to afford the corresponding fraction (fr. 3-1 to 3-9). Fr. 3-9 among the fractions accounted for about 50% by weight of the sample applied.

Preparative TLC——Fr. 3-2 was developed on the silica gel plate (solvent system, MeOH : $CHCl_3$ = 10 : 90) and found to consist of a major component (*Rf* 0.29, fr. 3-2-1) and two very minor components, judging from the potency of the spots on the plate. Fr. 3-3 was found to consist of two major components (*Rf* 0.38, fr. 3-3-1; *Rf* 0.67, fr. 3-3-2) and a very minor component. These major components (frs. 3-2-1, 3-3-1, and 3-3-2) were purified by preparative TLC (solvent system, MeOH : $CHCl_3$ = 10 : 90, 5 : 95, and 1 : 99, respectively), and they showed anti-platelet aggregating activity. Frs. 3-2-1 (compound II) and 3-3-1 (compound I) were chromatographically pure on silica gel (*Rf* values: see above) and HPLC analysis (conditions: same as for preparative HPLC; r.v.: 14.8 and 17.8 ml, respectively). On the other hand, fr. 3-3-2 was not homogeneous on silica gel TLC with repeated development, though it gave a single peak (r.v.: 19.2 ml) on HPLC analysis. Fr. 3-3-2 was further subjected to preparative TLC (solvent system: $CHCl_3$ only) and developed six times, resulting in the separation of yellow needles (fr. 3-3-2a: compound III) and a colorless oily substance (fr. 3-3-2b: compound IV). Both fractions showed anti-platelet aggregating activity. Total yields of compounds I, II, and III were 1.81, 1.10, and 0.33 mg, respectively, from 100 mg of fr. 3. Compound V (colorless oily substance) was also isolated from fr. 3-4 by preparative TLC (solvent system, MeOH : $CHCl_3$ = 10 : 90) and showed anti-platelet aggregating activity. Compounds IV and V gave very low yields. Esculetin (4.8 mg) was isolated from fr. 6 (50 mg) by preparative TLC (solvent system, MeOH : $CHCl_3$: AcOH = 8 : 20 : 1), as it was confirmed by HPLC that fr. 6 contained mainly esculetin.

Compound I (2-(3-Methoxy-4-hydroxyphenyl)ethanol)——Colorless oil; MS *m/z*: 168.0821 (M^+, $C_9H_{12}O_3$ = 168.0786), 137, 122, 94. IR ν_{max}^{KBr} cm^{-1}: 1601. UV λ_{max}^{MeOH} nm: 228, 281, 287 sh. ^1H NMR ($CDCl_3$) δ: 2.80, 3.83 (each 2H, t, J = 7 Hz), 3.89 (3H, s), 6.70 (1H, dd, J = 2 and 8 Hz), 6.73 (1H, d, J = 2 Hz), 6.87 (1H, d, J = 8 Hz).

Compound II (2-(*p*-Hydroxyphenyl)ethanol)——Recrystallized from $CHCl_3$ to give colorless needles, mp 92 °C; MS *m/z*: 138.0678 (M^+, $C_8H_{10}O_2$ = 138.0678), 120, 107. IR ν_{max}^{KBr} cm^{-1}: 1603, 1596. UV λ_{max}^{MeOH} nm: 224, 278, 285 sh. ^1H NMR (CD_3OD) δ: 2.71, 3.68 (each 2H, t, J = 7 Hz), 6.69, 7.02 (each 2H, d, J = 9 Hz).

Compound III (2,6-Dimethoxy-*p*-benzoquinone)——Recrystallized from hexane–EtOAc to give yellow needles, sublimes; MS *m/z*: 168.0424 (M^+, $C_8H_8O_4$ = 168.0423), 138, 80, 69. IR ν_{max}^{KBr} cm^{-1}: 1669, 1642, 1621, 1596. UV λ_{max}^{MeOH} nm: 286, 294 sh, 382. ^1H NMR ($CDCl_3$) δ: 3.82 (6H, s), 5.85 (2H, s).

Esculetin——Recrystallized from EtOAc to give needles. This was identified by direct comparison (mp, IR, MS, ^1H NMR) with an authentic sample.

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