Antidiabetogenic Constituents from the Thai Traditional Medicine *Cotylelobium melanoxylon*

Hisashi MATSUDA,[a] Yasunobu ASAO,[a] Seikou NAKAMURA,[a] Makoto HAMAO,[a] Sachiko SUGIMOTO,[a] Masako HONGO,[a] Yutana PONGPIRIYADACHA,[b] and Masayuki YOSHIKAWA*,[a]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan: and* [b] *Faculty of Science and Technology, Rajamangala University of Technology Srivijaya; Thungyai, Nakhonsithammarat 80240, Thailand.*
Received January 6, 2009; accepted January 31, 2009; published online February 17, 2009

The methanolic extracts from the wood and bark of *Cotylelobium melanoxylon* were found to inhibit plasma glucose elevation after sucrose loading in rats and triglyceride elevation after olive oil loading in mice. A new stilbene dimer, melanoxylin A, together with the known stilbene dimers [(+)-ampelopsin F, (+)-isoampelopsin F, and (+)-ε-viniferin] and a trimer (vaticanol G) and a lignan [(+)-lyoniresinol] were isolated from the wood extract, and a new stilbene trimer, melanoxylin B, together with the known stilbene dimers [(+)-ε-viniferin and *cis*-(+)-ε-viniferin] and trimers (vaticanols A, E, and G) were isolated from the bark extract of *C. melanoxylon*. The principal constituents, vaticanols A, E, and/or G, inhibited plasma glucose and triglyceride elevation after sucrose loading in rats and olive oil loading in mice, respectively. In addition, vaticanols A, E, and/or G inhibited the enzyme activities of rat intestinal α-glucosidase, porcine pancreatic lipase, and rat lens aldose reductase.

Key words *Cotylelobium melanoxylon*; melanoxylin A; melanoxylin B; stilbene oligomer; plasma glucose elevation inhibitor; plasma triglyceride elevation inhibitor

The Dipterocarpaceae plant *Cotylelobium melanoxylon* is widely distributed in Southeast Asia. It has been used as an astringent, antilaxative, and blood coagulation agent in traditional Thai medicine.[1,2] In our previous study, we reported the isolation and structural elucidation of steroidal saponins with antihyperglycemic effects from the anthotaxy of *Borassus flabellifer*, the exuded juice of which is a raw material in palm sugar, a sweetener used by diabetic patients.[3] The bark of *C. melanoxylon* is used as an antiseptic and fermentation inhibitor in preparing palm sugar. However, the chemical constituents and biofunctional effects of this plant have not yet been investigated thoroughly.

In the course of our characterization studies on bioactive constituents from natural Thai medicines,[4—9] the methanolic (MeOH) extracts of the wood and bark of *C. melanoxylon* were found to inhibit increases in plasma glucose levels in sucrose-loaded rats and plasma triglyceride (TG) levels in olive oil-loaded mice. Furthermore, a new stilbene dimer, melanoxylin A (**1**), and a new stilbene trimer, melanoxylin B (**2**), together with nine known compounds were isolated from the wood and bark extract.

In the present report, we describe the chemical elucidation of **1** and **2**, together with the inhibitory effects of the principal constituents, vaticanols A (**3**), E (**4**), and G (**5**), on the increase in plasma glucose and TG levels after sucrose and olive oil loading, respectively, and the results of several *in vitro* bioassays such as inhibition of intestinal α-glucosidase, aldose reductase, and pancreatic lipase, and rat lens aldose reductase.

Isolation from the Wood and Bark of *C. melanoxylon* The wood of *C. melanoxylon* collected in Thailand was finely cut and extracted with MeOH under reflux to provide the MeOH extract (50.5% from the wood). Using the same method, the MeOH extract (43.4%) was obtained from the bark of *C. melanoxylon* collected in Thailand. Furthermore, the MeOH extract from the wood was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish the EtOAc-soluble (48.9%) and aqueous (1.6%) fractions. The EtOAc-soluble fraction from the wood was subjected to normal- and reversed-phase column chromatography and finally to HPLC to yield a new stilbene dimer, melanoxylin A (**1**, 0.0036%), together with four known stilbene derivatives, vaticanol G (**5**, 28.0%),[10] (+)-ampelopsin F (**6**, 0.131%),[11] (+)-isoampelopsin F (**7**, 0.0045%),[12] and (+)-ε-viniferin (**8**, 0.057%),[13] and a lignan, (+)-lyoniresinol (**10**, 0.0062%).[14] In the same manner, a new stilbene trimer, melanoxylin B (**2**, 0.0032%), five known stilbene derivatives, vaticanols A (**3**, 2.0%),[15] E (**4**, 2.9%),[16] and G (**5**, 7.3%), (+)-ε-viniferin (**8**, 0.10%), and *cis*-(+)-ε-viniferin (**9**, 0.0034%),[17] and an aromatic compound, *p*-hydroxybenzoic acid (0.0044%), were obtained from the MeOH extract from the bark of *C. melanoxylon*.

Structures of Melanoxylins A (1) and B (2) Melanoxylin A (**1**) was isolated as a yellow powder with negative optical rotation ($[\alpha]_D^{23}$ −63.9° in MeOH). The IR spectrum of **1** showed absorption bands at 3450, 1618, and 1519 cm^{-1} ascribable to the hydroxyl group and aromatic ring. In the electron ionization (EI)-MS of **1**, a molecular ion peak was observed at *m/z* 454 (M^+), and high-resolution EI-MS (HR-EI-MS) analysis revealed the molecular formula of **1** to be $C_{28}H_{22}O_6$. The ^{1}H- (acetone-d_6) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned based on the results of various NMR experiments,[18] showed signals assignable to a methylene [δ 3.20 (1H, dd, J=3.0, 17.2 Hz, H-8α), 3.60 (1H, dd, J=3.7, 17.2 Hz, H-8β)], three methines [δ 4.19 (1H, d, J=11.7 Hz, H-8′), 5.22 (1H, dd-like, H-7), 5.74 (1H, d, J=11.7 Hz, H-7′)], and 12 aromatic protons. The planar structure of **1** was confirmed in DQF COSY and HMBC experiments (Fig. 1). Thus the long-range correlations in the HMBC experiment on **1** were observed between the following proton and carbon pairs: H-2, -6 and C-4, -7; H-3, -5 and C-1; H-7 and C-8, -9, -9′, -10′; H-8 and C-1, -9, -10, -14; H-12 and C-10, -11, -13; H-14 and C-10, -12, -13; H-2′, -6′ and C-4′, C-7′; H-3′, -5′ and C-1′; H-7′ and C-9′; H-8′ and C-10, -1′, -9′; H-12′ and C-10′, -14′; and H-14′ and C-8′, -10′, -13′. The stereostructure of **1** was determined in a difference

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp
© 2009 Pharmaceutical Society of Japan

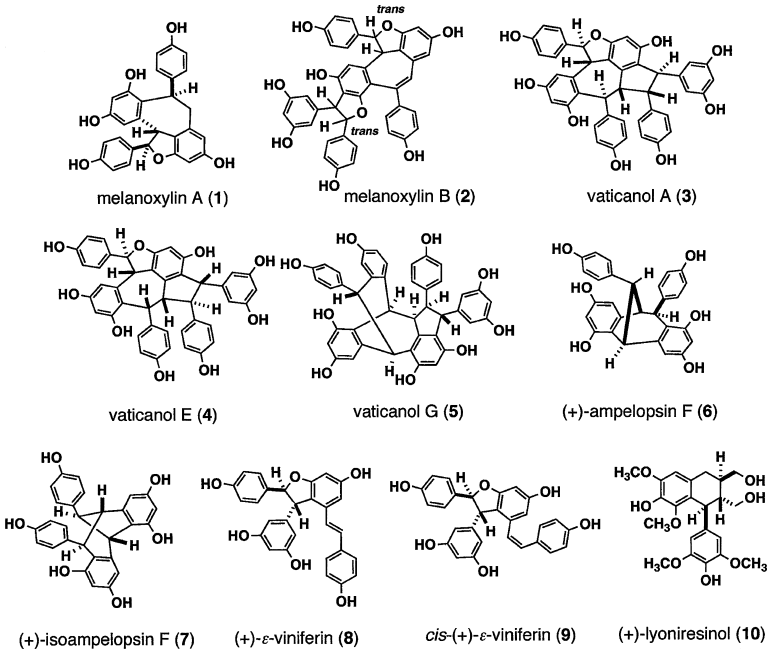

Chart 1

Table 1. ^{13}C-NMR (125 MHz) Data for **1** and **2**[a)]

Carbon	1	2	Carbon	1	2	Carbon	2
1	134.7	134.4	1′	131.0	132.8	1″	136.8
2	128.6	128.6	2′	130.0	127.9	2″	128.8
3	115.6	116.1	3′	116.0	116.3	3″	116.0
4	156.0	158.4	4′	157.2	157.9	4″	157.7
5	115.6	116.1	5′	116.0	116.3	5″	116.0
6	128.6	128.6	6′	130.0	127.9	6″	128.8
7	35.9	94.9	7′	88.3	86.3	7″	141.7
8	33.8	57.2	8′	49.3	53.1	8″	127.1
9	138.1	145.5	9′	142.6	145.7	9″	123.7
10	119.0	107.8	10′	122.8	119.0	10″	115.8
11	160.4	159.7	11′	158.7	161.5	11″	160.5
12	95.7	102.1	12′	101.5	130.8	12″	102.4
13	158.5	159.7	13′	156.6	158.3	13″	158.2
14	109.0	107.8	14′	105.4	102.5	14″	91.3

a) Measured in acetone-d_6.

nuclear Overhauser enhancement spectroscopy (NOESY) experiment, in which correlations were observed between the following proton pairs: H-2, -6 and H-8β, -8′β; H-7α and H-8α; H-8β and H-14; and H-8′β and H-2′, -6′. Thus the relative stereostructure of **1** was the same as that of (+)-ampelopsin B.[19)] Finally, the absolute configuration of **1** was characterized based on the circular dichroism (CD) spectra. The CD spectra of **1** showed a negative Cotton effect [210 nm ($\Delta\varepsilon$ −26.2), 236 nm ($\Delta\varepsilon$ −19.5), 286 nm ($\Delta\varepsilon$ −3.0)]. On the other hand, the CD spectra of (+)-ampelopsin B showed a positive Cotton effect [211 nm ($\Delta\varepsilon$ +28.1), 236 nm ($\Delta\varepsilon$ +24.3), 288 nm ($\Delta\varepsilon$ +4.0)]. On the basis of this evidence, melanoxylin A (**1**) is an enantiomer of (+)-ampelopsin B, and the structure of **1** was characterized as shown.

Melanoxylin B (**2**), obtained as a yellow powder with positive optical rotation ($[\alpha]_D^{25}$ +265.2° in MeOH), showed absorption bands assignable to the hydroxyl group and aromatic ring in the IR spectrum. The molecular formula $C_{42}H_{30}O_9$ was determined based on EI-MS [*m*/*z* 678 $(M)^+$] and HR-EI-MS measurement. The ^{1}H- and ^{13}C-NMR (Table 1) spectra[18)] of **2** indicated the presence of four methines [δ 4.09 (1H, d, J=4.3 Hz, H-8′), 4.62 (1H, d, J=8.6 Hz, H-8), 5.45 (1H, d, J=8.6 Hz, H-7), 6.43 (1H, d, J=4.3 Hz, H-7′)], an olefinic proton [δ 6.70 (1H, s, H-8″)], and 18 aromatic protons. The proton and carbon signals of **2**, except for the 1″—8″ positions in the ^{1}H- and ^{13}C-NMR spectra, were similar to those of gnemonol H.[20)] The planar structure of **2** was confirmed in DQF COSY and HMBC experiments (Fig. 1). Thus the long-range correlations in the HMBC experiment on **2** were observed between the following proton and carbon pairs: H-2, -6 and C-4, -7; H-3, -5 and C-1; H-7 and C-9; H-8 and C-9, -11′, -12′; H-10, -14 and C-8, -11, -12; H-12 and C-10, -14; H-2′, -6′ and C-4′, -7′; H-3′, -5′ and C-1′; H-7′ and C-9′; H-8′ and C-10′, -9″; H-14′ and C-10′, -12′; H-2″, -6″ and C-4″, -7″; H-3″, -5″ and C-1″; H-8″ and C-10′, -1″, -9″, -10″; and H-12″ and C-10″, -11″, -13″, -14″. NOESY experiments on **2** showed correlations between the following

proton pairs: H-2, -6 and H-8; H-7 and H-10, -14; H-2′, -6′ and H-8′; and H-7′ and H-14′. Therefore the geometries of the 7- and 8-protons and the 7′- and 8′-protons were determined to be a *trans*-orientation of the two dihydrofuran rings. On the basis of this evidence, the structure of melanoxylin B (**2**) was determined as shown.

Effects of MeOH Extracts and Vaticanols A (3), E (4), and G (5) on Plasma Glucose Elevation after Sucrose Loading in Rats Since the bark of *C. melanoxylon* has been used for preparing palm sugar, which is beneficial to diabetic patients, we examined the effects of the MeOH extracts of the wood and bark on elevated in plasma glucose levels in sucrose-loaded rats. Both extracts significantly inhibited the plasma glucose elevation at doses of 125 and 250 mg/kg, *per os* (*p.o.*). The principal constituents, vaticanols A (**3**) and G (**5**), also showed significant inhibition at a dose of 50 mg/kg, *p.o.* Vaticanol E (**4**, 50 mg/kg, *p.o.*) tended to inhibit the plasma glucose elevation, although the inhibition by **4** was not statistically significant (Table 2). These results indicate that compounds **3**—**5** are active compounds in the antihyperglycemic effects of this herb.

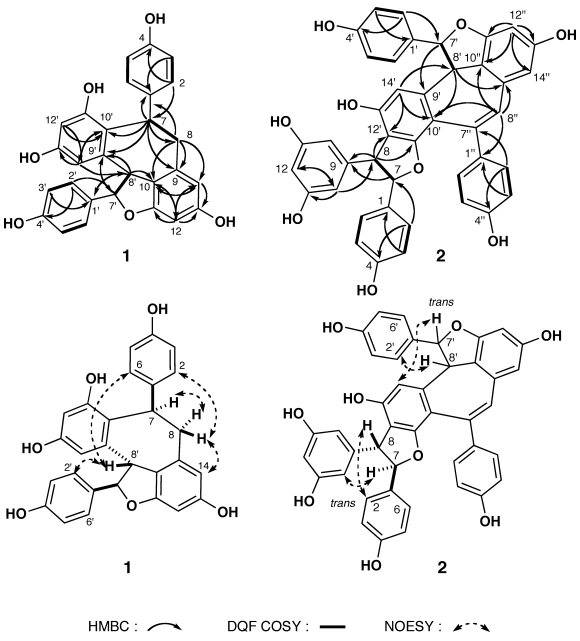

Fig. 1. Selected HMBC and NOE Correlations

Effects of MeOH Extracts and Vaticanols A (3), E (4), and G (5) on Plasma TG Elevation after Olive Oil Loading in Mice Next, the effects of the MeOH extracts of the wood and bark of *C. melanoxylon* on the increase in plasma TG levels in olive oil-loaded mice were examined. As shown in Table 3, both extracts significantly inhibited plasma TG elevation at doses of 250 and 500 mg/kg, *p.o.* Vaticanols A (**3**), E (**4**), and G (**5**) also showed inhibitory effects at 200 mg/kg, *p.o.* These results indicate that compounds **3**—**5** are active compounds in the antihyperlipidemic effects of this herb.

Effects of MeOH Extracts and Vaticanols A (3), E (4), and G (5) on Gastric Emptying in Mice In our previous study, we reported that inhibition of gastric emptying markedly inhibited plasma glucose and TG elevations in sucrose- and olive oil-loaded rats and mice, respectively.[21—23] Therefore the effects of the MeOH extracts of *C. melanoxylon* and principal constituents **3**—**5** on gastric emptying in mice after carboxylmethyl cellulose sodium salt (CMC-Na) solution loading were examined. As shown in Table 4, both extracts significantly inhibited gastric emptying at doses of 125—500 mg/kg, *p.o.*, 30 min after CMC-Na solution loading. Vaticanols A (**3**), E (**4**), and G (**5**) significantly inhibited gastric emptying at doses of 100 and 200 mg/kg, *p.o.* However, vaticanol G (**5**) did not show such effects at doses of 12.5—50 mg/kg, *p.o.* (data not shown). These findings suggest that the inhibition of gastric emptying is involved in the

Table 2. Inhibitory Effects of the MeOH Extracts from the Wood and Bark of *C. melanoxylon* and Vaticanols A (**3**), E (**4**), and G (**5**) on Plasma Glucose Elevation in Sucrose-Loaded Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Plasma glucose (mg/dl)[a]		
			0.5 h	1.0 h	2.0 h
Normal	—	6	70.4±2.8**	81.5±3.5**	80.3±3.5**
Control	—	8	188.3±6.4	146.1±3.7	124.4±2.7
MeOH ext. of wood	125	6	103.9±9.0**	127.4±6.9	131.2±2.7
	250	6	103.4±7.0**	125.2±7.4*	131.2±4.9
Normal	—	5	91.2±5.3**	94.5±3.7**	84.2±10.5**
Control	—	10	182.6±2.9	173.7±4.5	127.7±3.4
MeOH ext. of bark	125	6	124.0±4.4**	152.2±4.3**	129.9±4.1
	250	6	110.5±7.2**	139.5±5.1**	127.5±2.9
Vaticanol G (**5**)	12.5	6	175.8±3.3	186.8±3.2	128.5±5.0
	25	6	169.5±6.1	178.6±4.8	135.0±2.6
	50	6	127.2±4.3**	150.1±2.4**	145.6±3.0
Normal	—	6	74.3±2.7**	82.4±2.5**	77.1±2.8**
Control	—	8	178.1±6.2	134.8±3.6	108.0±1.9
Vaticanol A (**3**)	25	6	163.8±6.1	151.7±6.1	120.0±2.6
	50	6	147.3±6.0*	152.0±3.0	121.2±2.0
Vaticanol E (**4**)	25	6	177.2±8.7	152.3±3.7	121.0±2.7
	50	6	156.8±12.2	155.7±6.7	118.3±3.1
Acarbose	2.5	6	138.5±9.8**	135.5±7.7	117.5±3.4
	5	6	115.8±3.3**	126.3±3.8	114.7±3.5
	10	6	114.8±8.6**	117.2±5.2	109.2±2.5

a) Values represent mean±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

Table 3. Inhibitory Effects of the MeOH Extracts from the Wood and Bark of *C. melanoxylon* and Vaticanol G (**5**) on Plasma TG Elevation in Olive Oil-Loaded Mice

Treatment	Dose (mg/kg, *p.o.*)	*n*	Plasma TG (mg/dl)[a)]		
			2.0 h	4.0 h	6.0 h
Normal	—	11	133.6±12.2**	137.4±11.9**	130.9±11.0**
Control	—	8	535.6±39.5	474.1±97.6	405.6±91.1
MeOH ext. of wood	250	11	335.1±25.1**	461.7±59.9	321.2±34.1
	500	11	60.6±9.7**	67.7±23.5**	95.8±41.0**
Normal	—	6	90.1±6.9*	84.8±4.7**	92.1±10.2**
Control	—	6	399.4±43.3	309.4±31.3	233.5±13.1
MeOH ext. of bark	250	6	418.0±129.2	309.0±37.1	193.5±26.8
	500	6	91.9±11.7*	105.3±27.8**	120.8±32.5**
Normal	—	7	134.2±15.2**	116.8±17.4**	82.3±12.1**
Control	—	11	587.9±77.3	406.9±42.7	253.3±28.2
Vaticanol A (**3**)	100	9	605.8±47.0	406.2±46.3	256.8±30.6
	200	9	368.6±34.3*	365.6±43.6	247.8±29.4
Vaticanol E (**4**)	100	9	478.7±57.8	301.6±19.9	189.1±15.1
	200	9	326.3±54.6**	353.9±42.4	211.2±31.9
Normal	—	8	111.3±11.6**	109.5±9.7**	99.1±11.7**
Control	—	9	416.0±26.3	438.7±31.1	398.4±30.4
Vaticanol G (**5**)	50	10	516.2±66.1	472.9±62.9	268.5±23.7
	100	10	403.2±35.2	377.0±46.6	239.0±33.2*
	200	10	245.7±41.5*	282.2±41.7	269.4±70.3
Normal	—	7	91.9±9.4**	97.3±7.4**	90.6±9.4**
Control	—	9	440.3±60.2	393.2±60.1	263.3±45.0
Orlistat	5	7	371.3±41.5	297.0±67.4	171.9±24.9
	10	7	203.8±52.1**	160.4±47.7**	129.1±16.6**
	20	7	198.6±24.1**	131.0±16.8**	114.5±7.6**

a) Values represent mean±S.E.M. Significantly different from the control group, $*p<0.05$, $**p<0.01$.

Table 4. Inhibitory Effects of the MeOH Extracts from the Wood and Bark of *C. melanoxylon* and Vaticanol G (**5**) on Gastric Emptying in CMC-Na-Loaded Mice

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric emptying (%)[a)]	Inhibition (%)
Control	—	9	88.9±1.1	—
MeOH ext. of wood	125	7	72.2±2.9**	18.8
	250	7	55.1±4.9**	38.0
	500	7	38.3±0.7**	56.9
Control	—	11	77.7±4.0	—
MeOH ext. of bark	125	7	69.8±2.7	10.2
	250	7	54.9±3.2**	29.3
	500	7	44.9±2.1**	42.2
Control	—	7	84.8±2.6	—
Vaticanol A (**3**)	100	7	72.3±2.7**	14.7
	200	7	59.4±2.1**	30.0
Vaticanol E (**4**)	100	7	72.3±2.1*	14.7
	200	7	64.9±3.8**	23.4
Vaticanol G (**5**)	100	7	74.4±1.4*	12.3
	200	7	56.1±3.2**	33.8

a) Values represent mean±S.E.M. Significantly different from the control group, $*p<0.05$, $**p<0.01$.

antihyperglycemic and antihyperlipidemic effects of the extracts and of compounds **3**—**5** at higher doses.

Effects of MeOH Extracts and Vaticanol A (3), E (4), and G (5) on Enzyme Activities of Rat Intestinal α-Glucosidase and Porcine Pancreatic Lipase Next, the inhibitory effects of the MeOH extracts of *C. melanoxylon* and vaticanols A (**3**), E (**4**), and G (**5**) on enzyme activities of rat intestinal α-glucosidase (maltase and sucrase) and porcine pancreatic lipase were examined, and the results are summarized in Table 5. The MeOH extracts and compounds **3** and **4** moderately inhibited enzyme activities of rat intestinal maltase and sucrase, but compound **5** showed only very weak inhibition. The inhibitory effects of the MeOH bark extract were greater than that of the wood extract. These results suggest that the inhibition of sucrase activity is involved in the antihyperglycemic effects of the MeOH extracts and compounds **3** and **4**, but not of **5**. Pancreatic lipase enzyme activity was also inhibited by both extracts and compounds **3**—**5**. The wood extract showed greater effects than bark extract, similar to the results in olive oil-loaded mice. These results

Table 5. Inhibitory Effects of the MeOH Extracts from the Wood and Bark of *C. melanoxylon* and Vaticanols A (**3**), E (**4**), and G (**5**) on Enzyme Activities of α-Glucosidase, Lipase, and Aldose Reductase and Their DPPH and $O_2^{\cdot-}$-Scavenging Activities

	α-Glucosidase (IC_{50} μg/ml)		Lipase (IC_{50} μg/ml)	Aldose reductase (IC_{50} μg/ml)	DPPH (SC_{50} μg/ml)[a]	$O_2^{\cdot-}$ (IC_{50} μg/ml)
	Maltase	Sucrase				
MeOH ext. of wood	307	206	25	61	12	7.8
MeOH ext. of bark	208	99	39	17	14	6.4

	α-Glucosidase (IC_{50} μM)		Lipase (IC_{50} μM)	Aldose reductase (IC_{50} μM)	DPPH (SC_{50} μM)	$O_2^{\cdot-}$ (IC_{50} μM)
	Maltase	Sucrase				
Vaticanol A (**3**)	218	148	52	24	25	16
Vaticanol E (**4**)	342	89	86	31	13	9.5
Vaticanol G (**5**)	>400 (32%)[b]	>400 (38%)[b]	59	>100 (47%)[c]	35	37
Acarbose	2.0	1.7	—	—	—	—
Orlistat	—	—	0.056	—	—	—
Epalrestat	—	—	—	0.072	—	—
dl-α-Tocopherol	—	—	—	—	11	—
(+)-Catechin	—	—	—	—	6.0	1.5

a) Concentration required for 50% reduction of DPPH radical 40 μM. *b*) Inhibition (%) at 400 μM. *c*) Inhibition (%) at 100 μM.

suggest that the lipase inhibitory activity is involved in the inhibitory effects of the MeOH extracts of *C. melanoxylon* and **3**—**5** on blood TG elevation in olive oil-loaded mice.

Effects of MeOH Extracts and Vaticanols A (3), E (4), and G (5) on Enzyme Activity of Rat Lens Aldose Reductase and Their DPPH and $O_2^{\cdot-}$ Scavenging Activities Finally, the inhibitory effects of the MeOH extracts of *C. melanoxylon* and vaticanols A (**3**), E (**4**), and G (**5**) on rat lens aldose reductase were examined. Aldose reductase has been reported to catalyze the reduction of glucose to sorbitol as a key enzyme in the polyol pathway. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataracts.[24] In addition, a hyperglycemia-induced process of overproduction of superoxide anion radical ($O_2^{\cdot-}$) by the mitochondrial electron transport chain was reported to inhibit partially the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, thereby diverting upstream metabolites from glycolysis into the polyol pathway, advanced glycation end-product formation, *etc*.[25—27] In addition, active oxygen species were reported to be detected during the glycation processes with traces of metal ions, and strong antioxidants with a phenolic moiety scavenge active oxygen species derived from the glycation processes and inhibit advanced glycation processes.[28,29]

As shown in Table 5, both the MeOH extracts of *C. melanoxylon* and vaticanols A (**3**) and E (**4**) showed inhibitory effects on the enzyme activity of rat lens aldose reductase. The bark extract showed greater effects than the wood extract, and vaticanol G (**5**) showed very weak inhibition, similar to the inhibition of rat intestinal α-glucosidase. In addition, both the MeOH extracts and compounds **3**—**5** showed scavenging effects against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and $O_2^{\cdot-}$.

In conclusion, the MeOH extracts from the wood and bark of *C. melanoxylon* were found to inhibit plasma glucose elevation after sucrose loading in rats and blood TG elevation after olive oil loading in mice. A new stilbene dimer, melanoxylin A (**1**), together with the known stilbene dimers [(+)-ampelopsin F (**6**), (+)-isoampelopsin F (**7**), and (+)-ε-viniferin (**8**)], a trimer [vaticanol G (**5**)], and a lignan [(+)-lyoniresinol (**10**)] were isolated from the wood extract, and a new stilbene trimer, melanoxylin B (**2**), together with the known stilbene dimers [(+)-ε-viniferin (**8**) and *cis*-(+)-ε-viniferin (**9**)] and trimers [vaticanols A (**3**), E (**4**), and G (**5**)] were isolated from the bark extract. Compounds **3** and **5**, which are the main constituents of the extracts, significantly inhibited plasma glucose elevation after sucrose loading in rats. The MeOH extracts and **3**—**5** also inhibited plasma TG elevation after olive oil loading in mice. The extracts and compounds **3** and **4** inhibited the enzyme activities of rat intestinal maltase and sucrase and of rat lens aldose reductase. Furthermore, the MeOH extracts and compounds **3**—**5** inhibited the enzyme activity of porcine pancreatic lipase and exhibited DPPH radical- and $O_2^{\cdot-}$-scavenging activities. These results suggest that the MeOH extracts from the wood and bark of *C. melanoxylon* could be effective in the prevention of diabetes. Structure–activity relationships of the stilbene oligomers and their effects in diabetic animal models should be studied further.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and HR-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz), JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-10A refractive index and SPD-10A*vp* UV–VIS detectors; HPLC column, Inertsil ODS-3 (GL Sciences, Inc., 250×4.6 mm i.d.). YMC-Pack ODS-A and ODS-Ph (YMC, Inc., 250×4.6 mm i.d.) columns were used for analytical purposes, and Inertsil ODS-3 (GL Sciences, Inc., 250×20 mm i.d.) and YMC-Pack ODS-A and ODS-Ph (YMC, Inc., 250×20 mm i.d.) columns were used for preparative purposes.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reversed-phase silica

gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, 100—200 mesh); TLC, precoated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material The wood and bark of *C. melanoxylon* were collected in Thailand in 2006 (wood) and 2007 (bark) and identified by one of the authors (Y. P.).

Extraction and Isolation The wood (500 g) of *C. melanoxylon* was finely crushed and extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (252.4 g, 50.5%). Using a similar method, the MeOH extract (204.0 g, 43.4%) was obtained from the bark (470 g) of *C. melanoxylon*. Part of the MeOH extract (238.2 g) from the wood was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to yield an EtOAc-soluble fraction (230.5 g, 48.9%) and an aqueous fraction (7.7 g, 1.6%). Part of the EtOAc-soluble fraction (165.6 g) from the wood was subjected to ordinary-phase silica gel column chromatography {3.5 kg, $CHCl_3$–MeOH–H_2O [(10 : 3 : 1, v/v/v, lower layer)→(7 : 3 : 1, v/v/v, lower layer)→(6 : 4 : 1, v/v/v)]→MeOH} to give nine fractions {fr. 1 (5.2 g), fr. 2 (1.6 g), fr. 3 (2.1 g), fr. 4 (7.9 g), fr. 5 (2.3 g), fr. 6 [44.3 g, =vaticanol G (**5**)], fr. 7 (53.0 g), fr. 8 (35.7 g), and fr. 9 (10.4 g)}. Fr. 2 (1.6 g) was subjected to reversed-phase silica gel column chromatography [57 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give 10 fractions {fr. 2-1, fr. 2-2 [=(+)-lyoniresinol (**10**, 19.8 mg)], fr. 2-3, fr. 2-4 (121 mg), fr. 2-5 (414 mg), fr. 2-6 (90 mg), fr. 2-7, fr. 2-8, fr. 2-9, and fr. 2-10}. Fr. 2-4 (121 mg) was purified by HPLC [MeOH : H_2O (40 : 60), HPLC column: GL Sciences ODS-3] to give melanoxylin A (**1**, 11.6 mg). Fr. 2-5 (414 mg) was purified by HPLC [MeOH : H_2O (50 : 50), HPLC column: GL Sciences ODS-3] to give (+)-ε-viniferin (**8**, 174 mg). Fr. 2-6 (90 mg) was purified by HPLC [MeOH : H_2O (50 : 50), HPLC column: GL Sciences ODS-3] to give (+)-ε-viniferin (**8**, 8.3 mg). Fr. 3 (2.1 g) was subjected to reversed-phase silica gel column chromatography [65 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give 10 fractions {fr. 3-1, fr. 3-2 [=(+)-ampelopsin F (**6**, 457 mg)], fr. 3-3, fr. 3-4 (227 mg), fr. 3-5, fr. 3-6, fr. 3-7, fr. 3-8, fr. 3-9, and fr. 3-10}. Fr. 3-4 (227 mg) was purified by HPLC [MeOH : H_2O (40 : 60), HPLC column: GL Sciences ODS-3] to give (+)-isoampelopsin F (**7**, 15.6 mg). Part of fr. 7 (20 g) was subjected to reversed-phase silica gel column chromatography [600 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give three fractions [fr. 7-1, fr. 7-2 (=vaticanol G, **5**, 19.2 g), and fr. 7-3].

Part of the MeOH extract (185 g) from the bark was subjected to ordinary-phase silica gel column chromatography {3.7 kg, $CHCl_3$–MeOH–H_2O [(10 : 3 : 1, v/v/v, lower layer)→(7 : 3 : 1, v/v/v, lower layer)→(6 : 4 : 1, v/v/v)]→MeOH} to give 13 fractions {fr. 1 (325 mg), fr. 2 (2.4 g), fr. 3 (1.1 g), fr. 4 (565 mg), fr. 5 (943 mg), fr. 6 (1.1 g), fr. 7 (155 mg), fr. 8 (25.8 g), fr. 9 (42.9 g), fr. 10 (39.0 g), fr. 11 [30.9 g, =vaticanol G (**5**)], fr. 12 (10.7 g), and fr. 13 (21.0 g)}. Fr. 2 (2.4 g) was subjected to reversed-phase silica gel column chromatography [90 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give benzoic acid (18.9 mg). Fr. 5 (555 mg) was further subjected to ordinary-phase silica gel column chromatography {15 g, $CHCl_3$–MeOH–H_2O [(30 : 3 : 1, v/v/v, lower layer)→(20 : 3 : 1, v/v/v, lower layer)→(10 : 3 : 1, v/v/v, lower layer)]→MeOH} to give eight fractions {fr. 5-1, fr. 5-2, fr. 5-3, fr. 5-4, fr. 5-5 [262 mg, =(+)-ε-viniferin (**8**)], fr. 5-6, fr. 5-7, and fr. 5-8}. Part of fr. 6 (1.0 g) was subjected to reversed-phase silica gel column chromatography [40 g, MeOH–H_2O (40 : 60→50 : 50→60 : 40, v/v)→MeOH] to give 13 fractions [fr. 6-1, fr. 6-2, fr. 6-3, fr. 6-4, fr. 6-5, fr. 6-6 (86 mg), fr. 6-7, fr. 6-8, fr. 6-9, fr. 6-10, fr. 6-11, fr. 6-12, and fr. 6-13]. Fr. 6-6 (86 mg) was purified by HPLC [MeOH : H_2O (50 : 50), HPLC column: YMC-Pack ODS-A] to give *cis*-(+)-ε-viniferin (**8**, 14.1 mg). Fr. 7 (155 mg) was purified by HPLC [MeOH : H_2O (50 : 50), HPLC column: YMC-Pack ODS-A] to give melanoxylin B (**2**, 13.1 mg). Fr. 8 (350 mg) was purified by HPLC [MeOH : H_2O (45 : 55), HPLC column: YMC-Pack Ph] to give vaticanol A (**3**, 19.6 mg). Fr. 9 (6.7 g) was purified by HPLC [MeOH : H_2O (43 : 57), HPLC column: YMC-Pack Ph] to give vaticanol A (**3**, 1.16 g) and vaticanol E (**4**, 1.10 g). Part of fr. 10 (8.0 g) was subjected to reversed-phase silica gel column chromatography [260 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→50 : 50→60 : 40, v/v)→MeOH] to give four fractions [fr. 10-1, fr. 10-2 (6.7 g), fr. 10-3, and fr. 10-4]. Part of fr. 10-2 (800 mg) was purified by HPLC [MeOH : H_2O (43 : 57), HPLC column: YMC-Pack Ph] to give vaticanols A (**3**, 58 mg) and E (**4**, 137 mg). These known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values.[10—17]

Melanoxylin A (**1**): A yellow powder; $[\alpha]_D^{23}$ −63.9° (c=0.59, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)]: 211 (−26.2), 236 (−19.5), 286 (−3.0). UV [MeOH, nm (log ε)]: 282 (3.81). IR (KBr): ν_{max} 3450, 1618, 1519 cm^{-1}; ^{1}H-NMR (acetone-d_6, 600 MHz) δ: 3.20 (1H, dd, J=3.0, 17.2 Hz, H-8α), 3.60 (1H, dd, J=3.7, 17.2 Hz, H-8b), 4.19 (1H, d, J=11.7 Hz, H-8′), 5.22 (1H, dd-like, H-7), 5.74 (1H, d, J=11.7 Hz, H-7′), 6.06 (1H, s-like, H-12), 6.22 (1H, s-like, H-14′), 6.33 (1H, s-like, H-14), 6.43 (1H, s-like, H-12′), 6.65 (2H, d, J=8.9 Hz, H-3, -5), 6.77 (2H, d, J=8.3 Hz, H-3′, -5′), 6.94 (2H, d, J=8.9 Hz, H-2, -6), 7.10 (2H, d, J=8.3 Hz, H-2′, -6′); ^{13}C-NMR: see Table 1; EI-MS m/z: 454 $[M]^+$; HR-EI-MS: m/z 454.1409 (Calcd for $C_{28}H_{22}O_6$ $[M]^+$, 454.1416).

Melanoxylin B (**2**): A yellow powder; $[\alpha]_D^{23}$ +265.2° (c=0.28, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)]: 215 (−10.2), 259 (−18.6), 289 (+6.9), 315 (−3.2), 345 (+1.7). UV [MeOH, nm (log ε)]: 223 (4.73), 285 (4.19), 324 (4.14). IR (KBr): ν_{max} 3415, 1612, 1512, 1455, 1074 cm^{-1}; ^{1}H-NMR (acetone-d_6, 600 MHz) δ: 4.09 (1H, d, J=4.3 Hz, H-8′), 4.62 (1H, d, J=8.6 Hz, H-8), 5.45 (1H, d, J=8.6 Hz, H-7), 6.18 (1H, d, J=2.1 Hz, H-12″), 6.23 (2H, d, J=2.1 Hz, H-10, -14), 6.26 (1H, d, J=2.1 Hz, H-12), 6.32 (1H, d, J=2.1 Hz, H-14″), 6.43 (1H, d, J=4.3 Hz, H-7′), 6.67 (2H, d, J=8.6 Hz, H-3″, -5″), 6.70 (1H, s, H-8″), 6.75 (1H, s, H-14′), 6.83 (2H, d, J=8.4 Hz, H-3, -5), 6.85 (2H, d, J=8.4 Hz, H-3′, -5′), 6.90 (2H, d, J=8.6 Hz, H-2″, -6″), 7.27 (2H, d, J=8.4 Hz, H-2, -6), 7.33 (2H, d, J=8.4 Hz, H-2′, -6′); ^{13}C-NMR: see Table 1; EI-MS m/z: 678 $[M]^+$; HR-EI-MS: m/z 678.1885 (Calcd for $C_{42}H_{30}O_9$ $[M]^+$, 678.1889).

Bioassay Methods. Animals Male ddY mice and Wistar rats were purchased from Kiwa Laboratory Animal Co., Ltd. (Wakayama, Japan). The animals were housed at a constant temperature of 23±2 °C and fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 20—24 h prior to the beginning of experiments, but were allowed free access to tap water. All experiments were performed using conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Effects on Plasma Glucose Elevation in Sucrose-Loaded Rats The experiments were performed as described in our previous reports with a slight modification.[3,22,30—32] Each test sample was administered orally to fasted rats (body weight 130—170 g), and 20% (w/v) sucrose solution (5 ml/kg, *p.o.*) was administered 30 min thereafter. Blood samples (*ca.* 0.3 ml) were collected from the infraorbital venosus plexus under ether anesthesia 0.5, 1, and 2 h after the oral administration of sucrose. The collected blood was immediately mixed with heparin sodium (5 units/tube). After centrifugation of blood samples, the plasma glucose level was determined enzymatically using the Glucose CII test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan). The intestinal α-glucosidase inhibitor acarbose was used as a reference compound.

Effects on Plasma TG Elevation in Olive Oil-Loaded Mice The experiments were performed as described in our previous reports with a slight modification.[8,33—35] Each test sample was administered orally to fasted mice (body weight 25—30 g), and olive oil (5 ml/kg, *p.o.*) was administered 30 min thereafter. Blood samples (*ca.* 0.25 ml) were collected from the infraorbital venosus plexus under ether anesthesia 2, 4, and 6 h after the oral administration of olive oil. The collected blood was immediately mixed with heparin sodium (5 units/tube). After centrifugation of blood samples, plasma TG was determined enzymatically using the Triglyceride E test Wako (Wako Pure Chemical Industries). The pancreatic lipase inhibitor orlistat was used as a reference compound.

Gastric Emptying in Mice The experiments were performed as described in our previous reports.[22,23] A solution of 1.5% CMC-Na containing 0.05% phenol red as a marker was given intragastrically (0.3 ml/mouse) to conscious mice. Thirty minutes later, the mice were killed by cervical dislocation. The abdominal cavity was opened, the gastroesophageal junction and the pylorus were clamped, and then the stomach was removed, weighed, placed in 10 ml of NaOH 0.1 M, and homogenized. The suspension was allowed to settle for 1 h at room temperature, 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v), and then the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 ml of NaOH 0.5 M, and the amount of phenol red was determined based on the absorbance at 560 nm. Phenol red recovered from animals killed immediately after the administration of CMC-Na solution was used as the standard (0% emptying). The test sample was given orally *via* a metal orogastric tube 30 min prior to the administration of the test meals. Gastric emptying (%) in the 30-min period was calculated according to the following equation:

$$\text{gastric emptying (\%)} = (1 - \text{amount of test sample/amount of standard}) \times 100$$

Effects on Rat Intestinal α-Glucosidase The experiments were performed as described in our previous reports with a slight modification.[22,31,32] The rat small intestinal brush border membrane fraction was prepared and its suspension in maleate 0.1 M buffer (pH 6.0) was used to determine the small intestinal α-glucosidase enzyme activity of maltase and sucrase. The enzyme suspension was diluted to hydrolyze maltose and sucrose to produce *ca.* 0.30 and *ca.* 0.15 μmol/tube of D-glucose, respectively, in the following reaction. The substrate (maltose: 37 mM, sucrose: 37 mM), test compound, and the enzyme in maleate buffer 0.1 M (pH 6.0, 0.1 ml) were incubated together at 37 °C. After 30 min of incubation, 0.4 ml of water was added to the test tube, and the tube was immediately immersed in boiling water for 2 min to stop the reaction and then cooled with water. The glucose concentration was determined using the enzymatic method. Each test sample was dissolved in dimethyl sulfoxide (DMSO). Measurements were performed in duplicate, and IC_{50} values were determined graphically. The intestinal α-glucosidase inhibitor acarbose was used as a reference compound.

Effects on Rat Lens Aldose Reductase The experiments were performed as described in our previous reports.[32,36] The supernatant fluid of rat lens homogenate was used as a crude enzyme. The enzyme suspension was diluted to produce *ca.* 10 nmol/tube of β-nicotinamide adenine dinucleotide phosphate (NADP) in the following reaction. The incubation mixture contained phosphate buffer 135 mM (pH 7.0), Li_2SO_4 100 mM, NADPH 0.03 mM, DL-glyceraldehyde 1 mM as a substrate, and 100 μl of enzyme fraction, with 25 μl of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μl of HCl 0.5 M. Then, 0.5 ml of NaOH 6 M containing imidazole 10 mM was added, and the solution was heated at 60 °C for 20 min to convert NADP into a fluorescent product. Fluorescence was measured using a fluorescence microplate reader (FLUOstar OPTIMA, BMG Labtechnologies) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Each test sample was dissolved in DMSO. Measurements were performed in duplicate, and IC_{50} values were determined graphically. The aldose reductase inhibitor epalrestat was used as a reference compound.

Effects on Porcine Pancreatic Lipase Activity The experiments were performed as described in our previous reports with a slight modification.[9,37] A suspension of triolein (80 mg), phosphatidylcholine (10 mg), and sodium taurocholate (5 mg) in 9 ml of Tris–HCl buffer 0.1 M (pH 7.0) containing NaCl 0.1 M was homogenously emulsified using a homogenizer (straight Teflon pestle with a straight glass tube, volume 20 ml). The substrate suspension (0.1 ml) in a test tube was preincubated with 5 μl of test sample in DMSO and 95 μl of Tris–HCl buffer for 3 min at 37 °C. An aliquot of porcine pancreatic lipase (250 μg/ml, type II, Sigma Chemical Co.) (50 μl) or Tris–HCl buffer (50 μl) as a blank test was then added to start the reaction. After 30 min of incubation, the test tube was immediately immersed in boiling water for 2 min to stop the reaction and then cooled with water. The free fatty acid concentration was determined using a commercial kit (NEFA C-test Wako, Wako Pure Chemical Industries). Measurements were performed in duplicate, and IC_{50} values were determined graphically. The pancreatic lipase inhibitor orlistat was used as a reference compound.

DPPH Radical-Scavenging Activity The experiments were performed as described in our previous reports.[38,39] An ethanol solution of DPPH (100 μM, 1.0 ml) was mixed with different concentrations of each test compound (0.5 ml) and acetate buffer 0.1 M (pH 5.5, 1.0 ml), and the absorbance change at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, SC_{50}) of DPPH radical solution 40 μM was determined graphically. *dl*-α-Tocopherol and (+)-catechin were used as reference compounds.

$O_2^{\cdot-}$-Scavenging Activity The experiments were performed as described in our previous reports.[38,39] Briefly, a reaction mixture containing xanthine 100 μM, ethylenediaminetetraacetic acid (EDTA) 100 μM, water-soluble tetrazolium (WST)-1 25 μM, and xanthine oxidase *ca.* 1.9 mU/ml in sodium carbonate buffer 50 mM (pH 10.2) was incubated with each test sample for 20 min at 37 °C (total volume: 3.0 ml). After incubation, the solution was mixed with 0.1 ml of HCl 2 mM to stop the reaction. Formazan formation was monitored at 450 nm. Measurements were performed in duplicate, and IC_{50} values for WST-1 formazan formation were determined graphically. (+)-Catechin was used as a reference compound.

Statistics Values are expressed as mean±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used.

Acknowledgments This research was supported by the 21st COE Program, Academic Frontier Project, and a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

1) Nanthawan B., Oranuch C., "Samunprai Maipunban 1," Prachachon Publishing, Bangkok, 1996, p. 895.
2) Mahathad W., "Textbook of Thai Traditional Medicine," Phitak Agsorn Publishing, Bangkok, 1980, p. 396.
3) Yoshikawa M., Xu F., Morikawa T., Pongpiriyadacha Y., Nakamura S., Asao Y., Kumahara A., Matsuda H., *Chem. Pharm. Bull.*, **55**, 308—316 (2007).
4) Morikawa T., Xu F., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 1530—1534 (2006).
5) Matsuda H., Yoshida K., Miyagawa K., Asao Y., Takayama S., Nakashima S., Xu F., Yoshikawa M., *Bioorg. Med. Chem.*, **15**, 1539—1546 (2007).
6) Yoshikawa M., Morikawa T., Funakoshi K., Ochi M., Pongpiriyadacha Y., Matsuda H., *Heterocycles*, **75**, 1639—1650 (2008).
7) Morikawa T., Funakoshi K., Ninomiya K., Yasuda D., Miyagawa K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **56**, 956—962 (2008).
8) Asao Y., Morikawa T., Xie Y., Okamoto M., Hamao M., Matsuda H., Muraoka O., Yuan D., Yoshikawa M., *Chem. Pharm. Bull.*, **57**, 198—203 (2009).
9) Morikawa T., Xie Y., Asao Y., Okamoto M., Yamashita C., Muraoka O., Matsuda H., Pongpriyadacha Y., Yuan D., Yoshikawa M., *Phytochemistry*, in press.
10) Ito T., Tanaka T., Nakaya K., Iinuma M., Takahashi Y., Naganawa H., Ohyama M., Nakanishi Y., Bastow K. F., Lee K. H., *Tetrahedron*, **57**, 7309—7321 (2001).
11) Oshima Y., Ueno Y., Hisamichi K., Takeshita M., *Tetrahedron*, **49**, 5801—5804 (1993).
12) Luo H. F., Zhang L. P., Hu C. Q., *Tetrahedron*, **57**, 4849—4854 (2001).
13) Li W. W., Ding L. S., Li B. G., Chen Y. Z., *Phytochemistry*, **42**, 1163—1165 (1996).
14) Ouyang M. A., Wein Y. S., Su R. K., Kuo Y. H., *Chem. Pharm. Bull.*, **55**, 804—807 (2007).
15) Tanaka T., Ito T., Nakaya K., Iinuma M., Riswan S., *Phytochemistry*, **54**, 63—69 (2000).
16) Ito T., Tanaka T., Ido Y., Nakaya K., Iinuma M., Takahashi Y., Naganawa H., Riswan S., *Heterocycles*, **55**, 557—567 (2001).
17) Kim H. J., Chang E. J., Bae S. J., Shim S. M., Park H. D., Rhee C. H., Park J. H., Choi S. W., *Arch. Pharm. Res.*, **25**, 293—299 (2002).
18) The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double-quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation spectroscopy (HMBC) experiments.
19) Oshima Y., Ueno Y., Hikino H., Yang L. L., Yen K. Y., *Tetrahedron*, **46**, 5121—5126 (1990).
20) Iliya I., Ali Z., Tanaka T., Iinuma M., Furusawa M., Nakaya K., Murata J., Darnaedi D., *Helv. Chim. Acta*, **85**, 2538—2546 (2002).
21) Yoshikawa M., Matsuda H., *BioFactors*, **13**, 231—237 (2000).
22) Yoshikawa M., Nakamura S., Ozaki K., Kumahara A., Morikawa T., Matsuda H., *J. Nat. Prod.*, **70**, 210—214 (2007).
23) Shimoda H., Ninomiya K., Nishida N., Yoshino T., Morikawa M., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **13**, 223—228 (2003).
24) Terashima H., Hama K., Yamamoto R., Tsuboshima M., Kikkawa R., Hatanaka I., Shigeta Y., *J. Pharmacol. Exp. Ther.*, **229**, 226—230 (1984).
25) Nishikawa T., Edelstein D., Du X. L., Yamagishi S., Matsumura T., Kaneda Y., Yorek M. A., Beebe D., Oates P. J., Hammes H. P., Giardino I., Brownlee M., *Nature* (London), **404**, 787—790 (2000).
26) Du X. L., Edelstein D., Rossetti L., Fantus I. G., Goldberg H., Ziyadeh F., Wu J., Brownlee M., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 12222—12226 (2000).
27) Brownlee M., *Nature* (London), **414**, 813—820 (2001).
28) Sakurai T., Tsuchiya S., *FEBS Lett.*, **236**, 406—410 (1988).
29) Lou H., Yuan H., Yamazaki Y., Sasaki T., Oka S., *Planta Med.*, **67**,

345—349 (2001).
30) Yoshikawa M., Wang T., Morikawa T., Xie H., Matsuda H., *Chem. Pharm. Bull.*, **55**, 1308—1315 (2007).
31) Yoshikawa M., Morikawa T., Matsuda H., Tanabe G., Muraoka O., *Bioorg. Med. Chem.*, **10**, 1547—1554 (2002).
32) Yoshikawa M., Pongpiriyadacha Y., Kishi A., Kageura T., Wang T., Morikawa T., Matsuda H., *Yakugaku Zasshi*, **123**, 871—880 (2003).
33) Ninomiya K., Matsuda H., Shimoda H., Nishida N., Kasajima N., Yoshino T., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **14**, 1943—1946 (2004).
34) Yoshikawa M., Morikawa T., Yamamoto K., Kato Y., Nagatomo A., Matsuda H., *J. Nat. Prod.*, **68**, 1360—1365 (2005).
35) Morikawa T., Li X., Nishida E., Ito Y., Matsuda H., Nakamura S., Muraoka O., Yoshikawa M., *J. Nat. Prod.*, **71**, 825—835 (2008).
36) Xie H., Wang T., Matsuda H., Morikawa T., Yoshikawa M., Tani T., *Chem. Pharm. Bull.*, **53**, 1416—1422 (2005).
37) Yoshikawa M., Sugimoto S., Kato Y., Nakamura S., Wang T., Yamashita C., Matsuda H., *Chem. Biodiv.*, in press.
38) Morikawa T., Xie H., Matsuda H., Wang T., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 506—513 (2006).
39) Matsuda H., Morikawa T., Ishiwada T., Managi H., Kagawa M., Higashi Y., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 440—443 (2003).