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# Antidiabetogenic oligostilbenoids and 3-ethyl-4-phenyl-3,4dihydroisocoumarins from the bark of *Shorea roxburghii*

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## ABSTRACT

A methanol extract of the bark of *Shorea roxburghii* (Dipterocarpaceae) was found to inhibit plasma glucose elevation in sucrose-loaded mice. From the extract, three new 3-ethyl-4-phenyl-3,4-dihydroisocoumarins, 1'S-dihydrophayomphenol  $A_2$  (1) and phayomphenols  $B_1$  (2) and  $B_2$  (3), were isolated together with 24 known compounds including 20 stilbenoids and oligostilbenoids. The structures of 1–3 were determined on the basis of their spectroscopic properties as well as of chemical evidences. Among the isolates, (–)-hopeaphenol (6), hemsleyanol D (8), (+)- $\alpha$ -viniferin (15), and (–)-balanocarpol (18) showed inhibitory activity against plasma glucose elevation in sucrose-loaded rats at doses of 100–200 mg/kg, p.o. To clarify the mode of action of the antihyperglycemic property, effects of these oligostilbenoids on gastric emptying in mice, those on glucose uptake in isolated intestinal tissues as well as inhibitory activities against rat intestinal  $\alpha$ -glucosidase and rat lens aldose reductase were examined.

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#### 1. Introduction

Shorea roxburghii G. DON (Dipterocarpaceae) is widely distributed in Thailand and its neighboring countries such as Cambodia, India, Laos, Malaysia, Myanmar, and Vietnam, etc. The bark of S. roxburghii ('Phayom' in Thailand) has been used as an astringent or a preservative for traditional beverages in Thailand. In Indian folk medicine, they have been used for treatments of dysentery, diarrhea, and cholera, etc.<sup>1</sup> In the course of our exploratory studies on bioactive constituents in Thai natural medicines,<sup>2–13</sup> we have reported isolation and structural elucidation of dihydroisocoumarins, stilbenoids, and oligostilbenoids from a methanol extract of the bark of S. roxburghii.<sup>2</sup> We also revealed that the methanol extract and several oligostilbenoid constituents showed antihyperlipidemic effect in olive oil-loaded mice and pancreatic lipase inhibitory activity.<sup>2</sup> As a continuing study, we conducted further evaluations of the extract and/or constituents, and found that the methanol extract showed inhibitory activity against increase in plasma glucose levels in sucrose-loaded mice. By the intensive fractionalization of the extract, three new 3-ethyl-4-phenyl-3,4-dihydroisocoumarins named 1'S-dihydrophayomphenol  $A_2(1)$  and phayomphenols  $B_1(2)$  and  $B_2$ 

(3) have been isolated as minor constituents. This paper deals with the structural elucidation of these new dihydroisocoumarins (1-3) as well as inhibitory effects of the principal oligostilbenoid constituents on increase in plasma glucose levels in sucrose-loaded mice. To clarify the mechanism of action of the antidiabetogenic activity observed, effects of these oligostilbenoids on gastric emptying in mice, those on glucose uptake in isolated intestinal tissues as well as their inhibitory activities against rat intestinal  $\alpha$ -glucosidase and rat lens aldose reductase were also examined.

### 2. Results and discussion

# 2.1. Effect of methanol extract of the bark of *S. roxburghii* on plasma glucose elevation in sucrose-loaded mice

The bark of *S. roxburghii* (collected in Phatthalung Province, Thailand) was extracted with methanol under reflux to yield a methanol extract (15.6% from the dried bark). The methanol extract was subjected to Diaion HP-20 column chromatography ( $H_2O \rightarrow MeOH$ ) to give  $H_2O$ - and MeOH-eluted fractions (3.2% and 10.6%, respectively). As shown in Table 1, the methanol extract and its MeOH-eluted fraction showed inhibitory effect against increase in plasma glucose levels in sucrose-loaded mice at a dose of 500 mg/kg, p.o.



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Inhibitory effects of the methanolic extract from the bark of S. roxburghii and its methanol- and H2O-eluted fractions on plasma glucose levels in sucrose-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Plasma glucose (mg/dL) <sup>a</sup>		
			0.5 h	1.0 h	2.0 h
Normal	_	4	121.4 ± 14.7 <sup>c</sup>	124.9 ± 7.2 <sup>c</sup>	106.5 ± 3.9 <sup>b</sup>
Control	_	6	225.1 ± 12.5	229.9 ± 16.2	145.2 ± 7.2
MeOH extract	125	4	207.6 ± 22.6	213.4 ± 19.2	$156.0 \pm 7.4$
	250	4	188.1 ± 17.9	180.0 ± 23.2	146.9 ± 12.6
	500	4	154.4 ± 9.8 <sup>c</sup>	179.3 ± 12.1	167.6 ± 6.9
MeOH-eluted fraction	125	4	198.0 ± 13.9	185.8 ± 14.4	152.5 ± 3.4
	250	4	159.9 ± 17.2	172.7 ± 6.1	152.8 ± 3.4
	500	4	137.3 ± 4.4 <sup>c</sup>	$160.5 \pm 14.4^{a}$	162.3 ± 8.0
H <sub>2</sub> O-eluted fraction	250	4	176.7 ± 12.6	178.6 ± 14.0	142.7 ± 14.4
	500	4	230.6 ± 34.3	$210.0 \pm 20.9$	142.3 ± 14.6
Normal	_	6	124.8 ± 7.3 <sup>c</sup>	$143.0 \pm 5.4^{\circ}$	$131.8 \pm 6.4^{\circ}$
Control	_	9	218.7 ± 4.0	208.9 ± 6.8	163.7 ± 3.7
Acarbose	10	6	$162.4 \pm 11.7^{\circ}$	183.8 ± 3.8 <sup>b</sup>	151.5 ± 6.3
	20	6	153.8 ± 10.2 <sup>c</sup>	$185.4 \pm 8.1^{b}$	152.8 ± 3.8

<sup>a</sup> Each value represents the mean ± S.E.M.

<sup>b</sup> Significantly different from the control, p < 0.05.

<sup>c</sup> Significantly different from the control, *p* <0.01.

#### 2.2. Chemical constituents from the bark of S. roxburghii

In the preceding paper,<sup>2</sup> we reported the isolation and structure elucidation of two new dihydroisocoumarins, phayomphenols  $A_1$  (**4**) and  $A_2$  (**5**), relatively as the major constituents, the yield of which from the dried bark being 0.29% and 0.11%, respectively. By the intensive fractionation of the MeOH-eluted fraction in this study, three related compounds, 1'S-dihydrophayomphenol  $A_2$  (**1**, 0.0061%) and phayomphenols  $B_1$  (**2**, 0.0005%) and  $B_2$  (**3**, 0.0006%) were isolated as new constituents. Twenty kinds of stilbenoids and oligostilbenoids, (–)-hopeaphenol (**6**, 0.63%), (+)-isohopeaphe-

nol (**7**, 0.53%), hemsleyanol D (**8**, 0.30%), (–)-ampelopsin H (**9**, 0.015%), vaticanols B (**10**, 0.031%), C (**11**, 0.032%), A (**12**, 0.28%), E (**13**, 0.30%), and G (**14**, 0.042%), (+)-α-viniferin (**15**, 0.10%), pauciflorol A (**16**, 0.014%), hopeafuran (**17**, 0.012%), (–)-balanocarpol (**18**, 0.070%), (–)-ampelopsin A (**19**, 0.012%), malibatols A (**20**, 0.0029%) and B (**21**, 0.0007%), (+)-parviflorol (**22**, 0.0029%), *trans*-resveratrol 10-*C*-β-D-glucopyranoside (**23**, 0.90%), *cis*-resveratrol 10-*C*-β-D-glucopyranoside (**24**, 0.0081%), and piceid (**25**, 0.0098%), and a flavonol glycoside, quercetin 3-*O*-α-L-rhamnopyranoside (0.0050%), and a megastigmane glycoside, (6*S*,9*S*)-roseoside (0.0017%), were also isolated from the extracts of this plant (Chart 1).



Chart 1. Constituents from the bark of S. roxburghii.

# 2.3. Structures of 1'S-dihydrophayomphenol A<sub>2</sub> (1) and phayomphenols B<sub>1</sub> (2) and B<sub>2</sub> (3)

1'S-Dihydrophayomphenol  $A_2(1)$  was obtained as a white powder with positive optical rotation ( $[\alpha]_D^{25}$  +79.5 in MeOH). Its IR spectrum showed absorption bands at 3390, 1698, 1615, 1514, 1474, 1362, and 1258  $cm^{-1}$  ascribable to hydroxyls, lactone carbonyl functions, and an aromatic ring. The positive-ion FABMS spectrum of **1** showed a guasimolecular ion peak at m/z 339 (M+Na)<sup>+</sup>, and the molecular formula was determined as  $C_{17}H_{16}O_6$ , a two hydrogen homolog to compound 5, by high-resolution positive-ion FAB-MS measurement. The <sup>1</sup>H and <sup>13</sup>C NMR spectral properties of **1**<sup>14</sup> (Tables 2 and 3) were guite similar to those of 5 (Tables 4 and 5), except for signals due to 1' and 2' positions. Instead of a signal due to the carbonyl carbon  $[(\delta_c 205.4 \text{ in } CD_3OD)]$  observed in the <sup>13</sup>C NMR spectrum of **5**, a signal of methine carbon bearing an oxygen appeared at  $\delta_c$  69.9 (in CD<sub>3</sub>OD) in that of **1**. In the <sup>1</sup>H NMR spectrum of **1** (pyridine- $d_5$ , Tables 2 and 3), an additional methine signal appeared at  $\delta$  4.43 (1H, dq, I = 6.2, 6.6 Hz, H-1'), and thus the signal of the adjacent methyl appeared as a doublet at  $\delta$  1.52 (3H, d, I = 6.6 Hz, H<sub>3</sub>-2'). Therefore, the structure of **1** was speculated to be the reduced product of **5** at C-1' position. Actually oxidation of **1** with chromium trioxide (CrO<sub>3</sub>) in pyridine afforded phayomphenol  $A_2$  (5). The CD spectrum of the oxidized product was completely in accord with that of 5, thus the absolute stereochemistry at C-3 and C-4 being approved to be S and S, respectively.

The absolute configuration of the C-1' position in 1 was elucidated by the application of the modified Mosher's method.<sup>15</sup> Namely, 5,7,4"-trimethyl ester (1a), which was obtained from 1 upon reaction with trimethylsilyldiazomethane (TMSCHN<sub>2</sub>), was derived to 1'-(R)-MTPA ester (**1b**) by treatment with (R)-2-methoxy-2-trifluoromethylphenylacetic acid [(R)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP). On the other hand, 1'-(S)-MTPA ester (1c) was obtained from 1a using (S)-MTPA under the same conditions. As shown in Figure 2, signals due to the protons at C-3 and C-4 in **1c** were observed at lower field compared with those of **1b** [ $\Delta\delta$ : positive], while the signal due to the proton at C-1' in **1c** was observed at higher field compared with that of **1b**  $[\Delta\delta$ : negative]. Thus, the absolute configuration at C-1' of **1a** was determined to be S. Consequently, the absolute stereostructure of 1 was elucidated to be as shown. <sup>1</sup>H–<sup>1</sup>H COSY, HMQC, HMBC, and NOESY spectral properties also supported the depicted structure.

Phayomphenol B<sub>1</sub> (**2**),  $[\alpha]_D^{25}$  +162.9 (in MeOH), was obtained as a white powder. Its IR spectrum showed absorption bands at 3400, 1717, 1698, 1617, 1362, 1250, and 1125 cm<sup>-1</sup> ascribable to

Table	2
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<sup>1</sup>H NMR (500 MHz) data on 1'S-dihydrophayomphenol A<sub>2</sub> (1) and 1a-1c

Table	3
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 $^{13}\text{C}$  NMR (125 MHz) data on 1'S-dihydrophayomphenol A2 (1) and 1a

Position	1 <sup>a</sup>	1 <sup>b</sup>	1a <sup>b</sup>
	$\delta_{C}$	$\delta_{C}$	$\delta_{C}$
1	165.2	166.9	166.4
3	90.3	90.7	90.3
4	38.0	37.9	38.3
5	157.0	156.8	158.7
6	109.1	109.3	105.6
7	159.0	158.8	161.6
8	107.4	107.4	104.5
9	128.6	128.0	127.9
10	119.7	119.7	123.0
1′	69.5	69.9	70.1
2′	20.8	20.0	20.0
1″	134.2	134.2	135.3
2",6"	129.5	129.6	129.5
3",5"	116.5	116.3	115.0
4″	157.8	157.3	160.1
$5-OCH_3$			55.7
7-0CH <sub>3</sub>			56.1
4″-0CH <sub>3</sub>			56.4

<sup>a</sup> Measured in pyridine-*d*<sub>5</sub>.

<sup>b</sup> Measured in CD<sub>3</sub>OD.

Table 4		
<sup>1</sup> H NMR (CD <sub>3</sub> OD)	) data on phayomphenols $B_1$ (2), $B_2$ (3), $A_1$ (4), and	$IA_{2}(5)$

<b>2</b> <sup>a</sup>	<b>3</b> <sup>a</sup>	4 <sup>b</sup>	<b>5</b> <sup>b</sup>
$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H}$ (J Hz)
5.11 (d, 3.9)	5.27 (d, 1.2)	5.14 (d, 4.0)	5.28 (d, 1.5)
4.54 (d, 3.9)	4.74 (br s)	4.61 (d, 4.0)	4.80 (br s)
6.57 (d, 2.2)	6.55 (d, 2.2)	6.57 (d, 2.3)	6.55 (d, 2.3)
7.04 (d, 2.2)	6.98 (d, 2.2)	7.04 (d, 2.3)	6.99 (d, 2.3)
1.88 (3H, s)	2.22 (3H, s)	1.86 (3H, s)	2.33 (3H, s)
6.45 (d, 2.2)	6.54 (d, 2.2)	6.82 (d, 8.6)	6.96 (d, 8.6)
6.62 (d, 8.2)	6.70 (d, 8.2)	6.64 (d, 8.6)	6.71 (d, 8.6)
6.62 (d, 8.2)	6.70 (d, 8.2)	6.64 (d, 8.6)	6.71 (d, 8.6)
6.33 (dd, 2.2, 8.2)	6.51 (dd, 2.2, 8.2)	6.82 (d, 8.6)	6.96 (d, 8.6)
	$\begin{array}{c} {\bf 2}^{a} \\ \delta_{H} \ (J \ Hz) \\ \hline 5.11 \ (d, 3.9) \\ 4.54 \ (d, 3.9) \\ 6.57 \ (d, 2.2) \\ 7.04 \ (d, 2.2) \\ 1.88 \ (3H, s) \\ 6.45 \ (d, 2.2) \\ 6.62 \ (d, 8.2) \\ 6.62 \ (d, 8.2) \\ 6.63 \ (dd, 2.2, 8.2) \end{array}$	$\begin{array}{ccc} 2^{a} & 3^{a} \\ \delta_{H} \left( J  Hz \right) & \delta_{H} \left( J  Hz \right) \\ \hline 5.11 \left( d, 3.9 \right) & 5.27 \left( d, 1.2 \right) \\ 4.54 \left( d, 3.9 \right) & 4.74 \left( br  s \right) \\ 6.57 \left( d, 2.2 \right) & 6.55 \left( d, 2.2 \right) \\ 7.04 \left( d, 2.2 \right) & 6.98 \left( d, 2.2 \right) \\ 1.88 \left( 3H, s \right) & 2.22 \left( 3H, s \right) \\ 6.45 \left( d, 2.2 \right) & 6.54 \left( d, 2.2 \right) \\ 6.62 \left( d, 8.2 \right) & 6.70 \left( d, 8.2 \right) \\ 6.62 \left( d, 8.2 \right) & 6.51 \left( dd, 2.2, 8.2 \right) \\ 6.33 \left( dd, 2.2, 8.2 \right) & 6.51 \left( dd, 2.2, 8.2 \right) \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

<sup>a</sup> Measured at 700 MHz.

<sup>b</sup> Measured at 500 MHz.

hydroxyls, a carbonyl, and a lactone carbonyl functions, and an aromatic ring. The molecular formula determined by means of highresolution EIMS was  $C_{17}H_{14}O_7$ , a one oxygen homolog of **4**. Instead of *ortho*-coupled A<sub>2</sub>B<sub>2</sub>-type aromatic protons observed in the <sup>1</sup>H NMR spectrum of **4** [ $\delta$  6.64, 6.82 (2H each, both d, *J* = 8.6 Hz, H-3",5", 2",6")], *ortho*- and *meta*-coupled ABC-type aromatic protons

Position	<b>1</b> <sup>a</sup>	1 <sup>b</sup>	1a <sup>b</sup>	<b>1b</b> <sup>b</sup>	1c <sup>b</sup>
	$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H}$ (J Hz)	$\delta_{\rm H} (J  {\rm Hz})$	$\delta_{\rm H}$ (J Hz)
3	5.01 (br d, ca. 6)	4.39 (dd, 1.2, 6.9)	4.42 (dd, 1.2, 5.8)	4.58 (dd, 1.2, 6.9)	4.64 (dd, 1.2, 8.0)
4	5.10 (br s)	4.39 (br s)	4.43 (br s)	4.38 (br s)	4.47 (br s)
6	7.11 (d, 2.4)	6.58 (d, 2.3)	6.81 (d, 2.3)	6.68 (d, 2.3)	6.86 (d, 2.3)
8	7.42 (d, 2.4)	7.01 (d, 2.3)	7.21 (d, 2.3)	7.22 (d, 2.3)	7.22 (d, 2.3)
1′	4.43 (dq, 6.2, 6.6)	3.79 (dq, 6.9, 6.6)	3.82 (m)	5.29 (m)	5.25 (m)
2′	1.52 (3H, d, 6.6)	1.19 (3H, d, 6.6)	1.17 (3H, d, 6.6)	1.45 (3H, d, 6.6)	1.31 (3H, d, 6.3)
2",6"	7.46 (2H, d, 8.6)	6.89 (2H, d, 8.6)	6.96 (2H, d, 8.6)	6.91 (2H, d, 8.6)	6.95 (2H, d, 8.6)
3″,5″	7.09 (2H, d, 8.6)	6.68 (2H, d, 8.6)	6.81 (2H, d, 8.6)	6.80 (2H, d, 8.6)	6.82 (2H, d, 8.6)
5-0CH <sub>3</sub>			3.72 (3H, s)	3.68 (3H, s)	3.77 (3H, s)
7-0CH <sub>3</sub>			3.86 (3H, s)	3.89 (3H, s)	3.90 (3H, s)
4"-0CH <sub>3</sub>			3.73 (3H, s)	3.73 (3H, s)	3.74 (3H, s)
1'-OMTPA				3.50 (3H, s)	3.50 (3H, s)
				7.36-7.41 (3H, m)	7.39-7.42 (3H, m)
				7.45-7.47 (2H, m)	7.47-7.48 (2H, m)

<sup>a</sup> Measured in pyridine-*d*<sub>5</sub>.

<sup>b</sup> Measured in CD<sub>3</sub>OD.

Table 5  $^{13}$ C NMR (CD<sub>3</sub>OD) data on phayomphenols B<sub>1</sub> (2), B<sub>2</sub> (3), A<sub>1</sub> (4), and A<sub>2</sub> (5)

Position	<b>2</b> <sup>a</sup>	<b>3</b> <sup>a</sup>	$4^{\mathrm{b}}$	<b>5</b> <sup>b</sup>
	$\delta_{C}$	$\delta_{C}$	$\delta_{C}$	$\delta_{C}$
1	166.9	166.7	166.9	166.7
3	86.9	89.3	86.7	89.1
4	40.5	38.7	40.2	38.5
5	156.5	156.8	156.4	156.7
6	109.8	109.2	109.7	109.2
7	159.2	159.3	159.1	159.3
8	107.6	107.5	107.5	107.5
9	126.7	127.8	126.9	127.8
10	121.7	117.6	121.6	117.5
1′	207.8	205.4	207.6	205.4
2′	27.7	26.0	27.6	26.0
1″	129.1	133.2	128.3	132.4
2″	117.3	115.9	131.2	129.8
3″	146.2	146.4	116.1	116.3
4″	145.8	145.5	157.9	157.6
5″	116.2	116.5	116.1	116.3
6″	121.7	120.1	131.2	129.8

<sup>a</sup> Measured at 175 MHz.

<sup>b</sup> Measured at 125 MHz.



Phayomphenol B<sub>2</sub> (**3**) was also isolated as a white powder with positive optical rotation ( $[\alpha]_D^{25}$  +212.1 in MeOH). The molecular formula, C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>, obtained by the positive-ion high-resolution FABMS measurement, was found to be the same as that of **2**. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic properties of **3** (CD<sub>3</sub>OD, Tables 4 and 5) were quite similar to those of **2**, except for the signal due to the methyl group [ $\delta$  2.22 (3H, s, H<sub>3</sub>-2')]. The position of the new-ly introduced OH was determined in the same manner as was applied in the case for **2**, the *ortho*- and *meta*-coupled ABC-type aromatic protons [ $\delta$  6.51 (1H, dd, J = 2.2, 8.2 Hz, H-6"), 6.54 (1H, d, J = 2.2 Hz, H-2"), 6.70 (1H, d, J = 8.2 Hz, H-5")] and a cross-peak between the signal due to C-4 ( $\delta_c$  38.7) and the signal at  $\delta$  6.54 being observed in the <sup>1</sup>H NMR and HMBC spectra, respectively.



Figure 1. <sup>1</sup>H-<sup>1</sup>H COSY, HMBC, and NOESY correlations of 1-3.



Figure 2. Determination of absolute stereochemistry of 1. Reagents and conditions: (a) CrO<sub>3</sub>/pyridine, rt, 8 h, 29%. (b) TMSCHN<sub>2</sub>/MeOH, rt, 8 h, quant. (c) (*R*)- or (*S*)-MTPA, EDC·HCl,4-DMAP/CH<sub>2</sub>Cl<sub>2</sub>, *Δ*, 15 h, 1b: 43%, 1c: 66%.

Finally, the absolute stereochemistry of 2 and 3 were confirmed by their CD spectra, which showed a negative ( $\Delta \varepsilon$  –0.14 at 296 nm) and a positive Cotton effect ( $\Delta \varepsilon$  +4.37 at 294 nm), respectively. According to published CD data for 3,4-dihydroisocoumarins, these Cotton effects were assignable to  $n \rightarrow \pi^*$  transition of the lactone carbonyl group of dihydroisocoumarin chromophore.<sup>2,16,17</sup> This indicated that the 3-acetyl group in **3** was in  $\alpha$ -axial orientation, and accordingly the absolute configuration at C-3 of **3** was concluded to be  $S^{.18-23}$ . In the same manner, the absolute configuration at C-3 of 2 was confirmed to be R. These results indicated that the 3,4-dihydroxyphenyl moieties at C-4 in 2 and 3 were both in β-axial orientation, and their absolute configurations were also concluded to be S. On the basis of the foregoing evidences, the absolute stereostructures of 2 and 3 were confirmed as (3R.4S)and (3S,4S)-3-acetyl-5,7-dihydroxy-4-(3,4-dihydroxyphenyl)-3,4dihydroisocoumarin, respectively.

# 2.4. Antihyperglycemic effects of the chemical constituents from the bark of *S. roxburghii*

With respect to the principal constituents of *S. roxburghii*, phayomphenols A<sub>1</sub> (**4**) and A<sub>2</sub> (**5**), (–)-hopeaphenol (**6**), (+)-isohopeaphenol (**7**), hemsleyanol D (**8**), (+)- $\alpha$ -viniferin (**15**), (–)-balanocarpol (**18**), *trans*-resveratrol 10-*C*- $\beta$ -*p*-glucopyranoside (**23**), and resveratrol (**25a**),<sup>24–27</sup> effects on increase in plasma glucose levels in sucrose-loaded mice were examined. As shown in Table 6, oligostilbenoids, **6**, **8**, **15**, and **18** showed significant inhibitory activity against increase in plasma glucose levels at a dose of 100 or 200 mg/kg, p.o., and at 200 mg/kg, p.o. (*p* = 0.070), **7** tended to inhibit the elevation. The inhibitory effects of **8** and **15** were stronger than those of **6**, **7**, and **18**, while a stilbene monomer (**25a**) and its glycoside (**23**) showed weak effects. In this experiment, an intestinal  $\alpha$ -glucosidase inhibitor, acarbose, was employed as a positive control, which showed reasonable inhibition as was reported in the literature (Table 1).<sup>28</sup>

Next, the mode of action for the antihyperglycemic effects of the stilbenoids was studied. Plasma glucose levels are known to be regulated by many factors such as transport of sugar in the digestive tract, the secretion and release of hormones, and absorption of glucose through membranes of the small intestine.<sup>29</sup> Previously, we reported that inhibition of gastric emptying markedly inhibited plasma glucose and triglyceride elevations in sucroseand olive oil-loaded mice or rats, respectively.<sup>3,30–37</sup> Thus, effects of the oligostilbenes (6-8, 15, and 18) on the gastric emptying rate were examined. As shown in Table 7, compounds 6, 7, and 18 significantly suppressed the gastric emptying in mice at a dose of 200 mg/kg, p.o., and **15** also tended to suppress gastric emptying (p = 0.063) at the same dose. These findings suggest that these oligostilbenoids inhibited the plasma glucose elevation in orally sucrose-loaded mice possibly in part by suppressing the transfer of sucrose from the stomach to the small intestine.

Next, effects of these constituents on glucose uptake in rat small intestinal tissues<sup>38,39</sup> and also those on inhibitory activities<sup>28,31,40–42</sup> against rat small intestinal  $\alpha$ -glucosidases, maltase and sucrase, were examined. As shown in Table 8, principal oligostilbenoids (**6–8**, **15**, and **18**) did not inhibit glucose uptake at a concentration of 1 mM. On the other hand, most of the oligostilbenoids (**6–13**, **15**, **17**, **20**, and **21**) moderately inhibited the  $\alpha$ -glucosidase activities as shown in Table 9. These results suggest that the intestinal  $\alpha$ -glucosidase inhibitory activity is also involved in the inhibitory effects of the oligostilbenoids (**6–8** and **15**, but not **18**) against blood glucose elevation in sucrose-loaded mice.

Finally, inhibitory effects of these constituents including minor contents on rat lens aldose reductase were examined. Aldose reductase is known to be a key enzyme which catalyzes the reduction of glucose to sorbitol in the polyol processing pathway. Sorbitol dose not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataracts.<sup>27,43</sup> As shown in Table 9, several dihydroisocoumarins (**2–5**) and stilbenoids (**6**,

#### Table 6

Inhibitory effects of the constituents from the bark of S. roxburghii on plasma glucose levels in sucrose-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Plasma glucose (mg/dL) <sup>a</sup>		
			0.5 h	1.0 h	2.0 h
Normal	_	6	$126.6 \pm 5.2^{\circ}$	$139.8 \pm 5.6^{\circ}$	116.0 ± 3.2
Control	_	7	220.5 ± 16.9	221.4 ± 9.1	160.8 ± 3.5
Phayomphenol A <sub>1</sub> ( <b>4</b> )	100	7	189.3 ± 10.1	199.5 ± 10.1	159.2 ± 5.9
	200	7	184.1 ± 15.5	$178.1 \pm 11.0^{b}$	166.2 ± 7.0
Phayomphenol $A_2(5)$	100	6	231.2 ± 22.6	226.7 ± 19.8	179.4 ± 12.9
	200	7	$177.0 \pm 10.4$	197.3 ± 6.0	166.9 ± 8.0
Normal	_	7	$115.7 \pm 9.4^{\circ}$	$125.2 \pm 10.3^{\circ}$	129.9 ± 10.2 <sup>c</sup>
Control	_	10	232.9 ± 11.8	$229.0 \pm 9.6$	172.0 ± 11.3
(–)-Hopeaphenol ( <b>6</b> )	100	6	191.6 ± 13.9	194.9 ± 5.9	160.6 ± 7.3
	200	7	$158.3 \pm 5.6^{\circ}$	187.9 ± 6.1	179.4 ± 4.4
(+)-Isohopeaphenol (7)	100	6	182.5 ± 19.1	204.9 ± 7.3	191.6 ± 10.8
	200	7	179.7 ± 8.2	$209.4 \pm 11.0$	189.1 ± +6.8
Hemsleyanol D (8)	100	6	$173.5 \pm 16.8^{b}$	211.8 ± 24.3	174.1 ± 16.6
	200	7	$142.4 \pm 22.7^{c}$	201.7 ± 24.9	177.4 ± 15.2
(+)-α-Viniferin ( <b>15</b> )	100	6	$151.2 \pm 21.2^{c}$	219.8 ± 6.1	173.3 ± 9.1
	200	7	$153.5 \pm 19.2^{\circ}$	184.2 ± 7.7	167.2 ± 6.9
(–)-Balanocarpol ( <b>18</b> )	100	6	227.9 ± 12.6	210.6 ± 12.0	157.4 ± 5.1
	200	7	$169.2 \pm 14.5^{\circ}$	$192.0 \pm 10.8$	$156.4 \pm 9.7$
Normal	_	8	$119.4 \pm 4.8^{\circ}$	$131.9 \pm 2.7^{\circ}$	$114.7 \pm 3.9^{\circ}$
Control	_	8	226.5 ± 17.3	$200.9 \pm 4.3$	148.8 ± 6.3
trans-Resveratrol 10-C-Glc (23)	100	8	184.3 ± 17.8	$183.4 \pm 10.0$	140.9 ± 6.0
	200	8	188.7 ± 10.1	202.3 ± 12.4	$147.9 \pm 3.1$
Normal	_	5	$137.5 \pm 7.2^{\circ}$	$142.1 \pm 11.0^{c}$	135.9 ± 8.1 <sup>c</sup>
Control	_	10	230.9 ± 15.7	$203.0 \pm 9.3$	174.5 ± 7.1
Resveratrol ( <b>25a</b> )	100	7	218.3 ± 12.2	$210.9 \pm 14.0$	$181.4 \pm 4.5$
	200	6	$205.6 \pm 8.0$	192.7 ± 7.7	158.7 ± 7.5

<sup>a</sup> Each value represents the mean ± S.E.M.

<sup>b</sup> Significantly different from the control, p < 0.05.

<sup>c</sup> Significantly different from the control, *p* <0.01.

#### Table 7 Inhibitory effects of the constituents from the bark of S. roxburghii on gastric emptying in CMC-Na-loaded mice

Treatment	Dose (mg/kg, p.o.)	n	Gastric emptying (%) <sup>a</sup>	Inhibition (%)
Control	_	9	82.0 ± 3.1	_
(-)-Hopeaphenol ( <b>6</b> )	200	8	$58.2 \pm 2.7^{b}$	29.0
(+)-Isohopeaphenol (7)	200	8	64.7 ± 5.1 <sup>b</sup>	21.1
Hemsleyanol D ( <b>8</b> )	200	4	74.9 ± 6.5	8.7
$(+)-\alpha$ -Viniferin (15)	200	8	$69.4 \pm 3.4$	15.4
(-)-Balanocarpol ( <b>18</b> )	200	7	$61.7 \pm 2.3^{b}$	24.8

<sup>a</sup> Each value represents the mean ± S.E.M.

<sup>b</sup> Significantly different from the control, *p* <0.01.

# Table 8

Inhibitory effects of the constituents from the bark of S. roxburghii on glucose uptake in rats intestinal tissues

	Conc. (mM)	n	Glucose uptake <sup>a</sup> (dpm/100 mg tissue)	Inhibition (%)
Control	_	6	3172 ± 419	0.0
Phrolizin	1	6	$1606 \pm 103^{b}$	100.0
(-)-Hopeaphenol ( <b>6</b> )	1	6	2830 ± 259	21.8
(+)-Isohopeaphenol (7)	1	6	3038 ± 240	8.5
Control	_	10	3992 ± 370	0.0
Phrolizin	1	5	$1642 \pm 128^{b}$	100.0
Hemsleyanol D ( <b>8</b> )	1	5	3595 ± 311	16.9
$(+)-\alpha$ -Viniferin (15)	1	5	3564 ± 254	18.2
(–)-Balanocarpol ( <b>18</b> )	1	5	4088 ± 396	-4.1

 $^{\rm a}\,$  Each value represents the mean  $\pm$  S.E.M.

<sup>b</sup> Significantly different from the control, p < 0.01.

#### Table 9

Inhibitory effects of the methanolic extract from the bark of S. roxburghii and its methanol- and H<sub>2</sub>O-eluted fractions and the constituents on enzyme activities of α-glucosidases, and aldose reductase activities

	$\alpha$ -Glucosidase IC <sub>50</sub> <sup>a</sup> (µg/mL)		Aldose reductase $IC_{50}^{a}$ (µg/mL)
	Maltase	Sucrase	
MeOH extract	150	128	19.6
MeOH-eluted fraction	235	183	17.9
H <sub>2</sub> O-eluted fraction	>400 (4.8) <sup>2</sup> $\alpha$ -Chicosidase IC- $a^{a}$ (uM)	>400 (6.5)	$>400 (0.9)^{\circ}$
			Aluose reductase $IC_{50}$ (µW)
	Maltase	Sucrase	in a star and
1'S-Dihydrophayomphenol $A_2$ ( <b>1</b> )	>400 (31.4)	>400 (30.3) <sup>c</sup>	>100 (18.8)
Phayomphenol $B_1(2)$	>400 (20.9) <sup>c</sup>	ca. 400	32.5
Phayomphenol $B_2(3)$	>400 (40.4)	211	26.6
Phayomphenol $A_1$ ( <b>4</b> )	>400 (4.0) <sup>c</sup>	>400 (6.4) <sup>c</sup>	39.8
Phayomphenol A <sub>2</sub> ( <b>5</b> )	>400 (19.6) <sup>c</sup>	>400 (3.7) <sup>c</sup>	47.7
(–)-Hopeaphenol ( <b>6</b> )	338	195	69.0
(+)-Isohopeaphenol (7)	216	90.0	>100 (34.4) <sup>b</sup>
Hemsleyanol D ( <b>8</b> )	266	218	29.4
(–)-Ampelopsin H ( <b>9</b> )	178	97.1	50.2
Vaticanol B (10)	233	294	30.0
Vaticanol C (11)	140	94.2	21.2
Vaticanol A ( <b>12</b> ) <sup>31</sup>	218	148	23.7
Vaticanol E (13) <sup>31</sup>	342	88.9	30.8
Vaticanol G ( <b>14</b> ) <sup>31</sup>	>400 (32.2) <sup>c</sup>	>400 (37.8) <sup>c</sup>	>100 (46.7) <sup>b</sup>
(+)-α-Viniferin ( <b>15</b> )	172	234	7.8
Pauciflorol A (16)	>400 (44.0) <sup>c</sup>	55.7	29.5
Hopeafuran (17)	142 <sup>c</sup>	105	6.9
(–)-Balanocarpol ( <b>18</b> )	>400 (29.4) <sup>c</sup>	>400 (36.6) <sup>c</sup>	30.0
(-)-Ampelopsin A (19)	> 400 (-6.3)	>400 (0.4) <sup>c</sup>	68.8
Malibatol A (20)	362	231	35.6
Malibatol B (21)	262	143	10.0
(+)-Parviflorol (22)	>400 (6.8) <sup>c</sup>	>400 (-0.8) <sup>c</sup>	>100 (43.7) <sup>b</sup>
trans-Resveratrol 10-C-Glc (23)	>400 (20.1) <sup>c</sup>	>400 (14.7) <sup>c</sup>	>100 (30.3) <sup>b</sup>
cis-Resveratrol 10-C-Glc (24)	>400 (36.6)°	>400 (26.8)°	>100 (39.2) <sup>b</sup>
Piceid (25)	>400 (38.2)°	>400 (48.0)°	ca. 100
Resveratrol (25a)	>400 (36.9)°	>400 (35.7)°	25.0 <sup>27</sup>
Acarbose <sup>31</sup>	2.0	1.7	
Epalrestat <sup>31</sup>			0.072

<sup>a</sup> Each value represents the mean of 2–4 experiments.

 $^{b,c}$  Values in parentheses present inhibition % at 100 or 400  $\mu M.$ 

**8–13**, **15–21**, and **25a**) moderately inhibited the activity of rat lens aldose reductase.

In conclusion, the methanol extract of the bark of S. roxburghii was found to inhibit plasma glucose elevation in sucrose-loaded mice. Three new 3-ethyl-4-phenyl-3,4-dihydroisocoumarins, 1'Sdihydrophayomphenol  $A_2$  (1) and phayomphenols  $B_1$  (2) and  $B_2$ (3), were isolated together with 24 known compounds including 20 stilbenoids and oligostilbenoids. Among the isolates, (-)-hopeaphenol (6), hemsleyanol D (8), (+)- $\alpha$ -viniferin (15), and (–)-balanocarpol (18) significantly inhibited plasma glucose elevation in sucrose-loaded mice at doses of 100-200 mg/kg, p.o., and (+)-isohopeaphenol (7) tended to inhibit it at a dose of 200 mg/kg, p.o. As the possible mechanism of action responsible for the antihyperglycemic effect of the extract, following multiple modes were recursively induced on the basis of the observed activities of the constituents: (1) inhibition of gastric emptying: constituents 6, 7, and 18 significantly suppressed the gastric emptying, and 15 also acted moderately, (2)  $\alpha$ -glucosidase inhibitory activity: constituents **6–8** and 15 moderately inhibited enzyme activities of rat small intestinal sucrose, (3) aldose reductase inhibitory activity: constituents 2-6, 8-13, 15-21, and 25a moderately inhibited the rat lens aldose reductase. Inhibition of glucose uptake was excluded as a mode of the action because major constituents 6-8, 15, and 18 did not inhibit glucose uptake in rat small intestinal tissues.

# 3. Experimental

# 3.1. General

The following instruments were used to obtain spectral and 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; <sup>1</sup>H NMR spectra, JEOL JNM-ECA700 (700 MHz), JNM-ECA500 (500 MHz), and JNM-ECS400 (400 MHz) spectrometers; <sup>13</sup>C NMR spectra, JEOL JNM-ECA700 (175 MHz), JNM-ECA500 (125 MHz), and JNM-ECS400 (100 MHz) spectrometers, with tetramethylsilane as an internal standard; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; HPLC detector, Shimadzu SPD-10A UV–VIS detectors; HPLC column, Cosmosil 5C<sub>18</sub>–MS-II and  $\pi$ NAP (250 × 4.6 mm i.d. and 250 × 20 mm i.d. for analytical and preparative purposes, respectively).

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel 60 N (Kanto Chemical Co., Ltd, 63–210 mesh, spherical, neutral); reversed-phase silica gel CC, Diaion HP-20 (Nippon Rensui) and Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd, 100–200 mesh); TLC, pre-coated TLC plates with silica gel  $60F_{254}$  (Merck, 0.25 mm) (normal-phase) and silica gel RP-18  $F_{254S}$  (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF<sub>254S</sub> (Merck, 0.25 mm), detection was achieved by spraying with 1% Ce(SO<sub>4</sub>)<sub>2</sub>–10% aqueous H<sub>2</sub>SO<sub>4</sub>, followed by heating.

# 3.2. Plant material

The bark of *S. roxburghii* was collected in Phatthalung Province, Thailand on September 2006. The plant material was identified by one of the authors (Y.P.). A voucher specimen (2006.09. Raj-02) of this plant is on file in our laboratory.

# 3.3. Extraction and isolation

Dried barks of *S. roxburghii* (3.7 kg) were finely cut and extracted three times with MeOH under reflux for 3 h. Evaporation of the

combined extracts under reduced pressure provided a MeOH extract (575.7 g, 15.6%). An aliquot (525.7 g) was subjected to Diaion HP-20 CC (3.0 kg,  $H_2O \rightarrow$  MeOH, twice) to give  $H_2O$ -eluted (107.0 g, 3.2%) and MeOH-eluted (358.5 g, 10.6%) fractions. An aliquot (180.0 g) of the MeOH-eluted fraction was subjected to normal-phase silica gel CC [3.0 kg, CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (10:3:0.4  $\rightarrow$  7:3:0.5  $\rightarrow$  6:4:1, v/v/ v)  $\rightarrow$  MeOH] to give eight fractions [Fr. 1 (2.82 g), Fr. 2 (8.20 g), Fr. 3 (9.20 g), Fr. 4 (66.38 g), Fr. 5 (18.29 g), Fr. 6 (20.11 g), Fr. 7 (25.93 g), and Fr. 8 (31.25 g)]. The fraction 2 (8.20 g) was subjected to reversed-phase silica gel CC [250 g, MeOH-H<sub>2</sub>O (20:80  $\rightarrow$  $40:60 \rightarrow 50:50, v/v) \rightarrow MeOH$  to afford seven fractions [Fr. 2-1 (4.8 mg), Fr. 2-2 (41.1 mg), Fr. 2-3 (55.3 mg), Fr. 2-4 (774.3 mg), Fr. 2-5 (1256.3 mg), Fr. 2-6 (5540.5 mg), and Fr. 2-7 (1081.9 mg)] as reported previously.<sup>2</sup> The fraction 2-4 (500.4 mg) was purified by HPLC [Cosmosil 5C<sub>18</sub>-MS-II, MeOH–1% aqueous AcOH (20:80, v/v)] to give 1'S-dihydrophayomphenol  $A_2$  (1, 22.5 mg, 0.0021%) and phayomphenols B<sub>1</sub> (**2**, 5.1 mg, 0.0005%) and B<sub>2</sub> (**3**, 6.7 mg, 0.0006%) together with phayomphenols  $A_1$  (4, 6.4 mg, 0.0006%) and  $A_2$  (5, 442.6 mg, 0.040%). The fraction 2-5 (500.7 mg) was purified by HPLC [Cosmosil 5C<sub>18</sub>-MS-II, MeOH-1% aqueous AcOH (30:70, v/v)] to give 1 (9.9 mg, 0.0015%) together with 4 (123.0 mg, 0.018%) and 5 (165.4 mg, 0.025%).

#### 3.3.1. 1'S-Dihydrophayomphenol A<sub>2</sub> (1)

A white powder,  $[\alpha]_D^{25} + 79.5$  (*c* 0.17, MeOH). Positive-ion FABMS: *m*/*z* 339 (M+Na)<sup>+</sup>. High-resolution positive-ion FABMS: Calcd for C<sub>17</sub>H<sub>16</sub>O<sub>6</sub>Na (M+Na)<sup>+</sup>: 339.0845. Found: 339.0849. CD [MeOH, nm ( $\Delta \varepsilon$ )]: 228 (-22.20), 265 (+2.86), 330 (+1.25). UV [MeOH, nm ( $\log \varepsilon$ )]: 261 (3.72), 327 (3.45). IR (KBr): 3390, 1698, 1615, 1514, 1474, 1362, 1258, 1223, 1123, 1017 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, pyr-idine-*d*<sub>5</sub> and CD<sub>3</sub>OD)  $\delta$ : given in Table 2. <sup>13</sup>C NMR (125 MHz, pyridine-*d*<sub>5</sub> and CD<sub>3</sub>OD)  $\delta_{\varepsilon}$ : given in Table 3.

# 3.3.2. Phayomphenol B<sub>1</sub> (2)

A white powder,  $[\alpha]_D^{25}$  +162.9 (*c* 0.10, MeOH). EIMS *m/z* (%): 330 (M<sup>+</sup>, 11), 43 (100). High-resolution EIMS: Calcd for C<sub>17</sub>H<sub>14</sub>O<sub>7</sub> (M<sup>+</sup>): 330.0740. Found: 330.0733. CD [MeOH, nm ( $\Delta \varepsilon$ )]: 228 (-6.79), 267 (+2.56), 296 (-0.14). UV [MeOH, nm (log  $\varepsilon$ )]: 262 (3.53), 328 (3.30). IR (KBr): 3400, 1717, 1698, 1617, 1362, 1250, 1125 cm<sup>-1</sup>. <sup>1</sup>H NMR (700 MHz, CD<sub>3</sub>OD)  $\delta$ : given in Table 2. <sup>13</sup>C NMR (175 MHz, CD<sub>3</sub>OD)  $\delta_{c}$ : given in Table 3.

## 3.3.3. Phayomphenol B<sub>2</sub> (3)

A white powder,  $[\alpha]_D^{25}$ +212.1 (*c* 0.08, MeOH). Positive-ion FAB-MS: *m/z* 353 (M+Na)<sup>+</sup>. High-resolution positive-ion FABMS: Calcd for C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>Na (M+Na)<sup>+</sup>: 353.0637. Found: 353.0630. CD [MeOH, nm ( $\Delta \varepsilon$ )]: 227 (-13.63), 270 (+3.83), 294 (+4.37). UV [MeOH, nm (log  $\varepsilon$ )]: 260 (3.52), 324 (3.28). IR (KBr): 3400, 1732, 1717, 1541, 1509, 1362, 1123 cm<sup>-1</sup>. <sup>1</sup>H NMR (700 MHz, CD<sub>3</sub>OD)  $\delta$ : given in Table 2. <sup>13</sup>C NMR (175 MHz, CD<sub>3</sub>OD)  $\delta_c$ : given in Table 3.

#### 3.4. Chromium trioxide (CrO<sub>3</sub>) oxidation of 1

To a solution of **1** (3.5 mg) in pyridine (0.2 mL) was added  $CrO_3$  (6.6 mg)–pyridine (0.8 mL), and the mixture was stirred at 60 °C for 8 h. The reaction mixture was poured into saturated aqueous NaCl and the resulting mixture was extracted with EtOAc. The extract was washed with brine, and dried over anhydrous MgSO<sub>4</sub>. Removal of the solvent under reduced pressure gave a pale yellow oil, which was purified by HPLC [Cosmosil 5C<sub>18</sub>-MS-II, MeOH–1% aqueous AcOH (70:30, v/v)] to furnish **5** (1.0 mg, 29%) as a white powder.

#### 3.5. Methylation of 1

To a solution of **1** (8.5 mg) in MeOH (1.0 mL) was added trimethylsilyldiazomethane (TMSCHN<sub>2</sub>, 10% in hexane, ca. 0.5 mL). The mixture was stirred at room temperature for 8 h. Removal of the solvent under reduced pressure gave **1a** (9.6 mg, quant.).

#### 3.5.1. Compound 1a

A white powder,  $[\alpha]_D^{25}$  +69.6 (*c* 0.10, MeOH). Positive-ion FABMS: *m/z* 381 (M+Na)<sup>+</sup>. High-resolution positive-ion FABMS: Calcd for C<sub>20</sub>H<sub>22</sub>O<sub>6</sub>Na (M+Na)<sup>+</sup>: 381.1314. Found: 381.1309. UV [MeOH, nm (log  $\varepsilon$ )]: 258 (3.67), 319 (3.45). IR (KBr): 3400, 1717, 1609, 1512, 1458, 1362, 1252, 1132, 1046 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$ : given in Table 2. <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta_c$ : given in Table 3.

# 3.6. Preparation of (*R*)-MTPA ester (1b) and (*S*)-MTPA ester (1c) from 1a

A solution of **1a** (1.9 mg) in CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) was treated with (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA, 6.5 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 5.6 mg) and 4-dimethylaminopyridine (4-DMAP, 2.0 mg), and the mixture was heated under reflux for 15 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl, saturated aqueous NaHCO<sub>3</sub>, and brine, then dried over anhydrous MgSO<sub>4</sub> and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a pale yellow oil, which was purified by HPLC [Cosmosil 5C<sub>18</sub>-MS-II, MeOH–1% aqueous AcOH (70:30, v/v)] to give **1b** (1.3 mg, 43%). According to the similar procedure, **1c** (2.0 mg, 66%) was obtained from **1a** (1.9 mg) by using (S)-MTPA (6.5 mg), EDC·HCl (5.6 mg), and 4-DMAP (2.0 mg).

# 3.6.1. Compound 1b

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$ : given in Table 2.

### 3.6.2. Compound 1c

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$ : given in Table 2.

#### 3.7. Bioassay

# 3.7.1. Animals

Male ddY mice were purchased from Kiwa Laboratory Animal Co., Ltd, (Wakayama, Japan). The animals were housed at a constant temperature of  $23 \pm 2$  °C and were 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 a tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

#### 3.7.2. Effects on plasma glucose elevation in sucrose-loaded mice

The experiments were performed according to the method as described in our previous reports with a slight modification.<sup>3,30,31</sup> Thus, each test sample was administrated orally to the fasted male ddY mice (body weight 24–27 g), and 20% (w/v) sucrose solution (10 mL/kg, p.o.) was administrated 30 min thereafter. Blood samples (ca. 0.1 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 the blood samples, the plasma glucose level was determined enzymatically by the Glucose CII test Wako (Wako Pure Chemical Industries Ltd, Osaka, Japan). An intestinal  $\alpha$ -glucosidase inhibitor acarbose was used as a reference compound.

#### 3.7.3. Effect on gastric emptying in mice

The experiments were performed according to the method as described in our previous reports.<sup>3,30–37</sup> 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 latter, the mice were killed by cervical dislocation. The abdominal cavity was opened, the gastroesophageal junction and the pylorus were elamped, and then the stomach was removed, weighed, placed in 10 mL of 0.1 M NaOH, and homogenized. The suspension was allowed to settle for 1 h at room temperature, 1 mL of the supernatant was added to 0.1 mL of 20% (w/v) trichloroacetic acid, and then centrifuged at 3000 rpm for 20 min. The supernatant (0.1 mL) was mixed with 0.1 mL of 0.5 M NaOH, and the amount of the phenol red was determined on the basis of the optical density (OD) at 560 nm using a microplate reader (SH-1000 Lab., Corona Electric Co., Ltd). A phenol red solution (0.3 mL) was used as the standard (0% emptying). Test samples were given orally via a metal orogastric tube 30 min prior to the administration of the test meals. Gastric emptying rate (%) in the 30-min period was calculated according to the following equation:

### Gastric emptying rate (%)

= (1 – amount of the test sample/amount of the standard)  $\times$  100

## 3.7.4. Effects on glucose uptake in rat small intestinal tissues

The experiments were performed according to the method as described in our previous reports with a slight modification.<sup>38,39</sup> Thus, small fragments (0.10–0.15 g) of everted rat intestine were placed in 1 mL of modified Krebs–Henseleit solution, pH 7.4, with <sup>14</sup>C-U-glucose (2 mM,  $1 \times 10^5$  cpm/mL) with or without a test sample. Incubation was carried out at 30 °C for 6 min, then the pieces were washed two times for 3–5 s with the medium containing 1 mM phlorizin without <sup>14</sup>C-U-glucose, and placed on a filter paper to absorb the water from the tissue. The tissue was then weighed and dissolved using 2 M NaOH. After neutralization by 2 M HCl, the radioactivity was examined. Each test sample was dissolved in dimethyl sulfoxide (DMSO) and added to an incubation solution (final DMSO concentration was 0.5%). Inhibitions of the uptake by the vehicle only and 1 mM phrolizin were calculated to be 0% and 100%, respectively.

#### 3.7.5. Effects on rat intestinal $\alpha$ -glucosidase

The experiments were performed according to the method as described in our previous reports with a slight modification.<sup>18,27-</sup> <sup>30</sup> Thus, a rat small intestinal brush border membrane fraction was prepared and its suspension in 0.1 M maleate buffer (pH 6.0) was used to determine the small intestinal  $\alpha$ -glucosidase activity of maltase and sucrase. A mixture of a substrate (maltose: 37 mM, sucrose: 37 mM), a test compound, and the enzyme in 0.1 M maleate buffer (pH 6.0, 0.1 mL) were incubated 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 DMSO and the measurements were performed in duplicate, and IC<sub>50</sub> values were determined graphically. The concentration of each enzyme suspension was adjusted to produce ca. 0.30 and 0.15  $\mu mol/tube$  of <code>p-glucose</code> from the substrate maltose and sucrose, respectively. The intestinal  $\alpha$ glucosidase inhibitor acarbose was used as a reference compound.

### 3.7.6. Effects on rat lens aldose reductase

The experiments were performed according to the method as described in our previous reports.<sup>27,43</sup> Thus, the supernatant fluid

of a rat lens homogenate was used as a crude enzyme. The incubation mixture contained 135 mM phosphate buffer (pH 7.0), 100 mM Li<sub>2</sub>SO<sub>4</sub>, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100  $\mu$ L of enzyme fraction, with 25  $\mu$ L 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  $\mu$ L of 0.5 M HCl. Then, 0.5 mL of 6 M NaOH containing 10 mM imidazole was added, and the mixture was heated at 60 °C for 20 min to convert NADP into a fluorescent product. The fluorescence intensity 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 and the measurements were performed in duplicate, and IC<sub>50</sub> values were determined graphically. The concentration of enzyme suspension was adjusted to produce ca. 10 nmol/tube of β-nicotinamide adenine dinucleotide phosphate (NADP). An aldose reductase inhibitor epalrestat was used as a reference compound.

#### 3.8. Statistics

Values are expressed as means  $\pm$  S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis. Probability (*p*) values less than 0.05 were considered significant.

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