Bioactive Constituents from Chinese Natural Medicines. XXIV.[1] Hypoglycemic Effects of *Sinocrassula indica* in Sugar-Loaded Rats and Genetically Diabetic KK-A^y Mice and Structures of New Acylated Flavonol Glycosides, Sinocrassosides A_1, A_2, B_1, and B_2

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The methanolic extract from the whole plant of *Sinocrassula indica* (Crassulaceae) was found to inhibit the increase in serum glucose levels in oral administration of sucrose and glucose in rats at a dose of 250 mg/kg (*p.o.*). However, the extract did not inhibit the increase in serum glucose levels after intraperitoneal administration of glucose in these animals but did partly inhibit the gastric emptying. On the other hand, this extract significantly inhibited the increase in serum glucose levels after administration for 2 weeks in KK-A^y mice, a genetically type II diabetic mice, at a dose of 250 mg/kg/d (*p.o.*) without significant changes of the weights of body, liver, and visceral fat. From the extract, four new acylated flavonol glycosides, sinocrassosides A_1, A_2, B_1, and B_2, were isolated together with 11 flavonoids and 2 megastigmanes. The absolute stereostructures of the four new compounds were elucidated on the basis of chemical and physicochemical evidence.

Key words *Sinocrassula indica*; antidiabetic activity; type II diabetes; sinocrassoside; acylated flavonol glycoside; Crassulaceae

Sinocrassula indica (Crassulaceae) is a biennial plant distributed on the mountain areas in China (*e.g.*, Yunnan, Guangxi, Sichuan, Guizhou, and Hunan provinces). The whole plant of *S. indica* has been used for the treatment of hepatitis and otitis media in Chinese traditional medicine.[2] This plant also has been used as a vegetable and a herbal tea in Chinese local areas such as Guangxi province. However, chemical constituents from this herbal medicine were left uncharacterized. During the course of our studies on bioactive constituents from Chinese natural medicines,[1,3—18] we found that the methanolic extract of the whole plant of *S. indica* inhibited the increase in serum glucose levels in both sucrose- and glucose-loaded rats. From the methanolic extract, four new acylated flavonol glycosides, sinocrassosides A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**), were isolated together with 11 flavonoids (**5**—**15**) and 2 megastigmanes (**16**, **17**). This paper deals with antidiabetic activities of the methanolic extract from *S. indica* as well as the isolation of the constituents from this extract including the structure elucidation of **1**—**4**.

Hypoglycemic Effects of the Methanolic Extract from *S. indica* The dried whole plant of *S. indica* was extracted with methanol to give a methanolic extract (7.7% from the dried plant). As shown in Tables 1 and 2, the methanolic extract significantly inhibited the increase in serum glucose levels after oral administration of sucrose and glucose in rats at doses of 250 and 500 mg/kg (*p.o.*). However, the extract did not show significant effect on increase of these levels after intraperitoneal administration of glucose. These findings suggested that the absorption of glucose in the intestinal tract was suppressed by the extract.

The delay of sugar absorption after ingesting a meal has recently been found to be effective for type 2-diabetic patients, and the speed of gastric emptying is important in the regulation of glucose homeostasis.[19] The effect of the above extract on gastric emptying (GE) after administration of 10% glucose in rats was examined. As shown in Table 3, the inhibitory effect of the extract was observed, but it was weaker than the effects of lowering of blood glucose levels. These findings indicated that the extract inhibited not only gastric emptying but also glucose absorption *via* Na^+/glucose cotransporter,[20] although more detailed examination is needed.

The effect of the extract on serum glucose levels in KK-A^y mice, a genetically type II diabetic mice, was also examined. The extract (250 and 500 mg/kg/d, *p.o.*) significantly inhibited these levels and tended to reduce TG levels, but not serum T-Cho or FFA levels, after administration for 2 weeks under non-fasted conditions (Table 4).

On the other hand, serum glucose levels were lowered to normal (from *ca.* 530 to *ca.* 150 mg/dl) after the fasting, which was also reported previously,[21] and the extract did not affect blood glucose levels, body weight, weights of visceral fats (epididymal, mesenteric, and paranephric fats) and liver, TG, T-Cho, and FFA and L-TG or L-T-Cho levels in the fasted mice (Tables 5, 6). These results of experiments using diabetic mice as well as the results of experiments using rats described above suggested that inhibition or delay of sugar absorption is involved in the hypoglycemic effect of the extract, at least in part. The mechanism of action of the extract, however, requires further study.

Next, the methanolic extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (2.5%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (1.7%) and an H_2O-soluble fraction (3.4%). As shown in Table 7, the EtOAc- and *n*-BuOH-soluble fractions inhibited the increase in serum glucose levels in sucrose-loaded rats at a dose of 500 mg/kg (*p.o.*).

Isolation of Sinocrassosides (1—4) and Known Constituents To elucidate the constituents from the active fractions, the EtOAc- and *n*-BuOH-soluble fractions of the methanolic extract were subjected to normal- and reversed-phase column chromatographies, and finally to HPLC to give **1** (0.0013%), **2** (0.0012%), **3** (0.0009%), and **4** (0.0002%) together with 11 flavonoids, kaempferol[22] (**5**, 0.0026%),

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Table 1. Inhibitory Effects of the MeOH Extract from *S. indica* on Serum Glucose Levels in Sucrose-Loaded (*p.o.*) Rats[a)]

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1.0 h	2.0 h
Normal	—	5	56.2±2.0**	73.1±3.5**	68.8±4.5**
Control	—	5	166.3±3.7	149.1±7.2	121.3±8.2
MeOH ext.	125	5	146.8±12.2	147.3±13.0	123.7±5.8
	250	5	134.3±2.6*	140.5±13.4	115.7±16.2
	500	5	121.9±3.0*	132.0±4.4	117.1±2.7
Normal	—	6	81.4±2.8**	80.8±2.2**	84.7±2.4**
Control	—	7	169.6±6.7	138.6±3.7	118.5±3.0
Tolbutamide[36)]	12.5	6	152.6±2.8*	130.5±4.0	114.9±3.8
	25	6	138.1±3.5**	106.3±3.5**	99.5±2.1**

a) Each value represents the mean±S.E.M. Significantly different from the control: $*p<0.05$, $**p<0.01$.

Table 2. Inhibitory Effects of the MeOH Extract from *S. indica* on Serum Glucose Levels in Glucose-Loaded (*p.o.* or i.p.) Rats[a)]

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1.0 h	2.0 h
Glucose-loaded (0.5 g/kg, *p.o.*)					
Normal	—	6	80.0±3.3**	85.9±5.4**	86.4±2.6
Control	—	8	142.7±4.4	121.8±6.0	97.3±3.8
MeOH ext.	125	6	141.2±2.2	122.7±3.1	101.9±4.6
	250	6	125.3±5.5*	135.1±5.6	107.2±3.7
	500	6	94.2±3.7**	107.2±6.3	113.8±4.9
Glucose-loaded (0.5 g/kg, i.p.)					
Normal	—	6	76.2±3.9**	80.2±2.6**	75.0±2.1**
Control	—	7	122.8±6.0	113.1±4.0	102.6±3.8
MeOH ext.	125	6	129.6±4.6	126.6±3.3	103.5±3.0
	250	6	131.2±3.5	126.0±5.6	99.4±1.7
	500	6	123.1±9.8	112.2±7.6	99.5±5.7

a) Each value represents the mean±S.E.M. Significantly different from the control: $*p<0.05$, $**p<0.01$.

Table 3. Inhibitory Effect of the MeOH Extract from *S. indica* on Gastric Emptying (GE) in Rats[a)]

Treatment	Dose (mg/kg, *p.o.*)	*n*	GE (%)	Inhibition (%)
Control	—	7	61.8±1.6	—
MeOH ext.	125	6	49.3±2.6**	20.2
	250	6	44.0±1.1**	28.8
	500	6	46.2±2.8**	25.2

a) Each value represents the mean±S.E.M. Significantly different from the control: $**p<0.01$.

quercetin[22)] (**6**, 0.0046%), luteolin[22)] (**7**, 0.0020%), kaempferol 3-*O*-β-D-glucopyranoside[22)] (**8**, 0.0046%), quercetin 3-*O*-β-D-glucopyranoside[22)] (**9**, 0.0019%), kaempferol 7-*O*-α-L-rhamnopyranoside[23,24)] (**10**, 0.0070%), kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside[22)] (**11**, 0.021%), quercetin 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside[25)] (**12**, 0.0051%), multifloein B[26,27)] (**13**, 0.0052%), multinoside A[27,28)] (**14**, 0.0023%), and kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside[29)] (**15**, 0.0008%), two megastigmanes, *trans*-(3*S*,5*R*,6*R*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol 3-*O*-β-D-glucopyranoside[30)] (**16**, 0.0008%) and *trans*-(3*S*,5*R*,6*R*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol 9-*O*-β-D-glucopyranoside[30)] (**17**, 0.0018%), L-phenylalanine[31)] (0.0004%), and guanosine[31)] (0.0012%).

Absolute Stereostructures of Sinocrassosides A_1 (1), A_2 (2), B_1 (3), and B_2 (4) Sinocrassoside A_1 (**1**) was obtained as a yellow powder with negative optical rotation ($[\alpha]_D^{26}$ −26.3° in MeOH). The IR spectrum of **1** showed absorption bands at 1719, 1655, and 1597 cm^{-1} ascribable to ester carbonyl and γ-pyrone functions and broad bands at 3430 and 1026 cm^{-1}, suggestive of a glycoside structure. In the positive- and negative-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 503 $(M+H)^+$, *m/z* 525 $(M+Na)^+$, and *m/z* 501 $(M-H)^-$, and high-resolution FAB-MS analysis revealed the molecular formula of **1** to be $C_{25}H_{26}O_{11}$. The ^{1}H- (DMSO-d_6, Table 8) and ^{13}C-NMR (Table 9) spectra of **1**, which were assigned by various NMR experiments,[32)] showed signals assignable to two methyls [δ 1.16, 1.16 (each 3H, both d, J=7.0 Hz, 3‴, 4‴-H_3)], a methane [δ 2.63 (1H, qq, J=7.0 Hz, 2‴-H)], *meta*-coupled and *ortho*-coupled A_2B_2-type aromatic protons [δ 6.45, 6.86 (1H each, both d, J=1.9 Hz, 6, 8-H), 6.94, 8.10 (2H each, both d, J=8.9 Hz, 3′,5′, 2′,6′-H)], together with a rhamnopyranosyl moiety [δ 1.17 (3H, d, J=5.5 Hz, 6″-H_3), 5.59 (1H, br s, 1″-H)]. On alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v), kaempferol 7-*O*-α-L-rhamnopyranoside[23,24)] (**10**) was obtained together with isobutyric acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,3,5—7,10—13,15,17,18)] Comparison of the ^{13}C-NMR data for **1** with those for **10**[33)] revealed an acylation shift around the 3″-position of the rhamnopyranosyl

Table 4. Effects of the MeOH Extract from *S. indica* on Serum Glucose, Triglyceride (TG), Total Cholesterol (T-Cho), and Free Fatty Acids (FFA) Levels after Administration for 2 Weeks in KK-A^y Mice[a)]

Serum Glucose

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0 week	1 week	2 weeks
Control	—	11	379.2±28.4	459.3±28.1	527.5±23.8
MeOH ext.	250	10	351.4±24.9	374.1±37.0	404.5±46.1*
	500	10	359.6±25.0	382.2±33.8	374.7±32.2**
Troglitazone	100	10	367.9±23.9	329.5±26.4*	405.3±29.0*

Serum TG

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	Serum TG (mg/dl)	
			1 week	2 weeks
Control	—	11	339.3±14.2	524.8±21.4
MeOH ext.	250	10	347.1±22.7	451.1±39.2
	500	10	294.2±27.4	451.4±41.2
Troglitazone	100	10	287.5±19.0	429.5±50.3

Serum T-Cho

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	Serum T-Cho (mg/dl)	
			1 week	2 weeks
Control	—	11	142.1±6.0	165.0±7.5
MeOH ext.	250	10	155.5±7.6	172.5±10.0
	500	10	140.0±5.9	158.2±5.9
Troglitazone	100	10	155.5±7.2	166.5±8.3

Serum FFA

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	Serum FFA (mEq/l)	
			1 week	2 weeks
Control	—	11	3.0±0.6	2.9±0.7
MeOH ext.	250	10	3.2±0.8	2.8±0.6
	500	10	3.4±0.9	3.2±0.8
Troglitazone	100	10	2.3±0.6	2.7±0.7

a) Each value represents the mean±S.E.M. before fasting of mice. Significantly different from the control: $*p<0.05$, $**p<0.01$.

Table 5. Effects of the MeOH Extract from *S. indica* on Body Weight (BW), Visceral Fat, and Serum Glucose, Triglyceride (TG), Total Cholesterol (T-Cho), and Free Fatty Acids (FFA) Levels after Administration for 2 Weeks in KK-A^y Mice[a)]

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	BW (g)	Weight of visceral fat (mg)			Serum parameter			
				Epididymal fat	Mesenteric fat	Paranephric fat	Glucose (mg/dl)	TG (mg/dl)	T-Cho (mg/dl)	FFA (mEq/l)
Control	—	11	29.7±0.4	1056±46	754±50	434±49	154.3±7.0	172.5±21.7	135.6±4.2	0.9±0.1
MeOH ext.	250	10	29.5±1.0	1022±82	776±51	382±56	140.1±18.3	140.2±23.1	138.1±8.3	0.9±0.0
	500	10	29.6±0.7	920±50	662±43	336±62	117.4±5.5	178.6±15.6	133.9±6.9	1.0±0.1
Troglitazone	100	10	30.0±0.8	1106±80	740±75	528±27	155.4±13.1	170.7±8.6	136.9±13.3	0.6±0.1

a) Each value represents the mean±S.E.M. after fasting of mice.

moiety [**10**: δ_C 69.7 (2″-C), 69.9 (3″-C), 71.6 (4″-C); **1**: δ_C 67.2 (2″-C), 73.2 (3″-C), 68.6 (4″-C)]. Furthermore, in the heteronuclear multiple-bond correlations (HMBC) experiment of **1**, long-range correlations were observed between the following protons and carbons (1″-H and 7-C, 3″-H, 2‴-H and 1‴-C). Consequently, the position of isobutyryl ester moiety in **1** was determined to be the 3″-position and the structure of sinocrassoside A_1 was elucidated as kaempferol 7-*O*-α-L-(3″-isobutyryl)-rhamnopyranoside (**1**).

Sinocrassoside A_2 (**2**) was obtained as a yellow powder

Table 6. Effects of the MeOH Extract from *S. indica* on Liver Weight (LW), Liver Triglyceride (L-TG), and Total Cholesterol (L-T-Cho) Levels after Administration for 2 Weeks in KK-A^y Mice[a)]

Treatment	Dose (mg/kg/d, *p.o.*)	*n*	LW (mg)	L-TG (mg/g, wet tissue)	L-T-Cho (mg/g, wet tissue)
Control	—	11	1402±62	15.0±4.1	4.6±0.4
MeOH ext.	250	10	1316±57	12.6±1.4	4.3±0.4
	500	10	1302±57	12.6±1.1	3.8±0.2
Troglitazone	100	10	1762±99	15.0±2.9	3.7±0.2

a) Each value represents the mean±S.E.M. after fasting of mice.

Table 7. Inhibitory Effects of EtOAc, BuOH, and H_2O-Soluble Fractions of the MeOH Extract from *S. indica* on Serum Glucose Levels in Sucrose-Loaded (*p.o.*) Rats[a)]

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1.0 h	2.0 h
Normal	—	6	91.7±2.6**	95.1±3.1**	95.4±1.9**
Control	—	5	179.0±3.2	158.6±4.3	129.3±2.8
EtOAc-soluble fraction	500	5	112.8±6.8**	125.8±8.1*	122.9±10.5
BuOH-soluble fraction	500	5	113.9±5.7**	128.9±5.5*	134.7±6.3
H_2O-soluble fraction	500	5	177.2±4.2	175.4±8.0	152.6±3.9

a) Each value represents the mean±S.E.M. Significantly different from the control: * $p<0.05$, ** $p<0.01$.

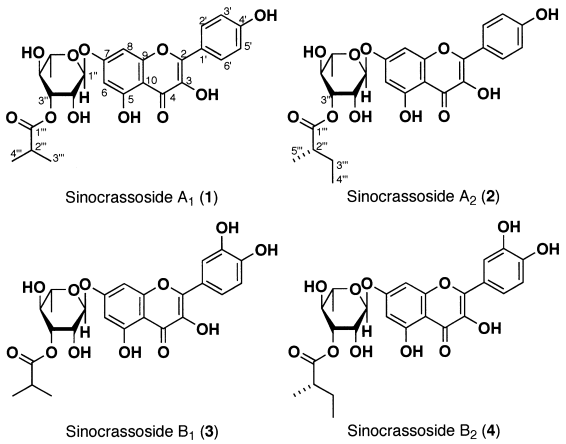

Chart 1

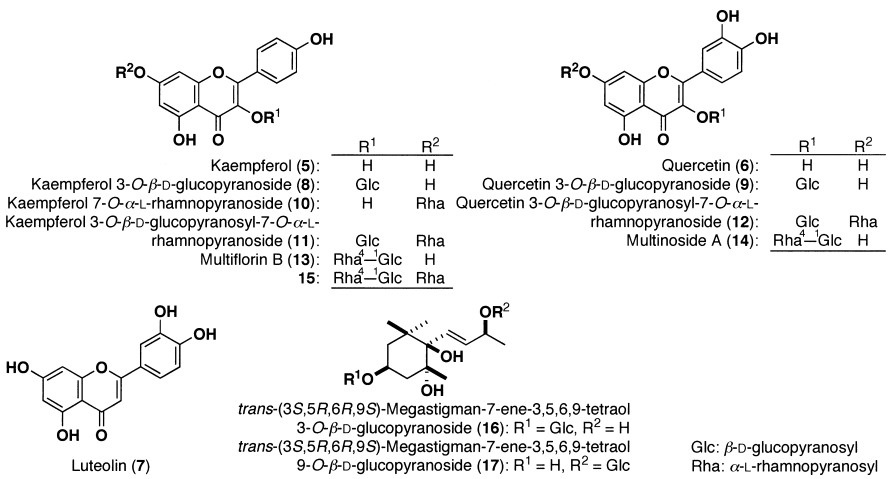

Chart 2

Table 8. ^{1}H-NMR Data (500 MHz) for Sinocrassosides A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**) in DMSO-d_6

Position	**1**	**2**	**3**	**4**
	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)
6	6.45 (d, 1.9)	6.44 (d, 2.2)	6.46 (d, 1.9)	6.43 (d, 2.1)
8	6.86 (d, 1.9)	6.86 (d, 2.2)	6.84 (d, 1.9)	6.81 (d, 2.1)
2′	8.10 (d, 8.9)	8.10 (d, 8.9)	7.71 (d, 1.9)	7.73 (d, 2.1)
3′	6.94 (d, 8.9)	6.94 (d, 8.9)		
5′	6.94 (d, 8.9)	6.94 (d, 8.9)	6.94 (d, 8.6)	6.90 (d, 8.6)
6′	8.10 (d, 8.9)	8.10 (d, 8.9)	7.63 (dd, 1.9, 8.6)	7.59 (dd, 2.1, 8.6)
5-OH	12.50 (br s)	12.49 (br s)	12.48 (br s)	12.46 (br s)
7-*O*-Rha				
1″	5.59 (br s)	5.59 (br s)	5.60 (br s)	5.57 (d, 1.7)
2″	4.04 (br s)	4.03 (br s)	4.07 (br s)	4.04 (br s)
3″	4.94 (dd, 3.4, 9.2)	4.96 (dd, 3.4, 9.5)	4.94 (dd, 3.4, 9.2)	4.96 (dd, 3.1, 9.2)
4″	3.56 (dd, 9.2, 9.5)	3.55 (dd, 9.2, 9.5)	3.59 (dd, 9.2, 9.5)	3.56 (dd, 9.2, 9.5)
5″	3.58 (qd, 5.5, 9.5)	3.58 (qd, 5.8, 9.2)	3.60 (qd, 6.2, 9.5)	3.59 (qd, 6.1, 9.5)
6″	1.17 (d, 5.5)	1.17 (d, 5.8)	1.19 (d, 6.2)	1.17 (d, 6.1)
Acyl				
2‴	2.63 (qq, 7.0, 7.0)	2.43 (qdd, 7.0, 7.0, 7.0)	2.63 (qq, 7.0, 7.0)	2.43 (qdd, 7.0, 7.0, 7.0)
3‴	1.16 (d, 7.0)	1.47 (qdd, 7.4, 7.0, 14.7)	1.16 (d, 7.0)	1.47 (qdd, 7.3, 7.0, 14.7)
		1.64 (qdd, 7.4, 7.0, 14.7)	1.16 (d, 7.0)	1.65 (qdd, 7.3, 7.0, 14.7)
4‴	1.16 (d, 7.0)	0.90 (dd, 7.4, 7.4)		0.90 (dd, 7.3, 7.3)
5‴		1.12 (d, 7.0)		1.12 (d, 7.0)

with negative optical rotation ($[\alpha]_D^{27}$ −78.8° in MeOH). The positive- and negative-ion FAB-MS of **2** showed quasimolecular ion peaks at m/z 539 $(M+Na)^+$ and m/z 515 $(M-H)^-$. High-resolution FAB-MS revealed the molecular formula of **2** to be $C_{26}H_{28}O_{11}$, and the IR spectrum showed absorption bands at 3410, 1720, 1655, 1597, and 1026 cm^{-1}, ascribable to hydroxyl, ester carbonyl, γ-pyrone, and ether functions. Alkaline hydrolysis of **2** with 10% KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) furnished **10**[23,24] together with (*S*)-2-methylbutyric acid, which was identified by HPLC analysis using an optical rotation detector.[34] The proton and carbon signals in the ^{1}H- (DMSO-d_6, Table 8) and ^{13}C-NMR (Table 9) spectra[32] of **2** were found to be similar to those of **1**, except for the signals due to an acyl group: [δ 0.90 (3H, dd, J=7.4, 7.4 Hz, 4‴-H_3), 1.12 (3H, d, J=7.0 Hz, 5‴-H_3), 1.47, 1.64 (1H each, both qdd, J=7.4, 7.0, 14.7 Hz, 3‴-H_2), 2.43 (1H, qdd, J=7.0, 7.0, 7.0 Hz, 2‴-H)]. The position of the acyl moiety in **2** was clarified by the HMBC experiments, in which long-range correlation was observed between the 3″-proton [δ 4.96 (1H, dd, J=3.4, 9.5 Hz)] and the 1‴-carbon (δ_C 175.5). Consequently, the structure of sinocrassoside A_2 was elucidated to be kaempferol 7-*O*-α-L-[3″-(*S*)-2-methylbutyryl]-rhamnopyranoside (**2**).

Sinocrassosides B_1 (**3**) and B_2 (**4**) were obtained as a yellow powder with negative optical rotations (**3**: $[\alpha]_D^{26}$ −21.8°; **4**: $[\alpha]_D^{25}$ −51.1°, both in MeOH). The molecular formulas of **3** ($C_{25}H_{26}O_{12}$) and **4** ($C_{26}H_{28}O_{12}$) were determined from the positive- and negative-ion FAB-MS and by high-resolution FAB-MS. Alkaline hydrolysis of **3** and **4** with 10% KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) gave quercetin 7-*O*-α-L-rhamnopyranoside (**3a**)[35] together with an organic acid, isobutyric acid from **3** and (*S*)-2-methylbutyric acid from **4**, which was identified by HPLC analysis, respectively.[1,3,5—7,10—13,15,17,18,34] The ^{1}H- (DMSO-d_6, Table 8) and ^{13}C-NMR (Table 9) spectra[32] of **3** and **4** indicated the presence of the following functions: a flavonol part [**3**: δ 6.46, 6.84 (1H each, both d, J=1.9 Hz, 6, 8-H), 6.94 (1H, d, J=8.6 Hz, 5′-H), 7.63 (1H, dd, J=1.9, 8.6 Hz, 6′-H), 7.71 (1H, d, J=1.9 Hz, 2′-H); **4**: 6.43, 6.81 (1H each, both d, J=2.1 Hz, 6, 8-H), 6.90 (1H, d, J=8.6 Hz, 5′-H), 7.59 (1H, dd, J=2.1, 8.6 Hz, 6′-H), 7.73 (1H, d, J=2.1 Hz, 2′-H)] and an α-L-rhamnopyranosyl moiety [**3**: δ 1.19 (3H, d, J=6.2 Hz, 6″-H_3), 5.60 (1H, br s, 1″-H); **4**: 1.17 (3H, d, J=6.1 Hz, 6″-H_3), 5.57 (1H, d, J=1.7 Hz, 1″-H)] together with an acyl

Table 9. ^{13}C-NMR Data (125 MHz) for Sinocrassosides A_1 (**1**), A_2 (**2**), B_1 (**3**), and B_2 (**4**) in DMSO-d_6

Position	**1** δ_C (mult.)	**2** δ_C (mult.)	**3** δ_C (mult.)	**4** δ_C (mult.)
2	147.5 (s)	147.5 (s)	147.8 (s)	147.9 (s)
3	136.0 (s)	136.0 (s)	136.0 (s)	136.1 (s)
4	176.0 (s)	176.0 (s)	176.5 (s)	175.9 (s)
5	160.4 (s)	160.4 (s)	160.3 (s)	160.4 (s)
6	98.7 (d)	98.7 (d)	98.8 (d)	98.8 (d)
7	161.0 (s)	161.0 (s)	161.0 (s)	161.1 (s)
8	94.4 (d)	94.4 (d)	94.4 (d)	94.3 (d)
9	155.6 (s)	155.6 (s)	155.6 (s)	155.7 (s)
10	104.8 (s)	104.8 (s)	104.7 (s)	104.8 (s)
1′	121.4 (s)	121.4 (s)	121.7 (s)	121.8 (s)
2′	129.6 (d)	129.6 (d)	115.0 (d)	115.2 (d)
3′	115.4 (d)	115.4 (d)	145.0 (s)	145.0 (s)
4′	159.3 (s)	159.3 (s)	147.5 (s)	147.6 (s)
5′	115.4 (d)	115.4 (d)	115.6 (d)	115.6 (d)
6′	129.6 (d)	129.6 (d)	120.3 (d)	120.1 (d)
7-*O*-Rha				
1″	98.1 (d)	98.1 (d)	98.0 (d)	98.3 (d)
2″	67.2 (d)	67.2 (d)	67.2 (d)	67.3 (d)
3″	73.2 (d)	73.1 (d)	73.1 (d)	73.1 (d)
4″	68.6 (d)	68.6 (d)	68.7 (d)	68.7 (d)
5″	70.0 (d)	70.1 (d)	70.0 (d)	70.1 (d)
6″	17.7 (q)	17.7 (q)	17.7 (q)	17.7 (q)
Acyl				
1‴	176.0 (s)	175.5 (s)	175.9 (s)	175.5 (s)
2‴	33.3 (d)	40.2 (d)	33.3 (d)	40.2 (d)
3‴	18.7 (q)	26.2 (t)	18.7 (q)	26.2 (t)
4‴	18.8 (q)	11.2 (q)	18.8 (q)	11.2 (q)
5‴		16.2 (q)		16.2 (q)

group {**3**: isobutyryl moiety [δ 1.16 (3H each, both d, J=7.0 Hz, 3‴, 4‴-H_3), 2.63 (1H, qq, J=7.0, 7.0 Hz, 2‴-H)], **4**: (*S*)-2-methylbutyryl moiety [δ 0.90 (3H, dd, J=7.3, 7.3 Hz, 4‴-H_3), 1.12 (3H, d, J=7.0 Hz, 5‴-H_3), 1.47, 1.65 (1H each, both qdd, J=7.3, 7.0, 14.7 Hz, 3‴-H_2), 2.43 (1H, qdd, J=7.0, 7.0, 7.0 Hz, 2‴-H)]}. In the HMBC experiment of **3**, long-range correlation was observed between the 3″-proton [δ 4.94 (1H, dd, J=3.4, 9.2 Hz)] and the 1‴-carbon (δ_C 175.9), while the long-range correlation in the HMBC experiment of **4** was observed between the 3″-proton [δ 4.96 (1H, dd, J=3.1, 9.2 Hz)] and the 1‴-carbon (δ_C 175.5). Thus, sinocrassosides B_1 and B_2 were elucidated as quercetin 7-*O*-α-L-(3″-isobutyryl)-rhamnopyranoside (**3**) and quercetin 7-*O*-α-L-[3″-(*S*)-2-methylbutyryl]-rhamnopyranoside (**4**), respectively.

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 high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

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, Ltd., Aichi, Japan, 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 whole plant of *S. indicum* cultivated in Guangxi province in China was purchased *via* Tochimoto Tenkaido Co., Ltd., Osaka, Japan. The plant material was identified by one of the authors (T. W.). A voucher specimen (2004.02. Guangxi-02) of this plant is on file in our laboratory.

Extraction and Isolation The dried whole plant of *S. indica* (9.9 kg) was extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (764 g, 7.7%), and an aliquot (725 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (237 g, 2.5%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (164 g, 1.7%) and an H_2O-soluble fraction (323 g, 3.4%).

The EtOAc-soluble fraction (100 g) was subjected to normal-phase silica gel column chromatography [2.5 kg, $CHCl_3$→$CHCl_3$–MeOH (9 : 1→8 : 2→7 : 3→6 : 4, v/v)→MeOH] to give eight fractions [Fr. 1 (13.2 g), Fr. 2 (5.8 g), Fr. 3 (4.2 g), Fr. 4 (48.2 g), Fr. 5 (5.3 g), Fr. 6 (14.2 g), Fr. 7 (4.7 g), and Fr. 8 (4.2 g)]. Fraction 4 (11.85 g) was subjected to reversed-phase silica gel column chromatography [350 g, H_2O→MeOH–H_2O (40 : 60→50 : 50→60 : 40→75 : 25→90 : 10, v/v)→MeOH] to afford seven fractions [Fr. 4-1 (1.63 g), Fr. 4-2 (0.36 g), Fr. 4-3 (0.26 g), Fr. 4-4 (0.29 g), Fr. 4-5 (1.13 g), Fr. 4-6 (2.00 g), and Fr. 4-7 (5.90 g)]. Fraction 4-2 (360 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give luteolin[22] (**7**, 20.2 mg, 0.0020%) and kaempferol[22] (**5**, 25.6 mg, 0.0026%). Fraction 4-3 (260 mg) was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give sinocrassosides A_1 (**1**, 12.6 mg, 0.0013%) and A_2 (**2**, 11.6 mg, 0.0012%). Fraction 5 (5.3 g) was subjected to reversed-phase silica gel column chromatography [180 g, H_2O→MeOH–H_2O (40 : 60→50 : 50→60 : 40→80 : 20→98 : 2, v/v)→MeOH] to afford six fractions [Fr. 5-1 (2.61 g), Fr. 5-2 (0.77 g), Fr. 5-3 (0.24 g), Fr. 5-4 (0.15 g), Fr. 5-5 (0.20 g), and Fr. 5-6 (1.31 g)]. Fraction 5-2 (770 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give sinocrassoside B_1 (**3**, 17.6 mg, 0.0009%) and quercetin[22] (**6**, 96.6 mg, 0.0046%). Fraction 5-3 (240 mg) was purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to give sinocrassoside B_2 (**4**, 7.1 mg, 0.0002%). Fraction 6 (14.2 g) was subjected to reversed-phase silica gel column chromatography [500 g, MeOH–H_2O (55 : 45→70 : 30→85 : 15→98 : 2, v/v)→MeOH] to afford five fractions [Fr. 6-1 (9.98 g), Fr. 6-2 (1.35 g), Fr. 6-3 (0.67 g), Fr. 6-4 (1.97 g), and Fr. 6-5 (0.22 g)]. Fraction 6-1 (1180 mg) was purified by HPLC [MeOH–H_2O (52 : 48, v/v)] to give kaempferol 3-*O*-β-D-glucopyranoside[22] (**8**, 14.0 mg, 0.0046%) and kaempferol 7-*O*-α-L-rhamnopyranoside[23,24] (**10**, 32.9 mg, 0.0070%). Fraction 7 (4.70 g) was subjected to reversed-phase silica gel column chromatography [140 g, MeOH–H_2O (55 : 45→70 : 30→85 : 15→98 : 2, v/v)→MeOH] to afford five fractions [Fr. 7-1 (3.44 g), Fr. 7-2 (0.34 g), Fr. 7-3 (0.27 g), Fr. 7-4 (0.31 g), and Fr. 7-5 (0.31 g)]. Fraction 7-1 (700 mg) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give quercetin 3-*O*-β-D-glucopyranoside[22] (**9**, 15.6 mg, 0.0019%), kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside[22] (**11**, 25.2 mg, 0.0031%), and multiflorin B[26,27] (**13**, 34.0 mg, 0.0042%).

The *n*-BuOH-soluble fraction (95.0 g) was subjected to normal-phase silica gel column chromatography [2.5 kg, $CHCl_3$–MeOH (9 : 1→8 : 2→7 : 3→6 : 4→5 : 5, v/v)→MeOH] to give eight fractions [Fr. 1 (7.1 g), Fr. 2 (31.6 g), Fr. 3 (5.3 g), Fr. 4 (17.4 g), Fr. 5 (12.5 g), Fr. 6 (5.1 g), Fr. 7 (11.3 g), and Fr. 8 (3.9 g)]. Fraction 4 (16.2 g) was subjected to reversed-phase silica gel column chromatography [490 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→50 : 50→60 : 40, v/v)→MeOH] to afford nine fractions [Fr. 4-1 (4.62 g), Fr. 4-2 (4.41 g), Fr. 4-3 (1.25 g), Fr. 4-4 (0.66 g), Fr. 4-5 (1.38 g), Fr. 4-6 (0.61 g), Fr. 4-7 (0.90 g), Fr. 4-8 (0.97 g), and Fr. 4-9 (1.10 g)]. Fraction 4-5 (1380 mg) was subjected to HPLC [MeOH–H_2O (45 : 55, v/v)] to give **11** (830.0 mg, 0.015%). Fraction 4-6 (610 mg) was purified by HPLC [MeOH–1% aqueous AcOH (40 : 60, v/v)] to give **11** (74.2 mg, 0.0015%), quercetin 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside[25] (**12**, 22.2 mg, 0.0004%), and **13** (48.4 mg, 0.0010%). Fraction 5 (12.0 g) was subjected to reversed-phase silica gel column chromatography [350 g, MeOH–H_2O (10 : 90→20 : 80→30 : 70→40 : 60, v/v)→MeOH] to afford ten fractions [Fr. 5-1 (3.69 g), Fr. 5-2 (0.40 g), Fr. 5-3 (0.45 g), Fr. 5-4 (0.71g), Fr. 5-5 (1.98 g), Fr. 5-6 (1.03 g), Fr. 5-7 (0.73 g), Fr. 5-8 (1.31 g), Fr. 5-9 (0.91 g), and Fr. 5-10 (0.78 g)]. Fraction 5-2 (400 mg) was purified by HPLC [MeOH–H_2O (5 : 95, v/v)] to give L-phenylalanine[31] (20.4 mg, 0.0004%) and guanosine[31] (60.8 mg, 0.0007%). Fraction 5-4 (510 mg) was further separated by HPLC [MeOH–H_2O (25 : 75, v/v)] to give *trans*-(3*S*,5*R*,6*R*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol 3-*O*-β-D-glucopyranoside[30] (**16**, 30.9 mg, 0.0008%) and *trans*-(3*S*,5*R*,6*R*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol 9-*O*-β-D-glucopyranoside[30] (**17**, 69.0 mg, 0.0018%). Fraction 5-6 (160 mg) was purified by HPLC [MeOH–H_2O (35 : 65, v/v)] to furnish **12** (40.9 mg, 0.0048%). Fraction 5-8 (300 mg) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to give **11** (17.9 mg, 0.0015%), **12** (35.3 mg, 0.0030%), multinoside A[27,28] (**14**, 27.6 mg, 0.0023%), and kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside[29] (**15**, 9.6 mg, 0.0008%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, UV, IR, MS, and ^{1}H- and ^{13}C-NMR) with reported values[22—30] or commercial samples.[31]

Sinocrassoside A_1 (**1**): A yellow powder, $[\alpha]_D^{26}$ −26.3° (c=0.60, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{25}H_{26}O_{11}Na$ $(M+Na)^+$ 525.1373; Found 525.1377. UV [λ_{max} (log ε), MeOH] 266 (4.20), 323 (3.99) nm. IR (KBr, cm^{-1}): 3430, 2962, 1719, 1655, 1597, 1026. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: given in Table 8. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 9. Positive-ion FAB-MS m/z: 503 $(M+H)^+$, 525 $(M+Na)^+$. Negative-ion FAB-MS m/z: 501 $(M-H)^-$.

Sinocrassoside A_2 (**2**): A yellow powder, $[\alpha]_D^{27}$ −78.8° (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{28}O_{11}Na$ $(M+Na)^+$ 539.1529; Found 539.1522. UV [λ_{max} (log ε), MeOH] 267 (4.14), 319 (4.10) nm. IR (KBr, cm^{-1}): 3410, 2962, 1720, 1655, 1597, 1026. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: given in Table 8. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 9. Positive-ion FAB-MS m/z: 539 $(M+Na)^+$. Negative-ion FAB-MS m/z: 515 $(M-H)^-$.

Sinocrassoside B_1 (**3**): A yellow powder, $[\alpha]_D^{26}$ −21.8° (c=1.20, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{25}H_{27}O_{12}$ $(M+H)^+$ 519.1503; Found 519.1509, Calcd for $C_{25}H_{26}O_{12}Na$ $(M+Na)^+$ 541.1322; Found 541.1328. UV [λ_{max} (log ε), MeOH] 256 (4.18) nm. IR (KBr, cm^{-1}): 3432, 2920, 1726, 1655, 1597, 1026. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: given in Table 8. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 9. Positive-ion FAB-MS m/z: 519 $(M+H)^+$, 541 $(M+Na)^+$. Negative-ion FAB-MS m/z: 517 $(M-H)^-$.

Sinocrassoside B_2 (**4**): A yellow powder, $[\alpha]_D^{25}$ −51.1° (c=0.50, MeOH).

High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{28}O_{12}Na$ $(M+Na)^+$ 555.1478; Found 555.1483. UV [λ_{max} (log ε), MeOH] 256 (4.22) nm. IR (KBr, cm^{-1}): 3432, 2936, 1719, 1655, 1597, 1059. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: given in Table 8. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 9. Positive-ion FAB-MS *m/z*: 533 $(M+H)^+$, 555 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 531 $(M-H)^-$.

Alkaline Hydrolysis of 1—4 A solution of **1**—**4** (each 5.0 mg) in 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v, 1.0 ml) was stirred at 37 °C for 1 h. Removal of the solvent under reduced pressure gave a reaction mixture. A part of the reaction mixture was dissolved in $(CH_2)_2Cl_2$ (2.0 ml) and the solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: CH_3OH–H_2O (70 : 30, v/v); detection: UV (254 nm); flow rate: 0.9 ml/min] to identify the *p*-nitrobenzyl esters of isobutyric acid (**a**, t_R 11.5 min) from **1** and **3** and 2-methylbutyric acid (**b**, t_R 17.5 min) from **2** and **4**. The rest of the reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product. A part of the product was subjected to HPLC analysis [column: YMC-Pack ODS-AQ, 250×4.6 mm i.d.; mobile phase: CH_3CN–0.1% aqueous H_3PO_4 (20 : 80, v/v); detection: optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; flow rate: 1.0 ml/min] to identify (*S*)-2-methylbutyric acid [**b**′, t_R 15.4 min (positive)] from **2** and **4**. The rest of the product was subjected to HPLC [MeOH–H_2O (50 : 50 v/v)] to give kaempferol 7-*O*-α-L-rhamnopyranoside[23,24] (**10**, 2.0 mg from **1**, 2.0 mg from **2**) and quercetin 7-*O*-α-L-rhamnopyranoside[35] (**3a**, 2.0 mg from **3**, 2.0 mg from **4**).

Bioassay Method. Animals Male Wistar rats (130—170 g) were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. Male KK-A^y mice (20—25 g) were purchased from CLEA Japan, Inc., Tokyo. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo). The rats were fasted for 24—26 h prior to the beginning of the experiment, but were allowed free access to tap water in sugar-loaded experiments. All of the experiments were performed with conscious rats and mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effects on Increase in Serum Glucose Levels in Surcose- or Glucose-Loaded Rats Effects of the methanolic extract and EtOAc-, *n*-BuOH-, and H_2O-soluble fractions from the whole plant of *S. indica* on increase in serum glucose levels in sugar-loaded rats were examined according to the method reported previously.[36—38] The test samples were suspended in 5% acacia solution (5 ml/kg), and then given orally to the rats. Thirty minutes thereafter, a water solution (5 ml/kg) of sucrose (1.0 g/kg) or glucose (0.5 g/kg) was administered orally (*p.o.*) or intraperitoneally (i.p.). Blood (*ca.* 0.4 ml) was collected from infraorbital venous plexus under ether anesthesia at 0.5, 1.0, and 2.0 h after administration of the sugar, and the serum glucose levels were determined by the glucose-oxidase method (Glucose CII-test Wako, Wako Pure Chemical Industries, Ltd.). Tolbutamide (Wako Pure Chemical Industries, Ltd.) was used as a reference compound.

Effect on Gastric Emptying in Rats Gastric emptying was determined by a modification of the phenol red method.[39] A solution of 10% glucose containing 0.05% phenol red as a marker was given orally (0.75 ml/rat) to rats. Thirty minutes later, the animals were sacrificed by cervical dislocation under ether anesthesia. The abdominal cavity was opened, and the gastroesophageal junction and pylous were clamped, then the stomach was removed, weighed and placed in 0.1 M NaOH (50 ml) and homogenized. The suspension was allowed to settle for 1 h at room temperature and 5 ml of the supernatant was added to 20% trichloroacetic acid (0.5 ml) and then centrifuged at 3000 rpm for 20 min. The supernatant (2 ml) was mixed with 0.5 M NaOH (2 ml), and the amount of phenol red was determined from the absorbance at 560 nm (Beckman, DU530). Phenol red recovered from animals sacrified immediately after the administration of a test meal was used as a standard (0% emptying). The gastric emptying (%) during the 30 min period was calculated by the following equation: Gastric emptying (%)=(1−amount of test sample/amount of standard)×100. The test sample was given orally by means of a metal orogastric tube 30 min prior to the administrating the test meal.

Hypoglycemic Effects of the Methanolic Extract in KK-A^y Mice Effects of the methanolic extract from the whole plant of *S. indica* on serum glucose levels in KK-A^y mice were examined according to the method reported previously with a modification.[40—43] Briefly, test sample suspended in 5% acacia solution and vehicle (5% acacia solution) were given orally to KK-A^y mice (5 weeks old) once a day (16:00—17:00) for 2 weeks. Blood glucose (*ca.* 0.2 ml) was collected from infraorbital venous plexus under ether anesthesia just before the experiment (0 d) and at the 7th and 14th day after the administration. Serum triglyceride (TG), serum total cholesterol (T-Cho), and serum free fatty acid (FFA) levels of non-fasted KK-A^y mice were determined using commercial kits (Triglyceride E-test Wako, Cholesterol E-test Wako, and NEFA C-test Wako, Wako Pure Chemical Industries, Ltd.). After final administration of the test sample, the mice were fasted for 20—24 h, and then a blood sample (*ca.* 0.4 ml) was collected and serum glucose, TG, T-Cho, and FFA were determined. In addition, body weight and weights of liver and visceral fat (epididymal, mesenteric, and paranephric fats) were measured. After removal of the liver, 200 mg of liver tissue was cut and homogenized with 10 ml of a mixture of chloroform and methanol (2 : 1, v/v). The homogenate was centrifuged (3000 rpm, 10 min), and 50 μl of supernatant was transferred to a test tube. The solvent was evaporated in a boiling water bath, and liver TG (L-TG) and liver T-Cho (L-T-Cho) were determined. Troglitazone was used as a reference compound.

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis.

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References and Notes

1) Part XXIII: Ninomiya K., Morikawa T., Zhang Y., Nakamura S., Matsuda H., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1185—1191 (2007).
2) Shanghai Scientific and Technologic Press, "Dictionary of Chinese Traditional Medicines," Shogakkan, Tokyo, 1985, pp. 364—365.
3) Matsuda H., Morikawa T., Tao J., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 208—215 (2002).
4) Morikawa T., Matsuda H., Toguchida I., Ueda K., Yoshikawa M., *J. Nat. Prod.*, **65**, 1468—1474 (2002).
5) Tao J., Morikawa T., Toguchida I., Ando S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 4005—4012 (2002).
6) Morikawa T., Tao J., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 638—645 (2003).
7) Tao J., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 654—662 (2003).
8) Matsuda H., Morikawa T., Xie H., Yoshikawa M., *Planta Med.*, **70**, 847—855 (2004).
9) Sun B., Morikawa T., Matsuda H., Tewtrakul S., Wu L. J., Harima S., Yoshikawa M., *J. Nat. Prod.*, **67**, 1464—1469 (2004).
10) Morikawa T., Sun B., Matsuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
11) Xie H., Wang T., Matsuda H., Morikawa T., Yoshikawa M., Tani T., *Chem. Pharm. Bull.*, **53**, 1416—1422 (2005).
12) Morikawa T., Xie H., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **69**, 881—886 (2006).
13) Morikawa T., Xie H., Matsuda H., Wang T., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 506—513 (2006).
14) Xie H., Morikawa T., Matsuda H., Nakamura S., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 669—675 (2006).
15) Yoshikawa M., Matsuda H., Morikawa T., Xie H., Nakamura S., Muraoka O., *Bioorg. Med. Chem.*, **14**, 7468—7475 (2006).
16) Matsuda H., Sugimoto S., Morikawa T., Kubo M., Nakamura S., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 106—110 (2007).
17) Yoshikawa M., Morikawa T., Zhang Y., Nakamura S., Muraoka O., Matsuda H., *J. Nat. Prod.*, **70**, 575—583 (2007).
18) Morikawa T., Zhang Y., Nakamura S., Matsuda H., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 435—441 (2007).
19) Matsuda H., Li Y., Yamahara J., Yoshikawa M., *J. Pharmacol. Exp. Ther.*, **289**, 729—734 (1999).
20) Asano T., Ogihara T., Katagiri H., Sakoda H., Ono H., Fujishiro M., Anai M., Kurihara H., Uchijima Y., *Curr. Med. Chem.*, **11**, 2717—2724 (2004).
21) Alberts P., Nilsson C., Selén G., Engblom L. O. M., Edling N. H. M., Norling S., Klingstrom G., Larsson C., Forsgren M., Ashkzari M., Nilsson C. E., Fiedler M., Bergqvist E., Öhman B., Björkstrand E., Abrahmsen L. B., *Endocrinology*, **144**, 4755—4762 (2003).
22) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahe-*

dron, **34**, 1389—1397 (1978).
23) Arisawa M., Minabe N., Saeki R., Takakuwa T., Nakaoki T., *Yakugaku Zasshi*, **91**, 522—524 (1971).
24) Murakami T., Kohno K., Kishi A., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 1673—1680 (2000).
25) Senatore F., D'Agostino M., Dini I., *J. Agric. Food Chem.*, **48**, 2659—2662 (2000).
26) Yamasaki K., Kasai R., Masaki Y., Okihara M., Tanaka O., *Tetrahedron Lett.*, **14**, 1231—1234 (1977).
27) Hasler A., Gross G.-A., Meier B., Sticher O., *Phytochemistry*, **31**, 1391—1394 (1992).
28) Seto T., Yasuda I., Akiyama K., *Chem. Pharm. Bull.*, **40**, 2080—2082 (1992).
29) Fang S.-H., Rao Y. K., Tzeng Y.-M., *Bioorg. Med. Chem.*, **13**, 2381—2388 (2005).
30) Otsuka H., Hirata E., Shinzato T., Takeda Y., *Phytochemistry*, **62**, 763—768 (2003).
31) Those known compounds were identified by comparison of their physical data with commercially obtained samples.
32) The ^{1}H- and ^{13}C-NMR spectra of **1**—**4** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), homocorrelation spectroscopy (^{1}H–^{1}H COSY), heteronuclear multiple quantum coherence (HMQC), and HMBC experiments.
33) Kaempferol 7-*O*-α-L-rhamnopyranoside (**10**): ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: 147.5 (C-2), 135.9 (C-3), 175.9 (C-4), 160.2 (C-5), 99.85 (C-6), 161.3 (C-7), 94.4 (C-8), 155.7 (C-9), 104.6 (C-10), 121.5 (C-1′), 129.6 (C-2′,6′), 115.5 (C-3′,5′), 159.2 (C-4′), 98.2 (C-1″), 69.7 (C-2″), 69.9 (C-3″), 71.6 (C-4″), 70.1 (C-5″), 17.8 (C-6″).
34) Morikawa T., Matsuda H., Ohgushi T., Nishida N., Ishiwada T., Yoshikawa M., *Heterocycles*, **63**, 2211—2215 (2004).
35) Awaad A. S., Maitland D. J., Soliman G. A., *Bioorg. Med. Chem. Lett.*, **16**, 4624—4628 (2006).
36) Yoshikawa M., Shimada H., Morikawa T., Yoshizumi S., Matsumura N., Murakami T., Matsuda H., Hori K., Yamahara J., *Chem. Pharm. Bull.*, **45**, 1300—1305 (1997).
37) Yoshikawa M., Morikawa T., Matsuda H., Tanabe G., Muraoka O., *Bioorg. Med. Chem.*, **10**, 1547—1554 (2002).
38) Yoshikawa M., Xu F., Morikawa T., Pongpiriyadacha Y., Nakamura S., Asao Y., Kumahara A., Matsuda H., *Chem. Pharm. Bull.*, **55**, 308—316 (2007).
39) Murakami T., Nakamura J., Kageura T., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 1720—1725 (2000).
40) Takeuchi H., Mooi L. Y., Inagaki Y., He P., *Biosci. Biotechnol. Biochem.*, **65**, 2318—2321 (2001).
41) Kuroda M., Mimaki Y., Nishiyama T., Mae T., Kishida H., Tsukagawa M., Takahashi K., Kawada T., Nakagawa K., Kitahara M., *Biol. Pharm. Bull.*, **28**, 937—939 (2005).
42) Miura T., Nosaka K., Ishii H., Ishida T., *Biol. Pharm. Bull.*, **28**, 2152—2154 (2005).
43) Miura T., Ueda N., Yamada K., Fukushima M., Ishida T., Kaneko T., Matsuyama F., Seino Y., *Biol. Pharm. Bull.*, **29**, 585—587 (2006).