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Medicinal Foodstuffs. VII.[1] On the Saponin Constituents with Glucose and Alcohol Absorption-Inhibitory Activity from a Food Garnish "Tonburi", the Fruit of Japanese *Kochia scoparia* (L.) SCHRAD.: Structures of Scoparianosides A, B, and C

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The methanolic extract of a food garnish "Tonburi", the fruit of Japanese *Kochia scoparia* (L.) SCHRAD. (Chenopodiaceae), was found to inhibit the increase in serum glucose in glucose-loaded rats. Through bioassay-guided separation, momordin Ic and its 2′-*O*-*β*-D-glucopyranoside were isolated as the active principles from this medicinal foodstuff together with three new saponins named scoparianosides A, B, and C. The structures of scoparianosides A, B, and C were elucidated on the basis of chemical and physicochemical evidence as 3*β*,22*α*-dihydroxyolean-12-en-28-oic acid (22*α*-hydroxyoleanolic acid), 3-*O*-*β*-D-xylopyranosyl (1→3)-*β*-D-glucopyranosiduronic acid, 3*β*-hydroxyolean-18-en-28-oic acid (morolic acid), 3-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid, and 3*β*-hydroxyolean-13(18)-en-28-oic acid, 3-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid.

Momordin Ic and its 2′-*O*-*β*-D-glucopyranoside, both of which are the principal saponin constituents of this medicinal foodstuff, were found to potently inhibit glucose and ethanol absorption in rats.

Key words scoparianoside; *Kochia scoparia*; glucose absorption inhibitor; alcohol absorption inhibitor; medicinal foodstuff; Tonburi

Kochia scoparia (L.) SCHRAD. (houkigi in Japanese, Chenopodiaceae), which is an annual herbaceous plant 50 to 150 cm tall, is cultivated in China, Korea, and Japan. The fruit of this plant is known as a Chinese natural medicine, Kochiae Fructus (Chinese name "地膚子"), which is listed as an upper grade (上薬) in Shen Nung's Herbal (神農本草経). Kochiae Fructus has been used as a tonic, diuretic, analgesic, and antidote and for the treatment of cutaneous pruritus in Chinese and Japanese traditional preparations. On the other hand, the fresh fruit of Japanese *Kochia scoparia*, which is commonly called "Tonburi" in Japanese, is used as a food garnish in Japanese-style dishes. In chemical studies on the constituents of *Kochia scoparia*, several phytoecdysteroids, saponins, and alkaloids were isolated from the seed, fruit, and aerial part of this plant.[2] Recently, we have found that the glycoside fraction of Kochiae Fructus, the fruit of Chinese *Kochia scoparia*, exerts an inhibitory effect on the cutaneous pruritus induced by Compound 48/80 or serotonin in mice.[3] Furthermore, we have isolated four saponins called kochianosides I, II, III, and IV from the glycoside fraction with antipruritogenic activity and elucidated their structure.[4]

As a part of our continuing studies on the bioactive constituents of medicinal foodstuffs,[1,5] the methanolic extract of the fruit of Japanese *Kochia scoparia* was found to inhibit the increase in serum glucose in glucose-loaded rats. By monitoring the inhibitory effect on the increase in serum glucose, we have isolated two principal saponins, momordin Ic (**4**) and its 2′-*O*-*β*-D-glucopyranoside (**5**), as the active principles of this medicinal foodstuff together with three new saponins called scoparianosides A (**1**), B (**2**), and C (**3**) and seven known saponins. This paper describes the structure elucidation of scoparianosides (**1**—**3**) and the inhibitory activity of major saponins (**4**—**7**) on the increase in serum glucose and on alcohol absorption.[6]

The fruit of *Kochia scoparia*, which is cultivated in Akita Prefecture, was extracted with methanol under reflux. The methanolic extract was found to inhibit the increase in serum glucose in glucose-loaded rats after a single oral administration of 500 mg/kg dose. The methanolic extract was partitioned in an ethyl acetate–water mixture to furnish the ethyl acetate-soluble fraction and the water phase. The water phase was further extracted with 1-butanol to give the 1-butanol-soluble fraction and the water-soluble fraction. Since the 1-butanol-soluble fraction (so-called glycoside fraction) inhibited the increase in serum glucose as shown in Table 1, it was subjected to ordinary silica-gel column chromatography to provide seven fractions (fr. 1—7), which were further separated by repeated HPLC to yield scoparianosides A (**1**, 0.0056% from the natural medicine), B (**2**, 0.0066%), and C (**3**, 0.0004%) together with momordins Ic[7] (**4**, 0.4530%) and IIc[7] (**6**, 0.1366%), 2′-*O*-*β*-D-glucopyranosyl momordin Ic[8] (**5**, 0.1635%), 2′-*O*-*β*-D-glucopyranosyl momordin IIc[8] (**7**, 0.0244%), kochianosides I[4] (0.0005%), II[4] (0.0021%), III[4] (0.0003%), and IV[4] (0.0003%), and oleanolic acid 3-*O*-glucuronide[9] (0.0006%).

Structures of Scoparianosides A (1), B (2), and C (3) Scoparianoside A (**1**) was isolated as colorless fine crystals of mp 204—206 °C from chloroform–methanol solution. In the IR spectrum of **1**, there were absorption bands at 1726 and 1719 cm^{-1} ascribable to a carboxyl

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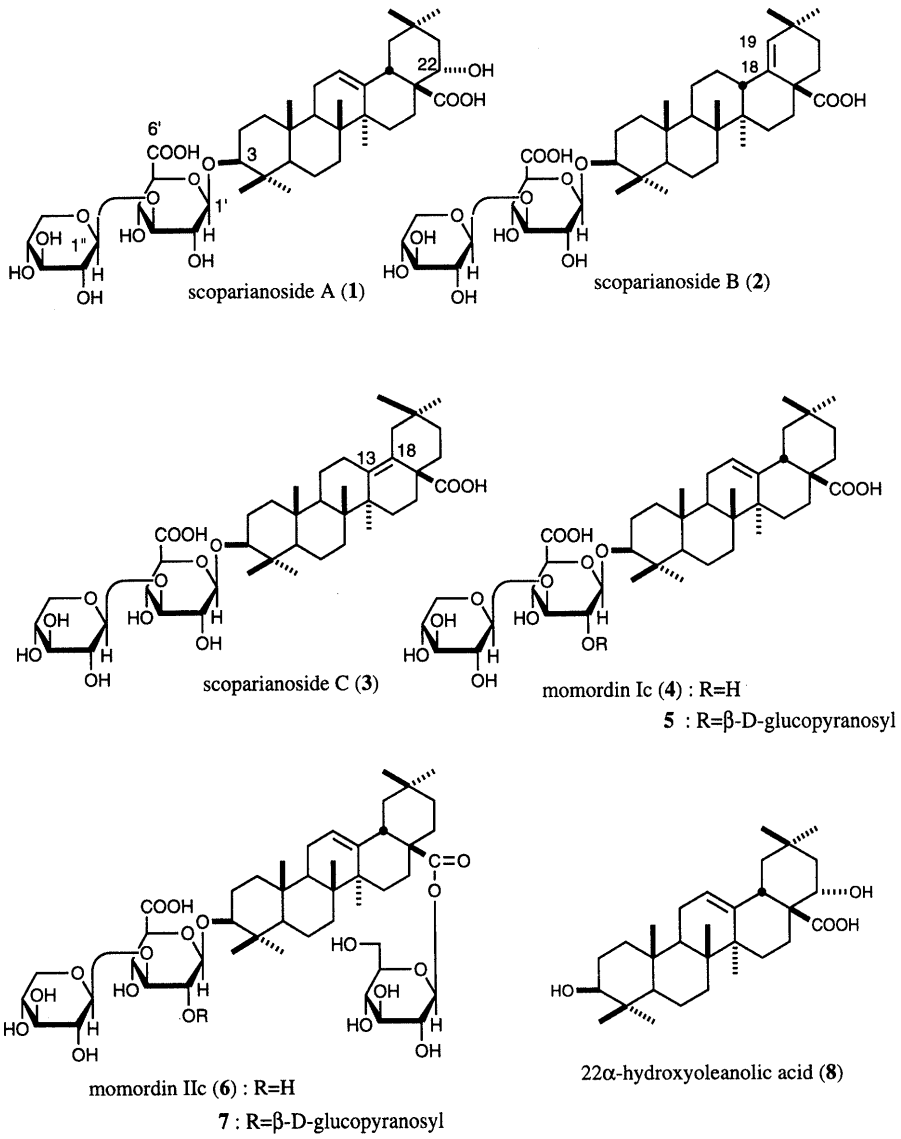

Chart 1

group and strong bands at 3438 and 1036 cm^{-1} suggestive of a glycosidic structure.

The molecular formula $C_{41}H_{64}O_{14}$ was determined from the positive-ion and negative-ion FAB-MS and by high-resolution FAB-MS measurement. Thus, in the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 803 $(M+Na)^+$, while the negative-ion FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 779 $(M-H)^-$. Furthermore, fragment ion peaks at *m/z* 647 $(M-C_5H_9O_4)^-$ and *m/z* 471 $(M-C_{11}H_{17}O_{10})^-$, which were derived by cleavage of the glycosidic linkage at the 3′ and 3-positions, were observed in the negative-ion FAB-MS of **1**. Acid hydrolysis of **1** with 5% sulfuric acid furnished D-glucuronic acid (D-glucuronolactone) and D-xylose, which were identified by GLC analysis of their thiazolidine derivatives.[10] Enzymatic hydrolysis of **1** with glycyrrhizinic acid hydrolase[11] liberated a new triterpene 22α-hydroxyoleanolic acid (**8**). The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[12] of **8** showed signals assignable to an olefin proton [δ 5.51 (dd, J=3.6, 3.6 Hz, 12-H)], and two axial methine protons bearing a hydroxyl group [δ 3.45 (dd, J=5.8, 10.3 Hz, 3-H), 4.58 (dd, J=6.1, 10.4 Hz, 22-H)] together with seven tertiary methyls, nine methylenes, and three methines. The position of two hydroxyl groups was clarified by a heteronuclear multiple bond connectivity (HMBC) experiment (J_{C-H}=8 Hz), which showed long-range correlations between the protons and carbons shown

Table 1. Inhibitory Effect of the Methanolic Extract and Fractions from the Fruit of *Kochia scoparia* on the Increase in Serum Glucose in Glucose-Loaded Rats

	Dose (mg/kg, *p.o.*)	*n*	Serum glucose concentration (mg/dl) 0.5 h
Control (normal)	—	5	65.0± 2.6**
Control (glucose-loaded)	—	6	171.0± 4.6
MeOH ext.	500	5	92.9± 0.8**
AcOEt fraction	200	5	132.4± 9.2**
BuOH fraction (glycoside fraction)	200	5	99.9± 6.1**
H_2O fraction	200	5	179.8±10.6

** $p<0.01$.

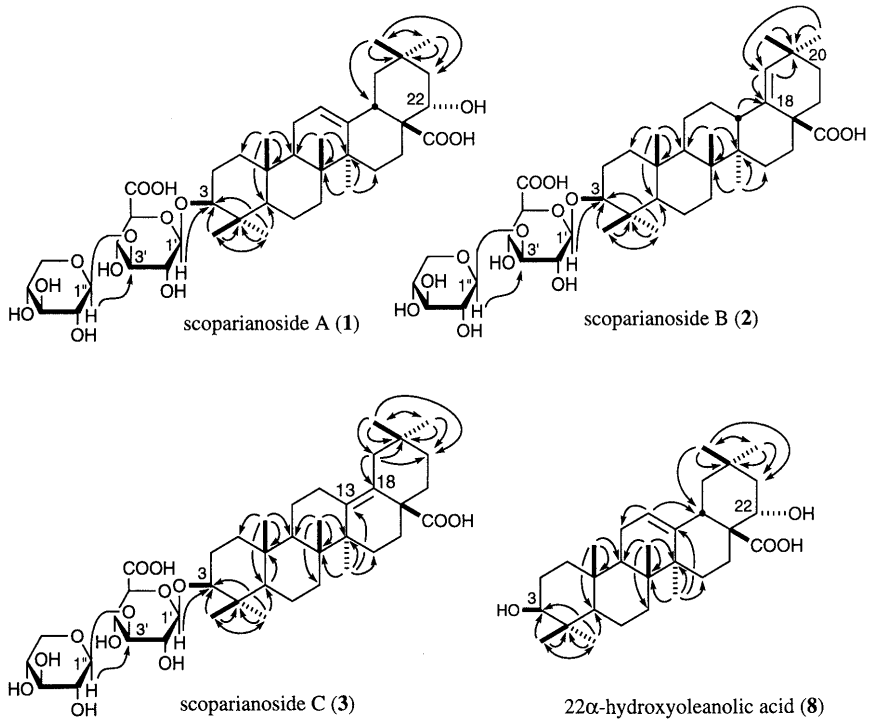

Fig. 1. Long-Range Correlations in the HMBC of **1**, **2**, and **3**

in Fig. 1. Furthermore, in the rotating frame NOE spectroscopy (ROESY) experiment involving **8**, nuclear Overhauser effect (NOE) correlations were observed between the 22β-proton and the 30-methyl protons and between the 30-methyl protons and the 18β-proton. Comparison of the NMR data for **8** with those for 22α-hydroxyhederagenin and 22β-hydroxyoleanene triterpene derivatives[13] led us to formulate the structure of **8**. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[12] of **1** indicated the presence of a 22α-hydroxyoleanolic acid moiety, a β-D-glucopyranosiduronic acid moiety [δ 5.05 (d, J=7.6 Hz, 1′-H)], and a β-D-xylopyranosyl moiety [δ 5.38 (d, J=7.2 Hz, 1″-H)]. In the HMBC experiment involving **1**, long-range correlations were observed between the 1″-proton and the 3′-carbon and between the 1′-proton and the 3-carbon. The carbon signals due to the oligosaccharide moiety in the ^{13}C-NMR spectrum of **1** were found to be superimposable on those of momordin Ic (**4**). On the basis of above mentioned evidence, the structure of scoparianoside A was formulated as 22α-hydroxyolean-12-en-28-oic acid 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid (**1**).

Scoparianoside B (**2**) was also isolated as colorless fine crystals of mp 211—214 °C and its IR spectrum showed absorption bands due to hydroxyl and carboxyl groups at 3475, 1726, 1701, and 1024 cm^{-1}. In the positive-ion FAB-MS of **2**, a quasimolecular ion peak was observed at m/z 787 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at m/z 763 $(M-H)^-$ in addition to fragment ion peaks at m/z 631 $(M-C_5H_9O_4)^-$ and m/z 455 $(M-C_{11}H_{17}O_{10})^-$. On acid hydrolysis of **2** followed by GLC analysis of the thiazolidine derivatives,[10] D-glucuronic acid (D-glucuronolactone) and D-xylose were identified. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[12] of **2** showed signals due to a sapogenol moiety [δ 0.75, 0.95, 0.98, 1.00, 1.06, 1.12, 1.26 (all s, 25, 24, 27, 26, 30, 29, 23-H$_3$), 2.68 (d-like, 13-H), 3.36 (dd, J=4.5, 11.9 Hz, 3-H), 5.28 (br s, 19-H)], a β-D-glucopyranosyl moiety [δ 4.97 (d, J=7.6 Hz, 1′-H)], and a β-D-xylopyranosyl moiety [δ 5.27 (d, J=7.3 Hz, 1″-H)]. The carbon signals of the disaccharide moiety in the ^{13}C-NMR (Table 2) spectrum of **2** were superimposable on those of **1** and **4**, whereas the carbon signals due to the sapogenol moiety of **2** were very similar to those of morolic acid glycosides.[4,14] The HMBC experiment involving **2** showed long-range correlations between the following protons and carbons (1″-H and 3′-C, 1′-H, 3-C, 30-H$_3$ and 19, 20-C, 29-H$_3$ and 19, 20-C, 19-H and 18, 20-C) shown in Fig 1. Consequently, the structure of scoparianoside B was characterized as morolic acid 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid (**2**).

Scoparianoside C (**3**), obtained as colorless fine crystals of mp 207—209 °C, showed absorption bands associated with the hydroxyl group and carboxyl groups in the IR spectrum. Here again, the molecular formula $C_{41}H_{64}O_{13}$, which was identical with that of **2**, was determined from its positive-ion FAB-MS [m/z 787 $(M+Na)^+$] and negative-ion FAB-MS [m/z 763 $(M-H)^-$, 631 $(M-C_5H_9O_4)^-$, and 455 $(M-C_{11}H_{17}O_{10})^-$] and by high-resolution MS measurement. Acid hydrolysis of **3** liberated D-glucuronic acid (D-glucuronolactone) and D-xylose. The ^{1}H-NMR (pyridine-d_5) spectrum of **3** showed signals due to a sapogenol moiety [δ 0.81, 0.86, 1.11 (3H each, all s, 25, 29, 26-H$_3$), 0.96, 1.30 (6H each, both s, 24, 30, 23, 27-H$_3$), 3.41 (dd, J=5.2, 12.2 Hz, 3-H)], a β-D-glucopyranosiduronic acid moiety [δ 5.02 (d, J=7.9 Hz, 1′-H)], and a β-D-xylopyranosyl moiety [δ 5.31 (d, J=7.3 Hz, 1″-H)]. The carbon signals due to the sapogenol

moiety in the ^{13}C-NMR (Table 2) of **3** resembled those of a 3β-hydroxyolean-13(18)-en-28-oic acid derivative,[15] whereas the carbon signals due to the oligoglycoside moiety were superimposable on those of **1**, **2**, and **4**. The HMBC experiment involving **3** showed long-range correlations between the following protons and carbons (1″-H and 3′-C, 1′-H and 3-C, 12-H_2 and 13-C, 19-H_2 and 18-C) as shown in Fig 1. Based on those findings, the structure of scoparianoside C was elucidated as a 3β-hydroxyolean-13(18)-en-28-oic acid 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid (**3**).

Table 2. ^{13}C-NMR Data for Scoparianosides A (**1**), B (**2**), and C (**3**) and 22α-Hydroxyoleanolic Acid (**8**) (125 MHz, pyridine-d_5)

	1	8	2	3
C-1	38.4	39.0	39.1	39.1
C-2	26.4	28.1	26.8	26.8
C-3	89.1	78.1	89.3	89.4
C-4	39.4	39.4	39.7	39.6
C-5	55.5	55.8	56.0	55.9
C-6	18.3	18.8	18.4	18.5
C-7	32.9	33.2	35.0	35.5
C-8	39.8	40.0	41.1	41.9
C-9	47.8	48.1	51.4	51.1
C-10	36.8	37.4	37.1	37.3
C-11	23.6	23.9	21.3	28.1
C-12	122.7	122.9	26.5	36.5
C-13	144.0	144.2	41.6	137.9
C-14	42.4	42.6	43.1	44.9
C-15	27.8	28.0	30.0	27.9
C-16	16.8	16.9	34.4	33.7
C-17	53.0	53.1	48.6	48.9
C-18	43.4	43.6	139.0	129.4
C-19	45.8	46.1	132.1	41.6
C-20	31.4	31.6	32.4	33.0
C-21	43.1	43.3	34.2	37.5
C-22	71.3	71.5	34.2	22.1
C-23	27.9	28.8	28.0	28.1
C-24	16.8	16.6	16.8	16.9
C-25	15.2	15.5	16.8	16.6
C-26	17.2	17.4	16.3	18.1
C-27	26.6	26.8	15.3	21.3
C-28	179.3	179.4	179.0	179.0
C-29	33.3	33.4	30.8	24.5
C-30	25.0	25.2	29.4	32.4
GlcA-1	106.8		106.8	106.9
2	74.5		74.6	74.6
3	86.3		86.6	86.6
4	71.4		71.4	71.4
5	77.4		77.5	77.5
6	172.1		172.0	172.1
Xyl-1	106.1		106.2	106.2
2	75.2		75.2	75.3
3	77.9		78.0	78.1
4	70.8		70.9	71.0
5	67.2		67.3	67.4

Inhibitory Effect of the Major Saponins (4, 5, 6, 7) from "Tonburi" on the Increase in Serum Glucose in Glucose-Loaded Rats Since the glycoside fraction inhibited the increase in serum glucose in glucose-loaded rats, we examined the inhibitory activity of the major saponins (**4**, **5**, **6**, **7**) isolated from the glycoside fraction of "Tonburi". As is apparent from Table 3, momordin Ic (**4**) and its 2′-*O*-β-D-glucopyranoside (**5**), both of which possessed the 28-carboxyl group and the 3-glucuronide moiety required for activity,[5a,16] showed potent inhibitory activity against the increase in serum glucose in glucose-loaded rats after a single oral dose of 100 mg/kg. On the other hand, the 3 and 28-bisdesmoside-type saponins, momordin IIc (**6**) and its 2′-*O*-β-D-glucopyranoside (**7**), lacked this activity.

Inhibitory Effect of the Major Saponins (4, 5, 6, 7) from "Tonburi" on Ethanol Absorption Previously, we have found that the saponins with hypoglycemic activity also inhibited ethanol absorption.[17] Now, we examined the inhibitory effect of the major saponins from "Tonburi" on ethanol absorption in rats. As shown in Table 4, momordin Ic (**4**) and its 2′-*O*-β-D-glucopyranoside (**5**) inhibited ethanol absorption, while momordin IIc (**6**) and its 2′-*O*-β-D-glucopyranoside (**7**) did not.

Experimental

The instruments used for obtaining the physical data and the experimental conditions for chromatography were as described in our previous paper.[1]

Isolation of the Saponin Constituents from the Fruit of Japanese *Kochia scoparia* The fruit of *Kochia scoparia* cultivated in Hinai-cho, Akita Prefecture (5 kg, obtained from the Akita Research Institute of Food and Brewing in 1995) was finely divided and extracted three times with methanol under reflux. Evaporation of the solvent from the extract under reduced pressure furnished the methanol extract (500 g). This extract (425 g) was partitioned in an AcOEt–H_2O (1 : 1) mixture. The aqueous layer was further extracted with 1-butanol. Removal of the solvent from

Table 3. Inhibitory Effect of Momordins Ic (**4**) and IIc (**6**) and Their 2′-*O*-β-D-Glucopyranosides (**5**, **7**) on the Increase in Serum Glucose in Glucose-Loaded Rats

	Dose (mg/kg, *p.o.*)	*n*	Serum glucose concentration (mg/dl)		
			0.5 h	1.0 h	2.0 h
Control (normal)	—	5	87.1±4.6**	101.6±7.7**	94.8±9.3*
Control (glucose-loaded)	—	7	149.7±5.2	137.2±4.8	115.0±3.1
			(62.6±5.2)	(35.6±4.8)	(20.2±3.1)
Momordin Ic (**4**)	100	5	98.9±2.0**	131.7±3.0	125.3±4.0
			(11.8±2.0**)	(30.1±3.0)	(30.5±4.0)
2′-*O*-β-D-Glucopyranosyl momordin Ic (**5**)	100	5	108.8±7.0**	119.7±6.1	110.6±4.0
			(21.7±7.0**)	(18.1±6.1)	(15.8±4.0)
Momordin IIc (**6**)	100	5	147.6±6.9	145.9±4.4	108.9±6.1
			(60.5±6.9)	(44.3±4.4)	(14.1±6.1)
2′-*O*-β-D-Glucopyranosyl momordin IIc (**7**)	100	5	137.4±8.2	142.5±4.1	113.1±4.4
			(50.3±8.2)	(40.9±4.1)	(18.3±4.4)

* $p<0.05$, ** $p<0.01$.

Table 4. Inhibitory Effect of Momordins Ic (**4**) and IIc (**6**) and Their 2′-*O*-*β*-D-Glucopyranosides (**5**, **7**) on the Ethanol Absorption

	Dose (mg/kg, *p.o.*)	*n*	Ethanol concentration in blood (mg/dl)		
			0.5 h	1.0 h	2.0 h
Control	—	5	0.693±0.067	0.625±0.079	0.207±0.027
Momordin Ic (**4**)	25	5	0.123±0.047**	0.222±0.044**	0.092±0.049
	50	4	0.062±0.031**	0.217±0.147**	0.075±0.027
	100	4	0.089±0.032**	0.122±0.035**	0.049±0.032
2′-*O*-*β*-D-Glucopyranosyl momordin Ic (**5**)	100	5	0.136±0.054**	0.250±0.049**	0.196±0.058
Momordin IIc (**6**)	100	5	0.611±0.120	0.602±0.047	0.202±0.055
2′-*O*-*β*-D-Glucopyranosyl momordin IIc (**7**)	100	5	0.612±0.121	0.529±0.049	0.215±0.045

** $p<0.01$.

the AcOEt-soluble, 1-butanol-soluble, and H_2O-soluble fractions under reduced pressure yielded the AcOEt extract (43 g), 1-butanol extract (180 g), and H_2O extract (200 g).

The 1-butanol extract (120 g) was subjected to ordinary-phase silica-gel column chromatography [2 kg, $CHCl_3$–MeOH–H_2O (7:3:1, lower phase-6:4:1)] and repeated HPLC (YMC-pack ODS, MeOH–AcOH) to provide scoparianosides A (**1**, 0.0056% from the natural medicine), B (**2**, 0.0066%), and C (**3**, 0.0004%), momordins Ic (**4**, 0.4530%) and IIc (**6**, 0.1366%), 2′-*O*-glucopyranosyl momordin Ic (**5**, 0.1635%), 2′-*O*-glucopyranosyl momordin IIc (**7**, 0.0244%), kochianosides I (0.0005%), II (0.0021%), III (0.0003%), and VI (0.0003%) and oleanolic acid 3-*O*-glucuronide (0.0006%). Nine known saponins were identified on the basis of the HPTLC, HPLC, ^{1}H- and ^{13}C-NMR spectra comparisons with authentic samples.[4)]

Scoparianoside A (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 204—206 °C, $[\alpha]_D^{24}$ +17.1° (c=1.7, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{64}O_{14}Na$ $(M+Na)^+$: 803.4194; Found: 803.4196. IR (KBr, cm^{-1}): 3438, 2926, 2887, 1726, 1719, 1655, 1036. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.79, 0.99, 1.03, 1.03, 1.13, 1.32, 1.38 (3H each, all s, 25, 24, 26, 29, 30, 23, 27-H$_3$), 3.36 (2H, m, 3, 18-H), 4.57 (1H, m, 22-H), 5.05 (1H, d, J=7.6 Hz, 1′-H), 5.38 (1H, d, J=7.2 Hz, 1″-H), 5.47 (1H, br s, 12-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS (m/z): 803 $(M+Na)^+$. Negative-ion FAB-MS (m/z): 779 $(M-H)^-$, 647 $(M-C_5H_9O_4)^-$, 471 $(M-C_{11}H_{17}O_{10})^-$.

Scoparianoside B (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 211—214 °C, $[\alpha]_D^{25}$ +5.1° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{64}O_{13}Na$ $(M+Na)^+$: 787.4244; Found: 787.4263. IR (KBr, cm^{-1}): 3475, 2944, 2867, 1726, 1701, 1655, 1024. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.75, 0.95, 0.98, 1.00, 1.06, 1.12, 1.26 (3H each, all s, 25, 24, 27, 26, 30, 29, 23-H$_3$), 2.68 (1H, d-like, 13-H), 3.36 (1H, dd, J=4.5, 11.9 Hz, 3-H), 4.97 (1H, d, J=7.6 Hz, 1′-H), 5.27 (1H, d, J=7.3 Hz, 1″-H), 5.28 (1H, br s, 19-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS (m/z) : 787 $(M+Na)^+$. Negative-ion FAB-MS (m/z): 763 $(M-H)^-$, 631 $(M-C_5H_9O_4)^-$, 455 $(M-C_{11}H_{17}O_{10})^-$.

Scoparianoside C (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 207—209 °C, $[\alpha]_D^{27}$ −7.7° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{64}O_{13}Na$ $(M+Na)^+$: 787.4245; Found: 787.4229. IR (KBr, cm^{-1}): 3456, 2940, 2908, 1736, 1719, 1655, 1038. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.81, 0.86, 1.11 (3H each, all s, 25, 29, 26-H$_3$), 0.96, 1.30 (6H each, both s, 24, 30, 23, 27-H$_3$), 3.41 (1H, dd, J=5.2, 12.2 Hz, 3-H), 5.02 (1H, d, J=7.9 Hz, 1′-H), 5.31 (1H, d, J=7.3 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS (m/z): 787 $(M+Na)^+$. Negative-ion FAB-MS (m/z): 763 $(M-H)^-$, 631 $(M-C_5H_9O_4)^-$, 455 $(M-C_{11}H_{17}O_{10})^-$.

Acid Hydrolysis of Scoparianosides A (1), B (2), and C (3) A solution of scoparianosides (**1**, **2**, **3**, 2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1:1, v/v, 1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the insoluble portion was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was separated on a Sep-Pack C18 cartridge column (H_2O, MeOH). The H_2O eluate was concentrated under reduced pressure to give a residue, which was treated with L-cystein methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) and the mixture was left standing at 60 °C for 1 h. The reaction solution was then treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) and the whole mixture was left standing at 60 °C for 1 h. The supernatant of the reaction mixture was subjected to GLC analysis to identify the thiazolidene derivatives of D-glucuronic acid (**i**) and D-xylose (**ii**). GLC conditions: column, Supelco SPB™-1, 0.25 mm(i.d.)×30 m; column temperature, 230 °C. t_R: **i**, 26.7 min; **ii**, 19.3 min.

Enzymatic Hydrolysis of Scoparianoside A (1) Giving 22-α-Hydroxyoleanolic Acid (8) A solution of **1** (9.8 mg) in 0.1 M acetate buffer (pH 4.4, 1.0 ml) was treated with glycyrrhizinic acid hydrolase (1.0 mg) and the solution was stirred at 44 °C for 3 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by ordinary-phase silica-gel column chromatography (1.0 g, $CHCl_3$: MeOH = 10 : 1) to give **8** (5.3 mg)

22α-hydroxyoleanolic Acid (**8**): Colorless fine crystals (MeOH), mp 271—273 °C, $[\alpha]_D^{24}$ +63.5° (c=0.3, pyridine). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{48}O_4Na$ $(M+Na)^+$: 495.3450; Found: 495.3437. IR (KBr, cm^{-1}): 3631, 3453, 2962, 1705, 1655. ^{1}H-NMR (pyridine-d_5) δ: 0.90, 1.02, 1.03, 1.08, 1.13, 1.25, 1.34 (3H each, all s, 25, 29, 24, 26, 30, 23, 27-H$_3$), 3.37 (1H, dd, J=3.7, 12.9 Hz, 18-H), 3.45 (1H, dd, J=5.8, 10.3 Hz, 3-H), 4.58 (1H, dd, J=6.1, 10.4 Hz, 22-H), 5.51 (1H, dd, J=3.6, 3.6 Hz, 12-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS (m/z): 495 $(M+Na)^+$. Negative-ion FAB-MS (m/z) : 471 $(M-H)^-$.

Bioassay of Hypoglycemic Activity in Rats Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 125—155 g were starved for 20—24 h but given water *ad libitum*. The test samples were suspended in 5% gum arabic solution (5 ml/kg), and then orally administered. After 30 min, an aqueous solution (5 ml/kg) of D-glucose (0.5 g/kg) was administered. Blood (*ca.* 0.4 ml) was collected from the jugular vein at 0.5, 1.0, and 2.0 h after D-glucose administration. The serum glucose concentration was assayed by the enzymatic glucose oxidase method (glucose C-II test, Wako). The statistical significance of differences was evaluated by analysis of variance (ANOVA) followed by Dunnett's test.[18)] Results were expressed as the mean±S.E. (Table 3).

Effects on Blood Ethanol Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama) weighing 130—170 g were fasted for 20—24 h but were given water *ad libitum*. The test samples were orally administered to the rats as suspensions in 5% gum arabic solution (5 ml/kg). After 1 h, 20% aqueous ethanol (5 ml/kg) was orally (*p.o.*) administered. Blood (*ca.* 0.4 ml) was collected from the jugular vein at 1, 2, and 3 h after ethanol administration and the ethanol blood concentration was assayed enzymatically (blood alcohol test "BMY", Boehringer-Mannheim Yamanouchi). Statistical significance was estimated by ANOVA followed by Dunnett's test.[18)] Results were expressed as the mean±S.E. (Table 4.)

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