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Medicinal Foodstuffs. III.[1] Sugar Beet. (1): Hypoglycemic Oleanolic Acid Oligoglycosides, Betavulgarosides I, II, III, and IV, from the Root of *Beta vulgaris* L. (Chenopodiaceae)

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Betavulgarosides I, II, III, and IV, oleanolic acid oligoglycosides having an unique acidic substituent, were isolated from the root of *Beta vulgaris* L. (sugar beet) together with betavulgarosides VI, VII, and VIII. The chemical structures of betavulgarosides I, II, III, and IV were identified from chemical and physicochemical evidence. Betavulgarosides II, III, and IV were found to exhibit hypoglycemic activity in an oral glucose tolerance test in rats.

Key words betavulgaroside; sugar beet; *Beta vulgaris*; medicinal foodstuff; hypoglycemic effect; oral glucose tolerance test

The roots of *Beta vulgaris* L. (sugar beet, Chenopodiaceae) have been used industrially as a raw material for sugar. The fresh roots and leaves of this plant, which is commonly called "satoudaikon" or "tensai" in Japanese, are used as a vegetable and food garnish in Japanese-style dishes. In Chinese traditional medicine, the roots of sugar beet have been known to exhibit sedative and emmenagogue-like effects. In chemical studies on the constituents of sugar beet, betacyanins and phenolic compounds have been reported[2] and recently, several oleanolic acid glycosides were isolated from the leaves of this plant.[3]

As a part of our continuing studies on the bioactive constituents of medicinal foodstuffs,[1,4] the saponin fraction obtained from the root of Japanese sugar beet was found to show an inhibitory effect on the elevation of plasma glucose levels in the oral glucose tolerance test in rats. We have so far characterized many saponins such as elatosides, camelliasaponins, escins, and senegasaponins, which exhibited potent inhibitory activities on ethanol and sugar absorption in rats, from *Aralia elata* SEEM. (root cortex, bark, and young shoot, Araliaceae),[4,5] *Camellia japonica* L. (seed, Theaceae),[6] *Aesculus hippocastanum* L. (seed, Hippocastanaceae),[7] and *Polygala senega* L. var. *latifolia* TORREY *et* GRAY (root, Polygalaceae).[8] Furthermore, by examination of the structure requirement for these activities, it has been found that the active saponins can be classified into the following three types of structure: 1) Oleanene-28-oic acid 3-*O*-monodesmosides (elatosides), 2) acylated polyhydroxy-oleanene-3-*O*-monodesmosides (camelliasaponins and escins), 3) oleanene acylated bisdesmosides (senegasaponins and senegins).[4–8] In the course of our screening program to find saponin constituents with hypoglycemic activity, we have isolated new saponins named betavulgarosides I (**1**),[9] II (**2**),[9] III (**3**),[9] IV (**4**),[9] VI,[10] VII,[10] and VIII[10] from the root of sugar beet using a bioassay-guided separation. On the other hand, betavulgarosides V,[9,11] IX,[11] and X[11] were isolated from the leaves of this plant. In this paper, we present a full account of the structure elucidation of betavulgarosides I (**1**), II (**2**), III (**3**), and IV (**4**) and of their hypoglycemic activity.[9]

The saponin constituents of the root were separated by the procedures shown in Chart 1. In the process of extraction and purification of betavulgarosides, it was found that betavulgarosides were partly converted to their 1‴-methyl esters during extraction with methanol under reflux, while the nonsubstituted derivative of betavulgarosides, chikusetsusaponin IVa (**7**),[12] was obtained by extraction with water under reflux.[9] After a preliminary examination to identify optimal extraction conditions, the fresh roots of sugar beet cultivated in Hokkaido Prefecture, Japan, were extracted with aqueous methanol and the extract was separated by reversed-phase silica-gel column chromatography (Chromatorex ODS). The methanol eluate from this chromatography was subjected to normal-phase silica-gel column chromatography to give a saponin fraction (fraction 6) with hypoglycemic activity. The saponin fraction was further separated by normal-phase silica-gel column chromatography and then HPLC to give betavulgarosides I (**1**, 0.0012% from the fresh root), II (**2**, 0.0004%), III (**3**, 0.0013%), IV (**4**, 0.0006%), VI (0.0001%), VII (0.0003%), and VIII (0.0001%).

Chemical Structures of Betavulgarosides I (1), II (2), III (3), and IV (4) Betavulgaroside I (**1**) was obtained as colorless fine crystals of mp 215—217 °C. The IR spectrum of **1** showed absorption bands at 1740 and 1736 cm^{-1} ascribable to carboxyls and ester, and strong absorption bands at 3429 and 3453 cm^{-1} suggestive of an oligoglycosidic structure. In the negative- and positive-mode FAB-Mass spectra of **1**, quasimolecular ion peaks were observed at *m/z* 953 $(M-H)^-$ and *m/z* 977 $(M+Na)^+$, and the high-resolution MS analysis of both quasimolecular ion peaks revealed the molecular formula of **1** to be $C_{47}H_{70}O_{20}$.

Methanolysis of **1** with 9% hydrogen chloride–dry methanol liberated oleanolic acid (**5**) as a sapogenol and methyl glycosides of D-glucose and D-glucuronic acid, while compound O (**6**)[13] was obtained upon the partial

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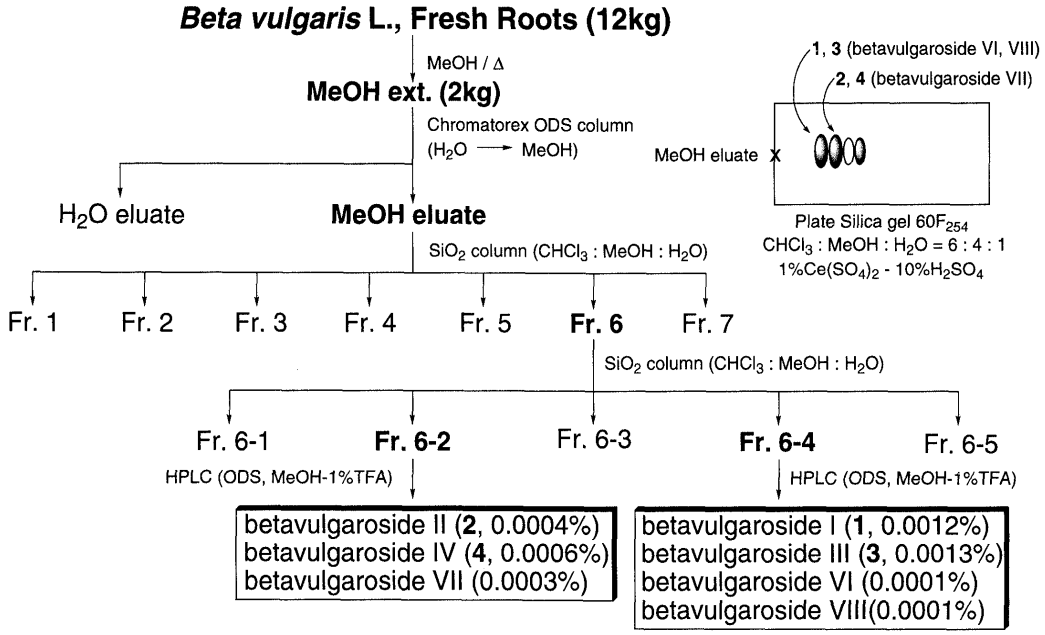


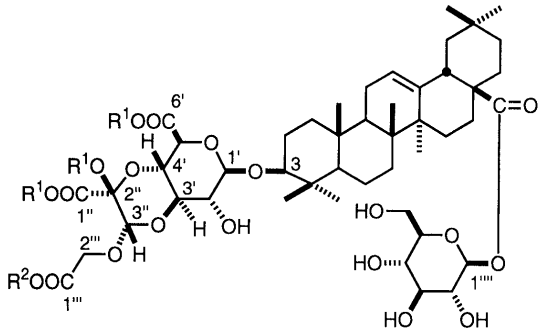
Chart I

hydrolysis of **1** with 2% aqueous sulfuric acid. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) of **1**, which were assigned by NMR analytical methods,[14] showed the presence of a glucuronide moiety having an acidic substituent composed of 3-oxopyruvic acid and glycolic acid [δ 5.00 (d, J=7.3 Hz, 1′-H), 4.23 (m, 2′-H), 4.85 (m, 3′-H), 5.38 (dd-like, 4′-H), 4.72 (m, 5′-H), 5.99 (s, 3″-H), 4.80 (m, 2‴-H_2)] together with an oleanolic acid moiety [δ 3.23 (dd-like, 3-H)] and a 28-*O*-β-D-glucopyranosyl moiety [δ 6.32 (d, J=7.9 Hz, 1⁗-H)]. The planar structure of the dioxolane part of the 3′ and 4′-positions of the 3-*O*-glucuronide moiety in **1** was confirmed by the HMBC experiment. Long-range correlations were observed between the following protons and carbons of **1**: 1′-H and 3-C, 3′-H and 3″-C, 3″-H and 3′-C, 3″-H and 2‴-C, 4′-H and 2″-C, 2‴-H_2 and 3″-C, 2‴-H_2 and 1‴-C.

Following treatment with methanol under reflux or standing for several days, **1** was readily converted into the 1‴-methyl ester (**1a**), which reverted to **1** upon weak alkaline hydrolysis with 2% aqueous potassium carbonate in acetonitrile at room temperature. The positive FAB-MS of **1a** showed a quasimolecular ion peak at m/z 991 $(M+Na)^+$, whose elemental composition was determined to be $C_{48}H_{72}NaO_{20}$ by high-resolution MS measurement. By comparison of the ^{1}H-NMR and ^{13}C-NMR data for **1a** with those for **1** and observation of the long-range correlation between the 1‴-OCH_3 protons and 1‴-C and between the 2‴-H_2 protons and 1‴-C in the HMBC experiment of **1a**, the 1‴-methylated structure of **1a** was determined, so that the glycolic acid part of **1** was found to be converted easily to the methyl ester in methanolic solution. On the other hand, methylation of **1** with excess diazomethane in methanol was found to give the 6′,1″,2″,1‴-tetra-*O*-methyl derivative (**1b**), whose structure was confirmed by ^{1}H-NMR and ^{13}C-NMR analysis [6′-$COOCH_3$: δ_C 168.7, 52.4, δ 3.72 (3H, s); 1″-$COOCH_3$: δ_C 166.8, 52.5, δ 3.85 (3H, s); 2″-OCH_3: δ_C 51.1, δ 3.85 (3H, s); 1‴-$COOCH_3$: δ_C 169.7, 51.6, δ 3.54 (3H, s)].[14] This evidence showed that the 2″-hydroxyl group of **1** was also methylated by excess diazomethane. Based on the above findings, the structure of betavulgaroside I (**1**) was identified except for the configurations of the ketal (C-2″) and acetal (C-3″) moiety. Recently, achyranthosides A and B were isolated as methyl derivatives from Achyranthis Radix and their structure were deduced from the X-ray crystallographic analysis of the prosapogenol methyl ester.[15] The 6′,1″,2″,1‴-tetra-*O*-methyl derivative (**1b**) was found to be identical with achyranthoside A trimethyl ester, so that the C-2″ and C-3″ configurations of **1** were established. Consequently, the total structure of betavulgaroside I (**1**) was determined to be as shown.

Betavulgaroside II (**2**), obtained as colorless fine crystals of mp 173—174 °C, showed absorption bands due to hydroxyl and carboxyl groups at 3432, 1741, 1731, 1080 cm^{-1} in the IR spectrum. The negative-mode FAB-MS of **2** showed a quasimolecular ion peak at m/z 791 $(M-H)^-$ and the molecular formula $C_{41}H_{60}O_{15}$ was determined by high-resolution MS measurement. The carbon signals assignable to the 3-*O*-sugar moiety having a dioxolane part and an oleanolic acid part in the ^{13}C-NMR spectrum (Table 1)[14] of **2** were shown to be superimposable on those of **1**. By comparison of the ^{1}H- and ^{13}C-NMR data for **2** with **1** and the detailed HMBC data of **2**, it was deduced that **2** lacked the 28-ester glucoside moiety of **1**. Finally, **2** was obtained by alkaline hydrolysis of **1** with 5% aqueous sodium hydroxide under reflux. Based on this evidence, the structure of betavulgaroside II (**2**) was characterized as shown.

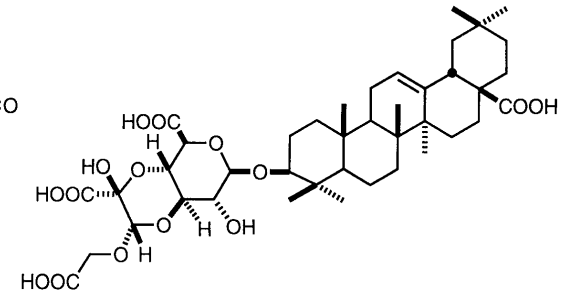
Betavulgaroside III (**3**) was also isolated as colorless fine crystals of mp 212—214 °C. The IR spectrum of **3** showed hydroxyl, ester, and carboxyl absorption bands. The molecular formula $C_{47}H_{72}O_{20}$ was determined from its positive and negative-mode FAB-MS [m/z 979 $(M+Na)^+$, m/z 955 $(M-H)^-$] and high-resolution MS



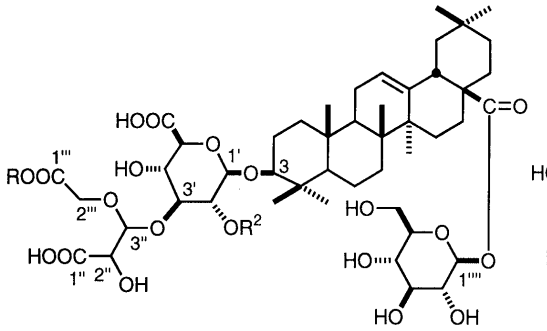
betavulgaroside I (**1**) : R^1=R^2=H

1a : R^1=H, R^2=CH_3

1b : R^1=R^2=CH_3

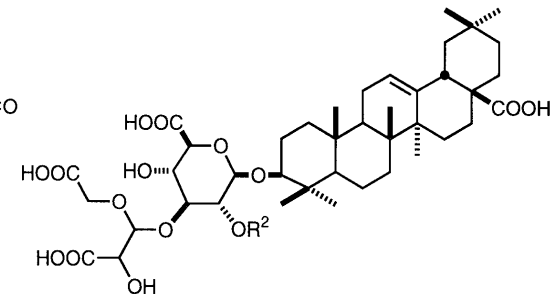


betavulgaroside II (**2**)

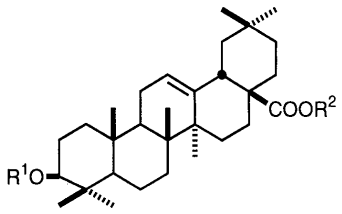


betavulgaroside III (**3**) : R=H

3a : R=CH_3



betavulgaroside IV (**4**)



oleanolic acid (**5**) : R^1=R^2=H

compound O (**6**) : R^1=H, R^2=β-D-Glc

chikusetsusaponin IVa (**7**) : R^1=β-D-GlcA, R^2=β-D-Glc

(β-D-Glc : β-D-glucopyranosyl, β-D-GlcA : β-D-glucuronopyranosyl)

Chart 2

measurement. The methanolysis of **3** furnished oleanolic acid (**5**), methyl glucoside, and methyl glucuronide, while compound O (**6**) was obtained by weak acid hydrolysis of **3** with 2% aqueous sulfuric acid.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **3** showed the presence of an acetal-type substituent composed of tartroaldehydic acid and glycolic acid at the 3′-hydroxyl group of the 3-*O*-glucuronic acid moiety [δ 3.34 (dd-like, 3-H), 4.99 (d, J=7.6 Hz, 1′-H), 4.51 (m, 3′-H), 5.34 (d, J=3.3 Hz, 2″-H), 6.30 (d, J=3.3 Hz, 3″-H), 5.06, 5.35 (ABq, J=16.5 Hz, 2‴-H)][14] together with the compound O moiety [δ 5.40 (br s, 12-H), 6.32 (d, J=6.9 Hz 1⁗-H)]. The structure of the acetal-type substituent and its connectivity to the 3-*O*-glucuronic acid moiety of **3** were confirmed by the HMBC experiment on **3**, in which long-range correlations were observed between the following protons and carbons: 1′-H and 3-C; 3″-H and 3′-C; 2″-H and 3″-C; 2″-H and 1″-C; 2‴-H_2 and 3″-C; 2‴-H_2 and 1‴-C. Methanol treatment of **3** provided the 1‴-methyl ester (**3a**) which reverted to **3** upon weak alkaline hydrolysis. The structure of the 1‴-methyl ester (**3a**) was corroborated by observation of a long-range correlation between the 1‴-methoxyl protons and 1‴-carbonyl carbon in the HMBC spectrum of **3a**. The above evidence and comparison of the ^{13}C-NMR data on **3** with **1** and **7** allowed us to identify the structure of betavulgaroside III (**3**).[16]

Betavulgaroside IV (**4**), isolated as colorless fine crystals of mp 186—187 °C, showed absorption bands of hydroxyl and carboxyl groups in the IR spectrum. Here again, the molecular formula $C_{41}H_{62}O_{15}$ of **4** was obtained from the quasimolecular ion peak [$(M-H)^-$, m/z 793] observed in the negative-mode FAB-MS and by high-resolution MS measurement. Comparison of the ^{1}H- and ^{13}C-NMR (Table 1) for **4** with **3** allowed us to identify the structure of **4**, which lacked the 28-*O*-glucosyl moiety

Table 1. ^{13}C-NMR Data for Betavulgarosides I (**1**), II (**2**), III (**3**), and IV (**4**), and the 1‴-Methyl Esters (**1a**, **3a**)

	1	**1a**	**2**	**3**	**3a**	**4**
C-1	38.4	38.5	38.5	38.6	38.6	38.6
C-2	26.4	26.5	26.5	26.6	26.6	26.6
C-3	89.2	89.4	89.3	89.2	89.2	89.2
C-4	39.3	39.5	39.5	39.5	39.5	39.5
C-5	55.5	55.6	55.7	55.7	55.7	55.7
C-6	18.3	18.5	18.4	18.5	18.5	18.4
C-7	33.0	33.0	33.3	33.1	33.1	33.1
C-8	39.7	39.9	39.7	39.9	39.9	39.7
C-9	47.8	47.9	47.9	48.0	48.0	48.0
C-10	36.7	36.9	36.9	36.9	36.9	36.9
C-11	23.2	23.4	23.8	23.4	23.4	23.4
C-12	122.7	122.9	122.5	122.8	122.8	122.5
C-13	144.0	144.1	144.8	144.1	144.1	144.8
C-14	42.0	42.1	42.1	42.1	42.1	42.2
C-15	28.1	28.2	28.3	28.2	28.2	28.3
C-16	23.6	23.7	23.8	23.7	23.7	23.8
C-17	46.8	47.0	46.6	47.0	47.0	46.7
C-18	41.6	41.8	42.0	41.7	41.7	42.0
C-19	46.0	46.0	46.4	46.2	46.2	46.4
C-20	30.6	30.8	31.0	30.7	30.8	31.0
C-21	33.8	34.0	34.2	34.0	34.0	34.2
C-22	32.4	32.5	33.2	32.5	32.5	32.1
C-23	27.9	28.0	28.1	28.1	28.1	28.2
C-24	16.7	16.9	16.9	16.9	16.9	16.9
C-25	15.3	15.5	15.4	15.5	15.5	15.4
C-26	17.3	17.4	17.3	17.4	17.4	17.4
C-27	26.0	26.1	26.2	26.1	26.1	26.2
C-28	176.3	176.4	180.1	176.4	176.4	180.2
C-29	33.0	33.0	33.3	32.1	33.1	33.1
C-30	23.5	23.6	23.8	23.6	23.6	23.8
3-*O*-GlcA						
C-1′	107.4	107.6	107.6	106.7	106.8	106.8
C-2′	71.9	72.0	72.1	74.8	74.7	74.8
C-3′	72.4	72.5	72.5	85.4	85.1	85.5
C-4′	70.0	70.1	70.1	72.3	72.3	72.4
C-5′	75.1	75.3	75.3	77.5	77.5	77.6
C-6′	171.4	171.4	171.6	172.4	172.4	172.4
6′-OMe						
C-1″	171.0	171.1	171.2	174.8	174.6	174.6
1″-OMe						
C-2″	93.8	93.8	94.0	74.2	74.2	74.2
2″-OMe						
C-3″	97.9	98.2	98.1	105.4	104.9	105.4
C-1‴	172.2	170.0	172.3	173.9	171.3	173.9
1‴-OMe		51.5			51.3	
C-2‴	64.7	64.5	64.9	65.1	64.2	64.3
28-*O*-Glc						
C-1⁗	95.7	95.7		95.7	95.7	
C-2⁗	74.0	74.1		74.1	74.1	
C-3⁗	78.7	78.9		78.8	78.9	
C-4⁗	70.9	71.1		71.1	71.1	
C-5⁗	79.1	79.3		79.3	79.3	
C-6⁗	62.0	62.2		62.2	62.2	

in **3**. Finally, the alkaline hydrolysis of **3** with 5% aqueous sodium hydroxide yielded **4** quantitatively. Consequently, the structure of betavulgaroside IV (**4**) was determined to be as shown.[16]

Hypoglycemic Activity of Betavulgarosides I (1), II (2), III (3), and IV (4) The inhibitory effects of betavulgarosides I (**1**), II (**2**), III (**3**), and IV (**4**) on the elevation of plasma glucose in the oral D-glucose tolerance test in rats are summarized in Table 2. Among them, betavulgaroside II (**2**) and IV (**4**) showed hypoglycemic activity after a single oral administration of 100 mg/kg. In the same bioassay, betavulgaroside III (**3**) also showed inhibitory activity but this was weaker than those of **2** and **4**, while betavulgaroside I (**1**) was found to exhibit no activity. We have reported that the oleanolic acid 3-*O*-monodesmoside structure is required for hypoglycemic activity from an examination of structure–activity relationships.[4,5] The evidence obtained from the experiments using betavulgarosides (**1**—**4**) was found to substantiate the previous above mentioned results of the structure–activity relationships for oleanolic acid 3-*O*-monodesmoside.

Table 2. Inhibitory Effects of the Saponin Fraction and Betavulgarosides I (**1**), II (**2**), III (**3**), and IV (**4**) from *Beta vulgaris* L. on the Elevation of Plasma Glucose Level by Oral Glucose Tolerance Test

	Dose (mg/kg, *p.o.*)	*n*	Plasma glucose concentration (mg/dl) 0.5 h	1 h	2 h
Control (normal)		5	82.1±4.2**	105.2±5.6**	106.0±5.3
Control		5	138.7±4.1	135.5±4.6	104.9±4.2
(glucose tolerance)			(56.6±4.1)	(30.3±4.6)	(−1.1±4.2)
Saponin fraction	200	5	117.2±6.0*	133.6±5.7	117.8±5.4
			(35.1±6.0*)	(28.4±5.7)	(11.8±5.4)
Control (normal)		10	72.4±3.3**	95.8±5.0**	90.6±4.8*
Control		9	148.6±4.7	138.3±4.6	107.9±4.1
(glucose tolerance)			(76.2±4.7)	(42.5±4.6)	(17.3±4.1)
1	100	5	153.5±5.9	144.7±4.7	114.0±5.3
			(81.1±5.9)	(48.9±4.7)	(23.4±5.3)
2	100	5	108.5±9.1**	137.7±5.4	121.8±4.9
			(36.1±9.1**)	(41.9±5.4)	(31.2±4.9)
3	100	5	139.3±3.3	135.1±4.9	103.0±1.9
			(66.9±3.3)	(39.3±4.9)	(12.4±1.9)
4	100	3	111.5±5.7**	125.8±5.9	114.0±0.6
			(39.1±5.7**)	(30.0±5.9)	(23.4±0.6)

* $p<0.05$, ** $p<0.01$. Values in parentheses show the difference in plasma glucose concentration between normals and each sample treatment.

Experimental

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

Isolation of Betavulgarosides I (1), II (2), III (3), IV (4), VI, VII, and VIII from the Fresh Roots of *Beta vulgaris* L. 1) The fresh roots of *Beta vulgaris* L. (12 kg, purchased at Yubari area, Hokkaido) were cut finely and extracted three times with 80% aqueous MeOH under reflux. After removal of the solvent under reduced pressure, the extract (2.0 kg) was subjected to reversed-phase silica-gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 4 kg), H_2O, MeOH] followed by evaporation *in vacuo* to furnish the MeOH eluate (14.9 g). Normal-phase silica-gel column chromatography {BW-200 (Fuji Silysia Chemical Ltd., 700 g), $CHCl_3$–MeOH–H_2O [10:3:1 (lower layer)→7:3:1 (lower layer)→6:4:1→5:5:1]} of the MeOH eluate afforded seven fractions [fr. 1 (2.0 g), fr. 2 (1.2 g), fr. 3 (0.7 g), fr. 4 (1.8 g), fr. 5 (0.6 g), fr. 6 (2.7 g), and fr. 7 (0.3 g)]. Fraction 6 was further purified by normal-phase silica-gel column chromatography {BW-200 (300 g), $CHCl_3$–MeOH–H_2O [7:3:1 (lower layer)→65:35:10 (lower layer)→6:4:1]} to give five fractions [fr. 6-1 (199 mg), fr. 6-2 (323 mg), fr. 6-3 (738 mg), fr. 6-4 (623 mg), fr. 6-5 (63 mg)]. HPLC {YMC-Pack ODS-A (YMC Co., Ltd., 250×20 mm, i.d.), MeOH–1% trifluoroacetic acid (TFA, 75:25, v/v)} separation of fraction 6-4 furnished **1** (147 mg, 0.0012%), **3** (150 mg, 0.0013%), betavulgarosides VI (17 mg, 0.0001%), and VIII (14 mg, 0.0001%). Fraction 6-2 was also separated by HPLC [YMC-Pack ODS-A (250×20 mm, i.d.), MeOH–1% TFA (85:15, v/v)] to give **2** (50 mg, 0.0004%), **4** (67 mg, 0.0006%), and betavulgaroside VII (24 mg, 0.0003%).

2) The fresh roots (9.5 kg) were cut finely and extracted with water under reflux. The extracted solution, after evaporation *in vacuo*, gave a syrup (2 kg) which was subjected to reversed-phase silica-gel column

chromatography (4 kg) followed by evaporation *in vacuo* to give the MeOH eluate (5.2 g). Normal-phase silica-gel column chromatography and HPLC separation of the MeOH eluate yielded **1** (0.0061%), **2** (0.0004%), **3** (0.0029%), **4** (0.0005%), betavulgaroside VI (0.0002%), VII (0.0003%), and chikusetsusaponin IVa (**7**, 0.0002%).

Betavulgaroside I (**1**): Colorless fine crystals, mp 215—217 °C, $[\alpha]_D^{28}$ +49.5° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{47}H_{70}NaO_{20}$ $(M+Na)^+$: 977.4358; Found: 977.4369. High-resolution negative-mode FAB-MS: Calcd for $C_{47}H_{69}O_{20}$ $(M-H)^-$: 953.4384; Found: 953.4375. IR (KBr) cm^{-1}: 3453, 3429, 1740, 1736, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.88, 1.07, 1.26, 1.28 (3H each, all s, 25, 30, 26, 23, 27-CH_3), 0.92 (6H, s, 24, 29-CH_3), 3.16 (1H, dd-like, 18-H), 3.23 (1H, dd-like, 3-H), 4.23 (1H, m, 2′-H), 4.72 (1H, m, 5′-H), 4.80 (2H, m, 2‴-H_2), 4.85 (1H, m, 3′-H), 5.00 (1H, d, J=7.3 Hz, 1′-H), 5.38 (1H, dd-like, 4′-H), 5.41 (1H, br s, 12-H), 5.99 (1H, s, 3″-H), 6.32 (1H, d, J=7.9 Hz, 1⁗-H). ^{13}C-NMR: as given in Table 1. Positive-mode FAB-MS (m/z): 977 $(M+Na)^+$. Negative-mode FAB-MS (m/z): 953 $(M-H)^-$.

Betavulgaroside II (**2**): Colorless fine crystals, mp 173—174 °C, $[\alpha]_D^{28}$ +70.1° (c=0.1, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{41}H_{59}O_{15}$ $(M-H)^-$: 791.3859; Found: 791.3832. IR (KBr) cm^{-1}: 3432, 1741, 1731, 1080. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.92, 1.01, 1.27, 1.32 (3H each, all s, 25, 24, 30, 23, 27-CH_3), 0.96 (6H, s, 26, 29-CH_3), 3.27 (1H, m, 18-H), 3.27 (1H, m, 3-H), 4.24 (1H, m, 2′-H), 4.73 (1H, m, 5′-H), 4.80 (2H, m, 2‴-H_2), 4.85 (1H, m, 3′-H), 5.00 (1H, d, J=7.3 Hz, 1′-H), 5.38 (1H, dd-like, 4′-H), 5.45 (1H, br s, 12-H), 5.99 (1H, s, 3″-H). ^{13}C-NMR: as given in Table 1. Negative-mode FAB-MS (m/z): 791 $(M-H)^-$.

Betavulgaroside III (**3**) : Colorless fine crystals, mp 212—214 °C, $[\alpha]_D^{28}$ +10.8° (c=0.1, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{47}H_{71}O_{20}$ $(M-H)^-$: 955.4539; Found: 955.4514. IR (KBr) cm^{-1}: 3429, 1742, 1736, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.82, 0.89, 0.91, 0.96, 1.08 (3H each, all s, 25, 30, 29, 24, 26-CH_3), 1.28 (6H, s, 23, 27-CH_3), 3.18 (1H, dd-like, 18-H), 3.34 (1H, dd-like, 3-H), 4.17 (1H, m, 2′-H), 4.51 (1H, m, 3′-H), 4.63 (1H, m, 5′-H), 4.65 (1H, m, 4′-H), 4.99 (1H, d, J=7.6 Hz, 1′-H), 5.06, 5.35 (2H, ABq, J=16.5 Hz, 2‴-H_2), 5.34 (1H, d, J=3.3 Hz, 2″-H), 5.40 (1H, br s, 12-H), 6.30 (1H, d, J=3.3 Hz, 3″-H), 6.32 (1H, d, J=6.9 Hz, 1⁗-H). ^{13}C-NMR: as given in Table 1. Positive-mode FAB-MS (m/z): 979 $(M+Na)^+$. Negative-mode FAB-MS (m/z): 955 $(M-H)^-$.

Betavulgaroside IV (**4**): Colorless fine crystals, mp 186—187 °C, $[\alpha]_D^{28}$ +10.4° (c=0.1, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{41}H_{61}O_{15}$ $(M-H)^-$: 793.4010; Found: 793.4023. IR (KBr) cm^{-1}: 3434, 1740, 1736, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 1.01, 1.28, 1.32 (3H each, all s, 25, 30, 23, 27-CH_3), 0.96 (9H, s, 24, 26, 29-CH_3), 3.28 (1H, dd-like, 18-H), 3.37 (1H, dd-like, 3-H), 4.17 (1H, m, 2′-H), 4.52 (1H, m, 3′-H), 4.65 (1H, m, 5′-H), 4.68 (1H, m, 4′-H), 5.00 (1H, d, J=5.9 Hz, 1′-H), 5.07, 5.38 (2H, ABq, J=16.7 Hz, 2‴-H_2), 5.34 (1H, br s, 2′-H), 5.46 (1H, br s, 12-H), 6.29 (1H, d-like, 3″-H). ^{13}C-NMR: as given in Table 1. Negative-mode FAB-MS (m/z): 793 $(M-H)^-$.

Methanolysis of Betavulgaroside I (1) A solution of **1** (2 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. The sapogenol constituent, oleanolic acid (**5**), was detected in the filtrate and was found to be identical with an authentic sample by TLC [$CHCl_3$–MeOH (10:1), benzene–acetone (3:1), hexane–AcOEt (1:2)] and HPLC [YMC-Pack ODS-A, MeOH–1% AcOH (85:15, v/v)] comparison. The sugar composition of the product was analyzed by GLC. A solution of the product in pyridine (0.1 ml) was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i) and methyl glucuronide (ii); GLC conditions: CBR1-M25-025, 0.25 mm (i.d.) × 25 m, capillary column; column temperature, 140—280 °C; He flow rate, 15 ml/min. t_R; i: 17.7 min, 17.9 min, ii: 18.4 min, 18.6 min.

Partial Acid Hydrolysis of Betavulgaroside I (1) Giving Compound O (6) A solution of **1** (20 mg) in 2% aqueous H_2SO_4 (3 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate, the residue was purified by normal-phase silica-gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (7:3:1, lower layer)] to give compound O (**6**, 11 mg). ^{1}H-NMR (pyridine-d_5) δ: 0.89, 1.03, 1.13, 1.23, 1.25 (3H each), 0.92 (6H) (all s, *tert*-CH_3 × 7), 3.20 (1H, dd-like, 3-H), 3.44 (1H, dd-like, 18-H), 5.45 (1H, br s, 12-H), 6.31 (1H, d, J=7.9 Hz, 1′-H). ^{13}C-NMR (pyridine-d_5) δ: aglycone moiety: 176.4, 144.1, 122.9, 78.1, 55.8, 48.1, 47.0, 46.2, 42.1, 41.8, 39.9, 39.4, 39.0, 37.4, 34.0, 33.1, 32.5, 30.8, 28.8, 28.2, 28.1, 26.1, 23.8, 23.7, 23.4, 18.8, 17.5, 16.5, 15.6; 28-*O*-β-D-glucosyl moiety: 95.7, 79.3, 78.9, 74.1, 71.1, 62.2, which was found to be identical with an authentic sample by normal-phase silica-gel TLC [$CHCl_3$–MeOH–H_2O (7:3:1, lower layer)], reversed-phase silica-gel TLC [MeOH–H_2O (1:1)], IR (KBr), ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) comparisons.

Methanol Treatment of Betavulgaroside I (1) Giving the 1‴-Methyl Ester (1a) 1) A solution of **1** (50 mg) in MeOH (10 ml) was stirred for 3 d at room temperature. After removal of the solvent *in vacuo*, the residue was separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d., MeOH–1% TFA (75:25, v/v)] to give **1a** (25 mg) and **1** (22 mg).

2) A solution of **1** (50 mg) in MeOH (10 ml) was heated under reflux for 3 h. After cooling, the solution was evaporated *in vacuo* to furnish a residue which was subjected to HPLC separation (same conditions as above) to give **1a** (30 mg).

1a: Colorless fine crystals, mp 200—202 °C, $[\alpha]_D^{28}$ +57.3° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{48}H_{72}NaO_{20}$ $(M+Na)^+$: 991.4515; Found: 991.4528. IR (KBr) cm^{-1}: 3474, 1743, 1736, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.79, 0.88, 1.08, 1.25, 1.29 (3H each, all s, 25, 30, 26, 23, 27-CH_3), 0.92 (6H, s, 24, 29-CH_3), 3.21 (1H, dd-like, 18-H), 3.28 (1H, dd-like, 3-H), 3.51 (3H, s, 1‴-OCH_3), 4.25 (1H, m, 2′-H), 4.63 (2H, m, 2‴-H_2), 4.72 (1H, m, 3′-H), 4.75 (1H, m, 5′-H), 5.00 (1H, d, J=7.6 Hz, 1′-H), 5.38 (1H, m, 4′-H), 5.40 (1H, br s, 12-H), 5.82 (1H, s, 3″-H), 6.33 (1H, d, J=7.9 Hz, 1⁗-H). ^{13}C-NMR: as given in Table 1. Positive-mode FAB-MS (m/z): 991 $(M+Na)^+$.

Methylation of Betavulgaroside I (1) with Diazomethane in MeOH Solution An ice-cooled solution of **1** (200 mg) in MeOH (10 ml) was treated with ethereal diazomethane (*ca.* 10 ml) until the yellow color persisted. The solution was stirred for 30 min, then the solvent was removed under reduced pressure to furnish a residue (235 mg). The residue was purified by HPLC [YMC-Pack ODS-A, MeOH–1% TFA (4:1, v/v)] to give **1b** (83 mg), which was found to be identical by comparing mp, $[\alpha]_D$, positive-mode FAB-MS, and ^{13}C-NMR (pyridine-d_5) with reported values.[15]

Weak Alkaline Hydrolysis of the 1‴-Methyl Ester (1a) with 2% Aqueous K_2CO_3 A solution of **1a** (20 mg) in CH_3CN (1 ml) was treated with 2% aqueous K_2CO_3 (1 ml) and the reaction mixture was stirred for 12 h at room temperature. The reaction solution was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. After evaporation of the solvent *in vacuo*, a residue was subjected to reversed-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., 1.0 g), $CHCl_3$–MeOH–H_2O (6:4:1)] to give **1** (15 mg), which was identified by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5) spectra as natural betavulgaroside I.

Alkaline Hydrolysis of Betavulgaroside I (1) Giving Betavulgaroside II (2) A solution of **1** (50 mg) in 5% aqueous NaOH (5 ml) was heated under reflux for 1 h. The reaction solution was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. After evaporation of the solvent *in vacuo*, a residue was purified by HPLC [YMC-Pack ODS-A, MeOH–1% TFA (4:1, v/v)] to give **2** (40 mg), which was found to be identical with authentic betavulgaroside II (**2**) by TLC [$CHCl_3$–MeOH–H_2O (65:35:10, lower layer), *n*-BuOH–AcOEt–H_2O (4:1:5, upper layer)], ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) comparisons.

Methanolysis of Betavulgaroside III (3) A solution of **3** (2 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. Oleanolic acid (**5**) in the filtrate was identified by TLC and HPLC co-chromatography (under the same conditions as described above for the methanolysis of **1**) with an authentic sample. A solution of the product in pyridine (0.1 ml) was treated with BSTFA (0.2 ml) for 1 h. The reaction mixture was subjected to GLC analysis (as described above for the methanolysis of **1**) to identify the TMS derivative of methyl glucoside and methyl glucuronide.

Partial Acid Hydrolysis of Betavulgaroside III (3) Giving Compound O (6) A solution of **3** (20 mg) in 2% aqueous H_2SO_4 (3 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with IRA-400 (OH^- form) and the resin was removed by filtration. Removal of the solvent *in vacuo* furnished a residue, which was purified by normal-phase silica-gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (7:3:1,

lower layer)] to give compound O (**6**, 10 mg). Compound O (**6**) obtained was shown to be identical with an authentic sample by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5) spectra comparisons.

Methanol Treatment of Betavulgaroside III (3) Giving the 1‴-Methyl Ester (3a) A solution of **3** (50 mg) in MeOH (10 ml) was heated under reflux for 3 h. After cooling, the solution was evaporated *in vacuo* to give a residue, which was separated by HPLC (under the same conditions as described above for the methanol treatment of **1**) to furnish **3a** (25 mg) and **3** (20 mg).

3a: Colorless fine crystals, mp 191—192 °C, $[\alpha]_D^{27}$ +13.5° (c=0.1, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{48}H_{73}O_{20}$ $(M-H)^-$: 970.4773; Found: 970.4706. IR (KBr) cm^{-1}: 3410, 1740, 1736, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.81, 0.88, 0.92, 0.96, 1.08 (3H each, all s, 25, 30, 29, 24, 26-CH_3), 1.27 (6H, s, 23, 27-CH_3), 3.18 (1H, dd-like, 18-H), 3.35 (1H, dd-like, 3-H), 3.51 (3H, s, 1‴-OCH_3), 4.13 (1H, m, 2′-H), 4.46 (1H, m, 3′-H), 4.60 (1H, m, 4′-H), 4.61 (1H, m, 5′-H), 4.92, 5.33 (2H, ABq, J=16.5 Hz, 2‴-H_2), 4.97 (1H, d, J=7.6 Hz, 1′-H), 5.40 (1H, m, 2″-H), 6.25 (1H, br s, 3″-H), 6.33 (1H, d, J=7.6 Hz, 1⁗-H). ^{13}C-NMR: as given in Table 1. Negative-mode FAB-MS (m/z): 969 $(M-H)^-$.

Weak Alkaline Hydrolysis of the 1‴-Methyl Ester (3a) with 2% Aqueous K_2CO_3 A solution of **3a** (50 mg) in CH_3CN (1 ml) was treated with 2% aqueous K_2CO_3 (1 ml) and the reaction mixture was stirred for 12 h at room temperature. The reaction solution was neutralized with Dowex HCR W×2 (H^+ form) and the resin was removed by filtration. After removal of the solvent *in vacuo*, the residue was subjected to reversed-phase silica-gel column chromatography (under same conditions as described above for the weak alkaline hydrolysis of **1a**) to give **3** (48 mg) which was identified by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5) spectra comparisons with natural betavulgaroside III.

Alkaline Hydrolysis of Betavulgaroside III (3) Giving Betavulgaroside IV (4) A solution of **3** (21 mg) in 5% aqueous NaOH (2 ml) was heated under reflux for 1 h. The reaction solution was neutralized with Dowex HCR W×2 (H^+ form) and the resin was removed by filtration. Following work-up of the filtrate, a residue was purified by HPLC (under the same conditions as described above for the alkaline hydrolysis of **1**) to give **4**, which was identified by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5) spectra comparisons as authentic betavulgaroside IV.

Bioassay for the Hypoglycemic Activity in Rats Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 125—155 g were starved for 20—24 h, but given water *ad libitum*. The test samples (**1**—**4**) were dissolved in water (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 orally. Blood (*ca.* 0.4 ml) was collected from the carotid at 0.5, 1.0, and 2.0 h after D-glucose administration. The plasma 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. Results were expressed as means ±S.E. (Table 2).

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