Medicinal Foodstuffs. XV.[1] Sugar Beet. (2): Structures of Betavulgarosides V, VI, VII, VIII, IX, and X from the Roots and Leaves of Sugar Beet (*Beta vulgaris* L., Chenopodiaceae)

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Following the elucidation of betavulgarosides I, II, III, and IV, betavulgarosides VI, VII, VIII were isolated from the roots of sugar beet (*Beta vulgaris* L.), while betavulgarosides V, IX, and X were isolated from the leaves of this plant. The structures of betavulgarosides V—X were determined on the basis of chemical and physicochemical evidence.

Key words betavulgaroside; *Beta vulgaris*; sugar beet; medicinal foodstuff; nortriterpene oligoglycoside; acidic acetal-type substituent

In the course of our studies in search of bioactive saponins and glycosides from medicinal foodstuffs,[1,2] and natural medicines,[3] we have found that the saponin fraction from the fresh roots of sugar beet (*Beta vulgaris* L., Chenopodiaceae) showed potent inhibitory effect on the increase of serum glucose levels in glucose-loaded rats. From the saponin fraction, we have isolated seven triterpene oligoglycosides called betavulgarosides I (**1**), II (**2**), III (**3**), IV (**4**), VI (**6**), VII (**7**), and VIII (**8**). In the preceding paper,[4] we reported the absolute stereostructures of betavulgarosides I (**1**)—IV (**4**) with a novel dioxolane-type or acetal-type substituent, both of which were presumed to be biosynthesized through an oxidative degradation process of a terminal monosaccharide moiety, and the inhibitory activity of betavulgarosides on the increase of serum glucose levels in glucose-loaded rats. As a continuation of that study, we isolated three new betavulgarosides V (**5**), IX (**9**), and X (**10**) from the leaves of sugar beet. This paper deals with the structure elucidation of the remaining six new triterpene oligoglycosides betavulgarosides V (**5**)—X (**10**) from the roots and leaves of sugar beet.

Betavulgarosides VI (6), VII (7), and VIII (8) from the Roots of Sugar Beet Betavulgaroside VI (**6**) was isolated as colorless fine crystals of mp 210—212 °C. The IR spectrum of **6** showed absorption bands at 1751 and 1735 cm^{-1} due to carboxyl and ester functions, and strong absorption bands at 3451 and 1075 cm^{-1} suggestive of an oligoglycosidic structure. The molecular formula $C_{47}H_{72}O_{21}$ was determined from the quasimolecular ion peaks observed in the positive- and negative-ion FAB-MS and by high-resolution MS analysis of both quasimolecular ion peaks. Namely, a quasimolecular ion peak was observed at *m/z* 995 $(M+Na)^+$ in the positive-ion FAB-MS, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 971 $(M-H)^-$.

Methanolysis of **6** with 9% hydrogen chloride in dry methanol liberated hederagenin (**13**)[5] as a sapogenol and methyl glycosides of D-glucose and D-glucuronic acid in a 1 : 1 ratio.[6] Upon partial acid hydrolysis of **6** with 2% aqueous sulfuric acid, hederagenin 28-*O*-β-D-glucopyranoside (**15**)[7] was obtained. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **6**, which were assigned with the aid of various NMR analytical methods,[8] showed the presence of a β-D-glucuronic acid moiety (C-1′–6′) having an acidic acetal-type substituent composed of a tartronaldehydic acid (C-1″–3″) and glycolic acid (C-1‴–2‴) [δ 5.20 (d, J=7.6 Hz, 1′-H), 4.35 (m, 3′-H), 5.30 (d, J=3.3 Hz, 2″-H), 6.25 (d, J=3.3 Hz, 3″-H), 5.03, 5.38 (both d, J=16.5 Hz, 2‴-H_2] together with the hederagenin 28-*O*-β-D-glucopyranoside moiety [δ 3.69 (dd-like, 3-H), 6.31 (d, J=7.9 Hz, 1⁗-H)]. The structure of the acetal-type substituent and its connectivity to the 3-*O*-β-D-glucuronic acid moiety of **6** were confirmed by a heteronuclear multiple bond correlation (HMBC) experiment on **6**, which showed long-range correlations 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. The carbon signals in the ^{13}C-NMR data of **6** were found to be similar to those of betavulgaroside III (**3**),[4b] except for the signals due to the 23-hydroxyl group in **6**: In particular, the carbon signals of the acetal-type substituent were completely superimposable on those of **3**. This evidence indicated that the stereostructure of the acetal-type substituent in

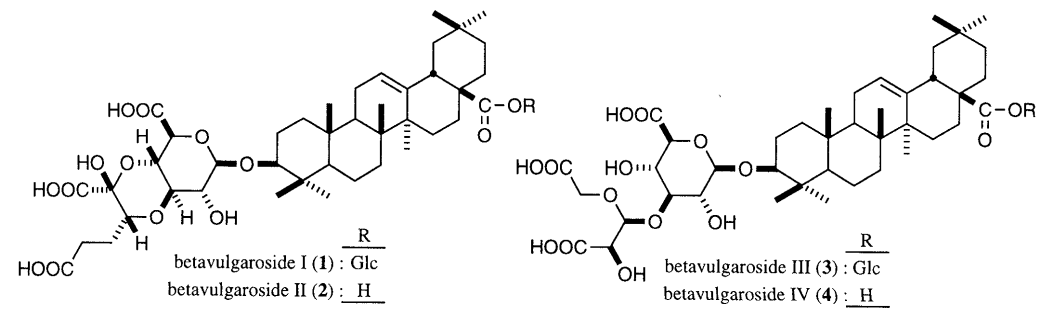

Chart 1

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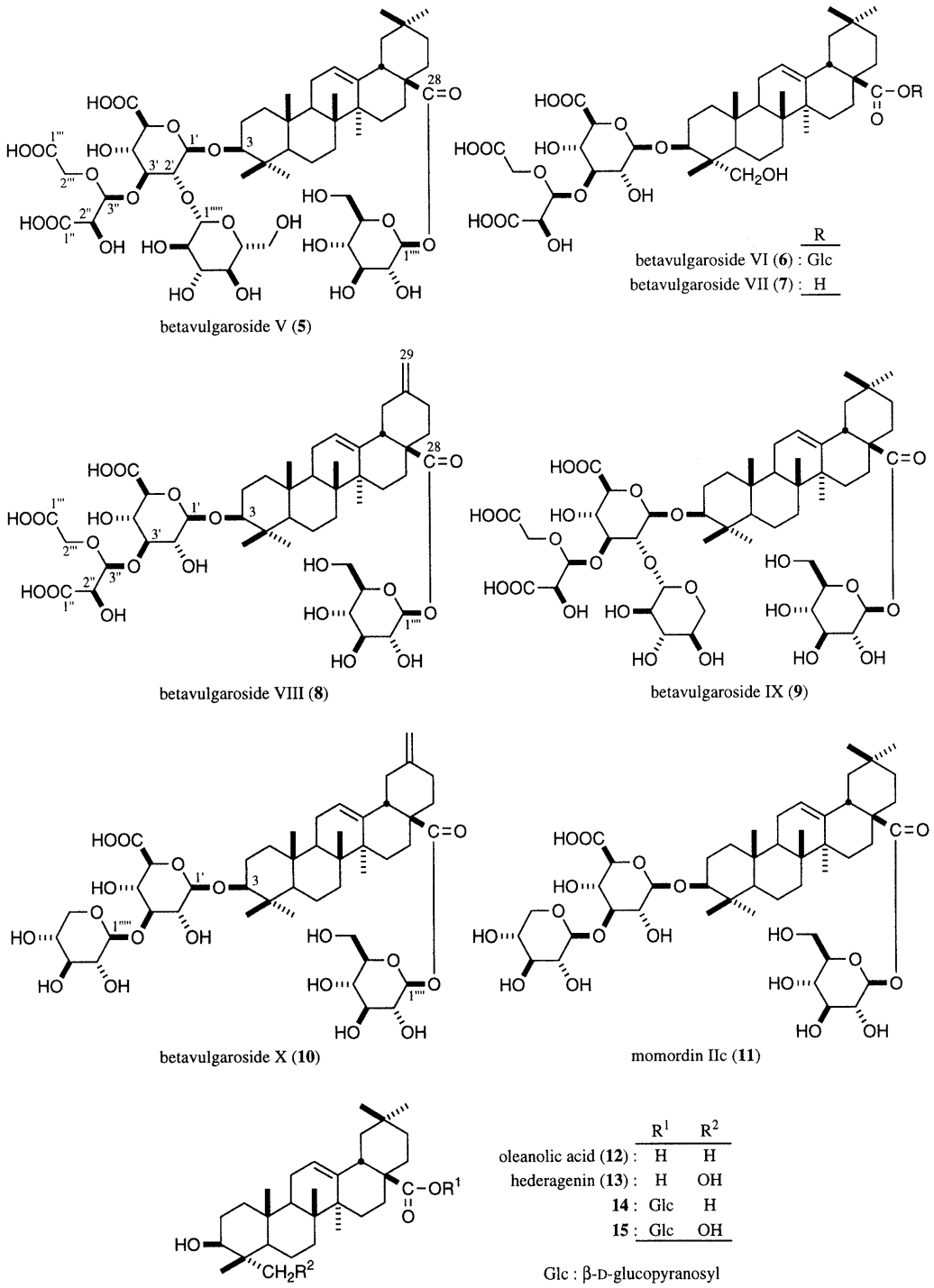


Chart 2

6 was same to that of **3**. Consequently, the structure of betavulgaroside VI (**6**) was characterized as shown.

Betavulgaroside VII (**7**) was also isolated as colorless fine crystals of mp 190—192 °C and its IR spectrum showed absorption bands due to hydroxyl and carboxyl functions. In the positive- and negative-ion FAB-MS of **7**, quasimolecular ion peaks were observed at *m/z* 833 $(M+Na)^+$ and *m/z* 809 $(M-H)^-$, respectively and the high-resolution MS analysis of both quasimolecular ion peaks revealed the molecular formula of **7** to be $C_{41}H_{62}O_{16}$. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **7** showed signals due to a β-D-glucuronic acid moiety with an acetal-type substituent [δ 5.29 (d, J=7.6 Hz, 1′-H), 5.30 (d, J=3.3 Hz, 2″-H), 6.27 (d-like, 3″-H), 5.03, 5.40 (both d, J=17.5 Hz, 2‴-H_2)] together with a hederagenin moiety. Comparison of the ^{1}H- and ^{13}C-NMR data for **7** with those for **6** allowed us to elucidate the structure of **7**, which lacked the 28-*O*-β-D-glucopyranosyl moiety in **6**. Finally, alkaline hydrolysis of **6** with 5% aqueous sodium hydroxide quantitatively furnished **7**. On the basis of this evidence, the structure of betavulgaroside VII (**7**) was determined to be as shown.

Betavulgaroside VIII (**8**), also obtained as colorless fine crystals of mp 215—217 °C, showed absorption bands ascribable to hydroxyl, carboxyl, ester, and olefin functions at

3449, 1751, 1735, 1657, 1076, and 889 cm^{-1} in the IR spectrum. Here again, the molecular formula $C_{46}H_{68}O_{20}$ of **8** was clarified from its positive- and negative-ion FAB-MS [quasi-molecular ion peaks: *m/z* 963 $(M+Na)^+$, *m/z* 939 $(M-H)^-$] and by high-resolution MS measurement. The 1H-NMR (pyridine-d_5) spectrum of **8** showed signals due to a β-D-glucuronic acid moiety [δ 5.00 (d, J=7.9 Hz, 1′-H)] with an acetal-type substituent [δ 5.31 (d, J=3.3 Hz, 2″-H), 6.32 (d, J=3.3 Hz, 3″-H), 5.07, 5.41 (both d, J=16.5 Hz, 2‴-H_2)], a β-D-glucopyranoside moiety [δ 6.27 (d, J=7.6 Hz, 1⁗-H)], and a nortriterpene sapogenol moiety [δ 0.81, 0.96, 1.06, 1.25, 1.28 (all s, 25, 24, 26, 27, 23-H_3), 3.12 (dd-like, 18-H), 3.37 (dd-like, 3-H), 4.69, 4.76 (both s, 29-H_2), 5.42 (br s, 12-H)]. The carbon signals due to the glycosidic structures in the ^{13}C-NMR data[8] of **8** were superimposable on those of **3** and **6**, whereas the signals of the sapogenol moiety were very similar to those of known akebonoic acid glycosides.[9] The HMBC experiment on **8** showed long-range correlations between the following protons and carbons suggestive of the 29-exo-methylene structure in the akebonoic acid moiety : 29-H_2 and 19, 21-C; 18-H and 17, 19-C (Fig. 1). These findings led us to elucidate the structure of betavulgaroside VIII (**8**) as shown.

Table 1. ^{13}C-NMR Data of Betavulgarosides VI (**6**)—VIII (**8**)

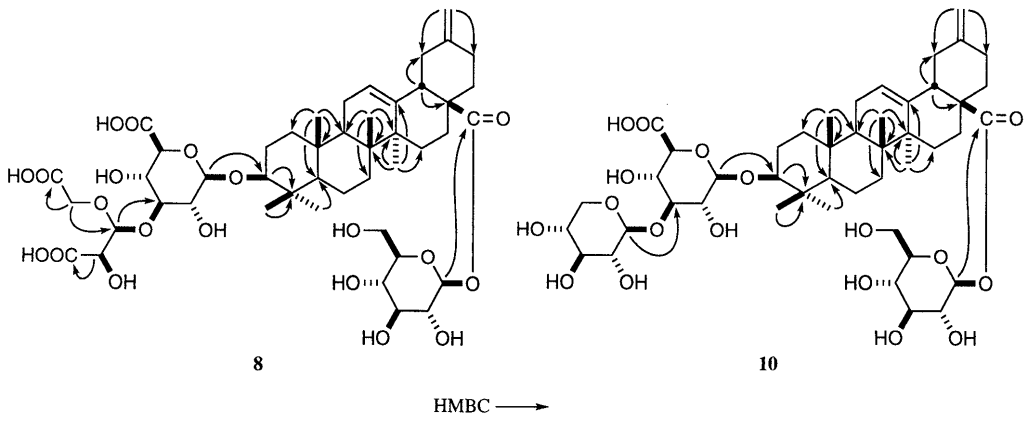
	6	7	8		6	7	8
C-1	38.7	38.6	38.7	C-25	16.1	16.0	15.5
C-2	26.1	26.2	26.6	C-26	17.5	17.4	17.4
C-3	82.0	82.0	89.2	C-27	26.1	26.2	26.1
C-4	43.5	43.0	39.5	C-28	176.4	180.2	175.8
C-5	47.4	47.4	55.7	C-29	33.1	33.2	107.3
C-6	18.2	18.1	18.5	C-30	23.7	23.8	
C-7	33.1	33.2	33.1	C-1′	106.0	106.0	106.8
C-8	39.9	39.8	39.9	C-2′	74.7	74.7	74.8
C-9	48.1	48.1	48.0	C-3′	85.4	85.4	85.5
C-10	36.8	36.8	36.9	C-4′	72.4	72.4	72.4
C-11	23.4	23.8	23.5	C-5′	77.6	77.6	77.6
C-12	122.8	122.6	122.3	C-6′	172.4	172.4	172.4
C-13	144.1	144.8	143.5	C-1″	174.8	174.8	172.8
C-14	42.1	42.2	42.1	C-2″	74.2	74.2	74.3
C-15	28.3	28.4	28.2	C-3″	105.3	105.4	105.4
C-16	23.8	23.8	23.7	C-1‴	173.9	173.9	173.9
C-17	47.0	46.6	47.3	C-2‴	64.8	64.9	65.1
C-18	41.7	42.0	47.7	C-1⁗	95.8		95.9
C-19	46.1	46.4	41.7	C-2⁗	74.2		74.1
C-20	30.8	30.9	148.5	C-3⁗	78.9		78.9
C-21	34.0	34.2	30.1	C-4⁗	71.1		71.2
C-22	32.6	32.9	37.6	C-5⁗	79.3		79.3
C-23	64.1	64.2	28.2	C-6⁗	62.6		62.4
C-24	13.7	13.7	16.9				

The spectra were taken in pyridine-d_5 at 68 MHz.

Betavulgarosides V (5), IX (9), and X (10) from the Leaves of Sugar Beet The methanolic extract of the leaves was subjected to octadecyl silica (ODS) column chromatography (Chromatorex DM1020T) in order to remove the sugar and lipid components. The methanol-eluted fraction was separated by silica gel and ODS column chromatography to give the saponin fraction, which was finally purified by HPLC (YMC-Pack R&D D-ODS-5-A, YMC-Pack ODS-A, YMC-Pack ODS) to provide betavulgarosides V (**5**, 0.0008%), IX (**9**, 0.0011%), and X (**10**, 0.0004%) and momordin IIc (**11**, 0.0002%).[3a)]

Betavulgaroside V (**5**) was isolated as colorless fine crystals of mp 205—206 °C and its IR spectrum showed absorption bands due to hydroxyl and carboxyl functions. In the positive- and negative-ion FAB-MS of **5**, quasimolecular ion peaks were observed at *m/z* 1141 $(M+Na)^+$ and *m/z* 1117 $(M-H)^-$ and the molecular formula $C_{53}H_{82}O_{25}$ was determined by their high-resolution MS measurement. Methanolysis of **5** liberated oleanolic acid (**12**)[2a)] and methyl glycosides of D-glucuronic acid and D-glucose in a 1 : 2 ratio,[6] while partial acid hydrolysis of **5** liberated compound O (**14**).[4,10] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[8] of **5** showed signals assignable to a β-D-glucuronic acid moiety [δ 4.99 (d, J=7.3 Hz, 1′-H)], a tartronaldehydic acid moiety [δ 5.35 (br s, 2″-H), 6.35 (br s, 3″-H)], a glycolic acid moiety [δ 4.95, 5.20 (both d, J=16.2 Hz, 2‴-H_2)], and a β-D-glucopyranosyl moiety [δ 5.72 (d, J=7.3 Hz, 1⁗-H)] together with the compound O moiety [δ 6.33 (d, J=8.4 Hz, 1⁗-H)]. The oligoglycoside structure including the acetal-type substituent of **5** was characterized by an HMBC experiment on **5**. That is, long-range correlations observed between the following protons and carbons: 1′-H and 3-C, 1⁗-H and 2′-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, 1⁗-H and 28-C. The carbon signals in the ^{13}C-NMR spectrum of **5** were very similar to those of **3**, except for the signals due to the 2′-*O*-β-D-glucopyranosyl moiety of **5**. This evidence allowed us to presume that the stereostructure of the acetal-type substituent in **5** was the same as **3**. Consequently, the structure of betavulgaroside V (**5**) was determined as shown.

Betavulgaroside IX (**9**), also isolated as colorless fine crys-



8 10

Fig. 1. HMBC Correlations of Betavulgarosides VIII (**8**) and X (**10**)

Table 2. ^{13}C-NMR Data of Betavulgarosides V (**5**), IX (**9**), X (**10**)

	5	9	10		5	9	10
C-1	38.6	38.7	38.7	C-28	176.4	176.4	175.8
C-2	26.4	26.6	26.6	C-29	33.1	33.2	107.3
C-3	89.5	89.7	89.3	C-30	23.4	23.7	
C-4	39.5	39.6	39.5	C-1′	105.1	105.3	106.8
C-5	55.8	55.9	55.7	C-2′	78.3	78.4	74.6
C-6	18.5	18.5	18.5	C-3′	83.9	83.8	86.5
C-7	33.1	33.1	33.1	C-4′	72.9	73.0	71.4
C-8	39.9	39.9	39.9	C-5′	77.2	77.3	77.6
C-9	48.0	48.1	48.0	C-6′	172.2	172.2	172.2
C-10	36.9	37.0	36.9	C-1″	174.5	174.5	
C-11	23.4	23.5	23.6	C-2″	73.0	73.0	
C-12	122.8	122.9	122.1	C-3″	105.2	105.2	
C-13	144.1	144.1	143.5	C-1‴	173.7	173.6	
C-14	42.1	42.2	42.1	C-2‴	65.7	65.8	
C-15	28.2	28.3	28.2	C-1⁗	95.7	95.8	95.9
C-16	23.6	23.8	23.7	C-2⁗	74.1	74.2	74.1
C-17	47.0	47.0	47.3	C-3⁗	78.8	78.9	78.8
C-18	41.7	41.8	47.7	C-4⁗	71.8	71.2	71.2
C-19	46.2	46.3	41.7	C-5⁗	79.2	79.3	79.3
C-20	30.7	30.8	148.5	C-6⁗	62.9	62.3	62.3
C-21	34.0	34.1	30.1	C-1⁗′	103.6	104.6	106.3
C-22	32.5	32.6	37.6	C-2⁗′	76.3	76.2	75.3
C-23	28.0	27.9	28.1	C-3⁗′	78.1	78.3	78.1
C-24	16.7	16.5	16.9	C-4⁗′	72.4	71.4	71.0
C-25	15.5	15.6	15.5	C-5⁗′	77.8	67.3	67.4
C-26	17.4	17.5	17.5	C-6⁗′	63.2		
C-27	26.1	26.1	26.1				

The spectra were taken in pyridine-d_5 at 125 MHz.

tals of mp 213—214 °C, liberated oleanolic acid (**12**) and methyl glycosides of D-glucuronic acid, D-xylose, and D-glucose in a 1 : 1 : 1 ratio on methanolysis.[6] The molecular formula $C_{52}H_{80}O_{24}$ was clarified from the quasimolecular ion peaks observed in the positive- and negative-ion FAB-MS [*m/z* 1111 $(M+Na)^+$, *m/z* 1089 $(M-H)^-$]. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[8] of **9** showed the presence of a β-D-glucuronic acid moiety [δ 4.98 (d, *J*=7.9 Hz, 1′-H), 4.49 (m, 3′-H)], a tartronaldehydic acid moiety [δ 5.43 (d, *J*=2.0 Hz, 2″-H), 6.37 (d, *J*=2.0 Hz, 3″-H), a glycolic acid moiety [δ 4.96, 5.20 (both d, *J*=16.4 Hz, 2‴-H_2)], and a β-D-xylopyranosyl moiety [δ 5.57 (d, *J*=7.6 Hz, 1⁗-H)] together with the compound O moiety [δ 6.33 (d, *J*=8.2 Hz, 1⁗-H)]. The glycosidic structure of **9** was characterized by the HMBC experiment, in which long-range correlations were observed between the following protons and carbons: 1′-H and 3-C, 1⁗-H and 2′-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, 1⁗-H and 28-C. The carbon signals in the ^{13}C-NMR data of **9** closely resembled those of **3** and **5**, except for the signals due to the 2′-*O*-β-D-xylopyranosyl moiety of **9**. On the basis of this evidence, the structures of betavulgaroside IX (**9**) was characterized as shown.

Betavulgaroside X (**10**), obtained as colorless fine crystals of mp 211—213 °C, liberated methyl D-glucuronide, methyl D-xyloside, and methyl D-glucoside in a 1 : 1 : 1 ratio on methanolysis.[6] The molecular formula $C_{46}H_{70}O_{18}$ was elucidated by the positive- and negative-ion FAB-MS [*m/z* 933 $(M+Na)^+$, *m/z* 909 $(M-H)^-$] and by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[8] of **10** showed signals assignable to a β-D-glucuronic acid moiety [δ 5.04 (d, *J*=7.6 Hz, 1′-H)], a β-D-xylopyranosyl moiety [δ 5.37 (d, *J*=7.6 Hz, 1⁗-H)], and a β-D-glucopyranosyl moiety [δ 6.28 (d, *J*=8.2 Hz, 1‴-H)] together with the akebonoic acid moiety [δ 3.38 (dd, *J*=4.6, 11.9 Hz, 3-H), 4.69, 4.76 (both s, 29-H_2), 5.43 (br s, 12-H)]. The carbon signals due to the 3-*O*-disaccharide moiety in ^{13}C-NMR spectrum of **10** were very similar to those of momordin II (**11**), whereas the carbon signals of the akebonoic acid 28-*O*-β-D-glucoside moiety closely resembled those of **8**. The HMBC experiment for **10** showed long-range correlations between the 1′-proton and the 3-carbon, between the 1⁗-proton and the 3′-carbon, and between the 1‴-proton and the 28-carbon. Consequently, the structure of betavulgaroside X (**10**) was determined as shown in Fig. 1.

Experimental

The following instruments were used to obtain physical data : melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (*l*=5 cm); GLC, Shimadzu GC-14A; IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM LA-500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM LA-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography : ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 60F_{254} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 60WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% Ce$(SO_4)_2$–10% aqueous H_2SO_4 and heating.

Isolation of Betavulgarosides V (5), VI (6), VII (7), VIII (8), IX (9), and X (10) from the Fresh Roots and Leaves of Sugar Beet Betavulgarosides VI (**6**), VII (**7**), and VIII (**8**) were isolated from the fresh roots of sugar beet cultivated in Yubari area, Hokkaido Prefecture, as described earlier.[4b]

Betavulgaroside VI (**6**): colorless fine crystals from $CHCl_3$–MeOH, mp 210—212 °C, $[\alpha]_D^{30}$+14.8° (*c*=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{47}H_{71}O_{21}$ $(M-H)^-$: 971.4488. Found: 971.4445. High-resolution positive-ion FAB-MS: Calcd for $C_{47}H_{72}O_{21}Na$ $(M+Na)^+$: 995.4464. Found : 995.4437. IR (KBr) : 3451, 1751, 1735, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 0.87, 0.87, 1.11, 1.22 (3H each, all s, 29, 30, 26, 27-H_3), 0.92 (6H, s, 24, 25-H_3), 3.17 (1H, dd-like, 18-H), 3.68, 4.32 (2H, ABq, *J*=11.4 Hz, 23-H_2), 3.69 (1H, dd-like, 3-H), 4.35 (1H, m, 3′-H), 5.20 (1H, d, *J*=7.6 Hz, 1′-H), 5.03, 5.38 (1H each, both d, *J*=16.5 Hz, 2‴-H_2), 5.30 (1H, d, *J*=3.3 Hz, 2″-H), 5.41 (1H, br s, 12-H), 6.25 (1H, d, *J*=3.3 Hz, 3″-H), 6.31 (1H, d, *J*=7.9 Hz, 1⁗-H). ^{13}C-NMR (pyridine-d_5) δc : given in Table 1. Negative-ion FAB-MS: *m/z* 971 $(M-H)^-$. Negative-ion FAB-MS: *m/z* 995 $(M+Na)^+$.

Betavulgaroside VII (**7**): colorless fine crystals from $CHCl_3$–MeOH, mp 190—192 °C, $[\alpha]_D^{30}$+59.4° (*c*=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{41}H_{61}O_{16}$ $(M-H)^-$: 809.3960. Found: 809.3914. High-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{62}O_{16}Na$ $(M+H)^+$: 833.3936. Found: 833.3952. IR (KBr): 3451, 1751, 1736, 1085 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.89, 1.27 (3H each, all s, 25, 27-H_3), 0.93, 1.00 (6H, s, 24, 29, 26, 30-H_3), 3.28 (1H, dd-like, 18-H), 3.71, 4.34 (1H each, both d, *J*=9.9 Hz, 23-H), 4.33 (1H, dd-like, 3-H), 4.34 (1H, m, 3′-H), 5.29 (1H, d, *J*=7.6 Hz, 1′-H), 5.03, 5.40 (1H each, both d, *J*=17.5 Hz, 2‴-H_2), 5.30 (1H, d, *J*=3.3 Hz, 2″-H), 5.45 (1H, br s, 12-H), 6.27 (1H, d-like, 3″-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 809 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 833 $(M+Na)^+$.

Betavulgaroside VIII (**8**): colorless fine crystals from $CHCl_3$–MeOH, mp 215—217 °C, $[\alpha]_D^{30}$+64.3° (*c*=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{46}H_{67}O_{20}$ $(M-H)^-$: 939.4225. Found: 939.4238. High-resolution positive-ion FAB-MS: Calcd for $C_{46}H_{68}O_{20}Na$ $(M+Na)^+$: 963.4202. Found: 963.4185. IR (KBr): 3449, 1751, 1735, 1657, 1076, 889 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.81, 0.96, 1.06, 1.25, 1.28 (3H each, all s, 25, 24, 26, 27, 23-H_3), 3.12 (1H, dd-like, 18-H), 3.37 (1H, dd-like, 3-H), 4.50 (1H, m, 3′-H), 4.69, 4.76 (1H each, both s, 29-H_2), 5.00 (1H, d,

J=7.9 Hz, 1′-H), 5.07, 5.41 (1H each, both d, J=16.5 Hz, 2‴-H_2), 5.31 (1H, d, J=3.3 Hz, 2″-H), 5.42 (1H, br s, 12-H), 6.27 (1H, d, J=7.6 Hz, 1⁗-H), 6.32 (1H, d, J=3.3 Hz, 3″-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Negative-ion FAB-MS: m/z 939 $(M-H)^-$. Positive-ion FAB-MS: m/z 963 $(M+Na)^+$.

The fresh leaves of sugar beet (2.6 kg, cultivated in Yubari area, Hokkaido Prefecture, Japan) were finely cut and extracted three times with aqueous MeOH under reflux. After removal of the solvent under reduced pressure, the extract (115 g) was subjected to ODS column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 500 g), H_2O→MeOH] to give the MeOH eluate (8.6 g), which was separated by silica gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., 250 g), $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1→5 : 5 : 1)] to afford five fractions [fr. 1 (4.2 g), fr. 2 (0.7 g), fr. 3 (0.6 g), fr. 4 (1.1 g), and fr. 5 (0.4 g)]. Fraction 2 was further separated by ODS column chromatography [40 g, MeOH–H_2O (30 : 70→60 : 40→80 : 20, v/v)] to give four fractions [fr. 2-1 (103 mg), fr. 2-2 (46 mg), fr. 2-3 (52 mg), and fr. 2-4 (144 mg)]. HPLC [YMC-Pack ODS (250×20 mm, i.d.), CH_3CN–1% aqueous TFA (40 : 60, v/v)] of fraction 2-2 furnished betavulgaroside X (**10**, 10.1 mg, 0.0004%). Fraction 2-3 was purified by HPLC [MeOH–1% aqueous TFA (80 : 20, v/v)] to give momordin IIc (**11**, 7.0 mg, 0.0003%). Fraction 4 was also purified by ODS column chromatography [50 g, H_2O→MeOH–H_2O (30 : 70, v/v)→MeOH] to provide three fractions [fr. 4-1 (311 mg), fr. 4-2 (639 mg), and fr. 4-3 (107 mg)]. Silica gel column chromatography [64 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer→65 : 35 : 10, lower layer→6 : 4 : 1→5 : 5 : 1)] of fr. 4-2 gave three fractions [fr. 4-2-1 (375 mg), fr. 4-2-2 (121 mg), and fr. 4-2-3 (109 mg)]. Fraction 4-2-2 was purified by HPLC [MeOH–1% aqueous TFA (75 : 25, v/v)] to furnish betavulgaroside IX (**9**, 24 mg, 0.0011%). Fraction 4-2-3 was purified by HPLC [MeOH–1% aqueous TFA (70 : 30, v/v)] to give betavulgaroside V (**5**, 9.5 mg, 0.0008%). Momordin IIc (**11**) was identical with an authentic sample[3a] by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5).

Betavulgaroside V (**5**): colorless fine crystals from $CHCl_3$–MeOH, mp 205—206 °C, $[\alpha]_D^{30}$+12.5° (c=0.6, MeOH). High-resolution negative-ion FAB-MS : Calcd for $C_{53}H_{81}O_{25}$ $(M-H)^-$: 1117.5122. Found: 1117.5094. High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{82}O_{25}Na$ $(M+Na)^+$: 1141.5042. Found: 1141.5034. IR (KBr): 3432, 1740, 1736, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.89, 0.91, 1.17, 1.18, 1.23, 1.26 (3H each, all s, 25, 30, 29, 24, 26, 23, 27-H_3), 3.18 (1H, dd-like, 18-H), 3.27 (1H, dd-like, 3-H), 4.46 (1H, m, 3′-H), 4.49 (1H, m, 4′-H), 4.99 (1H, d, J=7.3 Hz, 1′-H), 4.95, 5.20 (1H each, both d, J=16.2 Hz, 2‴-H_2), 5.35 (1H, br s, 2″-H), 5.40 (1H, br s, 12-H), 5.72 (1H, δ, J=7.3 Hz, 1⁗-H), 6.33 (1H, δ, J=8.4 Hz, 1⁗-H), 6.35 (1H, br s, 3″-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 2. Negative-ion FAB-MS: m/z 1117 $(M-H)^-$. Negative-ion FAB-MS: m/z 1141 $(M+Na)^+$.

Betavulgaroside IX (**9**): colorless fine crystals from $CHCl_3$–MeOH, mp 213—214 °C, $[\alpha]_D^{26}$+17.0° (c=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{52}H_{79}O_{24}$ $(M-H)^-$: 1087.4961. Found: 1087.4889. High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{80}O_{24}Na$ $(M+Na)^+$: 1111.4937. Found: 1111.4934. IR (KBr): 3432, 1740, 1736, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.82, 0.89, 0.91, 1.06, 1.09, 1.24, 1.27 (3H each, all s, 25, 30, 29, 24, 26, 23, 27-H_3), 3.18 (1H, dd-like, 18-H), 3.26 (1H, dd-like, 3-H), 4.47 (1H, m, 2′-H), 4.49 (1H, m, 3′-H), 4.71 (1H, m, 4′-H), 4.96, 5.20 (1H each, both d, J=16.4 Hz, 2‴-H_2), 4.98 (1H, δ, J=7.9 Hz, 1′-H), 5.41 (1H, br s, 12-H), 5.43 (1H, d, J=2.0 Hz, 2″-H), 5.57 (1H, d, J=7.6 Hz, 1⁗-H), 6.33 (1H, d, J=8.2 Hz, 1⁗-H), 6.37 (1H, d, J=2.0 Hz, 3″-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 2. Negative-ion FAB-MS: m/z 1087 $(M-H)^-$. Positive-ion FAB-MS: m/z 1111 $(M+Na)^+$.

Betavulgaroside X (**10**): colorless fine crystals from $CHCl_3$–MeOH, mp 211—213 °C, $[\alpha]_D^{26}$+46.9° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{46}H_{70}O_{18}Na$ $(M+Na)^+$: 933.4460. Found: 933.4514. IR (KBr): 3432, 1740, 1736, 1076, 891 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.81, 0.99, 1.07, 1.26, 1.31 (3H each, all s, 25, 24, 26, 27, 23-H_3), 3.12 (1H, dd, J=4.7, 13.9 Hz, 18-H), 3.38 (1H, dd, J=4.6, 11.9 Hz, 3-H), 4.18 (1H, m, 2′-H), 4.39 (1H, m, 3′-H), 4.57 (1H, t, J=9.5 Hz, 4′-H), 4.69, 4.76 (1H each, both s, 29-H_2), 5.04 (1H, d, J=7.6 Hz, 1′-H), 5.37 (1H, d, J=7.6 Hz, 1⁗-H), 5.43 (1H, br s, 12-H), 6.28 (1H, d, J=8.2 Hz, 1⁗-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 2. Negative-ion FAB-MS: m/z 909 (M-H)-. Positive-ion FAB-MS: m/z 933 $(M+Na)^+$.

Methanolysis of Betavulgarosides V (5), VI (6), VII (7), VIII (8), IX (9), and X (10) A solution of betavulgarosides (1 mg each of **5**, **6**, **7**, **8**, **9**, and **10**) 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 of each product was shown to be identical with an authentic sample [oleanolic acid (**12**)[2a] from **5** and **9**, hederagenin (**13**)[5] from **6** and **7**] by TLC [$CHCl_3$–MeOH (10 : 1), benzene–acetone (3 : 1), n-hexane–AcOEt (1 : 2)] and HPLC [YMC-Pack ODS-A (250×4.6 mm i.d.), MeOH–1% aqueous AcOH (85 : 15, v/v)]. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with N,O-bis(trimethylsilyl)trifluoroacetamide (0.02 ml) for 1 h. The reaction solution was subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glycosides [methyl glucuronide (**i**), methyl glucoside (**ii**), and methyl xyloside (**iii**): **i** from **7**, **i** and **ii** from **5**, **6**, and **8**, **i**, **ii**, and **iii** from **9** and **10**]. GLC conditions : column CBR1-M25-025 [Shimadzu Co., 0.25 mm (i.d.)×25 m], injector temperature: 140 °C, detector temperature: 280 °C, column temperature: 140—240 °C, 5 °C/min, initial time: 5 min, He flow rate: 15 ml/min, t_R (min): **i**: 18.4, 18.6, **ii**: 17.7, 17.9, **iii**: 15.6, 16.0.

Partial Acid Hydrolysis of Betavulgaroside VI (6) A solution of **6** (20 mg) in 2% aqueous H_2SO_4 (3 ml) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was purified by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer)] to yield hederagenin 28-O-β-D-glucopyranoside (**15**, 10.6 mg). **15** was identified by comparison of the physical data with reported values.[7]

Alkaline Hydrolysis of Betavulgaroside VI (6) A solution of **6** (50 mg) in 5% aqueous NaOH (5 ml) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized with Dowex HCR-W2 (H^+ form) and then filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by HPLC [MeOH–1% aqueous TFA (80 : 20, v/v)] to give betavulgaroside VII (**7**, 39 mg), which was identical with an authentic sample obtained from sugar beet by TLC, HPLC, ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5).

Partial Acid Hydrolysis of Betavulgaroside V (5) A solution of **5** (20 mg) in 2% aqueous H_2SO_4 (3 ml) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized with Amberlite IRA-400 (OH^- form) and then filtered. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was purified by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer)] to yield compound O (**14**, 9.8 mg). **14**, thus obtained, was identical with an authentic sample[4] by TLC, HPLC, ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5).

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