

A New Diterpenoid Glucoside and Two New Diterpenoids from the Fruit of *Vitex agnus-castus*

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A new labdane-type diterpenoid glucoside and two new labdane-type diterpenoids were isolated from the fruit (chasteberry) of *Vitex agnus-castus* L. (Verbenaceae) along with 14 known compounds comprising seven labdane-type diterpenoids, one halimane-type diterpenoid, two oleanane-type triterpenoids, two ursane-type triterpenoids, one aromadendrane-type sesquiterpenoid, and one flavonoid. Their structures were characterized on the basis of spectroscopic data as well as chemical evidence. Furthermore, the antioxidative activities of the flavonoid were evaluated using five different analyses.

Key words *Vitex agnus-castus*; Verbenaceae; chasteberry; diterpenoid; glucoside; antioxidative activity

Vitex agnus-castus L. (Verbenaceae) is widely distributed in Central Asia, the Mediterranean region, and Southern Europe. The fruit (chasteberry) of this plant is used as a dietary supplement for treating the hormone-imbalance syndrome in women.¹⁾ This fruit has been reported to contain essential oils, iridoids, flavonoids, and diterpenoids.^{1–9)} Further, linoleic acid isolated from this fruit was identified as an estrogenic compound.¹⁰⁾ In the course of our study on the constituents of Verbenaceae plants, we previously reported the isolation and structural elucidation of 14 diterpenoids, one each of norlabdane-type diterpenoid, aromadendrane-type sesquiterpenoid, and flavonoid from the fruit of *V. agnus-castus*.^{11,12)} As part of our continuing study on the constituents of this fruit, the present paper deals with the isolation and structural elucidation of a new labdane-type diterpenoid glucoside and two new labdane-type diterpenoids along with 14 known compounds comprising seven labdane-type diterpenoids, one halimane-type diterpenoid, two oleanane-type triterpenoids, two ursane-type triterpenoids, one aromadendrane-type sesquiterpenoid, and one flavonoid.

The fruit of *V. agnus-castus* was successively percolated with hexane, acetone, and methanol (MeOH) to give hexane extract, acetone extract, and MeOH extract. The acetone extract was subjected to silica gel, Chromatorex octadecyl silica (ODS), and Sephadex LH-20 column chromatography as well as HPLC on ODS to yield 16 compounds (2–17). The successive chromatography of the MeOH extract over Diaion HP20, Sephadex LH-20, Chromatrex ODS, and silica gel column yielded **1**.

Compounds **4**–**17** were identified as (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,16*S*)-6-acetoxy-9,13-epoxy-16-methoxy-labdane-15,16-olide (**4**),¹³⁾ (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,16*S*)-6-acetoxy-9,13-epoxy-16-methoxy-labdane-15,16-olide (**5**),¹³⁾ (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*)-6-acetoxy-9,13-epoxy-15-methoxy-labdane-16,15-olide (**6**),¹³⁾ (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*)-6-acetoxy-9,13-epoxy-15-methoxy-labdane-16,15-olide (**7**),¹³⁾ vitexilactone (**8**),¹⁴⁾ viteagnusin C (**9**),¹¹⁾ 8-*epi*-sclareol (**10**),¹¹⁾ vitetrolin D (**11**),¹⁴⁾ 2,3-dihydroxy-12-ursen-28-oic acid (**12**),¹⁵⁾

2*α*-hydroxyursolic acid (**13**),¹⁶⁾ 3-epimaslinic acid (**14**),¹⁷⁾ maslinic acid (**15**),¹⁶⁾ and 4*α*,10*α*-dihydroxyaromadendrane (**16**),¹⁸⁾ and casticin (**17**),¹⁹⁾ respectively, based on comparison of their physical and spectral data with authentic samples or those already reported (Fig. 1).

Compound **1**, tentatively named viteagnuside A, was obtained as an amorphous powder, and gave an [M+Na]⁺ ion peak at *m/z* 521 in the positive FAB-MS. The molecular formula of **1** was determined to be C₂₆H₄₂O₉ by high-resolution (HR)-positive FAB-MS. The ¹H-NMR spectrum of **1** showed

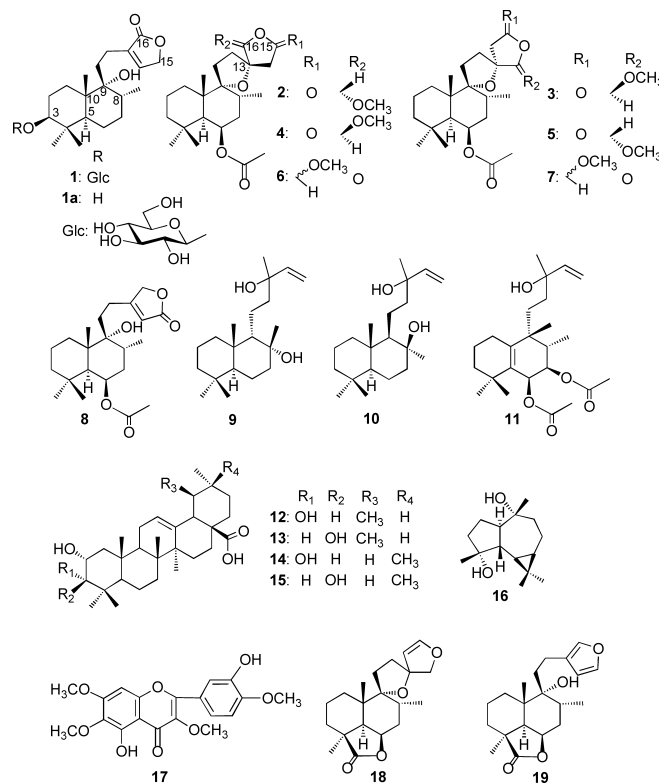


Fig. 1. Structures of **1**–**19** and **1a**

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Table 1. ^1H -NMR Spectral Data for **1** and **1a** (in $\text{C}_5\text{D}_5\text{N}$, 500 MHz)

	1	1a
1a	2.12 ddd (3.5, 10.5, 10.5)	ca. 1.95
1b	ca. 1.53	ca. 1.66
2a	2.37 dddd (3.5, 3.5, 4.0, 13.0)	2.18 m
2b	1.90 dddd (3.5, 10.5, 11.5, 13.0)	ca. 1.95
3	3.48 dd (4.0, 11.5)	3.52 dd (6.0, 10.0)
5	2.00 d (10.0)	2.00 dd (3.0, 12.5)
6a	ca. 1.59	ca. 1.66
6b	1.38 dddd (4.0, 10.0, 12.5, 12.5)	1.45 dddd (4.0, 12.5, 12.5, 12.5)
7a	1.69 dddd (4.0, 12.5, 12.5, 12.5)	1.73 dddd (4.0, 12.5, 12.5, 12.5)
7b	ca. 1.48	1.52 dddd like (2.5, 2.5, 2.5, 12.5)
8	ca. 1.75	ca. 1.80
11a	ca. 2.00	2.09 m
11b	ca. 1.76	ca. 1.82
12a	2.63 brt like (8.0)	2.68 ddd (1.5, 8.5, 8.5)
12b	2.63 brt like (8.0)	2.68 ddd (1.5, 8.5, 8.5)
14	7.22 s	7.20 dd (1.5, 1.5)
15a	4.76 d (1.5)	4.74 d (1.5)
15b	4.76 d (1.5)	4.74 d (1.5)
17	1.11 d (6.0)	1.13 d (6.5)
18	1.32 s	1.23 s
19	1.07 s	1.11 s
20	0.95 s	1.03 s
Glc-1	4.85 d (8.0)	
2	4.02 dd (8.0, 9.0)	
3	4.20 dd (9.0, 9.0)	
4	4.23 dd (9.0, 9.0)	
5	3.91 m	
6a	4.52 dd (2.5, 11.5)	
6b	4.40 dd (5.0, 11.5)	

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl.

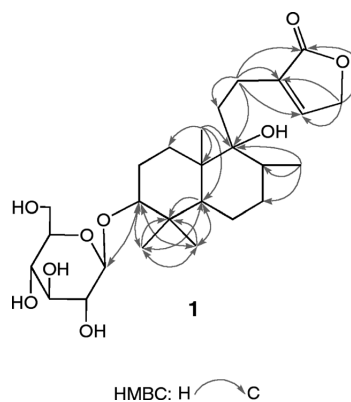
signals due to three tertiary methyl groups (δ 1.32, 1.07, 0.95), one secondary methyl group [δ 1.11 (d, $J=6.0$ Hz)], one olefinic proton [δ 7.22 (s)], and one anomeric proton [δ 4.85 (d, $J=8.0$ Hz)]. The ^{13}C -NMR spectrum of **1** gave 26 carbon signals including one each of carbonyl carbon (δ 174.8), oxygenated quaternary carbon (δ 76.7), oxygenated methine carbon (δ 89.1), oxygenated methylene carbon (δ 70.7), and glucopyranosyl group (δ 106.9, 75.7, 78.7, 71.7, 78.1, 62.9), and two olefinic carbons (δ 145.0, 134.8). These ^1H - and ^{13}C -NMR spectral signals were assigned with the aid of ^1H - ^1H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) techniques as shown in Tables 1 and 2, and the planar structure of **1**, which was a labdane-type diterpenoid glucoside possessing an α -substituted butenolide ring, could be characterized as illustrated in Fig. 2. In the nuclear Overhauser and exchange spectroscopy (NOESY) spectrum of **1**, the key nuclear Overhauser effects (NOEs) were observed between H-3 and H₃-18; H-5 and H₃-18; H-8 and H₃-20; and Ha-11 and H₃-20 (Fig. 3). Thus, relative configurations at C-3, C-5, C-8, C-9, and C-10 were concluded to be S^* , S^* , R^* , R^* , and S^* , respectively.

On acidic hydrolysis, **1** afforded D-glucose, which was identified by using optical rotation chiral detection in the HPLC analysis, along with several unidentified artificial aglycones. The glycosidic linkage was shown to be β by the coupling constant of the ^1H -NMR spectral signal due to anomeric proton. Enzymatic hydrolysis of **1** with β -glucosidase gave **1a**, whose ^1H -NMR spectrum exhibited signals due to three tertiary methyl groups (δ 1.23, 1.11, 1.03), one

Table 2. ^{13}C -NMR Spectral Data for **1**, **1a**, **2**, and **3** (125 MHz)

	1 ^{a)}	1a ^{a)}	2 ^{b)}	3 ^{b)}
1	30.6	30.7	32.5	34.1
2	26.8	28.4	19.0	18.7
3	89.1	78.1	44.0	43.8
4	39.6	39.5	34.2	34.1
5	45.8	45.7	48.8	48.4
6	21.7	22.0	70.5	70.4
7	31.6	31.7	36.3	36.6
8	37.0	37.1	31.6	31.0
9	76.7	76.8	95.0	94.9
10	43.2	43.6	42.8	43.1
11	33.1	33.2	28.9	28.3
12	23.1	23.1	37.0	37.6
13	134.8	134.9	86.0	85.8
14	145.0	144.9	39.1	39.9
15	70.7	70.6	173.6	173.6
16	174.8	—	108.0	109.2
17	16.5	16.6	16.9	16.5
18	28.6	29.1	32.9	33.0
19	17.0	16.7	23.7	23.8
20	16.7	16.5	19.7	19.9
1'			170.4	170.4
2'			21.9	21.9
OCH ₃			56.4	56.8
Glc-1	106.9			
2	75.7			
3	78.7			
4	71.7			
5	78.1			
6	62.9			

δ in ppm from TMS. a) In $\text{C}_5\text{D}_5\text{N}$. b) In CDCl_3 . Glc, glucopyranosyl. —, signal not recorded.

Fig. 2. HMBC Correlations Observed for **1**

secondary methyl group [δ 1.13 (d, $J=6.5$ Hz)], one olefinic proton [δ 7.20 (dd, $J=1.5, 1.5$ Hz)], one oxygenated methylene group [δ 4.74 (d, $J=1.5$ Hz)], and one oxygenated methine proton [δ 3.52 (dd, $J=6.0, 10.0$ Hz)]. Thus, **1a** was defined to be a genuine aglycone of **1**. In order to determine the absolute configuration of the aglycone, the ^{13}C -NMR spectral data of **1** were compared with those of **1a**. The glycosylation shifts were observed at C-2, C-3, and C-4 of the aglycone moiety of **1** with magnitudes -1.6 , $+11.0$, and $+0.1$ ppm, respectively, indicating the absolute configuration at C-3 of the aglycone to be S according to Kasai *et al.*²⁰⁾ and Seo *et al.*²¹⁾ Consequently, the structure of **1** was concluded to be (3*S*,5*S*,8*R*,9*R*,10*S*)-3,9-dihydroxy-13(14)-labdaen-16,15-olide 3- O - β -D-glucopyranoside.

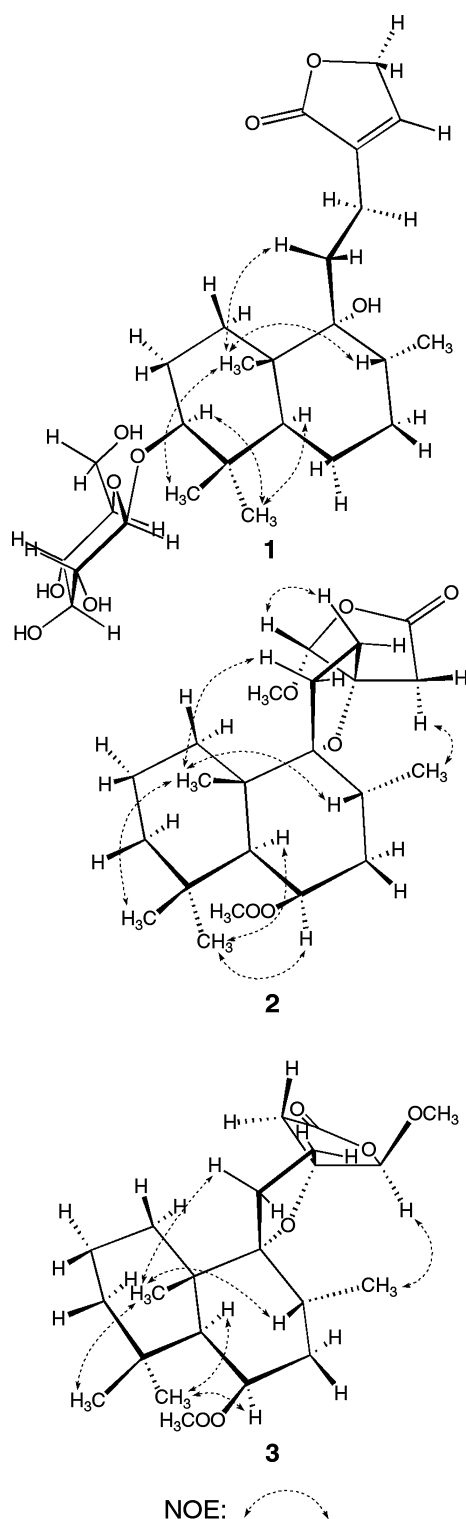


Fig. 3. Key NOE Correlations Observed for **1**, **2**, and **3**

Compound **2**, tentatively named viteagnusin I, was obtained as a colorless syrup. The positive FAB-MS of **2** indicated an $[M+Na]^+$ ion peak at m/z 431; the molecular formula of **2** was concluded to be $C_{23}H_{36}O_6$ by HR-positive FAB-MS. The 1H -NMR spectrum of **2**, which was closely analogous to that of **4**, exhibited signals due to three tertiary methyl groups (δ 1.22, 0.99, 0.97), one secondary methyl group [δ 0.82 (d, $J=6.5$ Hz)], one acetyl group (δ 2.04), and

Table 3. 1H -NMR Spectral Data for **2** and **3** (in C_5D_5N , 500 MHz)

	2	3
1a	ca. 1.62	ca. 1.34
1b	ca. 1.25	ca. 1.27
2a	ca. 1.67	ca. 1.64
2b	ca. 1.46	ca. 1.49
3a	ca. 1.33	ca. 1.31
3b	ca. 1.20	1.15 m
5	1.58 d (3.0)	1.58 d (3.0)
6	5.40 ddd (3.0, 3.0, 3.0)	5.39 ddd (3.0, 3.0, 3.0)
7a	ca. 1.72	ca. 1.70
7b	ca. 1.50	ca. 1.54
8	ca. 2.06	ca. 2.06
11a	ca. 2.17	2.17 m
11b	1.76 m	1.82 m
12a	ca. 2.06	ca. 2.09
12b	1.97 m	1.88 m
14a	3.10 d (16.5)	2.97 d (16.5)
14b	2.38 d (16.5)	2.41 d (16.5)
16	4.80 s	4.93 s
17	0.82 d (6.5)	0.82 d (6.5)
18	0.97 s	0.95 s
19	0.99 s	0.98 s
20	1.22 s	1.23 s
2'	2.04 s	2.04 s
OCH ₃	3.52 s	3.55 s

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses).

one methoxy group (δ 3.52). The ^{13}C -NMR spectrum of **2**, which could be superimposed on that of **4**, gave 23 carbon signals, including two carbonyl carbons (δ 173.6, 170.4), one acetal carbon (δ 108.0), one methoxy carbon (δ 56.4), one oxygenated methine carbon (δ 70.5), and two oxygenated quaternary carbons (δ 95.0, 86.0). These 1H - and ^{13}C -NMR spectral signals were examined in detail, and the planar structure of **2**, a labdane-type diterpenoid possessing one each of spiro-tetrahydrofuran ring, γ -spiro-lactone ring, methoxy group, and acetyl group, could be determined to be the same as that of **4**. The relative configurations were defined on the basis of the NOESY spectrum, in which correlations were observed between H-5 and H₃-18; H-6 and H₃-18; H-8 and H₃-20; Ha-11 and H₃-20; Ha-12 and H-16; and Hb-14 and H₃-17 (Fig. 3). Accordingly, **2** was defined as (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,16*R*)-6-acetoxy-9,13-epoxy-16-methoxy-labdane-15,16-olide.

Compound **3**, tentatively named viteagnusin J, was obtained as a colorless syrup, and the molecular formula of **3** was concluded to be the same as that of **2** by using HR-positive FAB-MS. The 1H - and ^{13}C -NMR spectra of **3** were analogous to those of **2**, and the planar structure of **3** was determined to be the same as that of **2** by using 2D-NMR techniques. In the NOESY spectrum of **3**, key NOEs were observed between H-5 and H₃-18; H-6 and H₃-18; H-8 and H₃-20; Ha-11 and H₃-20; and H-16 and H₃-17 (Fig. 3). Furthermore, the 1H - and ^{13}C -NMR spectra of **3** were different from those of **5**.¹³⁾ Therefore, **3** was defined as the 16-epimer of **5**.

Although, the absolute configurations of **2** and **3** have not been confirmed, they are probably the same as that of **1** from a biogenetic point of view. However, **2**—**7** might be artifacts produced from aldehydes during the isolation procedures, because of co-occurrence of epimers at C-16. Since Henderson and McCrindle reported that premarrubiin (**18**) was converted into marrubiin (**19**) on distillation *in vacuo*, heating

Table 4. Antioxidative Activity (%) of **17** on the Five Different Antioxidant Analyses

	17	Trolox	EDTA
O ₂ ⁻ radical scavenging activity	14.53±3.25	12.47±3.80	
NO scavenging activity	26.79±2.25	35.40±2.51	
DPPH radical scavenging activity	36.60±4.48	92.99±0.65	
H ₂ O ₂ scavenging activity	9.88±2.36	99.37±0.17	
Metal chelating activity	29.19±4.02		100.1±1.21

Data shown represent mean±S.D. derived from four determinations. The final concentration of each sample tested was 0.50 mM.

for 3 h in refluxing ethanol, or dissolution in CHCl₃,²² **1** might be also artifact.

During the course of our studies on natural antioxidants,^{23,24} the antioxidative properties of **17**, which was isolated as a major compound in this study, was evaluated using several measurements, *i.e.*, superoxide anion (O₂⁻) radical scavenging assay, nitrogen oxide (NO) scavenging assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, hydrogen peroxide (H₂O₂) scavenging assay, and metal chelating assay on ferrous ion. The scavenging activities on O₂⁻ radical, NO, DPPH radical, and H₂O₂ were *ca.* 1.2-fold, *ca.* 0.8-fold, *ca.* 0.4-fold, and *ca.* 0.1-fold of those of a standard sample, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), respectively, at a concentration of 0.50 mM and the activity of metal chelating effect was *ca.* 0.3-fold of that of a standard sample, ethylenediaminetetraacetic acid (EDTA) at a concentration of 0.50 mM (Table 4).

Experimental

All instruments and materials used were the same as cited in a previous report²⁴ unless otherwise specified.

Plant Material The fruit of *Vitex agnus-castus* L. was purchased in May 2006 from Charis Seijo Co., Ltd., a commercial supplier of herbs in Tokyo, Japan and identified by one of authors (T. Nohara). A voucher specimen has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Extraction and Isolation The powdered fruit of *V. agnus-castus* (1994 g) was percolated with hexane, acetone, and MeOH at room temperature, and each solvent was removed under reduced pressure to yield hexane extract (188.4 g), acetone extract (36.9 g), and MeOH extract (113.4 g), respectively. The acetone extract was subjected to silica gel column [Merck (Darmstadt, Germany), Art. 1.07734, hexane–acetone (20:1, 10:1, 5:1, 3:1, 1:1), CHCl₃–MeOH–H₂O (14:2:0.1, 7:3:0.5, 6:4:1, 0:1:0)] to give fractions (frs.) 1–9. The chromatography of fraction (fr.) 3 (6.0 g) over Chromatorex ODS column (Fuji Silysia Chemical Ltd., Aichi, Japan, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, and MeOH) gave frs. 3.1–3.11. Fr. 3.3 was chromatographed over silica gel column [Merck, Art. 1.09385, hexane–acetone (20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 0:1), MeOH] to yield frs. 3.3.1–3.3.12. Fr. 3.3.4 (196 mg) was subjected to HPLC (column, COSMOSIL 5C18 AR-II, Nacalai Tesque, Inc., Kyoto, Japan, 20 mm×250 mm; solvent, 80% MeOH) to afford **5** (9 mg), **4** (5 mg), **6** (33 mg), **7** (32 mg), **2** (8 mg), and **3** (16 mg). The chromatography of fr. 3.3.5 (574 mg) over silica gel column [Merck, Art. 1.09385, hexane–acetone (20:1, 10:1, 5:1, 3:1, 1:1, 0:1)] gave frs. 3.3.5.1–3.3.5.7. Frs. 3.3.5.1 (64 mg) and 3.3.5.2 (90 mg) were each subjected to HPLC (75% MeOH) under the conditions similar to those used for fr. 3.3.4 to afford **11** (8 mg) and **10** (3 mg) from fr. 3.3.5.1 and **9** (7 mg) and **10** (3 mg) from fr. 3.3.5.2. The chromatography of fr. 5 (7.6 g) over Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) produced frs. 5.1–5.10. Fr. 5.2 (44 mg) was chromatographed over silica gel column [Merck, Art. 1.09385, hexane–acetone (20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 0:1)] to give **16** (11 mg). The chromatography of fr. 5.4 (565 mg) over silica gel column [Merck, Art. 1.09385, hexane–acetone (20:1, 10:1, 7:1, 5:1, 1:1)] yielded **8** (4 mg). Frs. 5.7 (363 mg) and 5.8 (404 mg) were each subjected to HPLC under the same conditions as those used for fr. 3.3.4 to afford **14**

(4 mg) and **12** (13 mg) from fr. 5.7 and **15** (41 mg) and **13** (13 mg) from fr. 5.8. Fr. 6 (7.57 g) was recrystallized from MeOH to give **17** (734 mg) and fr. 6.1. Fr. 6.1 (6.82 g) was chromatographed over silica gel column [Merck, Art. 1.09385, hexane–acetone (7:1, 5:1, 3:1, 0:1), MeOH] to yield frs. 6.1.1–6.1.8. Fr. 6.1.6 (1331 mg) was subjected to Sephadex LH-20 column (Pharmacia Fine Chemicals, Uppsala, Sweden, MeOH) to give frs. 6.1.6.1–6.1.6.2 and **17** (776 mg). The MeOH extract was subjected to Diaion HP20 column chromatography (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan, H₂O, MeOH, acetone) to yield MeOH eluate (25.5 g) and acetone eluate (1.4 g). The MeOH eluate was chromatographed over Sephadex LH-20 column (MeOH) to yield frs. 10–14. The chromatography of fr. 12 (10881 mg) over silica gel column [Merck, Art. 1.09385, CHCl₃–MeOH–H₂O (14:2:0.1, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] gave frs. 12.1–12.15. Fr. 12.4 (1227 mg) was subjected to Chromatorex ODS column chromatography (50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to yield frs. 12.4.1–12.4.14 and **1** (37 mg).

Viteagnuside A (1): Amorphous powder, [α]_D²⁵ +2.8 (*c*=2.5, MeOH). Positive FAB-MS *m/z*: 521 [M+Na]⁺. HR-FAB-MS *m/z*: 521.2762 (Calcd for C₂₆H₃₆O₉Na: 521.2727). ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

Viteagnusin I (2): Colorless syrup, [α]_D¹⁹ –11.0 (*c*=0.7, CHCl₃). Positive FAB-MS *m/z*: 431 [M+Na]⁺. HR-FAB-MS *m/z*: 431.2415 (Calcd for C₂₃H₃₆O₆Na: 431.2410). ¹H-NMR spectral data: see Table 3. ¹³C-NMR spectral data: see Table 2.

Viteagnusin J (3): Colorless syrup, [α]_D¹⁹ –8.5 (*c*=0.5, CHCl₃). Positive FAB-MS *m/z*: 431 [M+Na]⁺. HR-FAB-MS *m/z*: 431.2408 (Calcd for C₂₃H₃₆O₆Na: 431.2410). ¹H-NMR spectral data: see Table 3. ¹³C-NMR spectral data: see Table 2.

Acidic Hydrolysis of 1 Compound **1** (5 mg) was heated in 2 M HCl (1 ml) at a temperature of 95 °C for 1 h. The reaction mixture was extracted with ethyl acetate (1 ml). The aqueous layer was neutralized with Amberlite MB-3 (Organo Co., Tokyo, Japan) and then evaporated under reduced pressure to give a monosaccharide fr. This fr. was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613, Showa Denko, Tokyo, Japan, 150 mm×6.0 mm; solvent, CH₃CN–H₂O (3:1); flow rate, 1.0 ml/min; column temperature, 70 °C; detector, JASCO OR-2090 plus, JASCO, Tokyo, Japan; pump, JASCO PU-2080; and column oven, JASCO CO-2060. The retention time and optical activity of the sample were identical with those [*t*_R (min): 7.1; optical activity: positive] of D-glucose. However, the ethyl acetate extract exhibited several spots by TLC, and the aglycone of **1** could not be obtained.

Enzymatic Hydrolysis of 1 Compound **1** (10 mg) was dissolved in CH₃COOH–CH₃COONa buffer solution (pH 5.5, 1 ml), and β -glucosidase (from Almonds Lot. 124H40281, Sigma Chemical Co., St. Louis, U.S.A., 30 mg) was added. The mixture was left to stand at 37 °C for 16 d. After removal of the solvent under reduced pressure, the residue was extracted with MeOH, and the MeOH extract was chromatographed over silica gel [Merck Art. 1.09385, CHCl₃–MeOH–H₂O (14:2:0.1, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1)] to give **1a** (0.2 mg) and **1** (8.5 mg).

1a: Amorphous powder, [α]_D¹⁷ +22.3 (*c*=0.03, MeOH). ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

Assay of Scavenging Effect on O₂⁻ Radical The O₂⁻ radical scavenging effect was measured by using the phenazine methosulfate (PMS) β -nicotinamide adenine dinucleotide (reduced form) (NADH) system according to previously described methods.^{25,26} Briefly, the reaction was started by the addition of NADH into the assay mixture containing nitroblue tetrazolium (NBT) plus test sample, then allowed to proceed for 10 min at room temperature. The absorbance of the resulting solution was measured at 570 nm. Trolox was used as a standard sample.

Assay of Scavenging Effect on NO The NO scavenging effect was measured by using the Griess method based on the spontaneous NO generation from sodium nitroprusside (SNP).^{26,27} Briefly, the reaction was started by the addition of SNP freshly prepared into the assay mixture containing test sample, allowed for 150 min incubation at room temperature. Thereafter, Griess reagent was added and the resulting solution was measured at 550 nm. Trolox was used as a standard sample.

Assay of Scavenging Effect on DPPH Radical The DPPH radical scavenging effect was measured based on the following method.²⁸ Briefly, the reaction was started by the addition of DPPH into the assay mixture containing test sample, then allowed to proceed for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm. Trolox was used as a standard sample.

Assay of Scavenging Effect on H₂O₂ The H₂O₂ scavenging effect was measured using the H₂O₂-dependent, horseradish peroxidase (HRP)-medi-

ated phenol red oxidation system.^{26,29)} Briefly, the reaction was initiated by the addition of phenol red and HRP into the assay mixture containing H₂O₂ plus test sample, allowed to proceed for 10 min at room temperature. The resulting solution, terminated with sodium hydroxide, was measured at 610 nm. Trolox was used as a standard sample.

Assay of Chelating Effect on Ferrous Metal Ions The ferrous-ion-chelating effect was measured based on the following method.³⁰⁾ Briefly, the reaction was started by the addition of ferrozine into the assay mixture containing test sample and ferrous chloride, then allowed to proceed for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. EDTA was used as a standard sample.

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