Four New Triterpene Glycosides from *Nigella damascena*

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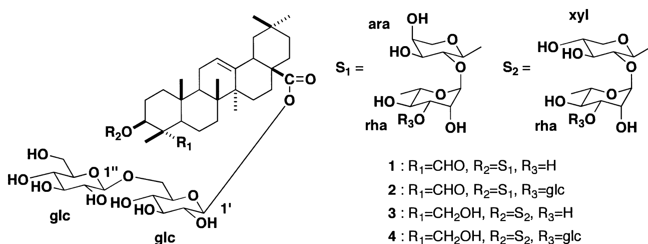
Four new triterpene glycosides, named nigellosides A, B, C, and D, were from the air-dried aerial parts of *Nigella damascena* L. (Ranunculaceae), and the structures were elucidated on the basis of spectroscopic data including 2D NMR spectra and chemical evidence. Their chemical structures have been characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl hederagenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, and 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl hederagenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Key words *Nigella damascena*; nigelloside; oleanene glycoside; Ranunculaceae

The genus *Nigella* with some 20 species belongs to Ranunculaceae. These are distributed from the southern area of Europa to the northern Africa, the southwestern Asia, and the Central Asia. *Nigella damascena* L. (Japanese name, kurotanesou) is cultivated as a garden plant. From its seeds, alkaloid,[1)] sesquiterpenes,[2)] and phenolic compounds[3)] were isolated. A survey of the literature showed no chemical work being done on the aerial parts of *N. damascena*. As part of our continuing investigation on the chemical constituents in the Ranunculaceous plants,[4—6)] this paper deals with structural elucidation of four new triterpene glycosides, named nigellosides A (**1**), B (**2**), C (**3**), and D (**4**).

Results and Discussion

The methanolic extract of the air-dried aerial parts of *N. damascena* was partitioned into a chloroform–water solvent system. The water-soluble portion was separated by MCI gel CHP20P, octadecyl silica gel (ODS), and silica gel column chromatographies, and finally HPLC to give nigellosides A (**1**), B (**2**), C (**3**), and D (**4**) together with eight triterpene glycosides, 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**5**),[7)] dipsacoside B (**6**),[8)] 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-β-D-glucopyranosyl ester (**7**),[9)] 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**8**),[10)] 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl oleanolic acid 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**9**),[11)] 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**10**),[12)] anhuienside C (**11**),[13)] and hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**12**).[14)]



The molecular formula of nigelloside A (**1**) was determined as $C_{53}H_{84}O_{22}$ by high-resolution (HR)-ESI-MS showing a $[C_{53}H_{84}O_{22}Na]^+$ ion at *m/z* 1095.5361. The ^{1}H-NMR spectrum revealed signals due to six quaternary methyls at δ 0.87, 0.88, 0.89, 1.05, 1.21, and 1.39, an olefinic proton at δ 5.39 (1H, br s), an aldehyde proton at δ 9.67 (s), and four anomeric protons at δ 4.72 (d, J=5.8 Hz), 5.01 (d, J=7.6 Hz), 6.06 (br s), and 6.22 (d, J=7.9 Hz). The ^{13}C-NMR spectrum displayed signals due to six quaternary carbon at δ 30.8, 36.1, 40.1, 42.2, 47.0, and 55.4, an oxygen-bearing methine carbon at δ 80.2, a set of olefinic carbon at δ 122.6 and 144.2, an ester carbonyl carbon at δ 176.5, an aldehyde carbon at δ 207.6, and four anomeric carbons at δ 95.7, 101.4, 102.0, and 105.3. A detailed analysis of these spectral data indicated that **1** was the 3,28-bisdesmoside of gypsogenin,[15)] having four monosaccharide units. The configuration of the hydroxyl group at C-3, bearing a saccharide moiety, was determined to be β from the coupling constants of the proton (dd, J=4.6, 11.5 Hz, H-3). Hydrolysis of **1** afforded L-arabinose, D-glucose, and L-rhamnose, the structure of which was confirmed by the ^{1}H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis, together with gypsogenin. The NMR data could be assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connection (HMBC) experiments. The anomeric centers of the arabinose and glucose moieties were determined to be α- and β-configurations, respectively, from each $^3J_{H1-H2}$ value. The anomeric configuration of rhamnose could not be deduced from the $^3J_{H1-H2}$ value. However, the ^{13}C shifts of the rhamnose were superimposable on those of methyl α-L-rhamnopyranoside. The presence of a 2-substituted α-L-arabinopyranosyl ($^4C_1$) unit, a 2-substituted β-D-glucopyranosyl ($^4C_1$) unit, a terminal β-D-glucopyranosyl ($^4C_1$) unit, and a terminal α-L-rhamnopyranosyl ($^1C_4$)

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Table 1. ^{13}C-NMR Data for **1**—**4** (in Pyridine-d_5, 125 MHz, δ ppm)

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C- 1	38.3	38.4	39.2	39.1	3-*O*-	ara	ara	xyl	xyl
2	25.2	25.5	26.5	26.6	C-1	102.0	102.9	105.3	105.5
3	80.2	80.6	80.9	81.2	2	75.3	74.8	77.8	76.9
4	55.4	55.6	43.5	43.6	3	73.8	74.6	79.7	79.6
5	48.4	48.4	47.8	47.6	4	68.7	69.4	71.5	71.5
6	20.6	20.6	18.2	18.1	5	65.0	66.1	67.0	67.0
7	32.5	32.5	32.9	32.8		rha	rha	rha	rha
8	40.1	40.2	39.9	39.9	C-1	101.4	101.3	101.8	101.4
9	47.9	47.9	48.2	48.2	2	72.4	71.9	72.3	71.7
10	36.1	36.1	36.9	36.9	3	72.5	83.3	72.6	83.1
11	23.7	23.7	23.9	23.8	4	74.2	73.1	74.2	73.0
12	122.6	122.6	122.9	122.9	5	69.7	69.6	69.7	69.8
13	144.2	144.2	144.1	144.1	6	18.6	18.5	18.7	18.6
14	42.2	42.2	42.2	42.1			glc		glc
15	28.2	28.2	28.3	28.3	C-1‴		106.8		106.9
16	23.3	23.3	23.4	23.4	2‴		76.1		75.9
17	47.0	47.0	47.1	47.0	3‴		78.4		78.4
18	41.7	41.7	41.7	41.7	4‴		71.5		71.5
19	46.2	46.2	46.2	46.2	5‴		78.8		78.7
20	30.8	30.8	30.7	30.7	6‴		62.5		62.5
21	34.0	34.0	34.0	34.0	28-*O*-	glc	glc	glc	glc
22	32.4	32.4	32.6	32.6	C-1′	95.7	95.7	95.7	95.7
23	207.6	207.6	63.8	63.9	2′	73.9	73.9	73.9	73.9
24	10.6	10.8	14.1	14.2	3′	78.7	78.6	78.7	78.6
25	15.7	15.7	16.2	16.2	4′	70.9	71.0	71.0	71.0
26	17.5	17.5	17.6	17.5	5′	78.0	78.0	78.0	78.0
27	26.0	26.1	26.1	26.1	6′	69.4	69.5	69.4	69.4
28	176.5	176.5	176.5	176.5		glc	glc	glc	glc
29	33.1	33.1	33.1	33.1	C-1″	105.3	105.3	105.3	105.3
30	23.7	23.7	23.7	23.7	2″	75.2	75.2	75.2	75.2
					3″	78.4	78.5	78.5	78.4
					4″	71.5	71.6	71.6	71.5
					5″	78.4	78.4	78.4	78.5
					6″	62.7	62.7	62.3	62.6

unit was shown by comparison of the ^{13}C shifts for each monosaccharide. In the HMBC experiments, long-range correlations were observed between the anomeric proton (δ 4.72) of 2-substituted arabinosyl moiety and the C-3 (δ 80.2) of gypsogenin, the anomeric proton (δ 5.01) of terminal glucosyl moiety and the C-6′ (δ 69.4) of 6-substituted glucosyl moiety, the anomeric proton (δ 6.06) of terminal rhamnosyl moiety and the C-2 (δ 75.3) of 2-substituted arabinosyl moiety, and the anomeric proton (δ 6.22) of 6-substituted glucosyl moiety and the C-28 (δ 176.5) of gypsogenin. From the above evidence, the structure of **1** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The HR-ESI-MS of nigelloside B (**2**) showed a peak at *m/z* 1257.5864 corresponding to the molecular formula $[C_{59}H_{94}O_{27}Na]^+$ (Calcd for 1257.5880). The ^{1}H-NMR data were similar to those of **1** except for the new additional anomeric proton at δ 5.64 (d, J=8.1 Hz). In the ^{13}C-NMR spectrum of **2**, signals due to the aglycon moiety and the saccharide moiety, attached to the carboxyl group at C-28, were in good agreement with those of **1**, although the signals due to the saccharide moiety, attached to the hydroxyl group at C-3, were not identical. Hydrolysis of **2** afforded gypsogenin, L-arabinose, D-glucose, and L-rhamnose. Meanwhile, the molecular formula of **2** was higher by $C_6H_{10}O_5$ (hexose unit) than that of **1**. The above data indicated that an additional glucosyl unit was linked to the saccharide moiety attached to the hydroxyl group at C-3. In the HMBC experiment, the anomeric proton signals at δ 4.65 (d, J=6.3 Hz, ara H-1), 6.17 (br s, rha H-1), and 5.64 (glc H-1‴) showed long-range correlations with the carbon signals at δ 80.6 (C-3), 74.8 (ara C-2), and 83.3 (rha C-3), respectively. Therefore, the structure of **2** was formulated as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Nigelloside C (**3**) showed a clustered molecular ion at *m/z* 1097.5492 $[C_{53}H_{86}O_{22}Na]^+$ in the HR-ESI-MS. The ^{1}H-NMR spectrum revealed signals due to six quaternary methyls at δ 0.84, 0.85, 0.97, 1.11, 1.12 and 1.17, an olefinic proton at δ 5.39 (1H, dd, J=2.9, 3.5 Hz), and four anomeric protons at δ 5.01 (d, J=8.0 Hz), 5.04 (d, J=7.5 Hz), 6.23 (d, J=8.1 Hz), and 6.49 (d, J=1.2 Hz). The ^{13}C-NMR spectrum displayed signals due to six quaternary carbon at δ 30.7, 36.9, 39.9, 42.2, 43.5, and 47.1, an oxygen-bearing methane carbon at δ 80.9, a set of olefinic carbon at δ 122.9 and 144.1, an ester carbonyl carbon at δ 176.5, and four anomeric carbons at δ 95.7, 101.8, 105.3, and 105.3. These spectral data indicated that **3** was the 3,28-bisdesmoside of hederagenin,[7] possessing four monosaccharide units. The configuration of the hydroxyl group at C-3 bearing a saccharide moiety was determined to be β from the coupling constants of the proton (dd, J=4.5, 11.5 Hz, H-3). Hydrolysis of

3 afforded hederagenin, D-glucose, L-rhamnose, and D-xylose. The anomeric center of the xylose moiety was determined to be β-configuration from the $^3J_{H1-H2}$ value. The $^4C_1$-conformation of xylose was shown by comparison of the ^{13}C shifts for monosaccharide. Meanwhile, the NMR data of **3** showed that the glucose and rhamnose moieties had the identical anomeric centers and conformations to **1**, respectively. In the HMBC experiments, the anomeric proton signals at δ 5.01 (glc H-1″), 5.04 (xyl H-1), 6.23 (glc H-1′), and 6.49 (rha H-1) showed long-range correlations with the carbon signals at δ 69.4 (glc C-6′), 80.9 (C-3), 176.5 (C-28), and 77.8 (xyl C-2), respectively. Thus, the structure of **3** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl hederagenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The molecular formula of nigelloside D (**4**) was higher by $C_6H_{10}O_5$ than that of **3**. A comparative study of the ^1H-NMR spectrum of **4** with that of **3** revealed them to be identical except for the appearance of the new additional anomeric proton at δ 5.50 (d, J=8.0 Hz). Furthermore, a detailed comparison of the ^{13}C-NMR spectrum of **4** with that of **3** showed the signal due to C-3 of rhamnose, which was shifted remarkably downfield by 10.5 ppm, and additional six carbon signals (106.9, 78.7, 78.4, 75.9, 71.5, 62.5). Hydrolysis of **4** afforded hederagenin, D-glucose, L-rhamnose, and D-xylose. The foregoing evidence indicated the presence of an additional glucosyl unit, which was linked to the hydroxyl group at C-3 of the rhamnose moiety, in **4**. The amomeric proton signals at δ 5.50 (glu H-1‴) showed long-range correlations with the carbon signals at δ 83.1 (rha C-3). Consequently, **4** was characterized as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl hederagenin 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

In regard to the chemical constituents of *N. damascena*, alkaloid,[1] sesquiterpenes,[2] and phenolic compounds[3] have been characterized from the seeds, but no report on the aerial parts (leaves and stems) has been published to date. To our knowledge, these triterpene glycosides (**1**—**12**) are first isolated from *N. damascena*. Meanwhile, a sugar sequence of 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl or 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl has been isolated from the Araliaceae, Caprifoliaceae, Dipsacaceae, Ranunculaceae, Umbelliferae and Valerianaceae plants. A sugar sequences of 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl has been isolated from the *Anemone anhuiensis* (Ranunculaceae)[13] and *Scabiosa tschiliensis* (Dipsacaceae),[11] respectively. These saccharide moiety containing D-xylose are rare sugar sequence.

Experimental

General Procedure Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL ECA 500 NMR spectrometer. The NMR samples of compounds **1**—**12** were prepared by pyridine-d_5. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The HR-ESI-MS was recorded with a JEOL JMS-T100LP spectrometer. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×30 cm) column with a Tosoh CCPM pump, Tosoh RI-8010 detector, and JASCO OR-2090 detector. TLC was performed on pre-coated Kieselgel 60 F_{254} plates (Merck), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), ODS (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.).

Plant Material The plant seeds, defined as the seeds of *N. damascena*, were provided by Sakata Seed Corp., Kanagawa, Japan. The plant seeds were cultivated at the Botanical Garden of Kumamoto University.

Extraction and Isolation The air-dried aerial parts (leaves and stems) of *N. damascena* (1.0 kg) was extracted with MeOH at room temperature for one month. The MeOH extract (52 g) was partitioned between chloroform-soluble (9 g) and water-soluble (43 g) portions. The water-soluble portion was subjected to MCI gel CHP20P column chromatography (MeOH/H_2O, 1 : 4→4 : 1) to afford five fractions [Fractions 1 (3.7 g), 2 (286 mg), 3 (334 mg), 4 (212 mg), and 5 (101 mg)]. Fraction 1 (3.7 g) was further separated by ODS column chromatography (MeOH/H_2O, 2 : 3→7 : 3) to afford four fractions [Fr. 1-1 (72 mg), Fr. 1-2 (442 mg), Fr. 1-3 (1.9 g), and Fr. 1-4 (413 mg)]. Fraction 1-3 (1.9 g) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 6 : 4 : 1), followed by HPLC (MeOH/H_2O, 13 : 7), to furnish compounds **5** (493 mg) and **6** (782 mg). Fraction 1-4 (413 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 6 : 4 : 1), followed by HPLC (MeOH/H_2O, 7 : 3), to furnish compounds **3** (160 mg) and **4** (142 mg). Fraction 2 (286 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 6 : 4 : 1), followed by HPLC (MeOH/H_2O, 7 : 3), to furnish compound **2** (98 mg). Fraction 3 (334 mg) was further separated by ODS column chromatography (MeOH/H_2O, 1 : 1→7 : 3) to afford three fractions [Fr. 3-1 (66 mg), Fr. 3-2 (22 mg), and Fr. 3-3 (196 mg)]. Fraction 3-1 (66 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 7 : 3), to furnish compound **1** (46 mg). Fraction 3-2 (22 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 7 : 3), to furnish compound **7** (6 mg). Fraction 3-3 (196 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 3 : 1), to furnish compound **8** (158 mg). Fraction 4 (212 mg) was further separated by ODS column chromatography (MeOH/H_2O, 3 : 2→7 : 3) to afford two fractions [Fr. 4-1 (74 mg) and Fr. 4-2 (105 mg)]. Fraction 4-1 (74 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 3 : 1), to furnish compound **9** (54 mg). Fraction 4-2 (105 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 3 : 1), to furnish compounds **10** (42 mg) and **11** (15 mg). Fraction 5 (101 mg) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 12 : 8 : 1), followed by HPLC (MeOH/H_2O, 4 : 1), to furnish compound **12** (7 mg).

Nigelloside A (**1**): A white powder, $[\alpha]_D^{25}$ −0.67° (c=0.98, MeOH). HR-ESI-MS (m/z): 1095.5361 (M+Na; Calcd for $C_{53}H_{84}O_{22}Na$: 1095.5352). ^1H-NMR (pyridine-d_5) δ: 0.87 (3H, s, H-29), 0.88 (3H, s, H-30), 0.89 (3H, s, H-25), 1.05 (3H, s, H-26), 1.21 (3H, s, H-27), 1.39 (3H, s, H-24), 1.67 (3H, d, J=6.3 Hz rha H-6), 3.17 (1H, dd, J=3.8, 13.5 Hz, H-18), 3.71 (1H, br d, J=9.7 Hz ara H-5a), 3.86 (1H, m, glc H-5″), 3.98 (1H, dd, J=7.6, 9.1 Hz, glc H-2″), 4.08 (1H, dd, J=4.6, 11.5 Hz, H-3), 4.08 (1H, overlapped, glc H-5′), 4.10 (1H, dd, J=7.9, 9.1 Hz, glc H-2′), 4.16 (1H, dd, J=9.1, 9.1 Hz, glc H-3″), 4.18 (1H, dd, J=9.1, 9.1 Hz, glc H-3′), 4.18 (1H, overlapped, ara H-3), 4.19 (1H, dd, J=9.1, 9.1 Hz, glc H-4″), 4.20 (1H, overlapped, ara H-4), 4.25 (1H, dd, J=9.2, 9.2 Hz, rha H-4), 4.26 (1H, dd, J=4.6, 13.5 Hz, ara H-5b), 4.32 (1H, dd, J=9.1, 9.1 Hz, glc H-4′), 4.33 (each 1H, dd, J=4.6, 11.4 Hz, glc H-6′a and glc H-6″a), 4.43 (1H, dd, J=5.8, 7.5 Hz, ara H-2), 4.46 (1H, dd, J=1.7, 11.4 Hz, glc H-6″b), 4.53 (1H, m, rha H-5), 4.57 (1H, dd, J=3.4, 9.2 Hz, rha H-3), 4.65 (1H, br d, J=3.4 Hz, rha H-2), 4.69 (1H, br d, J=10.3 Hz, glc H-6′b), 4.72 (1H, d, J=5.8 Hz, ara H-1), 5.01 (1H, d, J=7.6 Hz, glc H-1″), 5.39 (1H, br s, H-12), 6.06 (1H, br s, rha H-1), 6.22 (1H, d, J=7.9 Hz, glc H-1′), 9.67 (1H, s, H-23). ^{13}C-NMR (pyridine-d_5) δ: given in Table 1.

Nigelloside B (**2**): A white powder, $[\alpha]_D^{25}$ −1.74° (c=0.35, MeOH). HR-ESI-MS (m/z): 1257.5864 (M+Na; Calcd for $C_{59}H_{94}O_{27}Na$: 1257.5880). ^1H-NMR (pyridine-d_5) δ: 0.87 (3H, s, H-29), 0.88 (3H, s, H-30), 0.89 (3H, s, H-25), 1.05 (3H, s, H-26), 1.22 (3H, s, H-27), 1.47 (3H, s, H-24), 1.60 (3H, d, J=6.3 Hz rha H-6), 3.18 (1H, dd, J=4.0, 13.7 Hz, H-18), 3.70 (1H, br d, J=10.4 Hz ara H-5a), 3.87 (1H, m, glc H-5″), 3.99 (1H, dd, J=8.0, 9.1 Hz, glc H-2″), 4.03 (1H, m, glc H-5‴), 4.09 (1H, overlapped, ara H-3), 4.10 (1H, overlapped, glc H-5′), 4.11 (each 1H, dd, J=8.1, 9.1 Hz, glc H-2′ and glc H-2‴), 4.16 (1H, br s, ara H-4), 4.19 (each 1H, dd, J=9.1, 9.1 Hz, glc H-3″ and glc H-4″), 4.21 (1H, dd, J=9.1, 9.1 Hz, glc H-3′), 4.25 (1H, dd, J=3.4, 12.1 Hz, ara H-5b), 4.27 (1H, dd, J=9.1, 9.1 Hz, glc H-3‴), 4.31 (each 1H, dd, J=9.1, 9.1 Hz, glc H-4′ and glc H-4‴), 4.35 (each 1H, dd, J=4.9, 11.4 Hz, glc H-6′a and glc H-6″a), 4.39 (1H, dd, J=4.9, 11.3 Hz, glc H-6‴a), 4.44 (1H, dd, J=6.3, 8.5 Hz, ara H-2), 4.47 (1H, br d, J=11.4 Hz, glc H-

6″b), 4.48 (1H, dd, J=1.8, 11.3 Hz, glc H-6‴b), 4.48 (1H, dd, J=9.1, 9.1 Hz, rha H-4), 4.58 (1H, m, rha H-5), 4.65 (1H, d, J=6.3 Hz, ara H-1), 4.71 (1H, br d, J=9.8 Hz, glc H-6′b), 4.81 (1H, dd, J=2.9, 9.1 Hz, rha H-3), 4.95 (1H, br d, J=2.9 Hz, rha H-2), 5.03 (1H, d, J=8.0 Hz, glc H-1″), 5.39 (1H, br s, H-12), 5.64 (1H, d, J=8.1 Hz, glc H-1‴), 6.17 (1H, d, J=1.2 Hz, rha H-1), 6.24 (1H, d, J=8.0 Hz, glc H-1′), 9.78 (1H, s, H-23). ^{13}C-NMR (pyridine-d_5) δ: given in Table 1.

Nigelloside C (**3**): A white powder, $[\alpha]_D^{25}$ −10.5° (c=0.93, MeOH). HR-ESI-MS (m/z): 1097.5492 (M+Na; Calcd for $C_{53}H_{86}O_{22}Na$: 1097.5508). ^{1}H-NMR (pyridine-d_5) δ: 0.84 (3H, s, H-29), 0.85 (3H, s, H-30), 0.97 (3H, s, H-25), 1.11 (3H, s, H-26), 1.12 (3H, s, H-27), 1.17 (3H, s, H-27), 1.67 (3H, d, J=6.3 Hz rha H-6), 3.16 (1H, dd, J=4.0, 13.7 Hz, H-18), 3.54 (1H, t, J=10.9 Hz, xyl H-5a), 3.72 (1H, d, J=9.8 Hz, H-23a), 3.98 (each 1H, dd, J=9.1, 9.1 Hz, xyl H-3 and glc H-2″), 3.86 (1H, m, glc H-5″), 4.09 (each 1H, overlapped, xyl H-4 and glc H-5′), 4.10 (1H, dd, J=8.1, 9.1 Hz, glc H-2′), 4.16 (1H, dd, J=9.1, 9.1 Hz, glc H-3″), 4.17 (1H, dd, J=9.1, 9.1 Hz, glc H-3′), 4.19 (1H, dd, J=9.1, 9.1 Hz, glc H-4″), 4.20 (1H, dd, J=7.5, 9.1 Hz, xyl H-2), 4.23 (1H, overlapped, H-23b), 4.25 (1H, dd, J=5.2, 10.9 Hz, xyl H-5b), 4.27 (1H, dd, J=4.5, 11.5 Hz, H-3), 4.30 (1H, dd, J=9.2, 9.2 Hz, rha H-4), 4.32 (1H, dd, J=9.1, 9.1 Hz, glc H-4′), 4.33 (each 1H, dd, J=4.6, 11.5 Hz glc H-6′a and glc H-6″a), 4.45 (1H, br d, J=10.3 Hz, glc H-6″b), 4.66 (1H, dd, J=3.5, 9.2 Hz, rha H-3), 4.68 (1H, dd, J=1.7, 11.5 Hz, glc H-6′b), 4.76 (1H, m, rha H-5), 4.79 (1H, br d, J=3.5 Hz, rha H-2), 5.01 (1H, d, J=8.0 Hz, glc H-1″), 5.04 (1H, d, J=7.5 Hz, xyl H-1), 5.39 (1H, dd, J=2.9, 3.5 Hz, H-12), 6.23 (1H, d, J=8.1 Hz, glc H-1′), 6.49 (1H, d, J=1.2 Hz, rha H-1). ^{13}C-NMR (pyridine-d_5) δ: given in Table 1.

Nigelloside D (**4**): A white powder, $[\alpha]_D^{25}$ −14.0° (c=0.96, MeOH). HR-FAB-MS (m/z): 1259.6053 (M+Na; Calcd for $C_{59}H_{96}O_{27}Na$: 1259.6037). ^{1}H-NMR (pyridine-d_5) δ: 0.85 (3H, s, H-30), 0.86 (3H, s, H-29), 0.97 (3H, s, H-25), 1.11 (3H, s, H-26), 1.18 (3H, s, H-24), 1.18 (3H, s, H-27), 1.62 (3H, d, J=6.3 Hz rha H-6), 3.17 (1H, dd, J=4.1, 13.8 Hz, H-18), 3.53 (1H, dd, J=10.6, 10.9 Hz, xyl H-5a), 3.86 (1H, m, glc H-5″), 3.93 (1H, d, J=10.3 Hz, H-23a), 3.96 (1H, m. glc H-5‴), 3.99 (1H, dd, J=7.5, 9.1 Hz, glc H-2″), 4.09 (1H, dd, J=8.0, 9.1 Hz, glc H-2‴), 4.10 (1H, dd, J=8.0, 9.1 Hz, glc H-2′), 4.11 (each 1H, overlapped, xyl H-3, xyl H-4, and glc H-5′), 4.17 (1H, dd, J=8.0, 9.1 Hz, xyl H-2), 4.18 (each 1H, dd, J=9.1, 9.1 Hz, glc H-3″ and glc H-4″), 4.20 (1H, dd, J=9.1, 9.1 Hz, glc H-3′), 4.24 (each 1H, overlapped, xyl H-5b, glc H-3‴ and glc H-4‴), 4.26 (1H, dd, J=4.6, 10.3 Hz, glc H-6‴a), 4.30 (1H, dd, J=4.6, 11.5 Hz, H-3), 4.31 (1H, dd, J=9.1, 9.1 Hz, glc H-4′), 4.35 (each 1H, dd, J=5.2, 11.5 Hz, glc H-6′a and glc H-6″a), 4.39 (1H, d, J=10.3 Hz, H-23b), 4.45 (each 1H, br d, J=12.1 Hz, glc H-6″b and glc H-6‴b), 4.51 (1H, dd, J=9.1, 9.1 Hz, rha H-4), 4.70 (1H, br d, J=9.8 Hz, glc H-6′b), 4.76 (1H, m, rha H-5), 4.89 (1H, dd, J=2.9, 9.1 Hz, rha H-3), 5.00 (1H, br d, J=2.9 Hz, rha H-2), 5.01 (1H, d, J=7.5 Hz, glc H-1″), 5.03 (1H, d, J=8.0 Hz, xyl H-1), 5.39 (1H, dd, J=3.4, 3.5 Hz, H-12), 5.50 (1H, d, J=8.0 Hz, glc H-1‴), 6.24 (1H, d, J=8.0 Hz, glc H-1′), 6.49 (1H, d, J=1.2 Hz, rha H-1). ^{13}C-NMR (pyridine-d_5) δ: given in Table 1.

Sugar Analysis A solution of each compound (**1**, **2**, **3**, or **4**) (2 mg) in 2 N HCl/dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH_3CN/H_2O (3 : 1, 250 μl). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa Denko, Tokyo, Japan); solvent, CH_3CN/H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detection, optical rotation (OR). The t_R (min) of the sugars was detected as follows. **1**: L-rhamnose 4.7 (−), L-arabinose 6.3 (+), D-glucose 7.4 (+), **2**: L-rhamnose 4.7 (−), L-arabinose 6.3 (+), D-glucose 7.4 (+), **3**: L-rhamnose 4.7 (−), D-xylose 5.5 (+), D-glucose 7.4 (+), **4**: L-rhamnose 4.7 (−), D-xylose 5.5 (+), D-glucose 7.4 (+). [Reference: L-rhamnose 4.7 (negative optical rotation: −), D-xylose 5.5 (positive optical rotation: +), L-arabinose 6.3 (positive optical rotation: +), D-glucose 7.4 (positive optical rotation: +)].

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