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 $\rightarrow$ 2)- α -L-arabinopyranosyl gypsogenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester, 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl gypsogenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester, 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl hederagenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester, and 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl hederagenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 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, kurotanosou) is cultivated as a garden plant. From its seeds, alkaloid,¹⁾ sesquiterpenes,²⁾ and phenolic compounds³⁾ 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 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl hederagenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (5),⁷⁾ dipsacoid B (6),⁸⁾ 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl hederagenin 28- β -D-glucopyranosyl ester (7),⁹⁾ 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl oleanolic acid 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (8),¹⁰⁾ 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl oleanolic acid 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (9),¹¹⁾ 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl oleanolic acid 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (10),¹²⁾ anhuienside C (11),¹³⁾ and hederagenin 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside (12).¹⁴⁾

The molecular formula of nigelloside A (1) was determined as C₅₃H₈₄O₂₂ by high-resolution (HR)-ESI-MS showing a [C₅₃H₈₄O₂₂Na]⁺ ion at *m/z* 1095.5361. The ¹H-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, brs), 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 (brs), and 6.22 (d, *J*=7.9 Hz). The ¹³C-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 ¹H-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 ¹H–¹H correlation spectroscopy (COSY), ¹H-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 ³*J*_{H1–H2} value. The anomeric configuration of rhamnose could not be deduced from the ³*J*_{H1–H2} value. However, the ¹³C shifts of the rhamnose were superimposable on those of methyl α -L-rhamnopyranoside. The presence of a 2-substituted α -L-arabinopyranosyl (⁴C₁) unit, a 2-substituted β -D-glucopyranosyl (⁴C₁) unit, a terminal β -D-glucopyranosyl (⁴C₁) unit, and a terminal α -L-rhamnopyranosyl (¹C₄)

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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 $\rightarrow$ 2)- α -L-arabinopyranosyl gypsogenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 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 $[\text{C}_{59}\text{H}_{94}\text{O}_{27}\text{Na}]^+$ (Calcd for 1257.5880). The ^1H -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 $\text{C}_6\text{H}_{10}\text{O}_5$ (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 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl gypsogenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester.

Nigelloside C (**3**) showed a clustered molecular ion at m/z 1097.5492 $[\text{C}_{53}\text{H}_{86}\text{O}_{22}\text{Na}]^+$ in the HR-ESI-MS. The ^1H -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 $^3J_{\text{H1-H2}}$ value. The $^4\text{C}_1$ -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 $\rightarrow$ 2)- β -D-xylopyranosyl hederagenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester.

The molecular formula of nigelloside D (**4**) was higher by $\text{C}_6\text{H}_{10}\text{O}_5$ 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 anomeric 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 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl hederagenin 28- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester.

In regard to the chemical constituents of *N. damascena*, alkaloid,¹⁾ sesquiterpenes,²⁾ and phenolic compounds³⁾ 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 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl or 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 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 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl and 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 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. $\times$ 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 F₂₅₄ 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/C₁₈, Waters) and MCI gel CHP20P (Mitsubishi Chemi-

cal 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/H₂O, 1:4 $\rightarrow$ 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/H₂O, 2:3 $\rightarrow$ 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 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 6:4:1), followed by HPLC (MeOH/H₂O, 13:7), to furnish compounds **5** (493 mg) and **6** (782 mg). Fraction 1-4 (413 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 6:4:1), followed by HPLC (MeOH/H₂O, 7:3), to furnish compounds **3** (160 mg) and **4** (142 mg). Fraction 2 (286 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 6:4:1), followed by HPLC (MeOH/H₂O, 7:3), to furnish compound **2** (98 mg). Fraction 3 (334 mg) was further separated by ODS column chromatography (MeOH/H₂O, 1:1 $\rightarrow$ 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 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 7:3), to furnish compound **1** (46 mg). Fraction 3-2 (22 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 7:3), to furnish compound **7** (6 mg). Fraction 3-3 (196 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 3:1), to furnish compound **8** (158 mg). Fraction 4 (212 mg) was further separated by ODS column chromatography (MeOH/H₂O, 3:2 $\rightarrow$ 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 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 3:1), to furnish compound **9** (54 mg). Fraction 4-2 (105 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 3:1), to furnish compounds **10** (42 mg) and **11** (15 mg). Fraction 5 (101 mg) was subjected to silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 12:8:1), followed by HPLC (MeOH/H₂O, 4:1), to furnish compound **12** (7 mg).

Nigelloside A (1): A white powder, $[\alpha]_{\text{D}}^{25} -0.67^\circ$ ($c=0.98$, MeOH). HR-ESI-MS (m/z): 1095.5361 (M+Na; Calcd for $\text{C}_{53}\text{H}_{84}\text{O}_{22}\text{Na}$: 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, brd, $J=9.7$ 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, brd, $J=3.4$ Hz, rha H-2), 4.69 (1H, brd, $J=10.3$ 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, brs, H-12), 6.06 (1H, brs, 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, $[\alpha]_{\text{D}}^{25} -1.74^\circ$ ($c=0.35$, MeOH). HR-ESI-MS (m/z): 1257.5864 (M+Na; Calcd for $\text{C}_{59}\text{H}_{94}\text{O}_{27}\text{Na}$: 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, brd, $J=10.4$ 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, brs, 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, brd, $J=11.4$ 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, $[\alpha]_D^{25} -10.5^\circ$ ($c=0.93$, MeOH). HR-ESI-MS (m/z): 1097.5492 (M+Na; Calcd for $\text{C}_{53}\text{H}_{86}\text{O}_{22}\text{Na}$: 1097.5508). ^1H -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, $[\alpha]_D^{25} -14.0^\circ$ ($c=0.96$, MeOH). HR-FAB-MS (m/z): 1259.6053 (M+Na; Calcd for $\text{C}_{59}\text{H}_{96}\text{O}_{27}\text{Na}$: 1259.6037). ^1H -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 H₂O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C₁₈ cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH₃CN/H₂O (3 : 1, 250 μ l). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d. $\times$ 150 mm, Showa Denko, Tokyo, Japan); solvent, CH₃CN/H₂O (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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