Two New Antiinflammatory Triterpene Saponins from the Egyptian Medicinal Food Black Cumin (Seeds of *Nigella sativa*)

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An extensive phytochemical investigation of the polar fractions of a methanolic extract of Egyptian medicinal food, black cumin (seeds of *Nigella sativa* L.) led to the isolation of two new triterpene saponins, named sativosides A and B (**1**-**2**), along with four known saponins (**3**-**6**). Sativoside A (**1**) is the first example of saponins containing 18-ene triterpene aglycon not only in this *Nigella* genus but also in the family Ranunculaceae. The structure of the new saponins was elucidated mainly by a combination of 1D and 2D NMR data, together with HRFABMS and acid hydrolysis. Three compounds (**1**-**3**) showed the significant inhibition effect of phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-induced production of IL-6 in a human mast cell (HMC-1) line.

Key Words: *Nigella sativa* L, Sativosides A and B, 18-Ene triterpene, Pro-inflammatory cytokine

Introduction

The seeds of *Nigella sativa* L (black cumin) are an amazing medicinal food. In Arabic countries it is called Al-Haba-El-Sauda or Habat-Al-Baraka. *Nigella sativa* is an annual dicotyledon herbaceous plant of the family Ranunculaceae. It grows in Middle east area and it is believed to be indigenous to the Arabic countries and the Mediterranean region but has been cultivated in other parts of the world including northern Africa and parts of Asia.[1-2] For thousands of years, black cumin has been used in many Asian, Middle Eastern and Far Eastern countries as an additive in spices,[3] flavored and aromatic substances,[4] and food preservative.[1] These seeds are commonly eaten alone or in combination with honey and it is also used as condiments in many food preparations (curries, pastries, bread and Mediterranean cheese).[5-6] In addition, hot aqueous extract of seeds is also widely used in Egypt as warm drink. *N. sativa* extract can be used in the preservation of food and prevention of food poisoning.[7] It has long been used in traditional folk medicine as a protective and health remedy for a wide range of illnesses, including headache, bronchial asthma, abdominal pains, hypertension, gastrointestinal problems, diarrhea and rheumatism, especially in the Southeast Asia and Middle East.[8] Black cumin is one of the most extensively pharmacologically studied medicinal foods. Many biological studies on the effect of *N. sativa* seed extract or its active compounds including antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, antiulcer, anti-parasitic, antihistaminic, antiviral, antifungal, antibacterial, anti-helminthic, and immunomodulatory effects have been reported. The seeds have very rich and diverse chemicals which contain fixed and volatile oils, proteins, amino acids, alkaloids and saponins.[5,9-10] Several constituents were characterized in volatile oil, including carvone, carvene, α-pinene, *p*-cymene, and the crystalline active principle, nigellone. Moreover, thymoquinone, dithymoquinone, thymohydroquinone, and thymol are the pharmacologicaly active components in the volatile oil. Two indazole alkaloids, nigellicine and nigellidine, an isoquinoline alkaloid, nigellimine and its N-oxide,[9-10] and eight new dolabellane-type diterpene alkaloids, nigellamines A_1, A_2, B_1, B_2, A_3, A_4, A_5, and C were isolated from black cumin.[11-12] Four known hedragenin saponins have been previously reported from the seeds of *N. sativa*, two monodesmosidic glycosides with disaccharide (α-hederen) and trisacharide chains at C-3 of the aglycon through an ether bond,[13] in addition to two bisdesmosidic saponins with two sugar chains at C-3 and C-28 of the hedragenin triterpene.[14]

The polar extracts of the seeds of *N. sativa* showed many biological activities, including anticancer, antiulcer, antimicrobial, anti-inflammatory and spasmolytic effects[5,9-10] and saponins were the main chemical constituents in the polar fractions of our methanolic extract. Moreover, the importance of saponins is increasing in food, cosmetics, and pharmaceutical sectors because of their physicochemical (surfactant) properties and mounting evidence on their biological activity.[15-17] These observations prompted us to continue in our research aimed at finding biologically active and/or novel saponins from plant origin.[18-23] Recently, we investigated the suppression effect of the production of cytokine interleukin (IL)-6 in human mast cell (HMC-1) on the methanolic extract of the seeds of *N. sativa*, which may contribute to the treatment of asthmatic inflammation. Through bioactivity and ^{1}H NMR monitoring guided fractionation of the methanolic extract of the seeds of *N. sativa*, six triterpene glycosides were isolated composed of two new compounds, sativosides A (**1**) and B (**2**) and four known ones. Herein, we report the isolation and structure elucidation of two new triterpene saponins and the evaluation of inhibition effect of the isolated compounds on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-induced production of cytokine IL-6 in HMC-1 line.

Experimental

General methods. Optical rotations were measured on a JASCO P-1010 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. The 1D and 2D NMR spectra were obtained on a Varian VNMRS 500 spectrometer working at 500 MHz for ^{1}H and 125 MHz for ^{13}C. All NMR chemical shifts were referenced to CD_3OD at δ_H 3.30 and δ_C 49.0 as an internal standard. HRFAB mass spectra were acquired with a Jeol JMS-700, supported by Korea basic science Institute Daegu Center. HPLC was performed with a Varian RI detector using the YMC ODS-A column (10 × 250 mm, S-5 μm) and the Varian polaris NH_2 column (4.6 × 250 mm, S-5 μm). All solvents were distilled prior to use and authentic sugars were purchased from Fluka and Sigma.

Table 1. NMR Spectral Data of the Aglycon Portion of sativosides A (**1**) and B (**2**) in CD_3OD

no	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1a	0.93[a]	40.1, *t*	1.07[a]	39.5, *t*
1b	1.73[a]		1.68[a]	
2a	1.76[a]	26.8, *t*	1.77[a]	26.0, *t*
2b	1.89[a]		1.91[a]	
3	3.61[a]	82.3, *d*	3.87[a]	82.1, *d*
4		44.1, *s*		56.3, *s*
5	1.21[a]	48.6, *d*	1.32[a]	49.4, *d*
6a	1.36[a]	18.7, *t*	0.89[a]	21.4, *t*
6b	1.45[a]		1.48[a]	
7a	1.43[a]	35.2, *t*	1.22[a]	33.3, *t*
7b	-		1.50[a]	
8		41.9, *s*		41.0, *s*
9	1.35[a]	52.6, *d*	1.68[a]	48.6, *d*
10		37.8, *s*		37.0, *s*
11a	1.33[a]	22.2, *t*	1.91[a]	24.5, *t*
11b	1.58[a]		-	
12	1.25[a]	27.1, *t*	5.25 (1H, bs)	123.5, *d*
	1.57[a]			
13	2.28 (1H, d, 11.7)	42.3, *d*		144.9, *s*
14		43.7, *s*		43.0, *s*
15a	1.18[a]	30.5, *t*	1.05[a]	28.9, *t*
15b	1.70[a]		1.75[a]	
16a	1.60[a]	34.2, *t*	1.69[a]	24.0, *t*
16b	2.02[a]		2.04[a]	
17		49.9, *s*		48.0, *s*
18		138.3, *s*	2.85 (1H, dd, 4.1,18.3)	42.8, *d*
19	5.14 (1H, bs)	134.0, *d*	1.13[a]	47.2, *t*
			1.70[a]	
20		33.0, *s*		31.6, *s*
21a	1.33[a]	34.5, *t*	1.22[a]	34.9, *t*
21b	1.44[a]		1.37[a]	
22a	1.45[a]	34.6, *t*	1.60[a]	33.2, *t*
22b	2.22[a]		1.70[a]	
23	3.33[a]	64.6, *t*	9.5 (1H, bs)	209.3, *d*
	3.51[a]			
24	0.68 (3H, s)	13.7, *q*	1.13 (3H, s)	10.8, *q*
25	0.93 (3H, s)	17.8, *q*	1.00 (3H, s)	16.2, *q*
26	1.01 (3H, s)	16.8, *q*	0.79 (3H, s)	17.8, *q*
27	0.81 (3H, s)	15.6, *q*	1.17 (3H, s)	26.3, *q*
28		177.0, *s*		178.2, *s*
29	0.99 (3H, s)	30.9, *q*	0.91 (3H, s)	33.5, *q*
30	0.97 (3H, s)	29.4, *q*	0.94 (3H, s)	24.1, *q*

[a]Overlapped with other signals. *q* = CH_3, *t* = CH_2, *d* = CH, *s* = C.

Plant material. Seeds of *N. sativa* were purchased from an herbal market in Elarish, Egypt in May 2008. The voucher specimen is at the Department of Food Sciences and Technology, Faculty of Environmental Agricultural Sciences, Suez Canal University.

Extraction and isolation. The dried and powdered seeds of *N. sativa* (500 g) were extracted successively with hexane (3 × 1000 mL, 122 g), CH_2Cl_2 (3 × 1000 mL, 18.7 g), EtOAc (3 × 1000 mL, 12.6 g) and MeOH (3 × 1000 mL, 25 g) at room temperature with constant stirring for 24 h for each solvent. The MeOH extract (20 g) was fractionated by flash column chromatography (Si gel 60, 0.015 ~ 0.040 mm, Merck) by using a gradient of increasing polarity with CH_2Cl_2, EtOAc, and MeOH as the solvent, to yield 21 fractions (500 mL each). Fractions 14, 15, 16, and 17 were selected by the moderate inhibition effect of IL-6 in the HMC-1 line and combined for further separation. This mixture was concentrated to a brown viscous residue under reduced pressure at less than 40 °C, and then dissolved in H_2O (250 mL) and partitioned with water-saturated *n*-BuOH. After solvent removal, the residue of the *n*-BuOH fraction (8.1 g) was dissolved in a small amount of MeOH (50 mL) and purified by precipitation with Et_2O (2 × 250 mL), yielding a crude saponin mixture (7.12 g). This mixture was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A 12 nm S-75 μm) using step mixtures of the H_2O/MeOH solvent system (100/0, 30/70, and 0/100). The 70% MeOH (2.1 g) subfraction was further purified by reversed-phase semipreparative HPLC eluting with a 65% aqueous MeOH solvent in a flow rate of 2 mL/min to afford sativoside A (**1,** 14 mg, t_R = 18 min), sativoside B (**2**, 13 mg, t_R = 33.1 min), **3** (600 mg, t_R = 21.9 min), **4** (60 mg, t_R = 24.4 min), **5** (15 mg, t_R = 28.3 min), and **6** (6 mg, t_R = 31.2 min).

Sativoside A (**1**) : $[a]_D^{27}$ +24.6° (*c* 0.1, MeOH); IR (film) 3389 (OH), 2931 (CH), 1728 (C = O), 1644 (C = C), 1027-1039 (C – O) cm^{-1}; ^{1}H and ^{13}C NMR are given in Tables 1 and 2; HRFABMS *m/z*:1375.6517 (calcd for $C_{64}H_{104}O_{30}Na$:1375.6510).

Sativoside B (**2**) : $[a]_D^{27}$ + 7.2° (*c* 0.1, MeOH); IR (film) 3366 (OH), 2929 (CH), 1717 (C = O), 1624 (C = C), 1027-1039 (C – O) cm^{-1}; ^{1}H and ^{13}C NMR are given in Tables 1 and 2; HRFABMS *m/z*:1373.6346 for $[M+Na]^+$ (Calcd for $C_{64}H_{102}O_{30}Na$:1373.6354).

Sugar analysis. Each compound (each 5.0 mg) in H_2O (2 mL) and 2 N CF_3COOH (5 mL) were refluxed on a water bath at 75 °C for 6 h. Then, the reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with H_2O and evaporated to dryness to afford the aglycon. The aqueous layer was repeatedly evaporated to dryness with MeOH and the residue was dissolved in CH_3CN-H_2O (1:1) followed by HPLC analysis, using an isocratic elution of CH_3CN-H_2O (85:15) with RI detection in a flow rate of 0.9 mL/min. Coelution experiments with standard sugar samples allowed the identification of rhamnose (t_R = 7.13 min), xylose (t_R = 8.06 min), arabinose (t_R = 8.86 min), and glucose (t_R = 11.35 min). Co-injection of each hydrolysate with standard L-rhamnose, D-xylose, L-arabinose, and D-glucose yielded consistent peaks.

And then each of these elutes were individually collected, evaporated to dryness and dissolved in H_2O and the optical rotation was then recorded at room temperature.

Cell culture. The HMC-1 cells were grown in IMDM and supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 with 95% humidity. The HMC-1 cells were treated with new triterpene saponin compounds for 1 h. The cells were then stimulated with 50 nM of phorbol 12-myristate 13-acetate (PMA) plus 1 μM of A23187 and incubated at 37 °C for 8 h.

MTT assay for cell viability. Cell viability was examined by an MTT assay. After HMC-1 aliquots were seeded (3×10^5) in microplate wells, The compounds (5, 10, 50, and 100 μg/mL) were added to each well and incubated with 10 μL of an MTT solution (5 mg/mL) for 4 h at 37 °C under 5% CO_2 and 95% air. Then, the supernatant was removed and the formazone crystals were dissolved by the addition of 100 μL of DMSO. An automatic microplate reader was used to read the absorbance of each well at 590 nm.

Cytokine assay. The HMC-1 cells were pretreated with two concentrations of six compounds (5 and 10 μg/mL) for 1 h before PMA plus A23187-stimulation. We then used the enzyme-linked immunosorbent assay (ELISA) method to assay the culture supernatants for the IL-6 protein levels. To measure the cytokine IL-6, we used a modified ELISA method. We first conducted a sandwich ELISA for IL-6 in duplicate in 96-well ELISA plates (Nunc, Denmark). Then, the supernatant was decanted into a new microcentrifuge tube and the cytokine was quantitated by ELISA. ELISA plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) were coated overnight at 4 °C with anti-human IL-6 monoclonal antibody diluted in coating buffer (0.1 M carbonate, pH 9.5) and then washed four times with phosphate buffered saline (PBS) containing 0.05% Tween 20. The nonspecific protein binding sites were blocked with assay diluent (PBS containing 10% FBS, pH 7.0) for at least 1 h. After washing the plates again, the test sample or recombinant IL-6 standards were added. After incubation for 2 h, a working detector (biotinylated anti-human IL-6 monoclonal antibodies and streptavidin-horseradish peroxidase reagent) was added and incubated for 1 h. Accordingly, substrate solution (tetramethylbenzidine) was added to the wells and incubated for 30 min in the dark before the reaction was stopped with a solution of 2 N H_3PO_4. The absorbance was recorded at 450 nm. All subsequent steps took place at room temperature, and all standards and samples were assayed in duplicate.

Statistical analysis. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons, and Student's test for single comparisons. The data from the experiments are presented as means ± S.E.M.

Table 2. NMR Spectral Data of the Sugar Portion of sativosides A (**1**) and B (**2**) in CD_3OD

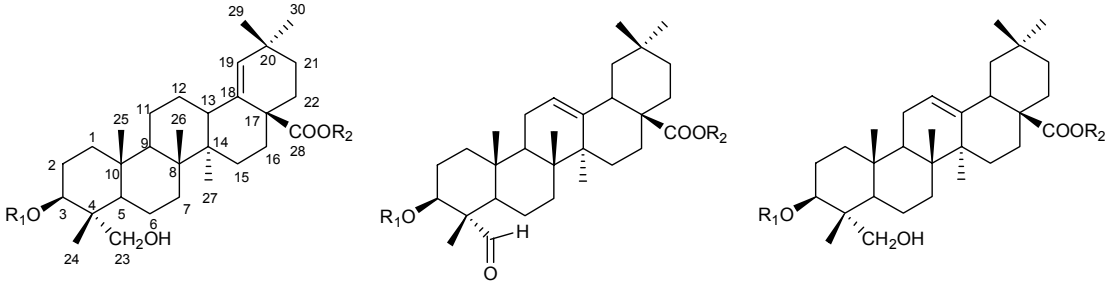
no	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
C-3				
Ara				
1	4.50 (1H, d, 5.4)	104.7, *d*	4.22 (1H, d, 3.5)	103.0, *d*
2	3.69[a]	76.0, *d*	3.63[a]	75.3, *d*
3	3.68[a]	74.2, *d*	3.62[a]	74.4, *d*
4	3.75 (1H, bs)	69.7, *d*	3.70[a]	69.6, *d*
5a	3.51[a]	65.5, *t*	3.45 (1H, bs)	65.7, *t*
5b	3.84 (1H, d, 3.7)		3.80 (1H, d, 3.6)	
Rha I				
1	5.23 (1H, bd, 1.2)	101.2, *d*	5.20 (1H, bd, 1.5)	100.9, *d*
2	4.07[a]	71.6, *d*	4.00 (1H, t, 1.5)	71.9, *d*
3	3.83[a]	82.1, *d*	3.87[a]	81.9, *d*
4	3.56 (1H, bd, 3.9)	72.7, *d*	3.53 (1H, dd, 3.9, 3.9)	72.9, *d*
5	3.92 (1H, d, 3.4)	69.9, *d*	3.87[a]	69.6, *d*
6	1.24 (3H, d, 6.1)	17.8, *q*	1.25 (3H, d, 6.1)	17.8, *q*
Xyl				
1	4.47 (1H, d, 7.3)	106.5, *d*	4.58 (1H, d, 7.1)	106.6, *d*
2	3.29[a]	75.2, *d*	3.28[a]	75.3, *d*
3	3.41[a]	78.3, *d*	3.38[a]	78.2, *d*
4	3.49 (1H, m)	71.0, *d*	3.47[a]	71.1, *d*
5a	3.21[a]	66.9, *t*	3.24[a]	67.0, *t*
5b	3.87 (1H, d, 5.3)		3.85 (1H, d, 5.3)	
C-28				
Glc I				
1	5.46 (1H, d, 8.3)	95.7, *d*	5.32 (1H, d, 8.1)	95.8, *d*
2	3.30[a]	76.7, *d*	3.31[a]	75.3, *d*
3	3.54[a]	78.0, *d*	3.51 (1H, d, 3.4)	78.1, *d*
4	3.46 (1H, dd, 2.7, 2.7)	70.8, *d*	3.38 (1H, dd, 2.7)	70.9, *d*
5	3.32[a]	77.5, *d*	3.32 (1H, bs)	77.7, *d*
6a	3.81[a]	69.7, *t*	3.78[a]	69.4, *t*
6b	4.07 (1H, d, 11.5)		4.07 (1H, d, 11.5)	
Glc II				
1	4.35 (1H, d, 7.8)	104.7, *d*	4.39 (1H, d, 8.1)	104.2, *d*
2	3.23[a]	75.2, *d*	3.22 (1H, bs)	75.3, *d*
3	3.29[a]	76.7, *d*	3.27[a]	76.8, *d*
4	3.53[a]	79.5, *d*	3.51 (1H, d, 3.4)	79.5, *d*
5	3.46[a]	76.6, *d*	3.45 (1H, bs)	76.7, *d*
6a	3.65(1H, d, 4.2)	61.8, *t*	3.63[a]	61.8, *t*
6b	3.79[a]		3.78[a]	
Rha II				
1	4.84 (1H, bs)	102.8, *d*	4.82 (1H, bs)	102.9, *d*
2	3.83[a]	72.3, *d*	3.82 (1H, d, 2.7)	72.4, *d*
3	3.63 (1H, t, 3.5)	72.1, *d*	3.61(1H, d, 3.2)	72.3, *d*
4	3.40[a]	73.6, *d*	3.39[a]	73.9, *d*
5	3.95 (1H, d, 3.4)	70.6, *d*	3.96 (1H, d, 3.4)	70.6, *d*
6	1.26 (3H, d, 6.3)	17.7, *q*	1.26 (3H, d, 6.3)	17.8, *q*

[a]Overlaped with other signals. *q* = CH_3, *t* = CH_2, *d* = CH.

Results and Discussion

The *n*-butanol fractions obtained from the methanolic extract of the seeds of *N. sativa* were suspended in MeOH and purified by precipitation with Et_2O yielding a crude saponin mixture. This extract was further fractionated by a combination of silica gel and RP-18 flash column chromatography, followed by semi-preparative reversed-phase HPLC to afford two new saponins, sativosides A (**1**) and B (**2**), together with four known compounds (Figure 1).

Sativoside A (**1**) was isolated as a white amorphous powder and its molecular formula was established as $C_{64}H_{104}O_{30}$ on the



I **II** **III**

compounds	aglycon	R_1	R_2
1	**I**	β-D-Xyl (1 → 3)-α-L-Rha I (1 → 2)-α-L-Ara	α-L-Rha II (1 → 4)-β-D-Glu II (1 → 6)-β-D-Glu I
2	**II**	β-D-Xyl (1 → 3)-α-L-Rha I (1 → 2)-α-L-Ara	α-L-Rha II (1 → 4)-β-D-Glu II (1 → 6)-β-D-Glu I
3	**III**	β-D-Xyl (1 → 3)-α-L-Rha I (1 → 2)-α-L-Ara	α-L-Rha II (1 → 4)-β-D-Glu II (1 → 6)-β-D-Glu I
4	**III**	α-L-Rha I (1 → 2)-α-L-Ara	α-L-Rha II (1 → 4)-β-D-Glu II (1 → 6)-β-D-Glu I
5	**III**	β-D-Xyl (1 → 3)-α-L-Rha (1 → 2)-α-L-Ara	β-D-Glu II (1 → 6)-β-D-Glu I
6	**III**	α-L-Rha (1 → 2)-α-L-Ara	β-D-Glu II (1 → 6)-β-D-Glu I

Figure 1. Structures of Saponins **1-6** isolated from *Nigella sativa* L.

basis of a combination of a peak at *m/z* 1375.6517 $[M+Na]^+$ (calcd 1375.6510) of HRFABMS and the ^{13}C NMR spectrum. The IR spectrum showed strong absorption bands at 3389 and 1728 cm^{-1}, typical of the hydroxyl and carbonyl group, respectively. The 1D NMR spectra (^1H, ^{13}C and DEPT) of **1** displayed the presence of eight methyls, one double bond and one carboxylic group. All signals observed in the upfield (0.5 ~ 2.5 ppm) and midfield (3.0 ~ 5.5 ppm) region of the ^1H NMR spectrum deduced compound **1** to be a triterpene glycoside, together with anomeric signals corresponding to six monosaccharide residues in the HSQC spectrum. From careful analysis with 1D and 2D NMR data (COSY, TOCSY, HSQC and HMBC), the aglycon part of **1** was revealed to be a triterpene of 3, 23-dihydroxyolean-18-en-28-oic acid, characteristic of six methyl singlets at δ 0.68, 0.81, 0.93, 0.97, 0.99, and 1.01, two diastereotopic protons at δ 3.33 and 3.51 ascribable to a CH_2OH group and a broad singlet at δ 5.14. The position of the double bond at $\Delta^{18(19)}$ was evident from the HMBC correlations of two methyl protons at δ 0.97 and 0.99 with the olefinic carbon at δ 134.0 (C-19) and of the proton at δ 5.14 with two carbons at δ 42.3 (C-13) and 49.9 (C-17). The linkage of the CH_2OH and the carbonyl carbon groups to two quaternary carbons at C-4 and C-17, respectively, was also secured by the HMBC correlations between two methylene protons at δ 3.33 and 3.51 and the carbon at δ 44.1 (C-4) and between the proton at δ 2.02 (H-16) and the carbonyl carbon at δ 177.0 (C-28). The relative stereochemistry of all chiral centers in the aglycon part of **1** was achieved by the ROESY experiment. The configuration of the glycosylated OH group at C-3 was assigned to be β from the NOE peak between H-3 and H-5. And the NOE effect between H_3-24 and H_3-25 indicated that the methyl group at C-24 was in a β position. The carbonyl group at C-28 was established to be oriented in the β-axial direction based on the observation of the NOE peak between H-16b and H-22b.[24-25]

The monosaccharide residues in the glycosidic part of **1** were assigned by a combination of COSY, TOCSY and HSQC spectra, starting from the well-separated anomeric protons at δ 4.35 (d, J = 7.8 Hz), 4.47 (d, J = 7.3 Hz), 4.50 (d, J = 5.4 Hz), 4.84 (bs), 5.23 (bd, 1.2), and 5.46 (d, J = 8.3 Hz). Based on the evaluation of spin-spin couplings and ROESY data, the existence of two β-glucopyranosyl (Glc), one β-xylopyranosyl (Xyl), one α-arabinopyranosyl (Ara), and two α-rhamnopyranosyl (Rha) residues were recognized. This is confirmed by both HPLC analysis with authentic sugars after acid hydrolysis of **1** and optical rotation measurement for each isolated residue, indicating that the sugar part was composed of rhamnose (L-Rha), xylose (D-Xyl), arabinose (L-Ara), and glucose (D-Glu) in a ratio of 2:1:1:2. Then, the sequence of monosaccharide units in the glycosidic part was established from the HMBC and ROESY spectra. In the HMBC experiment (Figure 2), the downfield shifted carbon at C-3 of the aglycon showed long-range correlation with the proton at δ 4.50 (H-1) of the Ara residue, of which C-2 was in turn connected to the H-1 of Rha I via an ether linkage from the correlations between the anomeric proton at δ 5.23 (H-1) of Rha I and C-2 (δ 76.0) of Ara unit. An additional HMBC correlation between the H-1 (δ 4.47) of Xyl and C-3 (δ 82.1) of Rha I completed the linkage of the trisaccharide chain at C3 of the aglycon part. Followed by the same analysis, the 28-*O*-triglycoside structure of **1** was characterized on the basis of the HMBC cross-peaks between H-1 (δ 5.46) of Glc I and C-28 (δ 177.0) of the aglycon part, H-1 (δ 4.35) of Glc II

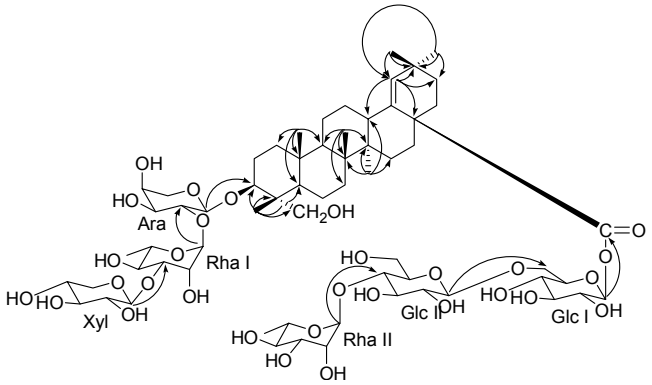


Figure 2. key HMBC correlations for Sativoside A (**1**).

Table 3. Effect of compounds **1-6** on IL-6 production

control		IL-6 (pg/mL)	Inhibition rate (%)	IC_{50} (μg/mL)
		3 ± 1		
PMA + A23187		679 ± 4		
1	10 μg/mL	386 ± 8	43	12.6
	5 μg/mL	572 ± 22	16	
2	10 μg/mL	265 ± 21	61	8.5
	5 μg/mL	510 ± 7	25	
3	10 μg/mL	271 ± 18	60	7.6
	5 μg/mL	422 ± 25	38	
4	10 μg/mL	549 ± 15	19	30.9
	5 μg/mL	695 ± 24	0	
5	10 μg/mL	678 ± 11	0	-
	5 μg/mL	675 ± 6	0	
6	10 μg/mL	533 ± 10	22	23.4
	5 μg/mL	674 ± 3	0	

and C-6 (δ 69.7) of Glc, H-4 (δ 3.53) of Glc II and C-1 (δ 102.8) of Rha II, and H-1 (δ 4.84) Rha II and C-4 (δ 79.5) of Glc II. The unequivocal sequence of two trisaccharide chains was also supported from ROESY peaks between the anomeric protons and the proton in the adjacent residue. The triterpene glycoside isolated from *Oreopanax guatemalensis* is the closest relative.[25] Thus, the structure of **1** was determined as 3-*O*-[β-D-xylopyranosyl-(1 → 3)-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl] 3β, 23-dihydroxy olean-18-en-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-*O*-βD-glucopyranosyl- (1 → 6)-β-D-glucopyranosyl] ester. To the best of our knowledge, compared with common bisdesmosidic saponins with trisaccharide chains at C-3 and C-28 of the aglycon, the aglycon of **1** possesses a rare olean-18-en moiety. This is the first reported occurrence of an olean-18-en type saponin in family Ranunculacea.

Sativoside B (**2**) exhibited a quasimolecular ion peak at *m/z* 1373.6346 $[M + Na]^+$ (calcd 1373.6354) consistent with the molecular formula $C_{64}H_{102}O_{30}$ in the HRFABMS. As described previously, the signal assignment of **2** from extensive 1D and 2D NMR spectroscopic studies suggested a bisdesmosidic saponin with two trisaccharides at the C-3 and C-28 position of the aglycon with the olean-12-en type, gypsogenin.[26] And the acid hydrolysis followed by HPLC analysis and optical activity measurement revealed sugar components to be the same with those of **1**. The feature of **2** indicated the presence of an aldehydic group, confirmed by the signal at δ 9.50 in the ^{1}H NMR spectrum and by the signal at δ 209.3 in the ^{13}C NMR spectrum. This group was connected to the position of C-4 from the HMBC correlation from the downfield shifted aldehydic proton at δ 9.50 to the quaternary carbon at δ 56.3 (C-4). Accordingly, compound **2** was identified as a 3-*O*-[β-D-xylopyranosyl-(1 → 3)-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl] gypsogenin 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester. The survey showed that the elucidated structure of **2** was very similar to that of **3** reported recently from the seeds of *N. sativa*.[14] Compound **2** may be inferred to be oxidized from **3** in the biosynthesis pathway.

Compounds **4-6** were determined as the known triterpene glycosides by analysis of the 2D NMR data by the same procedure as **1**; **4** was suggested to be a 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl] hedragenin 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester **5**,[14] a 3-*O*-[β-D-xylopyranosyl-(1 → 3)-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl] hedragenin 28-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, previously reported from *Cephalaria pastricensis*.[27] and **6** as a 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl] hedragenin 28-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, isolated from *C. pastricensis* and *N. glandulifera* Freyn.[28] However, the latter saponins **5** and **6** were not previously reported from the *Nigella* genus.

We examined the inhibition effect of the six isolated compounds on PMA plus calcium ionophore A23187-induced production of pro-inflammatory cytokine IL-6 in the HMC-1 line, which may be important in developing future anti-inflammatory therapies. Although weak cytotoxicity at high concentration of 100 μg/mL, all compounds were found not to affect HMC-1 cells viability at concentrations of 5, 10, 50 and 100 μg/mL (data not shown). As shown in Table 3, the levels of IL-6 was considerably increased after stimulation with PMA plus A23187 in HMC-1. Treatment of cell with compounds **1-3** (5 and 10 μg/mL) significantly inhibited IL-6 production in a concentration-dependent and statistically significant manner.

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