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Two Novel Anti-Inflammatory 21-Nordammarane Saponins from Tetraploid Jiaogulan (*Gynostemma pentaphyllum*)

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Supporting Information

ABSTRACT: Two novel 20-oxo-21-nordammar-22, 24-diene saponins, 21-norgypenosides A (**1**) and B (**2**), were characterized from the aerial parts of tetraploid Jiaogulan (*Gynostemma pentaphyllum*), a popular tea ingredient. Their structures, including the absolute configurations, were comprehensively elucidated by HRESIMS, 1D and 2D NMR data, chemical degradation, and through comparison of the experimental and calculated electronic circular dichroism (ECD) spectra. The two compounds suppressed the expression of interleukin (IL)-1β, cyclooxygenase (COX)-2, and tumor necrosis factor (TNF)-α mRNAs in the lipopolysaccharide-induced RAW 264.7 mouse macrophage cells at 10 and 100 μg/mL, suggesting their potential anti-inflammatory effects.

KEYWORDS: *Gynostemma pentaphyllum, 21-nordammarane saponins, anti-inflammation, NMR, structure identification*

INTRODUCTION

Gynostemma pentaphyllum is a perennial creeping plant with a common Chinese name of Jiaogulan. It is widely distributed in China, Japan, and Southeast Asian countries. Jiaogulan was first recorded as a vegetable and food in a book "Herbs for Famine" published during the Chinese Ming Dynasty (1368−1644 A.D.). It has also been used for a functional tea in China and sweet tea in Japan.[1] Previous chemical investigation has detected saponins, flavonoids, sterols, and megastigmane glycosides in *G. pentaphyllum*.[2−4] Jiaogulan saponins (gypenosides) are considered responsible for several health benefits of *G. pentaphyllum*. These beneficial effects may include anti-inflammatory, antiproliferative, hypolipemic, and immune-potentiating activities.[5,6] To date, more than 170 saponins with diverse carbon skeletons have been isolated from *G. pentaphyllum*, which has attracted increasing interest during the past few decades.[7]

Recently, our studies showed that tetraploid *G. pentaphyllum* may have greater saponin content than its diploid counterpart,[8] and the two new dammarane-type saponins isolated from tetraploid *G. pentaphyllum* showed significant anti-inflammatory activities.[9] To further investigate the individual bioactive constituents from tetraploid *G. pentaphyllum*, this study was performed to isolate and characterize two novel 21-nordammarane saponins, 21-norgypenosides A (**1**) and B (**2**), and to investigate their possible anti-inflammatory activities. The results from this study may expand our knowledge on the bioactive components of *G. pentaphyllum* and promote the value-added utilization of *G. pentaphyllum* for improving human health.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured at 589 nm in CH_3OH on a Jasco P-2000 polarimeter (Tokyo, Japan) with a sodium lamp. Circular dichroism spectra were received in CH_3OH on a Jasco J-815 spectrometer (Tokyo, Japan). IR spectra were recorded using a Nicolet 6700 FTIR spectrophotometer (Madison, WI). NMR spectra were obtained from a Bruker DRX500 or Bruker Avance III 500 spectrometer (Rheinstetten, Germany) with δ in ppm related to TMS and J in Hz. HR-ESIMS analyses were performed on a Waters Xevo G_2 Q-TOF mass spectrometer (Milford, MA). Semipreparative HPLC was carried out on a Waters liquid chromatograph system equipped with a 1525 pump, a 2489 detector, and a Waters SunFire OBD-C_{18} column (250 mm × 19 mm i.d., 5 μm). The leucine derivatives of sugars were identified using an Agilent 7890A gas chromatograph system (Santa Clara, CA). Medium-pressure column separations were performed on a Buchi Sepacore chromatography system (Flawil, Switzerland). Silica gel (200−300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, Shandong, China) and ODS (S-50 and 75 μm, YMC Co., Ltd., Kyoto, Japan) were used for regular and medium-pressure column chromatography fractionations. An IScript Advanced cDNA Synthesis kit was purchased from Bio-Rad Laboratories (Hercules, CA), while an AB Power SYBR Green PCR Master Mix was obtained from ABI (Applied Biosystems, Carlsbad, CA).

Cells and Reagents. The mouse RAW 264.7 macrophage cell line was bought from the Chinese Academy of Sciences (Shanghai, China). Lipopolysaccharide (LPS, from *Escherichia coli* O111:B4) was obtained from Millipore (Billerica, MA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and 1× PBS (pH 7.4) were purchased from

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Table 1. NMR Spectroscopic Data for 21-Norgypenosides A (1) and B (2)

	1		2	
positions	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	40.1, CH_2	1.49, 0.85, m	40.1, CH_2	1.59, 0.86, m
2	27.4, CH_2	2.27, 1.85, m	27.3, CH_2	2.05, 1.86, m
3	89.2, CH	3.39, dd, 11.6, 4.1	88.6, CH	3.30, dd, 11.7, 4.2
4	40.2, C		40.3, C	
5	57.1, CH	0.74, m	57.1, CH	0.76, m
6	18.9, CH_2	1.47, 1.37, m	18.9, CH_2	1.47, 1.38, m
7	36.0, CH_2	1.48, 1.30, m	36.1, CH_2	1.51, 1.34, m
8	40.7, C		40.7, C	
9	51.6, CH	1.40, m	51.6, CH	1.45, m
10	37.6, C		37.6, C	
11	33.4, CH_2	1.98, 1.42, m	33.5, CH_2	2.03, 1.50, m
12	72.0, CH	3.89, m	72.1, CH	3.88, m
13	54.7, CH	2.63, m	54.6, CH	2.64, m
14	52.2, C		52.2, C	
15	33.6, CH_2	1.87, 1.18, m	33.6, CH_2	1.85, 1.18, m
16	29.0, CH_2	2.16, 1.87, m	29.0, CH_2	2.15, 1.89, m
17	51.6, CH	3.42, m	51.6, CH	3.40, m
18	16.3, CH_3	1.00, s	16.3, CH_3	1.02, s
19	17.2, CH_3	0.78, s	17.2, CH_3	0.81, s
20	205.4, C		205.3, C	
22	129.4, CH	6.58, d, 15.0	129.4, CH	6.57, d, 15.0
23	138.5, CH	7.79, dd, 15.0, 11.6	138.4, CH	7.79, dd, 15.0, 11.6
24	125.5, CH	6.02, d, 11.6	125.5, CH	6.03, d, 11.6
25	147.0, C		146.9, C	
26	26.9, CH_3	1.69, s	26.9, CH_3	1.70, s
27	19.2, CH_3	1.67, s	19.1, CH_3	1.68, s
28	28.4, CH_3	1.26, s	28.5, CH_3	1.23, s
29	17.4, CH_3	1.18, s	17.3, CH_3	1.14, s
30	17.7, CH_3	1.00, s	17.6, CH_3	1.02, s
	Glc		**Ara**	
1′	105.5, CH	4.89, d, 7.5	105.3, CH	4.89, d, 5.6
2′	77.4, CH	4.31, m	75.3, CH	4.68, m
3′	90.1, CH	4.25, m	82.8, CH	4.35, m
4′	70.4, CH	4.11, m	68.8, CH	4.57, brs
5′	78.5, CH	3.94, m	65.5, CH_2	4.26, 3.77, m
6′	63.2, CH_2	4.53, 4.29, m		
	Rha		**Rha**	
1″	102.2, CH	6.54, brs	102.5, CH	6.19, brs
2″	73.0, CH	4.86, brs	73.0, CH	4.76, brs
3″	73.02, CH	4.64, m	73.1, CH	4.62, m
4″	74.4, CH	4.35, m	74.4, CH	4.30, m
5″	70.4, CH	4.79, m	70.5, CH	4.62, m
6″	19.2, CH_3	1.72, d, 6.2	19.1, CH_3	1.65, d, 6.1
	Glc		**Glc**	
1‴	104.4, CH	5.15, overlap	105.2, CH	5.13, d, 7.8
2‴	75.7, CH	4.05, m	75.5, CH	3.97, m
3‴	78.9, CH	4.23, m	78.8, CH	4.20, m
4‴	72.0, CH	4.14, m	72.0, CH	4.18, m
5‴	79.2, CH	4.07, m	79.1, CH	3.94, m
6‴	62.9, CH_2	4.60, 4.28, m	63.1, CH_2	4.52, 4.34, m

Gibco (Life Technologies, Carlsbad, CA). TRIzol reagent was obtained from Invitrogen (Life Technologies). All solvents used for isolation were of analytical grade, for HPLC analysis were of chromatographic grade, and for UPLC-Q-TOF-MS analysis were of mass spectrometry grade and used without further purification.

Plant Material. The aerial parts of tetraploid *Gynostemma pentaphyllum* (Thunb.) Makino were collected from the Dabashan area of Pingli, Shaanxi province of China in 2010 and were identified by the Asian Citrus Holdings Ltd. (Hong Kong). Botanicals were kept at 4 °C before use. A voucher specimen (No. 2010005) has been deposited in our laboratory,

School of Agriculture and Biology, Shanghai Jiao Tong University.

Extraction and Isolation. The aerial parts of tetraploid *G. pentaphyllum* (3.8 kg) were dried, powdered, and extracted with methanol three times (20 L, 2 h for each time) at 70 °C. The combined extracts were concentrated under reduced pressure to obtain a dark brown residue (800 g). The residue was suspended into deionized water (5 L) and extracted with petroleum ether, ethyl acetate, and *n*-butanol, sequentially. The *n*-butanol fraction (Fraction B, 390 g) was fractionated further on a silica gel (200−300 mesh) column chromatography with CH_2Cl_2-MeOH (100:1, 20:1, 10:1, 5:1, 1:1, and 1:2, v/v) to obtain four subfractions. Fraction B-3 (223 g) was further separated on a silica gel (200−300 mesh) column chromatography with EtOAc-MeOH (10:1, 5:1, 2:1, 1:1, and 1:2, v/v) to obtain five fractions: B-3-1 to B-3-5. Fraction B-3-4 (12 g) was subjected to ODS medium pressure column chromatography and washed with MeOH-H_2O (1:19, 1:4, 2:3, 3:2, 4:1, and pure methanol, v/v) to afford 10 fractions. Fraction B-3-4-7 (1200 mg) was separated on a silica gel (200−300 mesh) column chromatography with $CHCl_3$-MeOH (5:1 and 1:1, v/v) to yield 11 fractions, and the fraction B-3-4-7-6 (50 mg) was further purified by semipreparative HPLC (ACN-H_2O, 37:63, v/v; 4 mL/min; detection wavelength, 205 nm) to obtain two new saponins, compounds **1** (5 mg; t_R 69 min) and **2** (8 mg; t_R 77 min).

*21-Norgypenoside A (**1**).* White amorphous powder; $[\alpha]_D^{22}$ + 16.9° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 295 (4.15) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 354 (−0.65), 303 (+9.46), 243 (−0.49), 219 (+1.90) nm; IR (KBr): ν_{max} 3439, 2930, 1662, 1629, 1384, 1075 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR (pyridine-d_5) data are presented in Table 1; HRESIMS *m*/*z*: $[M - H]^-$ calcd for $C_{47}H_{75}O_{17}$, 911.5004; found 911.5009; 749.4484 $[M - H - Glc]^-$; 603.3903 $[M - H - Glc - Rha]^-$; and 441.3371 $[M - H - Glc - Rha - Glc]^-$ (Figure S1).

*21-Norgypenoside B (**2**).* White amorphous powder; $[\alpha]_D^{22}$ + 10.0° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 295 (4.23) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 354 (−0.49), 302 (+ 6.91), 244 (−0.57), 220 (+1.53) nm; IR (KBr): ν_{max} 3440, 2928, 1661, 1630, 1384, 1074 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR (pyridine-d_5) data are presented in Table 1; HRESIMS *m*/*z*: $[M - H]^-$ calcd for $C_{46}H_{73}O_{16}$, 881.4899; found 881.4903; 719.4379 $[M - H - Glc]^-$; 573.3788 $[M - H - Glc - Rha]^-$; and 441.3363 $[M - H - Glc - Rha - Ara]^-$ (Figure S2).

Acid Hydrolysis and GC Analysis. Compounds **1** and **2** (2 mg each) were hydrolyzed with 10% HCl/dioxane (1:1, v/v, 1 mL) at 80 °C for 4 h.[10] Dioxane and hydrochloric acid were removed using a nitrogen evaporator. The residue was diluted with 1 mL of distilled water and extracted with EtOAc (3 × 1 mL). After removal of solvent, the water layer was examined by TLC (10 × 20 cm) with $CHCl_3$/MeOH/H_2O (55:45:10, v/v/v) and compared with the authentic samples. The sugar spots were visualized by spraying with aniline hydrogen phthalate reagent, followed by heating at 110 °C for 10 min. The R_f values of monosaccharide were as follows: glucose (0.44), arabinose (0.52), and rhamnose (0.65).

The sugar residue was obtained by HCl hydrolysis as described above and dissolved in 1 mL of anhydrous pyridine. After 2 mg of L-leucine methyl ester hydrochloride (J&K Scientific Ltd., Beijing, China) was added, the mixture was kept at 60 °C for 1 h. The mixture was stirred with 2 mg of $NaBH_4$ for 1 h at ambient temperature, then 0.2 mL of Me_3SiCl (J&K Scientific Ltd., Beijing, China) was added to the mixture, and reaction was carried out at 60 °C for another 30 min. The leucine derivatives of sugars were further identified by GC analysis under the following conditions: HP-5 column (Agilent); injection temperature, 200 °C; column temperature was 150 °C for 2 min and increased to 310 °C at 10 °C/min; carrier gas was N_2 at a flow rate of 1 mL/min; the retention times of L-rhamnose, L-arabinose, and D-glucose derivatives were 7.43, 7.66, and 11.02 min, respectively.

ECD Calculation. Theoretical calculations of electronic circular dichroism (ECD) spectra for compound **1** were performed with the Gaussian 09 (Gaussian, Inc., Wallingford, CT) program package using a time-dependent density functional theory (TD-DFT).[11] Geometry for **1** was obtained and optimized by the MM2 force-field in Chem3D Ultra (version 9.0, 2005, CambridgeSoft, Cambridge, MA). Then, the conformation analysis was performed at the B3LYP/6-31+G(d, p) level of theory. The ECD calculations of **1** solution were performed at the B3LYP/6-31+G(d, p) level of theory. The calculated excitation energy (in nm) and rotatory strength *R*, in dipole velocity (R_{vel}) and dipole length (R_{len}) forms, were simulated into an ECD curve by using the following Gaussian function:

$$\Delta\varepsilon(E) = \sum_{i=1}^{n} \Delta\varepsilon_i(E) = \sum_{i=1}^{n} \left(\frac{R_i E_i}{2.29 \times 10^{-39} \sqrt{\pi\sigma}} \exp\left[-\left(\frac{E - E_i}{\sigma} \right)^2 \right] \right) \quad (1)$$

where σ is the width of the band at $1/e$ height, and E_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively. R_{vel} and σ were used with UV correction.

Potential Anti-Inflammatory Activities of 21-Norgypenosides A and B. Mouse RAW 264.7 macrophages were cultured overnight in 6-well plates to reach the confluence of 80% and were then incubated (1.5×10^5 cell/well) in DMEM supplemented with 10% FBS and 1% amphotericin B/streptomycin/penicillin under 5% CO_2 at 37 °C. The different concentrations of compounds **1** and **2** (10 and 100 μg/mL) were added to the cells. After a 24 h incubation, LPS was added into the media at the initial concentration of 10 ng/mL. After a 4 h induction, culture medium was discarded, cells were collected and washed with 1× PBS, and TRIzol reagent was added to the cells for total RNA isolation. IScript Advanced cDNA Synthesis kit was used to reverse transcribe cDNA, and real-time PCR was performed on an ABI 7900HT Fast Real-Time PCR System using AB Power SYBR Green PCR Master Mix.[12,13] Sequences of primers used in this study were as follows: IL-1β (forward, 5′-GTTGACGGACCCCAAAAGAT-3′; reverse, 5′-CCTCATCCTGGAAGGTCCAC-3′), COX-2 (forward, 5′-GGGAGTCTGGAACATTGTGAA-3′; reverse, 5′-GCACGTTGATTGTAGGTGGACTGT-3′), TNF-α (forward, 5′-CGAGTGACAAGCCTGTAGC-3′; reverse, 5′-GGTGTGGGTGAGGAGCACAT-3′). The mRNA amounts were normalized to an internal control, GAPDH mRNA (forward, 5′-CCTGGAGAAACCTGCCAAGTATG-3′; reverse, 5′-AGAGTGGGAGTTGCTGTTGAAGTC-3′). The amplification parameters used for PCR were as follows: 50 °C for 2 min, 95 °C for 10 min, and 46 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min.

Data Analysis. Data were reported as mean ± SD for triplicate determinations on an "as-it-is" botanical weight basis. One-way ANOVA and Tukey's tests were performed to identify differences in means. Statistics were analyzed using SPSS for Windows (version rel.16.0.0, 2007, SPSS Inc., Chicago, IL).

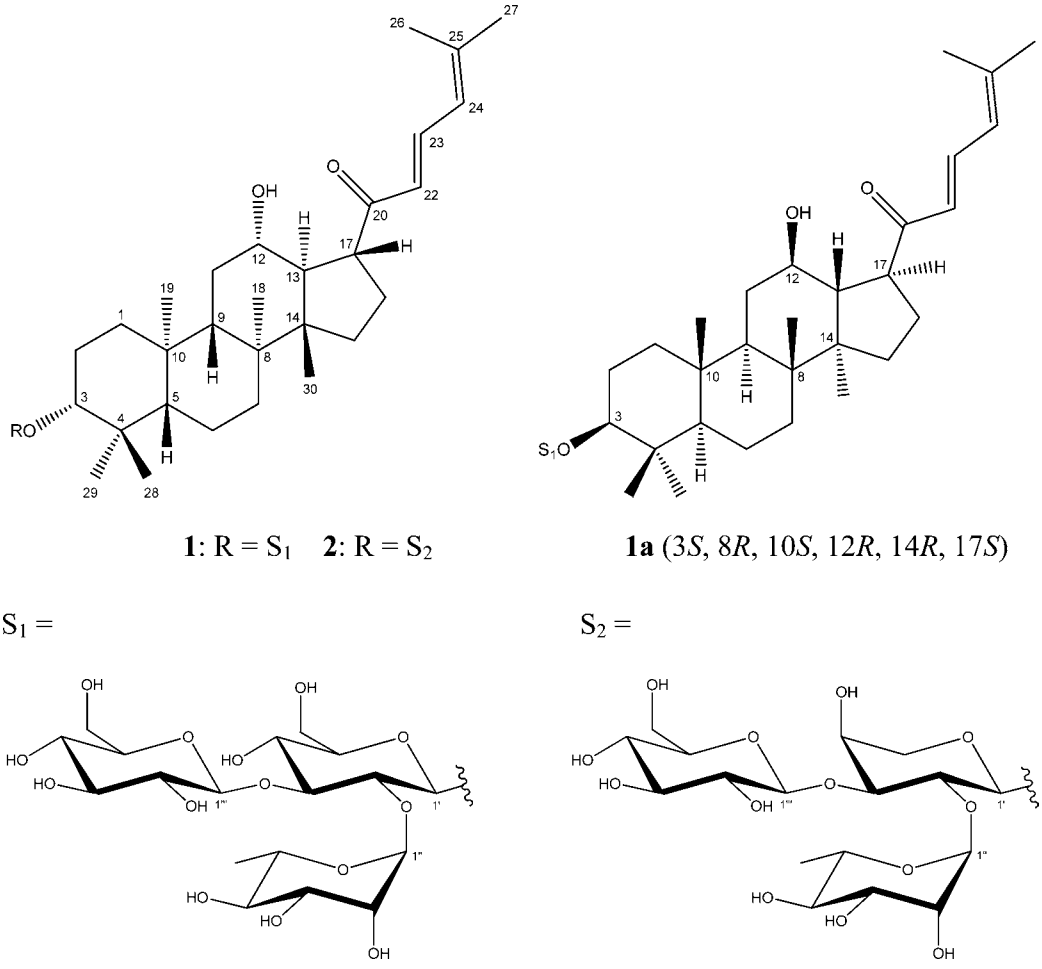

Figure 1. Compounds **1** and **2**, and **1a**, the model compound of **1** for TD-DFT ECD calculation.

Correlation was analyzed using a two-tailed Pearson's correlation test. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Structural Identification of the Novel Saponins. Repeated column chromatography over silica gel, ODS, and semipreparative HPLC of the methanol extract of the aerial parts of tetraploid *G. pentaphyllum* led to the isolation of two novel 20-oxo-21-nordammar-22, 24-diene saponins, 21-norgypenosides A (**1**) and B (**2**). Compound **1** was obtained as a white amorphous powder with an optical rotation of $[\alpha]_D^{22}$ + 16.9° (*c* 0.06, MeOH). The HR-ESI-MS peak at *m/z* 911.5009 $[M - H]^-$ (calculated as 911.5004), combined with the ^1H and ^{13}C NMR data (Table 1), indicated that the molecular formula of 1 was $C_{47}H_{76}O_{17}$. The IR spectrum gave typical absorption bands of hydroxyl (3439 cm^{-1}), carbonyl (1662 cm^{-1}), and alkenyl (1629 cm^{-1}) groups. The ^1H NMR spectrum of **1** demonstrated seven singlet methyl groups at δ_H 0.78, 1.00, 1.00, 1.18, 1.26, 1.67, and 1.69, respectively, and one duplet of methyl group at δ_H 1.72 (J = 6.2 Hz). The ^{13}C NMR spectrum of **1** showed 47 carbon resonances, which were further classified by DEPT spectrum as 8 methyl groups, 9 methylene groups including 2 oxygenated ones (δ_C 63.2, 62.9), 24 methane groups including 3 olefinic methane groups (δ_C 125.5, 129.4, 138.5), 3 ketal carbons (δ_C 102.2, 104.4, 105.5), 14 oxygenated methane groups, and 6 quaternary carbons including 1 keto carbon (δ_C 205.4) and 1 olefinic quaternary carbon (δ_C 147.0). By comparing the ^1H and ^{13}C NMR data of **1** with those saponins previously isolated from *G. pentaphyllum*,[14,15] the aglycon moiety of **1** was deduced as a dammarane triterpene possessing an unprecedented side chain at C-17.

The characteristic signals of three olefinic protons were δ 6.58 (1H, d, J = 15.0 Hz, H-22), 7.79 (1H, dd, J = 15.0, 11.6 Hz, H-23), and 6.02 (1H, d, J = 11.6 Hz, H-24), which suggested the existence of a conjugated diolefine group. Meanwhile, considering the key HMBC correlations of H-23/C-20, H_3-26/C-24, H_3-27/C-24, and H-13/C-20, the side chain at C-17 was unequivocally deduced as a −COCH═CH−CH═C$(CH_3)_2$ group (Figure 1). The UV maximum absorption of **1** was observed at 295 nm, which further supported the above deduction.

The MS^2 fragment ion peaks of compound **1** at *m/z* 749.4484 $[M - H - Glc]^-$, 603.3903 $[M - H - Glc - Rha]^-$, and 441.3371 $[M - H - Glc - Rha - Glc]^-$ indicated that the sugar moiety of **1** might consist of two hexoses (*m/z* 162) and one methyl pentose (*m/z* 146). Furthermore, GC analysis of the leucine derivatives of the sugars obtained from acid hydrolysis of **1** showed the presence of L-rhamnose and D-glucose in a molecular ratio of 1:2. With additional information from the ^1H and ^{13}C NMR data (Table 1), two β-D-glucopyranoses and one α-L-rhamnopyranose were characterized, and their sequence was unambiguously determined by HMBC spectrum.[16]

The relative configuration of aglycone in **1** was confirmed in accordance with a typical dammarane triterpene by ROESY analysis. The clear NOE correlations suggested that H-3, H_3-28, H-5, H-9, H-12, H_3-30, and H-17 were β-oriented, while H_3-29, H_3-19, H_3-18, and H-13 were α-oriented (Figure 2). Thus, the

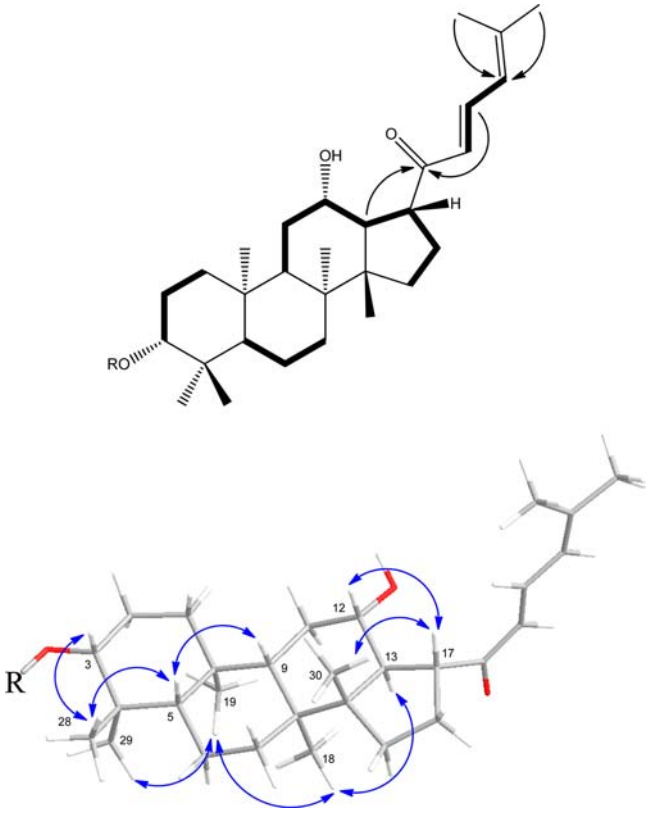

Figure 2. ^{1}H−^{1}H COSY (bold), selected HMBC (single arrows), and key NOE correlations (double arrows) of aglycon moiety of **1** and **2**.

structure of compound **1** was elucidated as (17α,22*E*)-3α,12α-dihydroxy-20-oxo-21-nordammar-22,24-diene-3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside, named 21-norgypenoside A.

Compound **2** was obtained as a white amorphous powder with an optical rotation of $[\alpha]_D^{22}$ + 10.0° (*c* 0.13, MeOH). The HRESIMS peak at *m*/*z* 881.4903 $[M - H]^-$ (calculated as 881.4899), along with the ^{1}H and ^{13}C NMR data, indicated that the molecular formula of **2** was $C_{46}H_{74}O_{16}$. The IR spectrum indicated typical absorption bands of hydroxyl (3440 cm^{-1}), carbonyl (1661 cm^{-1}), and alkenyl (1630 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for the sugar moiety. The MS^2 ion peaks at *m*/*z* 719.4379 $[M - H - Glc]^-$, 573.3788$[M - H - Glc - Rha]^-$, and 441.3363 $[M - H - Glc - Rha - Ara]^-$ for **2** and the results of GC analysis on acid hydrolysate of **2** suggested that the sugar moiety of **2** contained α-L-arabinopyranose, α-L-rhamnopyranose, and β-D-glucopyranose. The connection between the monosugars in **2** was determined by HMBC spectrum. The NOESY experiment revealed that **2** possessed the same configuration as **1**. Therefore, the structure of compound **2** was established as (17α,22*E*)-3α,12α-dihydroxy-20-oxo-21-nordammar-22,24-diene-3-*O*-[α-L-rhamnopyranosyl-(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside, named 21-norgypenoside B.

The absolute configuration of the aglycon moiety in **1** and **2** were established by comparison of their experimental ECD and calculated ECD spectra. The model compound **1a** with (3*S*, 8*R*, 10*S*, 12*R*, 14*R*, 17*S*) absolute configuration (Figure 1) was calculated by using the TD-DFT method at a B3LYP/6-31+G(d, p) level. The calculated result of **1a** produced a mirror image ECD curve of the experimental ECD (Figure 3) that

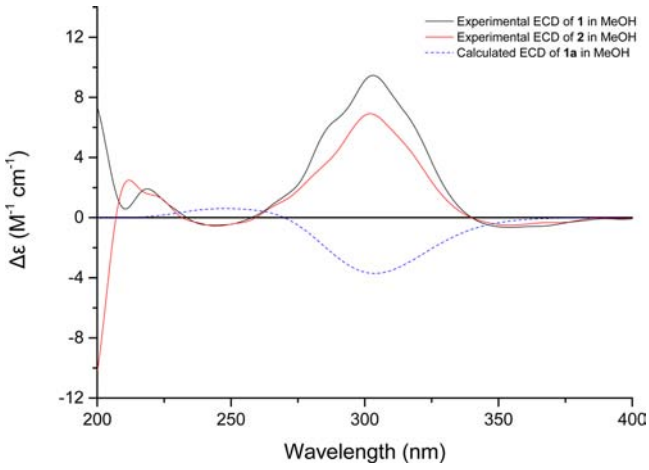

Figure 3. Experimental ECD spectra of **1** and **2** and calculated ECD spectrum of **1a** in MeOH.

permitted the assignment of the absolute configuration of **1** as (3*R*, 8*S*, 10*R*, 12*S*, 14*S*, and 17*R*). Since **2** shared an identical aglycon moiety with **1** and showed a consistent ECD spectrum with **1**, its absolute configuration was finally determined as 3*R*, 8*S*, 10*R*, 12*S*, 14*S*, and 17*R*.

Anti-Inflammatory Activities in the LPS-Induced RAW 264.7 Macrophage Cells. IL-1β and TNF-α are two key inflammatory mediators that may be involved in stimulation and progression of inflammation.[17] COX-2 is an isoform of the COX family and plays a major role in the development of inflammation.[18] In this study, compounds **1** and **2** had significant inhibitions on the mRNA expressions of IL-1β, COX-2, and TNF-α pro-inflammatory cytokines at both 10 and 100 μg/mL treatment concentrations in cultured LPS-induced RAW 264.7 macrophage cells (Figure 4). At 10 μg/mL, compound **2** showed a stronger suppression than compound **1** on IL-1β mRNA expression, whereas the two compounds had no difference in their inhibitory effects on COX-2 and TNF-α mRNA expressions. At a higher concentration of 100 μg/mL, compound **2** exhibited stronger inhibitory activities than compound **1** on the mRNA expression of three tested cytokines. Interestingly, compound **2** showed a dose-dependent inhibition on the three cytokines' mRNA expression, but compound **1** only exhibited a dose-dependent effect on suppressing IL-1β mRNA expression. The different anti-inflammatory abilities of compounds **1** and **2** might be explained by their structural difference in sugar moieties, suggesting a possible role of sugar moieties on the anti-inflammatory activities of gypenosides. It needs to be pointed out that no morphology change was observed in the RAW 264.7 macrophage cells after the treatment at both 10 and 100 μg/mL concentrations.

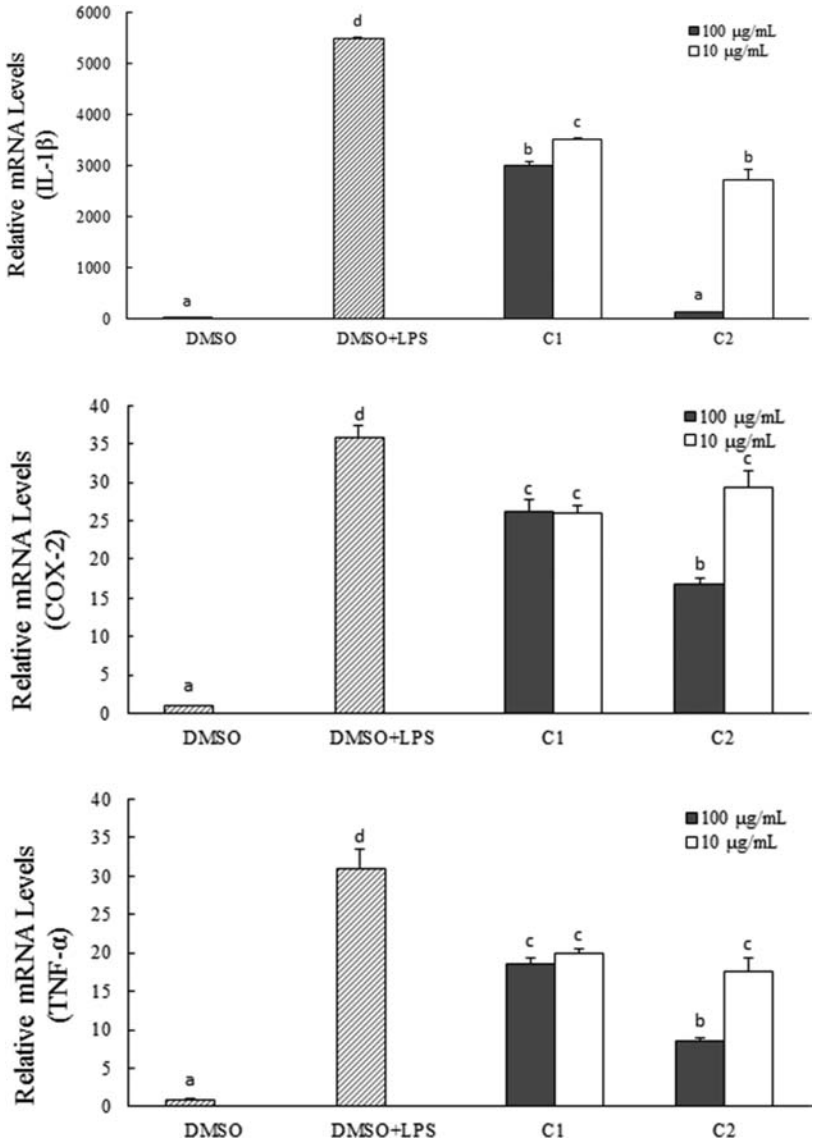

Figure 4. Inhibitory effects of compounds **1** and **2** on IL-1β, COX-2, and TNF-α mRNA expressions in LPS-induced mouse RAW 264.7 macrophage cells. C1 stands for compound **1**, and C2 is compound **2**. The vertical bars represent the standard deviation ($n = 3$) for each data point, and different letters represent significant differences ($P < 0.05$).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, HSQC, COSY, HMBC, and HRESIMS spectra of **1** and **2**. DEPT and ROESY spectra of **1**, NOESY spectrum of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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