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Sesquiterpenoids from the Leaves of *Dalbergia odorifera*

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Abstract: Two new sesquiterpenoids (1*R*,5*R*,6*R*)-11-hydroxyl-drocostuslactone (**1**) and (1*R*,10*S*)- gibberodione A (**2**) were acquired from the leaves of *Dalbergia odorifera*. The structures of the new compounds were elucidated by 1D and 2D NMR techniques, and X-ray crystallography analyses. The results of the bioactivity screening tests of the two new compounds revealed the potential anti-inflammatory effects of these two compounds, their IC50 values were 107.2 ± 4.02 and 54.64 ± 1.89 μg/mL, respectively. They could significantly decrease the production of NO ($P<0.01$) with 8~16 and 1~2 μg/mL and inhibited LDH ($P<0.01$) with 1~16 and 1~2 μg/mL in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, respectively.

Keywords: *Dalbergia odorifera* leaves; sesquiterpenoids; anti-inflammatory activity.

1. Introduction

Dalbergia odorifera T. Chen, which belongs to the family Fabaceae, is a vital TCM. Its heartwood was utilized in China and Korea to treat blood stasis, ischemic symptoms, welling, necrotic and rheumatic pains [1-2]. Phytochemical examination the heartwood of *D. odorifera* has identified substantial chemical substances, which involve neoflavonoids, flavonoids, furans, benzophenones and terpenoids [3-8]. The leaves of *D. odorifera* exhibited anti-inflammatory, antibacterial and antioxidant activities [9-10]. We studied the chemistry constituents of *D. odorifera*

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leaves.

Two new sesquiterpenoids were acquired from the leaves of *D. odorifera* (Figure 1). In addition, the bioactivity assay showed that two compounds had potential anti-inflammatory activity.

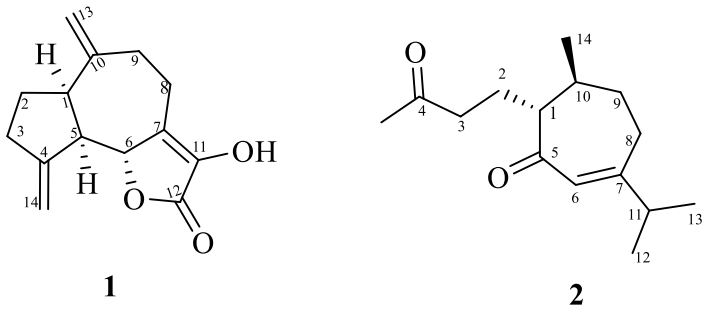

Figure 1. Structural features of chemical substances **1** and **2**

2. Materials and Methods

2.1. General Experimental Procedures

The nuclear magnetic resonance (NMR) spectra of the isolated compounds were acquired on a 400 MHz NMR spectrometer (Bruker Corporation, Switzerland). Ultraviolet (UV) patterns of the compounds were determined on a 210A double beam spectrophotometer (Shimadzu, Japan). High resolution mass spectrometry (HRMS) data were acquired on an LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Circular dichroism (CD) spectra were recorded using a JASCOJ-1500 spectropolarimeter (CA, USA). Optical rotations were measured using a JASCO P-1020 polarimeter (JASCO Corporation, Tokyo, Japan).

2.2. Plant Material

The leaves of *Dalbergia odorifera* were harvested from Shanya, Hainan Province in China, in July 2020, which was identified by associate researcher Zhou Hong. A voucher specimen (No20200704.) was preserved in Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Chinese Medicine.

2.2. Extraction and Isolation

The leaves of *Dalbergia odorifera* (24.0 kg) were isolated under reflux by 70% ethyl alcohol thrice. Ethyl alcohol was evaporated using a rotary evaporator at reduced pressure and 4.50 kg crude ethyl alcohol extract was obtained. The crude extract was subjected to D101 resin column using H_2O, EtOH-H_2O (15:85-95:5) as eluents to yield 7 fractions (Fr.1-Fr.7). The fraction 4 (282.8 g) was furtherly separated on silica gel column chromatography (CC) (CH_2Cl_2-MeOH, 500:1-0:1) to yield twelve fractions (Frs.4.A-Frs.4.L), and the Frs.4.C (6.9 g) was subjected to silica gel CC (petrol

ether-acetone, 200:1-1:1) to produce 3 fractions (Frs.4.C.1-Frs.4.C.3). The Frs.4.C.1 (301 mg) was subjected to Sephadex LH-20 CC with CH_2Cl_2-MeOH (1:1, v/v) solvent system and compound 1 (15 mg) was obtained. The Frs.4.D (2.8 g) was subjected to silica gel CC with (petrol ether-acetone, 200:1-1:1) solvent system and yielded to as 5 fractions as (Frs.4.D.1-Frs.4.D.5). By further separation of one of those fractions, Frs.4.D.3 (183.2 mg), on a silica gel column with CH2Cl2-MeOH (from 150:1-0:1, v/v) solvent system, compound **2** (8 mg) was isolated.

Table 1. The NMR data for **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	**1**		**2**	
	δ_H, mult. (*J* in Hz)	**δ_C**	**δ_H, mult. (*J* in Hz)**	**δ_C**
1	2.89 (1H, m)	48.9	2.31 (1H, m)	58.1
2	2.44 (2H, m)	30.7	1.94 (1H, m) 1.83 (1H, m)	24.0
3	2.56 (1H, m) 2.16 (1H, m)	31.1	2.49 (1H, ddd, *J* = 17.4, 9.0, 5.2Hz) 2.28 (1H, m, H-11)	42.0
4	-	149.5	-	208.9
5	2.60 (1H, d, *J* = 12.6 Hz)	51.8	-	205.7
6	4.61 (1H, d, *J* = 11.1 Hz)	79.4	5.84 (1H, d, *J* = 1.9Hz)	126.5
7	-	136.3	-	167.5
8	2.97 (1H, m) 2.35 (1H, m)	27.3	2.40 (1H, m) 2.25 (1H, m)	28.4
9	2.00 (1H, m) 1.81 (1H, m)	29.0	1.68 (1H, m) 1.64 (1H, m)	34.5
10	-	149.2	1.74 (1H, m)	34.8
11	-	137.1	2.35 (1H, m)	37.4
12	-	170.5	1.06 (3H, d, *J* = 2.6 Hz)	21.0
13	4.94 (1H, s) 4.91 (1H, s)	112.5	1.08 (3H, d, *J* = 2.6 Hz)	21.3
14	5.15 (1H, s) 5.10 (1H, s)	113.2	1.10 (3H, d, *J* = 6.5 Hz)	20.1
15	-	-	2.11 (3H, s)	30.1

*(1R,5R,6R)-11-hydroxyl-drocostuslactone (**1**)*: Colorless crystal (MeOH); $[\alpha]_D^{20}$= + 56.21 (*c* = 0.58, MeOH). UV (MeOH) λ_{max}: 240 nm, IR (KBr)ν_{max}: 3417, 1944, 1901, 1832, 1637, 1031 cm^{-1}. HR-ESI-MS *m/z* 233.1162 ([M+H] $^+$calcd. for $C_{14}H_{17}O_3$, 233.1172). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

*(1R,10S)-gibberodione A (**2**)*: Colorless oil (MeOH); $[\alpha]_D^{20}$= - 3.64 (*c* = 0.07, MeOH). UV (MeOH) λ_{max}: 240 nm, IR (KBr)ν_{max}: 2963, 2936, 1715, 1664 cm^{-1}. HR-ESI-MS *m/z* 237.1835 ([M+H] $^+$ calcd. for $C_{15}H_{25}O_2$, 237.1849). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as achromatic crystal, with a molecular formula of $C_{14}H_{16}O_3$ was deduced from the HR-ESI-MS peak at *m/z* 233.1162, in correspondence to seven levels of under saturation. The ^{1}H NMR spectral data of **1** displayed resonances for two olefinic protons [δ_H 5.15 (1H, s, H-14*α*), 5.10 (1H, s, H-14*β*), 4.94 (1H, s, H-13*α*), 4.91 (1H, s, H-13*β*)], three methines groups [δ_H 4.61 (1H, d, *J* = 11.1 Hz, H-6), 2.89 (1H, m, H-1), 2.60 (1H, d, *J* = 12.6 Hz, H-5)], one oxidative methine [δ_H 4.61 (1H, d, *J* = 11.1 Hz, H-6)], four methylenes [δ_H 2.97 (1H, m, H-8*α*), 2.56 (1H, m, H-3*α*), 2.44 (2H, m, H-2), 2.35 (1H, m, H-8*β*), 2.16 (1H, m, H-3*β*), 2.00 (1H, m, H-9*α*), 1.81 (1H, m, H-9*β*)]. The ^{13}C NMR spectra of **1** displayed 14 carbon resonances involving an ester carbonyl carbon [δ_C 170.5 (C-12)], six olefinic carbons [δ_C 149.5 (C-4), 149.2 (C-10), 137.1 (C-11), 136.3 (C-7), 113.2 (C-14), 112.5 (C-13)], one oxygenated methine [δ_C 79.4 (C-6)], two methines groups [δ_C 51.8 (C-5), 48.9 (C-1)], three methylenes [δ_C 31.1 (C-3), 30.7 (C-2), 29.0 (C-9), 27.3 (C-8)]. The data are closely comparable to those of dehydrocostuslactone [10]. The ^{1}H-^{1}H COSY correlation of H-14/H$_2$-3, H$_2$-3/H$_2$-2, H$_2$-2/H-1, H-13/H$_2$-9, H$_2$-9/H$_2$-8 showed the sequence =CH (1) −CH$_2$(2) −CH$_2$(3)/CH$_2$(8) −CH$_2$(9). The HMBC association of H-1 with C-13/C-10 /C-2/C-5/C-6, H-13 with C-1/C-10/C-9, H-14 with C-4/C-3/C-5. The relative stereochemistry was acquired based on the NOESY spectrum, where H-1 displayed association with H-5 revealing that H-1 was on the same side with H-5. However, no correlation was observed between H-5 and H-6. It reflects the trans orientation of H-5 and H-6 (Figure 2). The absolute stereochemistry of chemical substance **1** was identified as (1*R*,5*R*,6*R*)-11-hydroxyl-drocostuslactone via X-ray crystallographic analysis, as well (Figure 3).

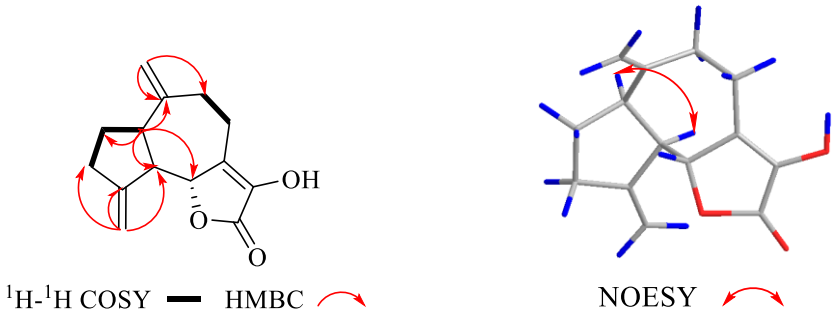

Figure 2. 2D NMR correlation of **1**

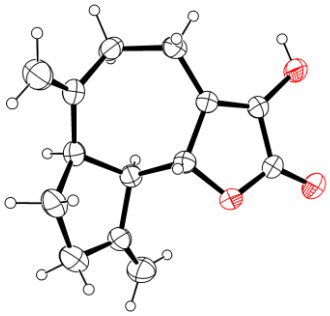

Figure 3. ORTEP drawing of compound **1**

Compound **2** was separated as achromatic oil. The molecular formula $C_{15}H_{24}O_2$ was determined by HR-ESI-MS data (*m/z* 235.1835 $[M+H]^+$). The ^1H NMR spectral data of **2** displayed resonances for an olefinic proton [δ_H 5.84(1H, d, *J*=1.9Hz, H-6)], three methines groups [δ_H 2.35 (1H, m, H-11), 2.31 (1H, m, H-1), 1.74 (1H, m, H-10)], four methylenes [δ_H 2.49 (1H, ddd, *J*=17.4, 9.0, 5.2Hz, H-3*α*), 2.28 (1H, m, H-11, H-3*β*), 2.40 (1H, m, H-8α), 2.25 (1H, m, H-8*β*), 1.94 (1H, m, H-2*α*), 1.83 (1H, m, H-2*β*), 1.68 (1H, m, H-9*α*), 1.64 (1H, m, H-9*β*)], and four methyl groups [δ_H 2.11 (3H, s, H-15), 1.10 (3H, d, *J*=6.5 Hz, H-14), 1.08 (3H, d, *J* = 2.6 Hz, H-13), 1.06 (3H, d, *J* = 2.6 Hz, H-12)]. Inspection of its ^{13}C-NMR spectra exhibited 15 carbon resonances assignable to two carbonyls carbon at [δ_C 208.9 (C-4), 205.7 (C-5)], one double bonds at [δ_C 167.5 (C-7), 126.5 (C-6)], three methines groups [δ_C 58.1 (C-1), 37.4 (C-11), 34.8 (C-10)], three methylenes [δ_C 24.0 (C-2), 42.0 (C-3), 28.4 (C-8), 34.5 (C-9)], and four methyl carbons [δ_C 30.1 (C-15), 21.3 (C-13), 21.0 (C-12), 20.1 (C-14)]. Systematic analyses of 2D NMR spectra, which involved HSQC, HMBC (Figure 4), allowed the total evaluation of every proton and carbon signal, and the establishment of the planar architecture of **2**. Those spectroscopy characteristics revealed that the architecture of **2** resembled that of gibberodione [11-12], except for the configuration. The relative configuration was assigned from the NOESY spectrum, in which H-1 [*δ*H 2.31 (1H, m, H-1)] showed correlation with H_3-14 [*δ*H 1.10 (3H, d, *J*=6.5 Hz, H-14)]. However, no correlation was observed between H-1 [*δ*H 2.31 (1H, m, H-1)] and H-10 [*δ*H1.74 (1H, m, H-10)]. It reflects the trans orientation of H-10 and H-1(Figure 4). To further reinforced the structure of 2, the ^{13}C NMR chemical shifts of four possible isomers (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** and (1*S*, 10*S*)/ (1*R*, 10*R*)-2 were calculated using the GIAO method at the mPW1PW91/6-311G (d, p) level with the Gaussian 09 software. The predicted chemical shifts for each isomer were weighted according to the Boltzmann distributions. The results showed that (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** exhibited a better coefficient of determination (R^2 = 0.9989) of linear correlation between the experimental and calculated ^{13}C NMR chemical shifts than (1*S*, 10*S*)/ (1*R*, 10*R*)-**2** (R^2 = 0.9978) (Figure 5). As shown in (Figure 6), the MAE (mean absolute error) and MD (maximum deviation) for (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** were 1.79 and 4.6 ppm, respectively, which are acceptable to meet MAE < 2.2 and MD < 5 [13]. The DP4 + probability analysis is a method that provides high probabilities to determine the relative stereochemistry of natural products [14]. The DP4 +

probability analysis showed that the best match of with the 100% with (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** isomer. The (1*R*,10*S*) of **2** was determined by ECD spectra (Figure 7). Accordingly, the architecture of **2** was revealed and named as (1*R*, 10*S*)-gibberodione A.

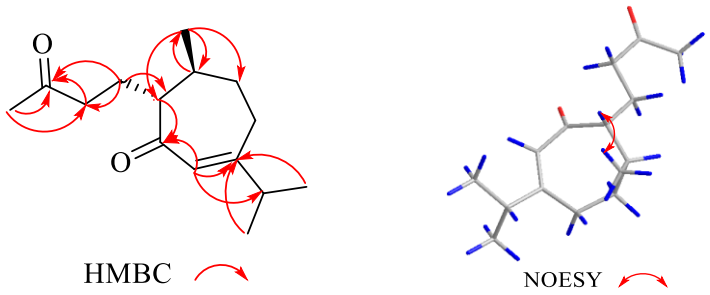

Figure 4. Selected 2D NMR correlation of **2**

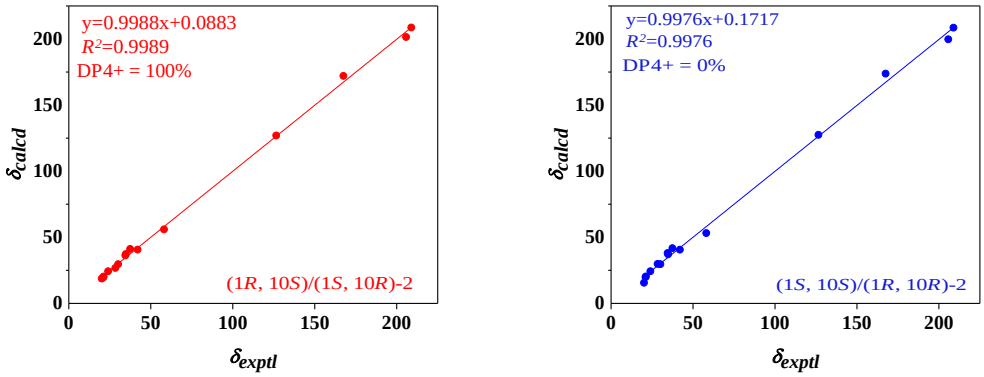

Figure 5. Linear correlation plots of calculated-experimental ^{13}C NMR chemical shift values for (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** and (1*S*, 10*S*)/ (1*R*, 10*R*) **2**

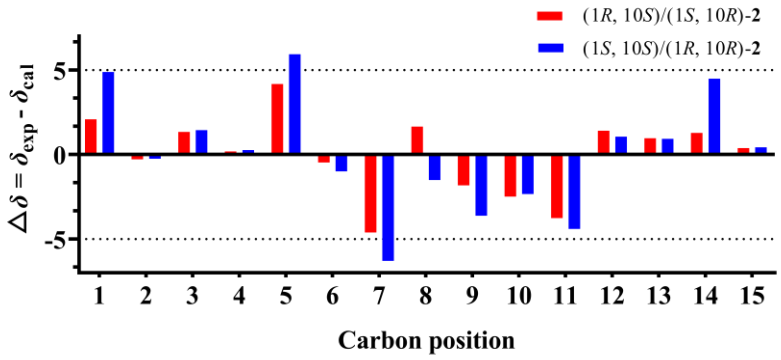

Figure 6. Relative errors between experimental and calculated ^{13}C NMR chemical shift of (1*R*, 10*S*)/ (1*S*, 10*R*)-**2** and (1*S*, 10*S*)/ (1*R*, 10*R*) **2**

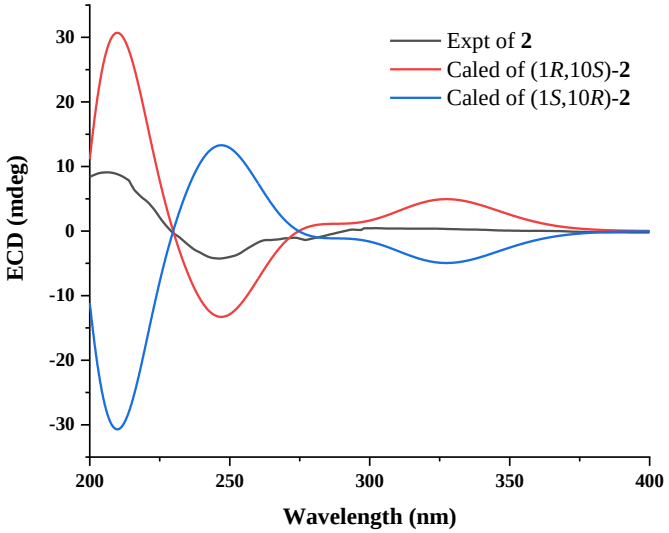

Figure 7. Experiment-derived and computed ECD spectra of compound

3.2. Anti-inflammatory Activity

Two new sesquiterpenoids were evaluated for their anti-inflammatory activity on the model of the LPS-stimulated RAW 264.7 macrophages. The cytotoxicity of compounds was tested by cell counting kit-8 (CCK-8) assay, using RAW 264.7 cells by using the same method in the literature [15-17]. The results of assays revealed that, in the range of 0~16 and 0~2 μg/mL, two compounds had no cytotoxicity to RAW 264.7, and the IC_{50} values with 107.2 ± 4.02 and 54.64 ± 1.89 μg/mL, respectively. We further investigated the effects of compounds in the LPS-activated release of the inflammation mediators nitric oxide (NO) and lactate dehydrogenase (LDH) activity by RAW 264.7 cells (Figure 8). Compound **1** had significantly decreased the production of NO with 2~4 μg/mL (P<0.05), and at 8~16 μg/mL, concentrations significantly decreased the production of NO (P<0.01), in contrast to the LPS group. In contrast to the LPS group, compound **1** at 1~16 μg/mL concentration levels significantly inhibited LDH activity (P<0.01). In addition, compound **2** at 1 and 2 μg/mL concentrations significantly decreased the production of NO and inhibited LDH activity (P<0.01). Both compounds showed anti-inflammatory activity by decreasing nitric oxide (NO) production and inhibited lactate dehydrogenase (LDH) activity in LPS-stimulated RAW 264.7.

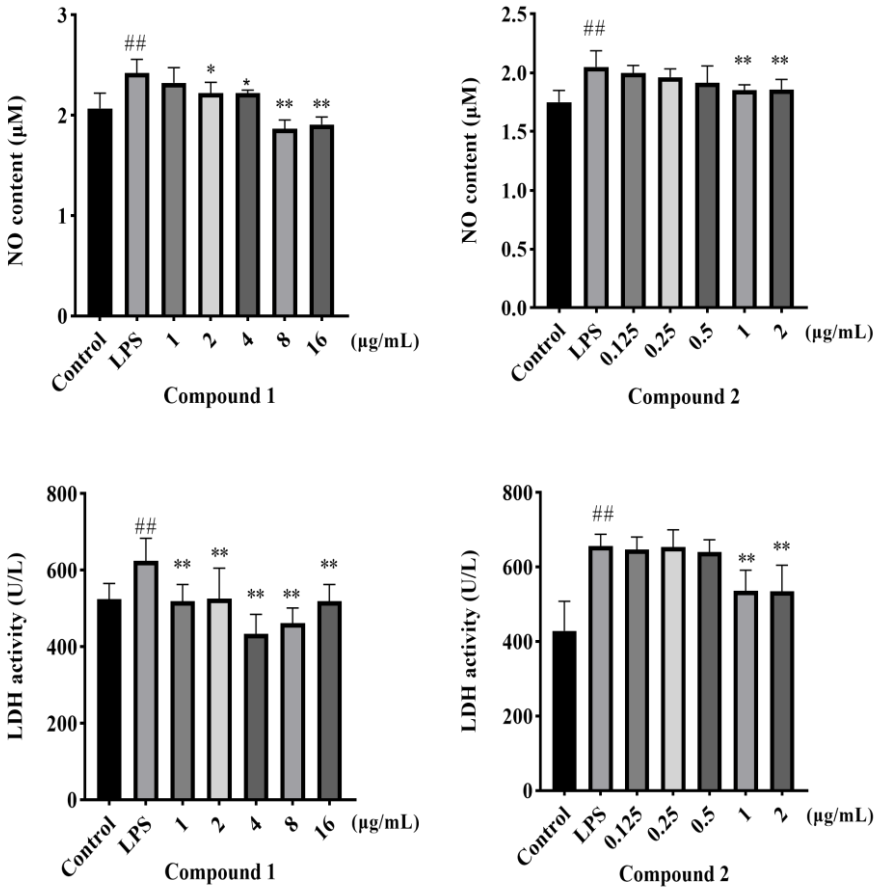

Figure 8. Effects of compounds on the production of NO and LDH in LPS-stimulated RAW264.7 cells
The values shown represent the mean (n=6). $^{\#\#}P<0.01$ compared to the control group; $^{*}P<0.05$ and $^{**}P<0.01$ compared to the LPS group

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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