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Lignan Glycosides from *Urena lobata*

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Abstract: Four new lignan glycosides; urenalignosides A–D (**1–4**), along with 12 known ones (**5–16**) were isolated from *Urena lobata*. Their structures were determined on the basis of extensive spectroscopic and spectrometric data (1D and 2D NMR; IR; CD; and HRESIMS). Compounds **2–4**; **6**; **7**; **10**; and **11** showed inhibition of nitric oxide production in lipopolysaccharide-induced RAW 264.7 macrophage cells with IC_{50} values in the range of 25.5–98.4 μM (positive control; quercetin; IC_{50} = 7.2 ± 0.2 μM).

Keywords: *Urena lobata* L.; lignan glycosides; urenalignosides A–D; nitric oxide production

1. Introduction

Urena lobata, belonging to the family Malvaceae, is an annually shrubby herbage widely distributed around the world, particularly in the tropical and subtropical areas of Asia, South America, and Africa [1]. This plant is also known as Caesar weed, Congo jute, and Bachita, the local name varies from region to region. In Africa, the leaves and flowers of *U. lobata* could be eaten as food during famine time and the bast fiber of *U. lobata* is used as cordage material [2]. More interestingly, *U. lobata* is also commonly used in folk medicines for the treatment of diabetes, abdominal colic, malaria, gonorrhea, dysentery, fever, rheumatism, and edema [3,4]. Pharmacological studies indicated that the extract of *U. lobata* showed significant antibacterial, antihyperglycemic, antinociceptive, antidiarrheal, anti-inflammatory, and wound healing activities [5–7]. In China, *U. lobata* is also named "Ditaohua," which is dominantly distributed in the south of China, such as Guangxi, Yunnan, and Guizhou provinces and clinically used to treat pathological leucorrhea and gonorrhea [8]. Promoted by these significant activities, great efforts have been made to clarify the bioactive constituents of *U. lobata* leading to the separation and elucidation of flavonoids, phenylethyl glycosides, lignans, coumarins, and triglycerides [1,9–13]. In our previous report, 16 megastigmane glycosides were identified from *U. lobata* [14]. As an ongoing study, four new lignan glycosides, urenalignosides A–D (**1–4**) together with 12 known ones (**5–16**) were obtained from *U. lobata* (Figure 1). Herein, the isolation and structural elucidation of the new compounds, as well as their inhibitory effects on NO production on LPS-stimulated RAW264.7 macrophage cells, are described.

2. Results

The 95% EtOH extracts of *U. lobata* were suspended in H_2O and extracted successively with petroleum ether (PE), EtOAc, and *n*-BuOH. The *n*-BuOH soluble fraction was separated by D_{101} macroporous adsorption resin, silica gel, and Sephadex LH-20 column chromatography and semi-preparative HPLC to afford four new lignan glycosides (**1–4**) together with 12 known ones (**5–16**) (Figure 1).

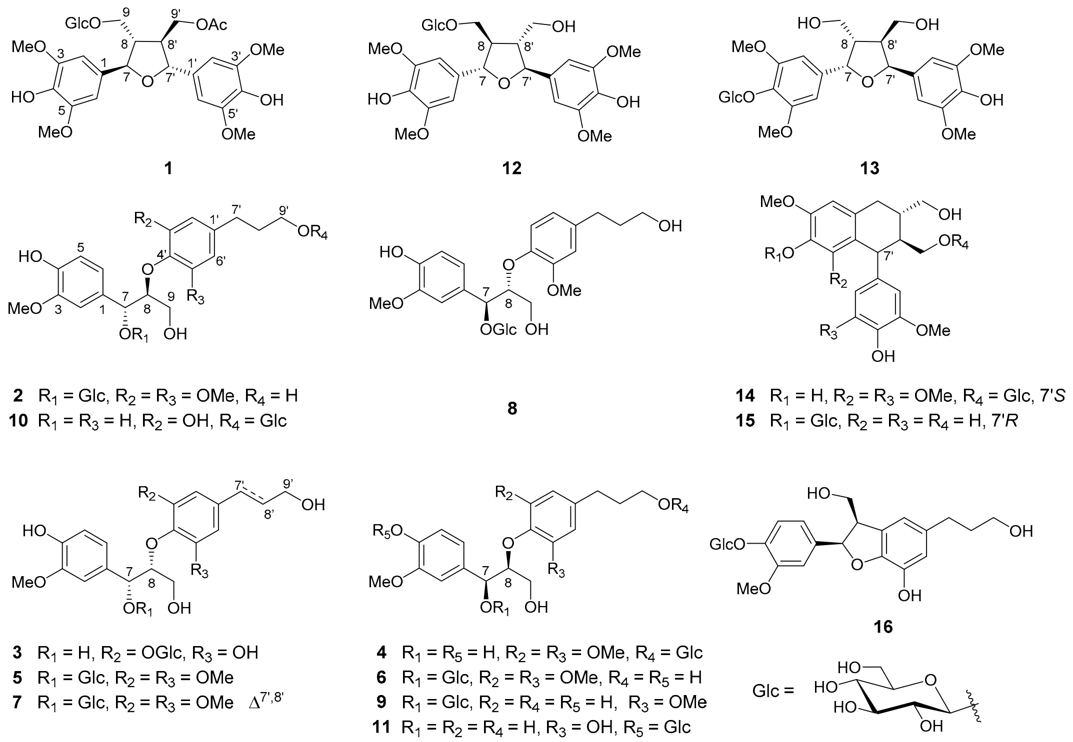

Figure 1. Structures of compounds **1**–**16** from *U. lobate*.

Compound **1** was obtained as a colorless powder. Its molecular formula was assigned as $C_{30}H_{40}O_{15}$ due to the presence of a $[M-H]^-$ ion at *m/z* 639.2282 (calcd for $C_{30}H_{39}O_{15}$, 639.2294) in the HRESIMS spectrum (Figure S1), which was also supported by the ^{13}C-NMR data (Table 1). The IR spectrum of **1** showed the absorption bands contributing to hydroxy group (3385 cm^{-1}), benzene ring (1615 and 1518 cm^{-1}), and ester carbonyl (1735 cm^{-1}) group. The NMR spectra of **1** (Figures S2 and S3) showed the presence of two 1,3,4,5-tetrasubstituted benzene moieties [δ_H 6.78 (2H, s, H-2,6), 6.80 (2H, s, H-2′,6′). δ_C 134.1 (C-1), 105.2 (C-2,6), 149.4 (C-3,5), 136.3 (C-4); 133.6 (C-1′), 104.8 (C-2′,6′), 149.3 (C-3′,5′), 136.4 (C-4′)], two oxygenated methines [δ_H 5.12 (1H, d, *J* = 8.0 Hz, H-7), 4.98 (1H, d, *J* = 9.0 Hz, H-7′). δ_C 85.6 (C-7), 84.2 (C-7′)], two *sp*^{3} methines [δ_H 2.75 (1H, m, H-8), 2.45 (1H, m, H-8′). δ_C 52.7 (C-8), 51.1 (C-8′)], two oxygenated methylenes [δ_H 4.36 (1H, overlapped, H-9′a) and 3.72 (1H, dd, *J* = 12.0, 5.0 Hz, H-9′b); 4.09 (1H, dd, *J* = 10.0, 5.5 Hz, H-9a) and 3.80 (1H, dd, *J* = 10.0, 4.5 Hz, H-9b). δ_C 69.3 (C-9), 64.8 (C-9′)], and four methoxyl groups [δ_H 3.93 (12H, s), δ_C 56.9]. Comparison of the above NMR data with those of icariol A_2 [15], a lignan previously isolated from *Epimedium sagittatum*, revealing the presence of an icariol A_2 moiety in **1**. In addition, signals due to an acetyl group [δ_H 1.95 (3H, s), δ_C 20.7, 172.8] and a glucopyranosyl moiety were also observed in the NMR spectra of **1**. The anomeric proton was presented at δ_H 4.36 (1H, d, *J* = 8.0 Hz), corresponding to the carbon at δ_C 104.6 assigned by HSQC experiment, and the relatively large coupling constant (*J* = 8.0 Hz) of the anomeric proton suggested that the glucopyranosyl moiety was in β configuration. Given that naturally occurring glucose is D-form, and limited by the small amount of **1**, we tentatively determined the glucopranosyl moiet in **1** was in D-form. In the HMBC spectra of **1**, the correlations between the anomeric proton δ_H 4.36 (1H, d, *J* = 8.0 Hz, H-1′′) and C-9 (δ_C 69.3) confirmed that the glucopyranosyl moiety was linked at C-9 (Figure 2). The acetyl group was linked at C-9′ determined by the HMBC correlation between H-9′ and the carbonyl carbon (δ_C 172.8). All the protons and carbons were unambiguously assigned (Table 1) by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments (Figures S4–S6).

The relative configuration of **1** was determined by NOESY spectrum (Figure S7), which showed the NOE correlations of H-7/H-8′ and H-7′/H-8. The CD spectra (Figure S8) of **1** showed the positive Cotton effect at 246 nm suggested that both C-7 and C-7′ were in *R* configuration [16,17], and thus the configuration of C-8, and C-8′ were assigned as 8*S*, 8′*S*. Accordingly, the structure of **1** was determined as shown in Figure 1, named as *urenalignoside A*.

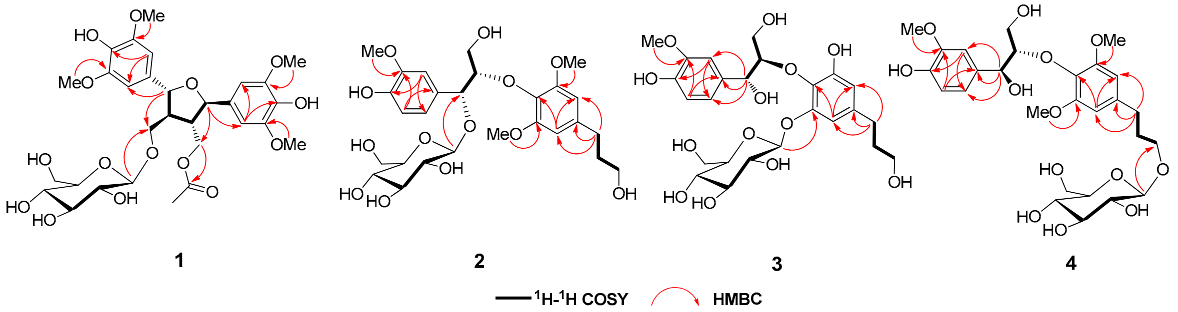

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**–**4**.

Compound **2** was obtained as colorless powder. Its molecular formula was assigned as $C_{27}H_{38}O_{13}$ by the [M + HCOO]$^-$ ion at *m/z* 615.2284 (calcd for $C_{28}H_{39}O_{15}$, *m/z* 615.2294) in the HRESIMS spectrum (Figure S9), which was also supported by the ^{13}C-NMR -NMR data (Table 1). The NMR spectra of **2** (Figures S10 and S11) showed the presence of a 1,3,4-trisubstituted [δ_H 7.28 (1H, d, *J* = 1.5 Hz, H-2), 6.83 (1H, d, *J* = 8.0 Hz, H-5), 6.96 (1H, dd, *J* = 8.0, 1.5 Hz, H-6). δ_C 130.8 (C-1), 113.3 (C-2), 148.7 (C-3), 147.1 (C-4), 115.5 (C-5), 122.1 (C-6)] and a 1,3,4,5-tetrasubstituted benzene moieties [δ_H 6.53 (2H, s, H-2′,6′). δ_C 135.1 (C-1′), 106.7 (C-2′,6′), 154.3 (C-3′,5′), 139.9 (C-4′)], two oxygen-bearing methines [δ_H 5.31 (1H, d, *J* = 3.0 Hz, H-7), 4.23 (1H, m, H-8). δ_C 77.7 (C-7), 86.8 (C-8)], two oxygen-bearing methylenes [δ_H 3.61 (2H, t, *J* = 6.4 Hz, H-9′), 3.16 (2H, m, H-9). δ_C 62.8 (C-9′), 61.4 (C-9)], two methylenes [δ_H 2.67 (2H, t, *J* = 7.5 Hz, H-7′), 1.86 (2H, m, H-8′). δ_C 33.4 (C-7′), 35.4 (C-8′)], and three methoxy groups [δ_H 3.89 (3H, s, 3-OCH_3), 3.74 (6H, s, 3′,5′-OCH_3). δ_C 56.4 (3, 3′, 5′-OCH_3)]. Comparison of the above-mentioned NMR data with those of 1-(4′-hydroxy-3′-methoxy-phenyl)-2-[4′′-(3-hydroxypropyl)-2′′,6′′-dimethoxyphenoxy] propane-1,3-diol, a lignan previously isolated from *Bursera tonkinensis* [18], suggested the occurrence of an 8-*O*-4′-neolignan moiety in **2**. In addition, signals due to a glucopyranosyl moiety were also observed in the NMR spectra of **2**. The relatively large coupling constant (*J* = 7.5 Hz) of the anomeric proton resonated at δ_H 4.23 (1H, d, *J* = 7.5 Hz, H-1′′) suggested the glucopyranosyl moiety was in β configuration. The linkage of the glucopyranosyl moiety was determined at C-7 by the HMBC correlation between the anomeric proton and C-7 (Figure 2). Unambiguous assignments of the protons and carbons (Table 1) were achieved by ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY experiments (Figures S12–S15).

It has been well reported that the relative configurations of C-7 and C-8 could be solved by the analysis of the coupling constant between H-7 and H-8. Regularly, a relatively small coupling constant (*J* = 3–4 Hz) between H-7 and H-8 defines the *erythro* configurations of C-7 and C-8, while a relatively large coupling constant (*J* = 6–8 Hz) give rise to the *threo* configurations of C-7 and C-8 [19–24]. Accordingly, the stereochemistry of C-7 and C-8 in **2** were assigned as *erythro* according to the small coupling constant (*J* = 3.0 Hz) between H-7 and H-8. The positive Cotton effect at 233 nm in the CD spectrum (Figure S16) of **2** suggested that the configuration of C-8 was *S* [22,24–26], and thus the configuration of C-7 was determined as *R*. Therefore, the structure of **2** namely *urenalignoside B* was elucidated as shown in Figure 1.

Compound **3** was obtained as a colorless powder, with a molecular formula of $C_{25}H_{34}O_{13}$ determined by the presence of a [M − H]$^-$ ion at *m/z* 541.1920 (calcd for $C_{25}H_{33}O_{13}$, *m/z* 541.1927) in the HRESIMS spectrum (Figure S17). The NMR data of **3** (Figures S18–S23) is comparable to those of **2**, except the absence of one methoxy group in **3**. In the HMBC spectrum of **3**, the correlation between the anomeric proton [δ_H 4.93 (1H, *J* = 7.5 Hz, H-1′′)] of the glucopyranosyl moiety and the C-3′ of the aglycon demonstrated that the glucopyranosyl moiety was linked at C-3′ in **3** (Figure 2). The large coupling constant (*J* = 8.5 Hz) between H-7 and H-8 suggested that the C-7 and C-8 were in *threo* orientation. The negative Cotton effect at 233 nm in the CD spectrum (Figure S24) of **3** suggested that the configuration of C-8 was *R* [23,24], and thus the configuration of C-7 was 7*R*. Therefore, the structure of **3** namely *urenalignoside C* was determined as shown in Figure 1.

Table 1. Data of compounds **1–4** (500 MHz for ^{1}H and 125 MHz for ^{13}C, CD_3OD, *J* in Hz).

No.	**1** [a]		**2** [a]		**3** [a]		**4** [a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		134.1		130.8		133.5		133.3
2	6.78, s	105.2	7.28, d, (1.5)	113.3	7.01, d, (1.5)	111.6	7.08, d, (1.5)	112.2
3		149.4		148.7		149.0		148.5
4		136.3		147.1		147.5		147.0
5		149.4	6.83, d, (8.0)	115.5	6.77, d, (8.5)	116.1	6.78, d, (8.0)	115.7
6	6.78, s	105.2	6.96, dd, (8.0, 1.5)	122.1	6.88, dd, (8.0, 1.5)	121.1	6.94, dd, (8.0, 1.5)	121.0
7	5.12, d, (8.0)	85.6	5.31, d, (3.0)	77.7	4.97, d, (8.5)	75.0	5.12, d, (7.0)	74.4
8	2.75, m	52.7	4.23, m	86.8	4.01, m	89.7	4.16, m	88.3
9	3.80, dd, (10.0, 4.5) 4.09, dd, (10.0, 5.5)	69.3	3.16, m	61.4	3.69, m	61.3	3.62, m	62.1
1′		133.6		135.1		140.0		133.3
2′	6.80, s	104.8	6.53, s	106.7	6.46, s	109.3	6.59, s	106.7
3′		149.3		154.3		152.0		153.9
4′		136.4		139.9		135.3		140.1
5′		149.3		154.3		152.0		153.9
6′	6.80, s	104.8	6.53, s	106.7	6.60, s	112.1	6.59, s	106.7
7′	4.98, d, (9.0)	84.2	2.67, t, (7.5)	33.4	2.56, t, (7.5)	33.0	2.69, t, (7.5)	33.4
8′	2.45, m,	51.1	1.86, m	35.4	1.80, m	35.2	1.87, m	35.4
9′	3.72, (dd,12.0, 5.0) 4.36, overlapped	64.8	3.61, t, (6.4)	62.8	3.56, t, (6.5)	62.2	3.81, dd, (11.0, 2.5) 3.93, dd, (11.0, 4.0)	69.2
Glu-1″	4.36, d, (8.0)	104.6	4.23, d, (7.5)	101.0	4.93, d, (7.5)	103.0	4.32, d, (8.0)	104.5
Glu-2″	3.26, overlapped	75.2	3.45, overlapped	75.2	3.48, overlapped	75.1	3.21, overlapped	75.4
Glu-3″	3.40, overlapped	78.1	3.45, overlapped	77.8	3.41, overlapped	78.0	3.25, overlapped	77.9
Glu-4″	3.33, overlapped	71.6	3.32, overlapped	71.9	3.40, overlapped	71.4	3.26, overlapped	71.8
Glu-5″	3.36, overlapped	78.2	3.45, overlapped	78.1	3.47, overlapped	78.3	3.28, overlapped	78.0
Glu-6″	4.32, overlapped 4.36, overlapped	62.8	3.87, overlapped 3.92, overlapped	62.2	3.68, overlapped 3.89, overlapped	62.5	3.68, overlapped 3.89, overlapped	62.9
<u>C</u>OCH$_3$		172.8						
CO<u>C</u>H$_3$	1.95 (3H, s)	20.7						
3-OCH$_3$	3.93 (3H, s)	56.9	3.89 (3H, s)	56.4	3.86, (3H, s)	56.4	3.89, (3H, s)	56.5
5-OCH$_3$	3.93 (3H, s)	56.9						
3′-OCH$_3$	3.93 (3H, s)	56.9	3.74 (3H, s)	56.4			3.89, (3H, s)	56.6
5′-OCH$_3$	3.93 (3H, s)	56.9	3.74 (3H, s)	56.4			3.89, (3H, s)	56.6

[a] Assignments were carried out based on HSQC and HMBC experiments.

Compound **4** was obtained as a colorless powder, with a molecular formula of $C_{27}H_{38}O_{13}$ by the $[M-H]^-$ ion *m/z* 569.2258 (calcd for $C_{27}H_{37}O_{13}$, *m/z* 569.2240) in the HRESIMS spectrum (Figure S25). Comparison of the NMR data of **4** (Figures S26–S31) with those of **2** revealed that these two compounds share a highly similar skeleton, except the significantly deshielded chemical shift of C-9′ (δ_C 69.2; $\Delta\delta_C$ + 6.3), suggesting that the *O*-glucopyranosyl moiety was linked at C-9′ in **4**, but not like that at C-7 in **2**. The deduction was confirmed by HMBC correlation between the anomeric proton [δ_H 4.32 (1H, *J* = 8.0 Hz, H-1″)] and C-9′ (Figure 2). The relatively large coupling constant (*J* = 7.0 Hz) between H-7 and H-8 suggested that the C-7 and C-8 were in *threo* orientation. The absolute configuration of C-8 was assigned as *S* based on the positive Cotton effect at 233 nm presented in the CD spectrum (Figure S32) of **4** [22,24–26], and thus the configuration of C-7 was assigned as *S*. Accordingly, the structure of **4** namely *urenalignoside D* was determined as shown in Figure 1.

By comparison of their spectroscopic and specific rotation data with those of the known compounds, the remaining 11 compounds were identified as (7*R*,8*R*)-*threo*-4,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-7-*O*-*β*-D-glucopyranoside (**5**) [21], rourinoside (**6**) [22], (7*R*,8*R*)-*threo*-guaiacylglycerol-8-*O*-4′-sinapyl ether-7-*O*-*β*-D-glucopyranoside (**7**) [23], (7*S*,8*R*)-*erythro*-4,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-7-*O*-*β*-D-glucopyranoside (**8**) [24], (7*S*,8*S*)-*threo*-4,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-7-*O*-*β*-D-glucopyranoside (**9**) [24], (–)-(7*R*,8*S*)-4,7,9,3′,9′-pentahydroxy-3-methoxy-8-*O*-4′-neolignan-9′-*O*-*β*-D-glucopyranoside (**10**) [25], (7*S*,8*S*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside

(**11**) [26], (7*S*,7′*S*,8*R*,8′*R*)-icariol A_2-9-*O*-*β*-D-glucopyranoside (**12**) [16], (7*S*,7′*S*,8*S*,8′*S*)-icariol A_2-4-*O*-*β*-D-glucopyranoside (**13**) [27], lyoniresinol-9′-*O*-*β*-D-glucopyranoside (**14**) [28], (−)-isolariciresinol 4-*O*-*β*-D-glucopyranoside (**15**) [29], and cedrusin-4′-*O*-*β*-D-glucopy ranoside (**16**) [30], respectively. Compounds **2–11** and **16** are neolignans which are classified as a subgroup of lignan family [31].

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were obtained on a Rudolph Autopol IV automatic polarimeter (Hackettstown, NJ, USA). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (Madison, WI, USA) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). NMR spectra were recorded on a Varian INOVA-500 spectrometer (Palo Alto, CA, USA) operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. HRESIMS was recorded on an LCMS-IT-TOF system, fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), LiChroprep RP-C_{18} gel (40–63 μm, Merck, Germany), D_{101} m acroporous adsorption resin (Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Qingdao Marine Chemical Inc., Qingdao, China) were used for open column chromatography (CC). HPLC was performed on a ShimadzuLC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with an SPD-M20A photodiode array detector monitoring at 254 nm. A semi-preparative HPLC column (YMC-Pack C_{18}, 250 × 10 mm, 5 μm) was employed for the isolation. TLC was performed using GF_{254} plates (Qingdao Marine Chemical Inc., Qingdao, China).

3.2. Plant Material

Urena lobata L. was collected in Guangxi Province, People's Republic of China, in September 2013. The plant material was authenticated by one of the authors (P.F. Tu) and a voucher specimen (DTH2013029) was deposited at the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China.

3.3. Extraction and Isolation

The air-dried *U. lobata* (13.6 kg) were refluxed with 95% EtOH for three times (3 × 180 L, each for 1 h). After removing the solvent under reduced pressure, the residue (1.35 kg) was suspended in water (6 L), and partitioned with petroleum ether (3 × 6 L), EtOAc (5 × 6 L), and *n*-BuOH (3 × 6 L), successively. The *n*-BuOH-soluble fraction (158 g) was subjected to D_{101} macroporous adsorption resin column and eluted with H_2O–EtOH (100:0, 90:10, 50:50, 20:80, 0:100) to yield five fractions (Fr. 1-5). Fr. 2 (20 g) and Fr. 3 (40 g) were combined and subjected to silica gel chromatography and eluted with a stepwise gradient of EtOAc-MeOH-H_2O from 30:2:1 to 5:2:1 to give five subfractions (Subfr. A–E). Subfr. B (8 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give six subfractions (Subfr. B1–B6). Subfr. B3 (1 g) was chromatographed on a silica gel column and eluted with gradient of CH_2Cl_2–MeOH (12:1, 10:1, 8:1, 5:1, 1:1, v/v) to give seven subfractions (Subfr. B3a–B3g). Subfr. B3d (0.2 g) was purified by semipreparative HPLC using 27% aqueous MeCN as the mobile phase to afford compound **7** (2.1 mg, t_R 34.5 min). Subfr. B3g (0.1 g) was applied to semi-preparative HPLC using 25% aqueous MeCN to obtain two compounds **8** (3.1 mg, t_R 23.0 min) and **9** (4.2 mg, t_R 48.5 min). Subfr. B4 (4 g) was subjected to RP-C_{18} open column and eluted with a stepwise gradient of MeOH–H_2O (1:4, 1:3, 1:2, 2:3, 1:0, v/v), to afford five fractions (Subfr. B4a–Subfr. B4e). Subfr. B4a (1.2 g) was applied to semi-preparative HPLC using 25% aqueous MeCN to give compound **1** (1.2 mg, t_R 28.5 min). Subfr. B4c (1.1 g) was further separated by ODS column chromatography and eluted with MeOH–H_2O (1:19→1:3) to obtain six fractions (Subfr. B4c1–B4c6). Subfr. B4c4 was repeatedly separated and purified by semi-preparative HPLC (27% aqueous MeCN) to give two fractions Subfr.

B4c4-1 (25.3 mg, t_R 40.0 min), Subfr. B4c4-2 (7.4 mg, t_R 49.0 min), and five compounds **3** (3.0 mg, t_R 44.5 min), **4** (2.1 mg, t_R 30.0 min), **5** (2.5 mg, t_R 36.0 min), **12** (7.5 mg, t_R 23.5 min), and **13** (2.5 mg, t_R 27.5 min). Subfr. B4c4-2 was purified by semi-preparative HPLC (30% aqueous MeOH) to give compounds **10** (1.8 mg, t_R 55.5 min) and **11** (2.0 mg, t_R 57.0 min). Subfr. B4c5 was applied to semi-preparative HPLC using 10% aqueous MeOH to give compounds **2** (2.5 mg, t_R 32.0 min), **6** (3.2 mg, t_R 37.0 min), **14** (2.1 mg, t_R 43.5 min), **15** (1.8 mg, t_R 54.0 min), and **16** (1.2 mg, t_R 55.5 min).

Urenalignoside A (**1**): Colorless powder, $[\alpha]_D^{25}$: −45.7 (*c* 0.1, MeOH); UV λ (log ε): 208 (4.49), 317 (4.31), 383 (3.91) nm; IR (KBr) ν_{max}: 3385, 2921, 1735, 1615, 1518, 1462, 1428, 1367, 1331, 1217, 1114, 1076, 1036 cm^{-1}; ^1H and ^{13}C-NMR data (see Table 1); negative-ion HRESIMS: *m/z* 639.2282 $[M - H]^-$ (calcd for $C_{30}H_{39}O_{15}$, 639.2294).

Urenalignoside B (**2**): Colorless powder, $[\alpha]_D^{25}$: −64.0 (*c* 0.1, MeOH); UV λ (log ε): 202 (4.14),226 (4.25), 277 (3.37), 298 (2.63), 317 (2.48), 329 (2.40), 341 (2.43), 348 (2.38) nm; IR (KBr) ν_{max}: 3423, 2926, 1630, 1384, 1253, 1119, 1076, 1037 cm^{-1}; ^1H and ^{13}C-NMR data (see Table 1); negative-ion HRESIMS: *m/z* 615.2284 $[M + HCOO]^-$ (calcd for $C_{28}H_{39}O_{15}$, 615.2294).

Urenalignoside C (**3**): Colorless powder, $[\alpha]_D^{25}$: −52.4 (*c* 0.1, MeOH); UV λ (log ε): 212 (4.58), 285 (4.00) nm; IR (KBr) ν_{max}: 3389, 2968, 2923, 2852, 1739, 1610, 1456, 1431, 1366, 1259, 1228, 1216, 1174, 1111, 1028 cm^{-1}; ^1H and ^{13}C-NMR data (see Table 1); negative-ion HRESIMS: *m/z* 541.1920 $[M - H]^-$ (calcd for $C_{25}H_{33}O_{13}$, 541.1927).

Urenalignoside D (**4**): Colorless powder, $[\alpha]_D^{25}$: −54.0 (*c* 0.1, MeOH); UV λ (log ε): 207 (4.62), 263 (4.70), 316 (4.23) nm; IR (KBr) ν_{max}: 3739, 3716, 3660, 3430, 2956, 2924, 2853, 1717, 1592, 1514, 1488, 1455, 1428, 1383, 1367, 1230, 1157, 1125, 1023 cm^{-1}; ^1H and ^{13}C-NMR data (see Table 1); negative-ion HRESIMS: *m/z* 569.2258 $[M - H]^-$ (calcd for $C_{27}H_{37}O_{13}$, 569.2240).

3.4. Biological Assays

The murine macrophage RAW264.7 cell line was purchased from Peking Union Medical College (PUMC) Cell bank (Beijing, China), and was cultured in DMEM supplemented with 10% Fetal Bovine Serum, 100U/mL penicillin G and 100 µg/mL streptomycin, in a humidified 5% CO_2 at 37 °C. Cell viability was evaluated using MTT assay. The NO concentration was detected by the Griess method. Briefly, RAW264.7 macrophage cells were seeded into 96-well plates at a density of 5×10^4 cells/well and stimulated with 0.5 µg/mL LPS (Sigma, St. Louis, MO, USA) in the presence or absence of test compounds. After incubation for 24 h at 37 °C, treated RAW264.7 macrophage cells were incubated with 100 µL MTT solution (0.5 mg/mL in medium) for another 4 h at 37 °C, subsequently, the supernatants were removed and residues were dissolved using 150 µL DMSO for each well; 50 µL of cell-free supernatant was mixed with 100 µL of Griess reagent containing equal volumes of 2% (*w/v*) sulfanilamide in 5% (*w/v*) phosphoric acid and 0.2% (*w/v*) *N*-(1-naphthyl) ethylenediamine solution to measure nitrite production. The absorbance was detected at 540 nm using a microplate reader (Thermo, Waltham, MA, USA). Compared with a calibration curve prepared using $NaNO_2$ standards. The experiments were performed in triplicate. quercetin was conducted as a positive control. All the compounds were prepared as stock solutions in DMSO (final solvent concentration less than 0.3% in all assays).

3.5. Bioactivity Evaluation

Compounds **1–16** were evaluated for their inhibitory effects on the NO production in LPS-stimulated RAW 264.7 macrophage cells. Quercetin was used as a positive control (IC_{50} = 7.2 ± 0.2 µM). Compounds **2–4**, **6**, **7**, **10**, and **11** exhibited weak inhibitory activity against NO production with IC_{50} values of 90.4 ± 3.2 µM, 74.3 ± 1.8 µM, 88.1 ± 2.2 µM, 98.4 ± 3.6 µM, 97.5 ± 2.6 µM, 97.7 ± 3.5 µM, 25.5 ± 1.2 µM, respectively.

Supplementary Materials: The following materials are available online: HRESIMS and NMR spectra data of compounds **1–4** as supporting information.

Author Contributions: S.S. designed and organized the study, Y.L. contributed the analysis and interpretation of data and wrote the draft, C.S. performed the isolation and structural elucidation of the chemicals and the bioassay experiments. N.D., B.Q., F.J., X.X., X.L., J.W. and X.W. contributed the analysis and interpretation of data, P.T. and S.S. reviewed and edited the manuscript.

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Sample Availability: Samples of the compounds **1–16** are available from the authors.