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New 8-O-4′ Neolignans and Their Antibacterial Activity from the Whole Plants of *Clematis lasiandra*

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Cite This: *ACS Omega* 2020, 5, 19661−19666

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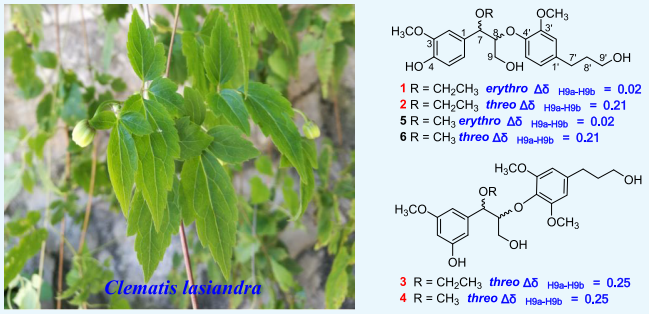
ABSTRACT: Four new 8-O-4′ neolignans, characterized at methoxy or ethoxy groups substituted at C-7, namely, (±) lasiandranins A−D (**1**−**4**), and two known analogs (±) pinnatifidanin BV (**5**) and (±) pinnatifidanin BVI (**6**) were isolated from the whole plants of *Clematis lasiandra* Maxim. The structures of **1**−**6** were determined by spectroscopic methods including 1D, 2D NMR, ECD, and HRESIMS analysis. Compounds **1** and **5** were determined as *erythro* configuration, while **2**−**4** and **6** were determined as *threo* configuration based on the chemical shift difference of H-9a and H-9b in CD_3OD. The 8-O-4′ neolignans were found from the genus *Clematis* for the first time. Compounds **1**−**6** were evaluated for their antibacterial activity against three plant pathogenic bacteria *Pseudomonas syringae* pv. *actinidiae*, *Ralstonia solanacearum*, and *Erwinia carotovora* by agar and broth dilution methods. Compounds **1**−**6** showed potent antibacterial activity against *R. solanacearum* with MIC values of 25−50 μg/mL and relatively lower activity against *P. syringae* pv. *actinidiae* with MIC values of 50−100 μg/mL, while they were inactive to *E. carotovora*.



New (±) 8-O-4′ neolignans (**1**−**4**) from *Clematis lasiandra*

1. INTRODUCTION

Natural products from plants, marine organisms, and microorganisms are important sources for discovery of new drug candidates and illustration for their exquisite biological activity.[1,2] 8-O-4′ neolignans are important natural products that showed antifungal,[3,4] anti-inflammatory,[5] antitumor,[6] antioxidant,[7] anti-HIV-1,[8] inhibitory of self-induced A*β* aggregation, and neuroprotective activities.[9] Naturally occurring 8-O-4′ neolignans have been isolated from *Myristica fragrans*,[10] *Virola surinamensis*,[11] *V. carinate*,[12] *V. pavonis*,[13] *Illicium difengpi*,[5] and *Crataegus pinnatifida*.[9] Due to the diverse bioactivity and limited sources for this kind of secondary metabolite from natural origin, a series of synthetic work for 8-O-4′ neolignans has been conducted.[3,7,14]

In the progress of our project to discover novel antimicrobial agents from terrestrial plants, the EtOAc partition of *Clematis lasiandra* Maxim was studied. *C. lasiandra* is a perennial herbaceous plant distributed widely in the north and south slopes of Qin Mountains, Shaanxi province of China.[15] Our previous studies on the *n*-BuOH partition from 70% EtOH extract led to identification of four new cytotoxic triterpenoid saponins,[16] two new phenolic glycosides, and one new lignanoid glycoside.[17] Triterpenoid saponins and phenolic glycosides were cytotoxic constituents for this species, while lignanoid glycosides can be recognized as nontoxic agent, since they just showed weak cytotoxicity. Thus, we try to evaluate the isolated (±) 8-O-4′ neolignans (**1**−**6**) for their antibacterial activity. Herein, we report the experimental details for isolation and structural elucidation of four new (±) 8-O-4′ neolignans (**1**−**4**) and two known analogs (±) pinnatifidanin BV (**5**) and (±) pinnatifidanin BVI (**6**) (Figure 1), as well as their antibacterial activity against three plant pathogenic bacteria *P. syringae* pv. *actinidiae*, *R. solanacearum*, and *E. carotovora*.

2. RESULTS AND DISCUSSION

Compound **1** was isolated as a yellow oil. The molecular formula of **1** was established as $C_{22}H_{30}O_7$ based on the

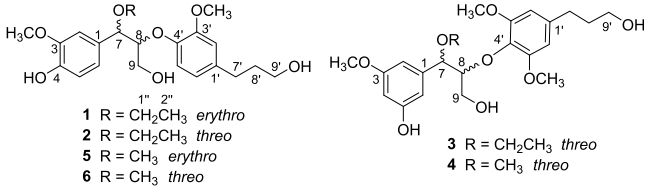


Figure 1. Chemical structures of compounds 1−6 from *Clematis lasiandra*.

Received: May 19, 2020
Accepted: July 16, 2020
Published: July 27, 2020

https://dx.doi.org/10.1021/acsomega.0c02339
ACS Omega 2020, 5, 19661−19666

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds 1−4 in CD_3OD[a]

	1		2		3		4	
no.	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)
1	131.9		131.8		132.4		131.6	
2	112.5	6.91 d (2.0)	112.3	6.97 d (1.9)	112.3	6.90 d (1.8)	112.2	6.89 d (1.8)
3	148.9		149.1		148.9		148.9	
4	147.4		147.5		115.7	6.76 d (1.8)	115.7	6.76 d (1.8)
5	119.5	6.70 d (8.2)	119.4	6.92 d (8.0)	147.2		147.2	
6	121.8	6.61 dd (8.2, 2.0)	121.5	6.82 dd (8.0, 1.9)	121.7	6.76 d (1.8)	121.8	6.76 d (1.8)
7	81.9	4.46 d (6.4)	82.6	4.53 d (5.9)	82.0	4.60 d (5.9)	83.9	4.49 d (5.8)
8	85.9	4.27 m	86.5	4.26 td (5.9, 5.9, 4.0)	86.8	4.15 m	86.8	4.15 m
9	62.3	3.82 m	62.4	3.63 dd (11.7, 4.0)	62.2	3.90 dd (12.1, 4.7)	62.2	3.88 m
		3.84 m		3.42 m		3.65 m		3.63 m
1′	137.9		137.8		139.7		139.8	
2′	114.2	6.75 d (2.0)	114.2	6.83 d (2.0)	107.1	6.50 brs	107.0	6.50 brs
3′	151.8		151.8		154.3		154.3	
4′	147.3		148.2		135.0		135.1	
5′	115.7	6.73 d (8.0)	116.0	6.77 d (8.1)	154.3		154.3	
6′	122.1	6.79 dd (8.0, 2.0)	121.9	6.69 dd (8.1, 2.0)	107.1	6.50 brs	107.0	6.50 brs
7′	32.6	2.58 m	32.7	2.62 m	33.4	2.62 m	33.4	2.62 m
8′	35.5	1.78 m	35.5	1.81 m	35.4	1.82 m	35.4	1.82 m
9′	62.2	3.53 t (6.5)	62.3	3.56 t (6.5)	61.4	3.56 t (6.5)	61.3	3.56 t (6.5)
1″	65.4	3.41 m	65.7	3.42 m	65.6	3.43 m		
2″	15.6	1.16 t (7.0)	15.6	1.13 t (7.0)	15.6	1.18 t (7.0)		
3-OCH_3	56.4	3.78 s	56.5	3.82 s	56.5	3.83 s	56.4	3.83 s
7-OCH_3							57.3	3.26 s
3′-OCH_3	56.5	3.75 s	56.6	3.83, s	56.6	3.75 s	56.6	3.76 s
5′-OCH_3					56.6	3.75 s	56.6	3.76 s

[a]Assignments aided by HSQC, ^{1}H-^{1}H COSY, and HMBC experiments.

HRESIMS of its quasi-molecular ion at m/z 429.1869 [M + Na]$^+$ (calcd for $C_{22}H_{30}O_7Na$, 429.1889). The ^{1}H NMR spectrum of **1** showed six aromatic proton signals at δ_H 6.91 (1H, d, J = 2.0 Hz), 6.70 (1H, d, J = 8.2 Hz), and 6.61 (1H, dd, J = 8.2, 2.0 Hz), along with δ_H 6.75 (1H, d, J = 2.0 Hz), 6.73 (1H, d, J = 8.0 Hz), and 6.79 (1H, dd, J = 8.0, 2.0 Hz), indicating two ABX system aromatic rings (Table 1). A 1,2,3-propane-triol moiety and a 3-propanol moiety were established by the COSY correlations from H-8 [δ_H 4.27 (1H, m)] to H-7 [δ_H 4.46 (1H, d, J = 6.4 Hz,)] and H_2−9 [δ_H 3.82 (1H, m) and 3.84 (1H, m)], together with correlations from H_2−8′ [δ_H 1.78 (2H, m)] to H_2−7′ [δ_H 2.58 (2H, m)] and H_2−9′ [δ_H 3.53 (2H, t, J = 6.5 Hz)]. Additionally, an ethoxy group at δ_H 3.41 (2H, m, H_2−1″) and 1.16 (3H, t, J = 7.0 Hz, H_3−2″) attached to the aliphatic chain at C-7 (δ_C 81.9) and two methoxy group singlets at δ_H 3.78 (3H, s, 3-OCH_3) and 3.75 (3H, s, 3′-OCH_3) attached to the aromatic rings at C-3 (δ_C 148.9) and C-3′ (δ_C 151.8), respectively, were established by HMBC correlations of H_2−1″/C-7, 3-OCH_3/C-3, and 3′-OCH_3/C-3′ (Figure 2). The ^{13}C NMR spectrum of **1** showed 22 carbon signals. Aside from one ethoxy and two methoxy groups, the remaining 18 carbons including 12 aromatic and six aliphatic carbons were attributed to two phenyl propanoid units on the basis of HMBC correlations from H-7 to C-2, C-6, C-8, and C-9 and from H_2−7′ to C-2′, C-6′, C-8′, and C-9′. Moreover, the correlation of H-8 and C-4′ in its HMBC spectrum confirmed that the two phenyl propanoid units formed an 8-O-4′ system neolignan.

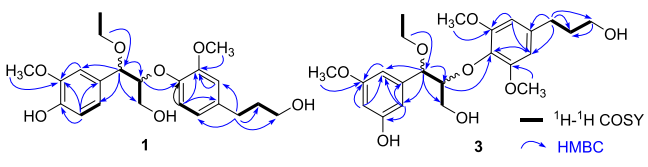


Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds 1 and 3.

For 8-O-4′ neolignans, the chemical shift difference between H-9a and H-9b ($\Delta\delta_{H9a-H9b}$) in CD_3OD has been reported as an accurate and concise approach to determine the relative configuration of H-7 and H-8.[18] A small chemical shift difference of H-9a and H-9b ($\Delta\delta_{H9a-H9b}$ = 0.02) as shown in Figure 3 determined the *erythro* configuration of C-7 and C-8 for **1**. 8-O-4′ neolignans usually existed in the form of enantiomer mixtures because of their biosynthetic properties based on previous studies.[9,19] Compared with similar 8-O-4′ neolignans, the small optical rotation (−8.1) and subtle Cotton effects in the ECD spectrum demonstrated that **1** should be a mixture of enantiomers.[9,19] The attempt to purify the racemic mixtures by chiral HPLC was unsuccessful due to the limited amount of sample. Thus, compound **1** with an *erythro* configuration was elucidated and named as (±) lasiandranin A.

Compound **2** displayed the same molecular formula of $C_{22}H_{30}O_7$ based on the quasi-molecular ion peak at m/z 429.1895 [M + Na]$^+$ (calcd for $C_{22}H_{30}O_7Na$, 429.1889) in its positive HRESIMS. The NMR spectroscopic data were similar to those of **1**, suggesting that **2** was an isomer of **1**, and they shared the same planar skeleton. A significant chemical shift difference of H-9a and H-9b ($\Delta\delta_{H9a-H9b}$ = 0.21) demonstrated the *threo* configuration of C-7 and C-8 for **2** (Figure 3). The small optical rotation (−4.1) and subtle Cotton effects in the ECD spectrum demonstrated that **2** should be a mixture of enantiomers.[9,19] Thus, the structure of **2** with a *threo*

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ACS Omega 2020, 5, 19661−19666

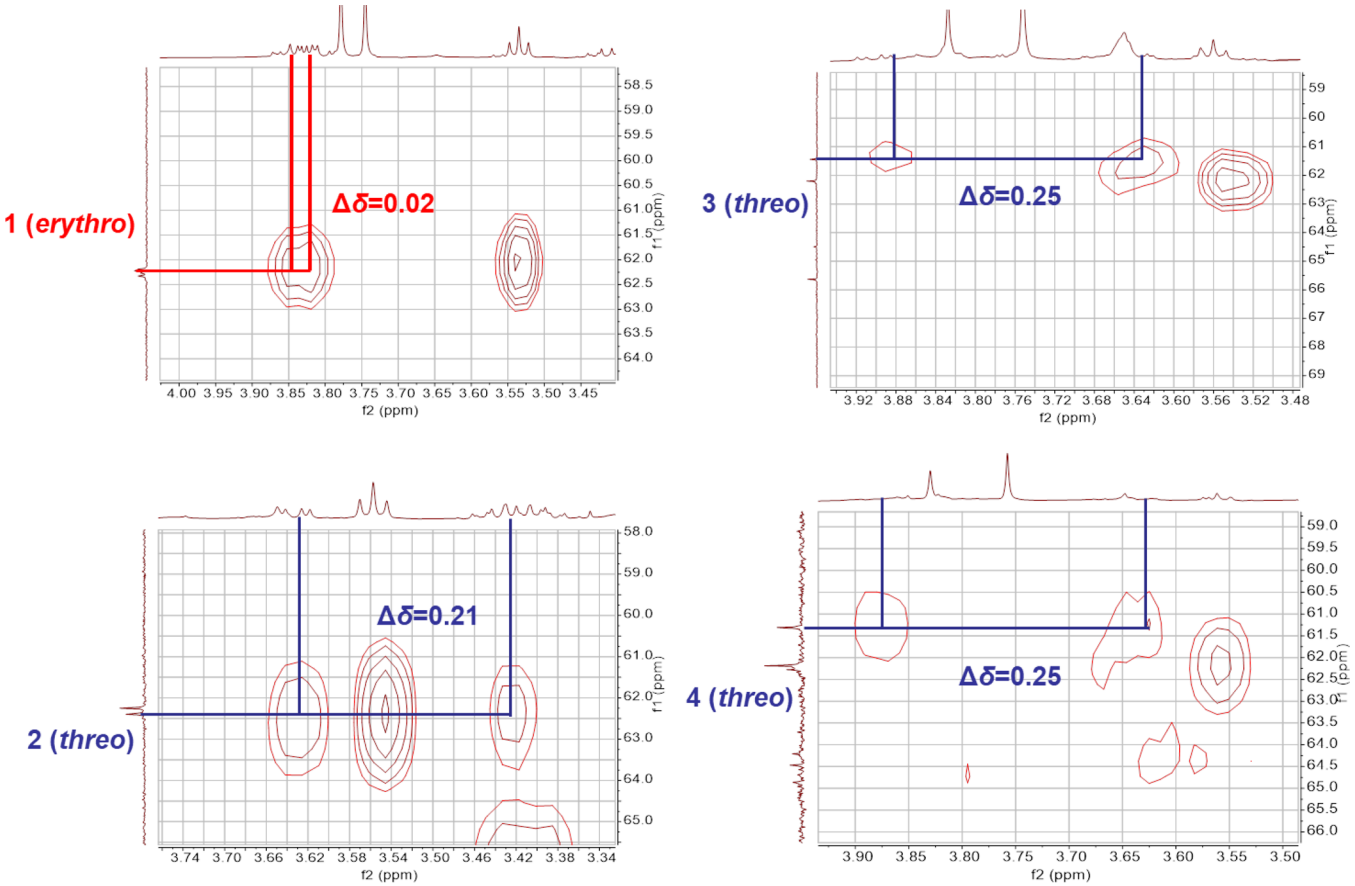

Figure 3. Chemical shift differences of H-9a and H-9b for compounds 1−4 in CD_3OD (red for *erythro*; blue for *threo*).

configuration was determined and named as (±) lasiandranin B.

Compound **3** was obtained as a yellow oil. Its molecular formula was determined as $C_{23}H_{32}O_8$ on the basis of a quasi-molecular ion peak at *m/z* 459.1973 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_8Na$, 459.1995) in the positive HRESIMS. In the ^1H NMR spectrum, compound **3** showed five aromatic protons at δ_H 6.90 (1H, d, J = 1.8 Hz), 6.76 (2H, d, J = 1.8 Hz), and 6.50 (2H, brs), revealing the presence of a 1,3,5-trisubstituted and 1,3,4,5-tetrasubstituted aromatic ring. A 1,2,3-propane-triol moiety and a 3-propanol moiety were established by COSY correlations from H-8 [δ_H 4.15 (1H, m)] to H-7 [δ_H 4.60 (1H, d, J = 5.9 Hz)] and H_2−9 [δ_H 3.90 (1H, d, J = 12.1, 4.7 Hz) and 3.65 (1H, m)], along with correlations from H_2−8′ [δ_H 1.82 (2H, m)] to H_2−7′ [δ_H 2.62 (2H, m)] and H_2−9′ [δ_H 3.56 (2H, t, J = 6.5 Hz)]. Furthermore, ethoxy group signals at δ_H 3.43 (2H, m, H_2−1″) and 1.18 (3H, t, J = 7.0 Hz, H_3−1″) attached to the aliphatic chain at C-7 (δ_C 82.0) and three methoxy group signals at δ_H 3.83 (3H, s, 3-OCH_3) and 3.75 (6H, s, 3′/5′-OCH_3) attached to the aromatic rings at C-3 (δ_C 148.9) and C-3′/C-5′ (δ_C 154.3) were established by HMBC correlations (Figure 2). The ^{13}C NMR spectrum of **3** showed 23 carbon signals. Except for one ethoxy and three methoxy groups, the remaining 18 carbons including 12 aromatic and six aliphatic carbons were attributed to two phenyl propanoid units on the basis of HMBC correlations from H-7 to C-2, C-6, C-8, and C-9, and from H_2−7′ to C-2′, C-6′, C-8′, and C-9′. Moreover, the HMBC correlation from H-8 to C-4′ confirmed that the two phenyl propanoid units in **3** formed an 8-O-4′ neolignan. The *threo* relative configuration of **3** was deduced by its chemical shift difference of H-9a and H-9b ($\Delta\delta_{H9a-H9b}$ = 0.25). The small optical rotation (−2.9) and subtle Cotton effects in the ECD spectrum demonstrated that **3** should be a mixture of enantiomers.[9,19] Accordingly, the structure of **3** with a *threo* relative configuration was determined and named as (±) lasiandranin C.

Compound **4** showed a molecular formula of $C_{22}H_{30}O_8$ based on the quasi-molecular ion peak at *m/z* 445.1812 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_8Na$, 445.1838) in its positive HRESIMS. The NMR spectral data of **4** were almost identical to those of **3**, except for one ethoxy group at C-7 that was replaced by a methoxy group. HMBC correlation from the methoxy group singlet at δ_H 3.26 (3H, s, 7-OCH_3) to C-7 (δ_C 83.7) indicated the above deduction. The relative configuration of **4** was determined as *threo* based on the significant chemical shift difference of H-9a and H-9b ($\Delta\delta_{H9a-H9b}$ = 0.25). The similar optical rotation (−3.0) and subtle Cotton effects in the ECD spectrum demonstrated that **4** should be a mixture of enantiomers.[9,19] Thus, the structure of **4** with a *threo* relative configuration was determined and named as (±) lasiandranin D.

The NMR data of **5** and **6** were similar to those of known compounds, pinnatifidanin BV and pinnatifidanin BVI, by comparison of their spectral data with the literature.[5] The chemical shift difference of H-9a and H-9b for **5** ($\Delta\delta_{H9a-H9b}$ = 0.02) and **6** ($\Delta\delta_{H9a-H9b}$ = 0.21) demonstrated that they possessed an *erythro* and *threo* relative configuration, respectively. Comparison of Cotton effects in the ECD spectra for compounds **1**−**6** demonstrated that **5** and **6** were also enantiomer mixtures. Thus, **5** with an *erythro* relative

https://dx.doi.org/10.1021/acsomega.0c02339
ACS Omega 2020, 5, 19661−19666

configuration of (±) pinnatifidanin BV and **6** with a *threo* relative configuration of (±) pinnatifidanin BVI were determined.

Furthermore, the literature reported that the coupling constant of H-7 in $CDCl_3$ ($J_{7,8}$≤5.0 Hz for *erythro*, $J_{7,8}$≥8.0 Hz for *threo*) could be used to determine the relative configuration of H-7 and H-8 for 8-O-4′ neolignans.[18] The ^{1}H-NMR data were also measured in $CDCl_3$ to verify the relative configuration of compounds **1**−**6** (Table 2).

Table 2. Values of $J_{7,8}$ (Hz) for Compounds 1−6 in CD_3OD and $CDCl_3$

solvents	1	2	3	4	5	6
CD_3OD	d, 6.4	d, 5.9	d, 5.9	d, 5.8	d, 6.3	d, 5.9
$CDCl_3$	d, 7.5	d, 7.3	d, 6.6	d, 6.3	d, 7.1	d, 7.4

Interestingly, there was no regulation and significant difference for their coupling constant of H-7 and H-8 ($J_{7,8}$ ranges from 6.3 to 7.5 Hz) in $CDCl_3$ due to the substitution of the methoxy or ethoxy group at C-7. Reconsideration of the coupling constant of H-7 and H-8 in CD_3OD demonstrated that **1** and **5** were *erythro* configurations with $J_{7,8}$ about 6.4 Hz, while **2**−**4** and **6** were *threo* configurations with $J_{7,8}$ about 5.9 Hz. The above information confirmed that $\Delta\delta_{H9a-H9b}$ is an accurate way to determine the relative configuration of H-7 and H-8 for 8-O-4′ neolignans, while the coupling constant of H-7 should be used cautiously when methoxy or ethoxy groups are substituted at C-7 for 8-O-4′ neolignans.

Compounds **1**−**6** were evaluated for their in vitro antibacterial activity against three plant pathogenic bacteria *P. syringae* pv. *actinidiae*, *R. solanacearum*, and *E. carotovora* by agar and broth dilution methods. MIC was defined as the lowest concentration showing no visible bacterial growth after incubation time. The results of the MIC values are shown in Table 3. Compounds **1**−**6** exhibited stronger antibacterial activity against *R. solanacearum* with the MIC values of 25−50 μg/mL, and they showed relatively lower activity against *P. syringae* pv. *actinidiae* with the MIC values of 50−100 μg/mL. However, they were inactive to *E. carotovora* with MIC values more than 200 μg/mL. The results indicated that the plant pathogenic bacterium *R. solanacearum* was the most sensitive species to be controlled by this series of compounds. Furthermore, compounds **1** and **5** characterized at the common *erythro* relative configuration showed stronger antibacterial activity against *R. solanacearum* and *P. syringae* pv. *actinidiae* than compounds **2**−**4** and **6** with the characterized *threo* relative configuration, indicating that *threo* configuration may be important for 8-O-4′ neolignans against plant pathogenic bacteria. However, opposite results verified that *threo* 8-O-4′ neolignans were considered to be more active than their *erythro* ones in their cytotoxic activities.[5] It is hard to determine which configuration is more important for their antifungal activities against different dermatophytes.[3] The above information demonstrated that bioactivity for 8-O-4′ neolignans may be related closely to their absolute configuration.

3. CONCLUSIONS

In summary, four new 8-O-4′ neolignans with a methoxy or ethoxy group substituted at C-7, namely, (±) lasiandranins A−D (**1**−**4**), and two known ones (±) pinnatifidanin BV (**5**) and (±) pinnatifidanin BVI (**6**) were identified from the whole plants of *C. lasiandra*. These 8-O-4′ neolignans were evaluated for their antibacterial activity against three plant pathogenic bacteria *P. syringae* pv. *actinidiae*, *R. solanacearum*, and *E. carotovora* by agar and broth dilution methods. The antibacterial activity assay presented in this study indicated that compounds **1** and **5** could be potential to be developed as antibacterial agents to control plant pathogenic bacteria *R. solanacearum*. However, as 8-O-4′ neolignans were minimal constituents from *C. lasiandra*, systematic studies on secondary metabolites from the genus *Clematis* or a synthetic work for this type of neolignan should be considered for their source to control plant pathogenic bacteria.

4. MATERIALS AND METHODS

4.1. General. Specific rotations were measured with a Perkin-Elmer 343 polarimeter (PerkinElmer, Waltham, USA). UV and electronic circular dichroism (ECD) spectra were recorded on a ZF-2 spectrometer (Shanghai Anting Electrical Instrument Co. Ltd., Shanghai, China) and Jasco-J-715 spectropolarimeter (Jasco Europe S.R.L, Cremella, Italy), respectively. The HRESIMS spectra were obtained with a Thermo Fisher Scientific Q-TOF mass spectrometer (Thermo Fisher Scientific, Waltham, USA). 1D and 2D NMR spectral experiments were performed in CD_3OD with a Bruker AVANCE-500 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with tetramethylsilane (TMS) as an internal standard. Separations and purifications were performed by column chromatography (CC) on silica gel H (10−40 μm, Qingdao Marine Chemical Inc., Qingdao, China) and a Sephadex LH-20 (GE Inc., USA). HPLC was carried out on a Shimadzu LC-10ATVP liquid chromatograph equipped with a SPD-10ADVP (UV-vis) detector (Shimadzu, Kyoto, Japan) at 206 nm using a YMC-Pack R&D ODS-A column (250 × 10 mm i.d., 5 μm, YMC Co., Ltd. Japan) for semi-preparation. TLC detection was achieved by spraying the silica gel plates (Qingdao Marine Chemical Inc., Qingdao, China) with 20% H_2SO_4 in EtOH followed by heating.

4.2. Plant Material. The whole plants of *Clematis lasiandra* Maxim were collected in the north of Qin Mountain, Shaanxi Province of China in September 2009 and were identified by Prof. Ji-Tao Wang from Shaanxi University of Chinese Medicine. A voucher specimen (No. 20090904) was deposited in the Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Xi′an, Shaanxi, PR China.

4.3. Extraction and Isolation. The air-dried whole plants of *C. lasiandra* (7.2 kg) were ground and then extracted with 70% EtOH (3 × 56 L) under reflux at room temperature. The

Table 3. MIC Values (μg/mL) of Compounds 1−6 against Plant-Pathogenic Bacteria In Vitro

strain	1	2	3	4	5	6	streptomycin sulfate
Pseudomonas syringae pv. *actinidiae*	50	100	100	100	50	100	12.5
Ralstonia solanacearum	25	50	50	50	25	50	12.5
Erwinia carotovora	>200	>200	>200	>200	>200	>200	200

https://dx.doi.org/10.1021/acsomega.0c02339
ACS Omega 2020, 5, 19661−19666

concentrated EtOH extract (1.4 kg) was suspended in H_2O and then partitioned successively with petroleum ether (3 × 6 L), EtOAc (3 × 6 L), and *n*-BuOH (5 × 6 L), respectively. The EtOAc extract (80.3 g) was separated into 16 fractions (Fr. A−P) on a silica gel column using a stepwise gradient elution of petroleum ether−EtOAc (100:1, 50:1, 30:1, 15:1, 10:1, 5:1, and 1:1) and then eluted with $CHCl_3$−MeOH (1:1) to give Fr. Q. Fr. Q (4.7 g) was subjected to CC over a silica gel column using a step gradient elution of $CHCl_3$−MeOH (10:1, 7:1, 5:1, 3:1, and 1:1) to give 11 subfractions (Fr. Q_1 − Fr. Q_{11}). Fr. Q_2 (342 mg) was eluted with $CHCl_3$−MeOH (1:1) on the Sephadex LH-20 to remove pigments and then was further purified by semi-preparative HPLC using MeOH−H_2O (40,60) as the mobile phase at a flow rate of 2.0 mL/min to afford compounds **1** (6.1 mg, t_R = 70.2 min), **2** (4.8 mg, t_R = 74.3 min), **3** (3.9 mg, t_R = 62.0 min), **4** (2.6 mg, t_R = 49.7 min), **5** (5.0 mg, t_R = 38.4 min), and **6** (2.7 mg, t_R = 40.5 min).

*4.3.1. (±) Lasiandranin A (**1**).* Yellow oil; $[\alpha]^{25}_D$ − 8.1 (*c* 0.06, MeOH); ECD (CH_3OH): − 0.9 (228 nm), + 1.0 (237); UV (CH_3OH) λ_{max} (log ε): 206 (3.24) nm, 233 (1.12) nm, 282 (0.51) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1; HRESIMS *m/z* 429.1869 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

*4.3.2. (±) Lasiandranin B (**2**).* Yellow oil; $[\alpha]^{25}_D$ − 4.1 (*c* 0.08, MeOH); ECD (CH_3OH): − 0.4 (228 nm), + 0.9 (237 nm); UV (CH_3OH) λ_{max} (log ε): 206 (3.12) nm, 234 (0.94) nm, 282 (0.47) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1; HRESIMS *m/z* 429.1895 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

*4.3.3. (±) Lasiandranin C (**3**).* Yellow oil; $[\alpha]^{25}_D$ − 2.9 (*c* 0.08, MeOH); ECD (CH_3OH): − 0.7 (228 nm), + 0.8 (237 nm); UV (CH_3OH) λ_{max} (log ε): 203 (1.38) nm, 232 (0.35) nm, 282 (0.20) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1; HRESIMS *m/z* 459.1973 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_8Na$, 459.1995).

*4.3.4. (±) Lasiandranin D (**4**).* Yellow oil; $[\alpha]^{25}_D$ − 3.0 (*c* 0.06, MeOH); ECD (CH_3OH): + 0.5 (228 nm); UV (CH_3OH) λ_{max} (log ε): 206 (2.40) nm, 232 (0.61) nm, 282 (0.30) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1; HRESIMS *m/z* 445.1812 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_8Na$, 445.1838).

4.4. Microorganisms and Media. Microorganisms of *Pseudomonas syringae* pv. *actinidiae*, *Ralstonia solanacearum*, and *Erwinia carotovora* were provided by College of Plant Protection, Northwest A&F University. The nutrient agar (NA) medium was composed of pepton (10 g), beef extract (3 g), NaCl (5 g), agar (15 g), and distilled water (1 L). Luria−Bertani (LB) broth consisted of yeast (5 g), tryptone (10 g), NaCl (10 g), and distilled water (1 L). The cryopreserved strains were inoculated on a NA solid medium, cultivated at 37 °C for 24 h, and placed in a refrigerator at 0−4 °C for further use. A single colony was picked from the activated strain, inoculated into the LB broth medium, and cultured on a shaker with a speed of 200 r/min for 12 h at 37 °C. The obtained bacterial solution was diluted with a spectrophotometer to 1 × 10^5−1 × 10^7 CFU/mL for use.

4.5. Antibacterial Assays. The antibacterial activities of the isolated compounds were evaluated with the agar and broth dilution methods.[20,21] The assays were carried out in 96-well microtiter plates. Compounds were dissolved in DMSO with the desired concentration of 40 mg/mL. Twofold serial dilutions of the compounds with the concentration ranging from 12.5 to 200 μg/mL were conducted by adding 2 μL of the test compounds to sterile 96-well plates containing 98 μL of inoculated LB medium with the density of the target bacteria of 1 × 10^6 CFU/mL. The final concentration of DMSO in the assay did not exceed 1%. The agricultural streptomycin sulfate was used as positive control, and the drug-free solution was used as blank control. The microtiter plates were incubated at 30 °C, except for *P. syringae* pv. *actinidiae* at 25 °C, for 24 to 48 h. Iodonitrotetrazolium chloride (INT) was used as the staining agent for bacteria. Each treatment was replicated three times.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02339.

HRESIMS, ^1H NMR, ^{13}C NMR, HSQC, HMBC, and COSY spectra in CD_3OD of compounds **1**−**4**. ^1H NMR and ^{13}C NMR in CD_3OD of compounds **5**−**6**. UV, ECD, and ^1H NMR ($CDCl_3$) spectra of compounds **1**−**6** (PDF)

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Author Contributions

[§]N.H. and L.H. contributed equally. N.H. and L.H. performed the isolation and bioassay work with the help of Y.L., J.L., X.T., and D.K. X.T. designed the study and prepared the manuscript. All authors discussed the results and commented on the manuscript at all stages.

Funding

The research work was financially supported by the National Key R&D Program of China (2017YFD0201105), the National Natural Science Foundation of China (31870331), and the Natural Science Foundation of Shaanxi Province, China (2017JM3015).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Mr. Hongli Zhang and Ms. Luqi Li for measurement of NMR and HRESIMS, respectively.

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https://dx.doi.org/10.1021/acsomega.0c02339
ACS Omega 2020, 5, 19661−19666