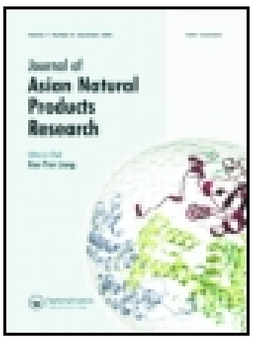

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Two new lignans from the fruits of *Forsythia suspensa*

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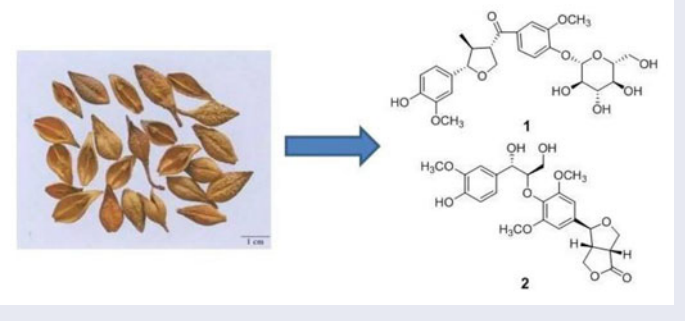
ABSTRACT

Two new lignans, wikstronoside B (**1**) and forsysesquinorlignan (**2**), were isolated from the fruits of *Forsythia suspensa*, along with two known sesquineolignans, hedyotol A and hedyotol C (**3** and **4**). The structures of new compounds were established via extensive spectroscopy techniques, including UV, IR, HRESIMS, NMR, and ECD. Compounds **3** and **4** were isolated from this plant for the first time. Their anti-inflammatory effects were evaluated via a detection model with LPS-induced murine macrophage RAW264.7 cells, and compound **3** showed a moderate activity.



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Oleaceae; *Forsythia suspensa*; sesquinorlignan

1. Introduction

As a traditional Chinese medicine, the fruits of *Forsythia suspensa* (Tunb.) Vahl are mainly used to treat inflammation, pyrexia and ulcers [1]. According to the previous studies, the compounds from *F. suspensa* are mainly phenylethanoid glycosides [2,3], triterpenoids [4], lignans [5], and flavonoids [6], in which some compounds show good pharmacological activities, such as anti-inflammatory [7–9], antioxidant [9], antibacterial [10], and neuroprotective effects [11]. In our continuing search for bioactive constituents from *F. suspensa*, four lignans (**1**–**4**) were isolated and identified. Compounds **1** and **2** were new lignans and compounds **3** and **4** were obtained from

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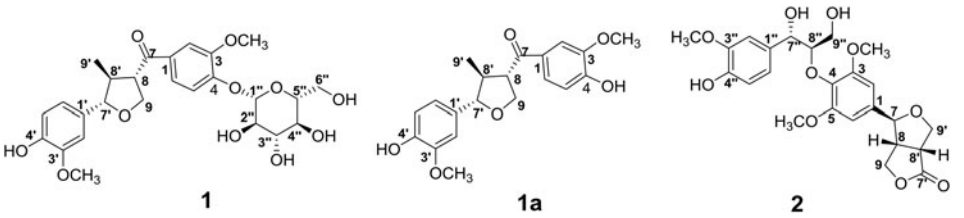

Figure 1. Chemical structures of compounds **1**, **1a**, and **2**.

the plant for first time (Figure 1). Their anti-inflammatory effects against LPS-induced NO production in murine macrophage RAW264.7 cells were estimated.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder, and its molecular formula was determined as $C_{26}H_{32}O_{11}$ by HRESIMS at *m/z* 521.2030 $[M+Na]^+$. The IR spectrum showed absorption bands attributed to hydroxy groups (3420 cm^{-1}), carbonyl groups (1669 cm^{-1}), and aromatic rings (1593, 1514, 1419 cm^{-1}). The ^{1}H-NMR spectrum of **1** revealed the presence of two sets of ABX system aromatic protons at δ_H 7.51 (1H, brs), 7.21 (1H, d, $J=8.5$ Hz), 7.62 (1H, brd, $J=8.5$ Hz), 6.87 (1H, brs), and 6.73 (2H, brs), two methoxy signals at δ_H 3.83 (3H, s) and 3.75 (3H, s), one methyl signal at δ_H 0.93 (3H, d, $J=6.5$ Hz), three methine signals at δ_H 4.28 (1H, d, $J=9.5$ Hz), 4.04–4.06 (1H, m), and 2.30–2.32 (1H, m), one methylene group at δ_H 4.19 (1H, t, $J=8.5$ Hz) and 3.92 (1H, dd, $J=15.0$, 8.5 Hz). In addition, one anomeric proton at δ_H 5.08 (1H, d, $J=7.0$ Hz) was also observed from the ^{1}H-NMR spectrum, and the remaining resonances were assigned to a glucosyl unit. After acid hydrolysis, the sugar unit of **1** was confirmed to be β-D-glucose by GC analysis after chiral derivatization (retention time of 29.6 min). The ^{13}C-NMR spectrum of **1** showed 12 aromatic carbons from δ_C 151.0 to 110.4, one carbonyl at δ_C 197.8 in the downfield region. With the exception of the glucosyl signals, two methoxy signals (δ_C 55.6 and 55.6), three methine signals (δ_C 87.9, 53.2, and 45.4), one oxymethylene signal (δ_C 69.6) and one methyl signal (δ_C 14.7) were observed in the upfield region. HMBC correlations of H-8/C-1, H-9/C-7, H-8′/C-1′, and H-9′/C-7′ revealed the presence of two C6-C3 units. Furthermore, the correlations of H-7′ with C-8 and C-9 indicated these two units formed a furan ring, and the correlation of H-1″/C-4 indicated the glucosyl unit was linked to C-4 (Figure 2). The relative configurations of H-7′/H-8′ and H-8/H-8′ were determined as *trans*-configurations via ROSEY correlations of H-9′ with H-8 and H-7′ (Figure 3). Considering the numerous conformations stemming from the single glucosyl bonds, aglycone (**1a**) was used for ECD calculation, and the calculated ECD data of (8*S*, 7′*S*, 8′*S*)-**1a** matched with the experimental data for **1a** (Figure 4). Thus, the absolute configuration of **1** was determined as 8*S*, 7′*S*, 8′*S*. Accordingly, the structure of **1** was assigned as shown and named wikstronoside B (Figure 1).

Compound **2** was obtained as a brown amorphous powder, and its molecular formula was determined as $C_{24}H_{28}O_{10}$ by HRESIMS at *m/z* 475.1603 $[M-H]^-$. The IR spectrum showed absorption bands attributed to hydroxy groups (3454 cm^{-1}), carbonyl groups (1767 cm^{-1}), and aromatic rings (1593, 1514, 1461 cm^{-1}). The ^{1}H-NMR

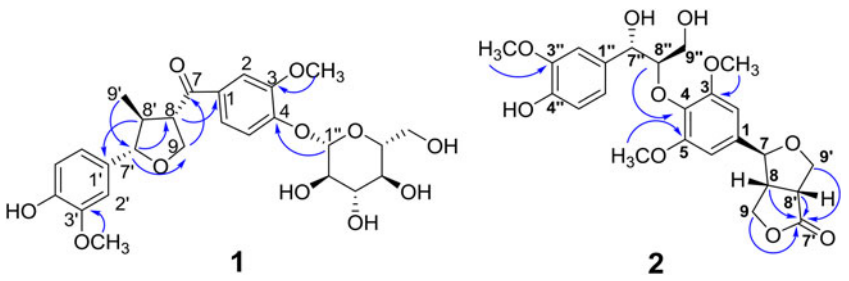

Figure 2. Key HMBC correlations of compounds **1** and **2**.

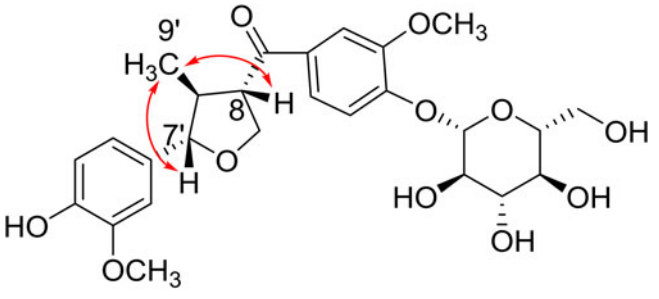

Figure 3. Key ROSEY correlations of compound **1**.

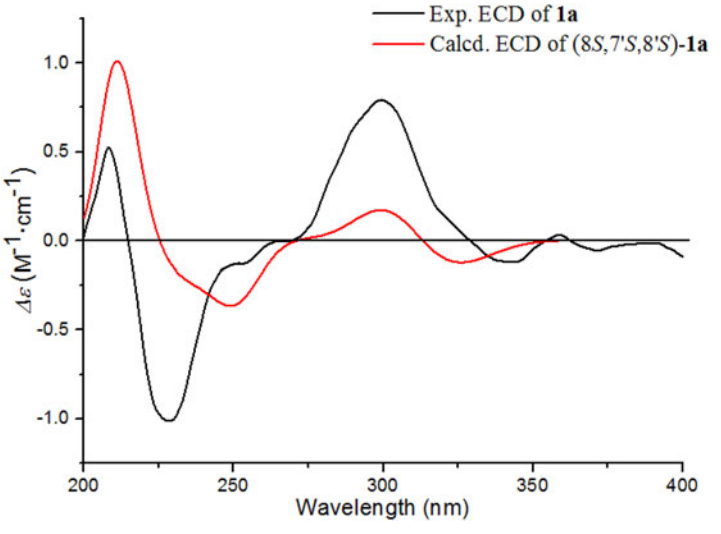

Figure 4. Experimental and calculated ECD spectrum of **1a**.

spectrum of **2** revealed the presence of one set of ABX system aromatic protons at δ_H 6.97 (1H, d, $J = 2.0$ Hz), 6.84 (1H, d, $J = 8.0$ Hz), and 6.72 (1H, dd, $J = 8.0$, 2.0 Hz) and two meta-substituted aromatic protons at δ_H 6.69 (2H, s). In addition, three methoxy signals at δ_H 3.87 (6H, s) and 3.82 (3H, s), three oxymethine signals at δ_H 4.97 (1H, d, $J = 6.5$ Hz), 4.72 (1H, d, $J = 6.0$ Hz), and 4.15 (1H, overlap), two methine signals at δ_H 3.54–3.56 (1H, m) and 3.16–3.18 (1H, m), three methylene groups at δ_H 4.57 (1H, dd, $J = 9.5$, 7.0 Hz), 4.43 (1H, dd, $J = 9.5$, 2.0 Hz), 4.31 (1H, dd, $J = 9.0$,

2.0 Hz), 4.12 (1H, dd, $J = 9.0$, 3.5 Hz), 3.78 (1H, dd, $J = 12.0$, 4.0 Hz), and 3.37 (1H, dd, $J = 12.0$, 3.5 Hz) were also observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **2** showed twelve aromatic carbons from δ_C 154.4 to 104.1, one carbonyl at δ_C 181.0 in the downfiled region, three methoxy signals (δ_C 56.7, 56.7, and 56.3), three oxymethine signals (δ_C 88.5, 87.5, and 74.2), two methine signals (δ_C 49.8 and 47.5), three oxymethylene signals (δ_C 72.0, 71.3, and 61.0) in the upfield region. The similar planar structure was established via comparing the NMR data with the corresponding those of hedyotol C [12], it's worth to note that compound **2** had one more carbonyl group and lacked one phenyl and methoxy group, that is the carbonyl in **2** replaced the aromatic ring in hedyotol C. The HMBC correlations of 3-OMe (δ_H 3.87) with C-3 (δ_C 154.3), 5-OMe (δ_H 3.87) with C-5 (δ_C 154.3), and 3″-OMe (δ_H 3.82) with C-3″ (δ_C 148.6) confirmed the three methoxy groups were located at C-3, C-5, and C-3″, respectively (Figure 2).

Comparison of the NMR data between **2** and hedyotol C demonstrated that they had same relative configuration [13], and the relative configuration of H-7″ and H-8″ was determined as *threo*-configuration via the chemical shift difference ($\Delta H_{9''} > 0.25$) [12], and the C-8/C-8′ of furofurans lignans in *F. suspensa* were all in *R*/*R* configurations. Thus, the configuration of compound **2** was determined as 7*S*,8*R*,8′*R*,7″*S**,8″*R**, and named forsysesquinorlignan (Figure 1).

In addition, compounds **3** and **4** were identified as hedyotol A and hedyotol C by comparison of spectroscopic data with those of the corresponding literature [13,14].

The detection model with LPS-induced murine macrophage RAW264.7 cells showed that their NO inhibition rates were 0.4%, 3.1%, 50.7%, and 9.9% at a concentration of 10 μM, respectively. Comparison with the positive control, curcumin (10 μM, 73.0%), showed that compound **3** had a moderate anti-inflammatory activity.

3. Experimental

3.1. General experimental procedures

The optical rotations, UV and ECD were measured on JASCO P-2000, JASCO V-650, and JASCO J-815 spectrometers (JASCO, Easton, MD, USA), respectively. IR spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method (Thermo Scientific, Waltham, MA, USA). NMR spectra were obtained on a Bruker AVIII-500 spectrometer (Bruker-Biospin, Billerica, MA, USA). HRESIMS were performed on an Agilent 6520 HPLC-Q-TOF (Agilent Technologies, Waldbronn, Germany). GC experiments were performed using an Agilent 7890A series system with a capillary column, HP-5 (60 m × 0.25 mm, with a 0.25 μm film; Dikma Technologies Inc., Beijing, China). The reversed-phase preparative HPLC was performed utilizing a Shimadzu LC-10AT instrument equipped with a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm; YMC Corp., Kyoto, Japan). Silica gel (100–200 mesh, Qingdao Marine Chemical Inc. Qingdao, China), RP-C_{18} (50 μm, YMC, Kyoto, Japan) and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) were employed for column chromatography. The HPLC experiments were performed using an Agilent 1260 system with an Apollo C18 column (250 × 4.6 mm, 5 μm, Grace Davison).

Table 1. ^{1}H and ^{13}C NMR Spectral data for compounds 1 and 2 (500/125 MHz, δ in ppm, J in Hz).

	1(DMSO-d_6)		**2**(CD_3OD)	
Position	δ_H	δ_C	δ_H	δ_C
1		130.5		137.6
2	7.51 brs	112.2	6.69 s	104.1
3		148.8		154.3
4		151.0		137.0
5	7.21 d (8.5)	114.2		154.3
6	7.62 brd (8.5)	122.8	6.69 s	104.1
7		197.8	4.72 d (6.0)	87.5
8	4.04–4.06 m	53.2	3.16–3.18 m	49.8
9	4.19 t (8.5) 3.92 dd (15.0, 8.5)	69.6	4.57 dd (9.5, 7.0) 4.43 dd (9.5, 2.0)	72.0
1′		131.3		
2′	6.87 brs	110.4		
3′		147.5		
4′		146.2		
5′	6.73 brs	115.1		
6′	6.73 brs	119.2		
7′	4.28 d (9.5)	87.9		181.0
8′	2.30–2.32 m	45.4	3.54–3.56 m	47.5
9′	0.93 d (6.5)	14.7	4.31 dd (9.0, 2.0) 4.12 dd (9.0, 3.5)	71.3
1″	5.08 d (7.0)	99.4		133.5
2″	3.26–3.28 m	73.0	6.97 d (2.0)	111.4
3″	3.27–3.29 m	76.8		148.6
4″	3.15–3.17 m	69.5		147.0
5″	3.36–3.38 m	77.2	6.84 d (8.0)	115.7
6″	3.65 brd (10.5) 3.42–3.44 m	60.5	6.72 d (8.0, 2.0)	120.6
7″			4.97 d (6.5)	74.2
8″			4.15 overlap	88.5
9″			3.78 dd (12.0, 4.0) 3.37 dd (12.0, 3.5)	61.0
3-OMe	3.83 s	55.6	3.87 s	56.7
5-OMe			3.87 s	56.7
3′-OMe	3.75 s	55.6		
3″-OMe			3.82 s	56.3

3.2. Plant material

The fruits of *Forsythia suspensa* were collected in December 2011 from Yuncheng City of Shanxi Province, China. The plant material was identified by associate professor Lin Ma (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences). A voucher specimen numbered ID-S-2597 was deposited at the Herbarium of the Department of Natural Medicinal Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3. Extraction and isolation

The fruits of *F. suspensa* (90.0 kg) were extracted with 75% EtOH (200 L) under reflux three times and filtered. The filtrate was evaporated *in vacuo* to give a crude extract (12.6 kg), which was suspended in H_2O and extracted successively with petroleum ether, EtOAc, and n-BuOH.

The n-BuOH portion (4 kg) was suspended in water (40 L) to obtain an water soluble layer, which was concentrated to yield an aqueous portion (1.5 kg). The portion was separated on a HP-20 resin column eluting with a mixture of EtOH-H_2O to obtain five fractions (0%, 15%, 30%, 50%, 95%). The 50% ethanol solution (392.0 g)

was separated on a HP-20 resin column eluting with a mixture gradient of EtOH-H_2O (0%, 10%, 20%, 30%, 40%, 50%, 95%) to obtain seven fractions (A–G). Separation of fraction G (65.0 g) was performed on a Sephadex LH-20 column in a mixture gradient of CH_3OH-H_2O (from 30% to 50%) to give 14 fractions (G1-G14). Fraction G13 (1.7 g) was further performed on a Sephadex LH-20 column in a mixture gradient of CH_3OH-H_2O (from 30% to 50%) to give nine fractions (G13.1-G13.9). Fraction G13.9 (54.0 mg) was purified using reversed-phase preparative HPLC with MeOH-H_2O (45:55) as the mobile phase to yield **1** (t_R 14.7 min, 4.0 mg).

The EtOAc fraction (3.0 kg) was chromatographically separated on a silica gel column eluted with EtOAc/acetone (from 100:0 to 0:100) and methanol to give 12 fractions (fractions 1–12). Fraction 10 (318.0 g) was further subjected to a silica gel column eluted with a gradient of CH_2Cl_2-MeOH (100:0–0:100), yielding fractions 10.1–10.15. Fractions 10.3 and 10.4 were further merged into three fractions (fractions A–C). Fraction B (1.1 g) was further chromatographed over ODS eluting with mixtures of MeOH-H_2O (15%, 25%, 30%, 35%, 40%, 45%, 70%, 100%) to yield fractions B1–B16. Fraction B4 (34.0 mg) was purified using reversed-phase preparative HPLC with MeOH-H_2O (35:65) as the mobile phase to yield **2** (t_R 19.3 min, 4.5 mg). Fraction B10 (47.0 mg) was purified using reversed-phase preparative HPLC with CH_3CN-H_2O (35:65) as the mobile phase to yield **3** (t_R 13.7 min, 10.0 mg). Fraction B14 (79.0 mg) was purified using reversed-phase preparative HPLC with CH_3CN-H_2O (35:65) as the mobile phase to yield **4** (t_R 15.3 min, 6.0 mg).

3.3.1. Wikstronoside B (1)

Yellow amorphous powder; $[\alpha]_D^{20}$ −30.0 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 275 (4.16), 223 (4.47) nm; ECD (CH_3OH) $\Delta\varepsilon_{208\,nm}$ +0.52, $\Delta\varepsilon_{228\,nm}$ −1.01, $\Delta\varepsilon_{299\,nm}$ +0.79; IR ν_{max} 3420, 2960, 1669, 1593, 1514, 1419, 1267, 1075 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESIMS: *m/z* 521.2030 $[M + Na]^+$ (calcd for $C_{26}H_{32}NaO_{11}$, 521.2017).

3.3.2. Sesquinorlignan (2)

Brown amorphous powder; $[\alpha]_D^{20}$ +25.0 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 279.2 (3.70), 231.6 (4.23), 205.2(4.81) nm; IR ν_{max} 3454, 1767, 1593, 1514, 1461, 1234, 1034 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESIMS: *m/z* 475.1603 $[M - H]^-$ (calcd for $C_{24}H_{27}O_{10}$, 475.1604).

3.4. Anti-inflammatory assay

The experimental method was carried out according to the literature [15].

Disclosure statement

No potential conflict of interest was reported by the authors.

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