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A New Lignan from the Herbaceous stems of *Ephedra intermedia* Schrenket C. A. Meyer.

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Abstract: A new lignan eplignan A (**1**), along with six konwn lignans (**2**–**7**) was isolated from the herbaceous stems of *Ephedra intermedia Schrenket C. A. Meyer*. Their structures were elucidated based on the spectroscopic evidence, mainly including NMR and HRESIMS data. The absolute configuration of **1** was determined by comparing calculated and experimental electronic circular dichroic (ECD) spectra. Moreover, all compounds (**1**–**7**) were evaluated for their protective effects against the BEAS-2B cells injury induced by TGF-β1 *in vitro*, and the results showed that compounds **1**, **2**, **4**, and **6** exhibited significantly protective activities at the concentration of 10 μM.

Keywords: Ephedra; *Ephedrae intermedia*; lignans; lung protective activity.

1. Introduction

Ephedra is a genus of non-flowering seed plants belonging to the Ephedraceae family including about 69 species distributed northern hemisphere [1]. Ephedrae Herba is usually defined as the herbaceous stems of *Ephedra sinica Stapf*, *Ephedra intermedia Schrenket C. A. Meyer* or *Ephedra equisetina Bunge* in the Chinese Pharmacopoeia 2020 edition [2], which was traditionally called “Ma huang” in China. As a Chinese folk medicine, Ephedrae Herba has been mainly used for the treatment of cold, asthma, cough and lack of sweating [3], which is mainly distributed in Xinjiang, Qinghai, Gansu province in northeast and northwest of China [4].

So far, various biological activities about *Ephedra sinica* have been reported, such as anti-microbial [5], lowering blood glucose [6], anti-inflammatory [7], anti-oxidant [8], and anti-immunity

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properties [9], due to its rich chemicals, which contain alkaloids [10], flavonoids [11], tannins [12], organic acids [13], and polysaccharides [9]. However, limited research has been done regarding phytochemical and biological properties of *E. intermedia.* As a part of our efforts to discover bioactive natural products, a new lignan, eplignan A (**1**), along with six known lignans (**2**–**7**) was isolated from the herbaceous stems of *E. intermedia*, whose structures were determined by detailed analysis of the spectroscopic evidence and HRESIMS data. Compounds **1**, **2**, **4**, and **6** showed extraordinarily protective activities against TGF-*β*1-induced BEAS-2B cells injury.

2. Materials and Methods

2.1 General Experiment Procedures

Optical rotations were performed on a Rudolph AP-IV Polarimeter (Rudolph, USA). IR spectra were obtained on a Thermo Nicolet IS 10 spectrometer (Thermo, USA). UV spectra were measured by using a Thermo EVO 300 spectrometer (Thermo, USA). NMR spectra were recorded on a BrukerAvance Ⅲ500 spectrometer (Bruker, Germany). HRESIMS spectra were carried out on a Bruker maXis HD mass spectrometer (Bruker, Germany). ECD spectra were obtianed using an Applied Photophysics Chirascan CD spectropolarimeter (AppliedPhotophysics, Leatherhead, Surrey, UK). Semi-preparative high performance liquid chromatography (HPLC) was conducted on a Saipuruisi LC 52 HPLC system (Saipuruisi, China), equipped an UV/vis 50 detector and a C_{18} column (10 × 250 mm, 5 μm; YMC, Kyoto, Japan). Toyopearl HW-40C, MCI gel CHP-20, ODS gel (50 μm) were obtained from TOSOH Corp (Tokyo, Japan) and Sephadex LH-20 (40–70 μm) were obtained from Amersham Pharmacia Biotech AB (Uppsala, Sweden). Silica gel (100–200 mesh, 200–300 mesh) was acquired from Marine Chemical Industry (Qingdao, China). The chemical reagents (analytical grade) were purchased from Hengxing Chemical Reagent Co. Ltd. (Tianjin, China). BEAS-2B cells purchased from Shanghai Institutes for Biological Sciences.

2.2. Plant Material

The herbaceous stems of *E. intermedia* were purchased from Xinjiang Xiyu Mocao Chinese Medicinal Materials Development Limited Company in October 2020, which was identified as the dry herbaceous stems of *E. intermedia* by Prof. Cheng-Ming Dong of Henan University of Chinese Medicine. The specimen (NO.20201111) was deposited at the Department of Natural Medicinal Chemistry, Henan University of Chinese Medicine, Zhengzhou, China.

2.3. Separation and Purification

The dried herbaceous stems of *E. intermedia* (45.0 kg) were cut into small segments, and extracted with 50% aqueous acetone (smashed tissue extraction) to give an extract (11.1 kg), which was dispersed in water, and sequentially extracted with CH_2Cl_2, EtOAc, and *n*-BuOH for fifteen times, respectively. The CH_2Cl_2 fraction (185.0 g) was separated by silica gel column chromatography (CC) eluted with a petroleum ether-EtOAc (v/v 50:1–1:1) gradient and obtained ten fractions (D1–D10).

Fraction D7 (17.2 g) was subjected to ODS gel CC eluted with a CH_3OH-H_2O (v/v 10:90–100:0) gradient system to give ten fractions (D7-1–D7-10). Fraction D7-2 (2.6 g) was rechromatographed by silica gel CC (CH_2Cl_2-CH_3OH v/v 350:1–20:1) to obtain five fractions (D7-2-1–D7-2-5). Then D7-2-2 (305.6 mg) was separated by Sephadex LH-20 CC eluted with CH_3OH and purified by semi-preparative HPLC (CH_3OH-H_2O v/v 47:53) to yield compound **1** (4.8 mg, t_R = 36.4 min). D7-2-3 (95.6 mg) was further purified by semi-preparative HPLC (CH_3CN-H_2O v/v 17:83) to provide compound **4** (3.1 mg, t_R = 49.6 min). D7-2-5 (120.6 mg) was performed on Toyopearl HW-40C eluted with CH_3OH to give three fractions (D7-2-5-1–D7-2-5-3), and the D7-2-5-2 (36.5 mg) was purified by semi-preparative HPLC with CH_3CN-H_2O (v/v 22:78) as the mobile phase to obtain compound **7** (3.4mg, t_R = 37.8 min).

Fraction D8 (12.0 g) was applied to MCI gel CHP-20 CC eluted with a gradient system of

CH_3OH-H_2O (v/v 10:90–100:0) to give six fractions (D8-1–D8-6). D8-4 (1.1 g) was further separated by silica gel CC eluted with a CH_2Cl_2-CH_3OH (v/v 300:1–10:1) gradient system to give nine fractions (D8-4-1–D8-4-9). D8-4-3 (231.1 mg) was rechromatographed with Sephadex LH-20 CC eluted with CH_3OH to afford five fractions (D8-4-3-1–D8-4-3-5). Then D8-4-3-3 (50.6 mg) was purified by semi-preparative HPLC (CH_3CN-H_2O v/v 25:75) to produce compound **2** (17.7 mg, t_R = 42.5 min). D8-5 (1.4 g) was passed through silica gel CC eluted with a CH_2Cl_2-MeOH (v/v 250:1–10:1) gradient system to obtain seven fractions (D8-5-1–D8-5-7). Compound **3** (6.1 mg, t_R = 46.9 min) was obtained from D8-5-1 (302.5 mg) using Toyopearl HW-40C (CH_3OH-H_2O v/v 70:30) and semi-preparative HPLC (CH_3OH-H_2O v/v 46:54). Compounds **5** (3.37 mg) and **6** (23.6 mg) were produced from D8-5-2 (170.4 mg) using silica gel CC (CH_2Cl_2-CH_3OH v/v 70:1 50:1 30:1).

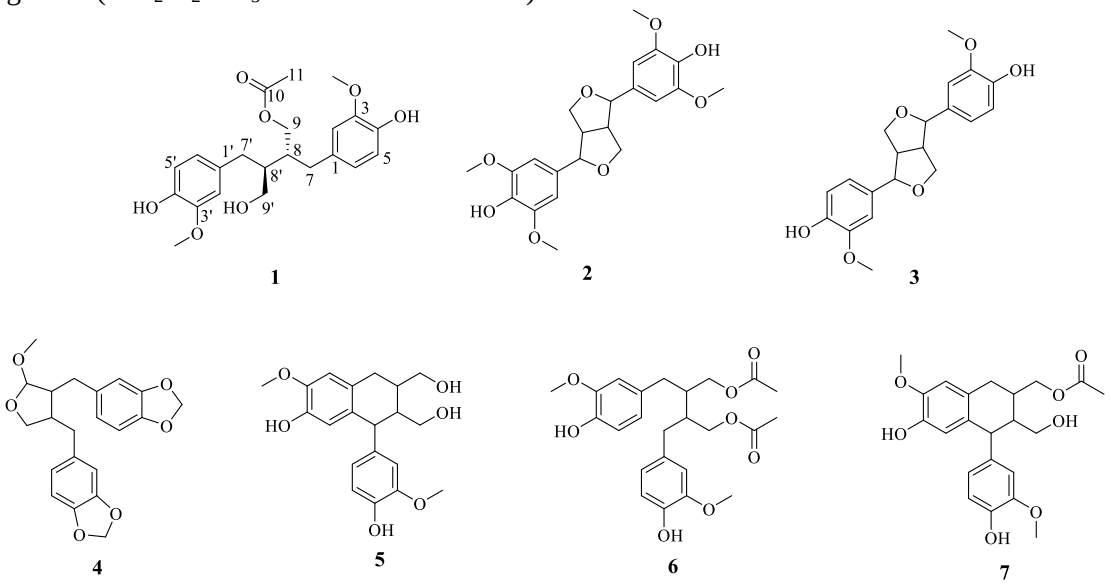

Figure 1. The structures of compounds **1**–**7**

2.4. Biological Activity

The BEAS-2B cells were plated into 96-well flat-bottomed cultured plates at a concentration of 4×10^3 cells per well in fresh culture medium and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Then divided into normal group (CON), model group (TGF-β1, 1 ng/mL) and each compound group [compounds **1**–**7**, 10 μM + TGF-β1, 1 ng/mL]. 20 μL MTT (5 mg/mL) was added to each well and the plates were incubated for 4 h after incubation for 24 h. Then the solution was removed before dimethyl sulfoxide (DMSO, 150 μL) was added. The optical density (OD) values were measured at 490 nm with a microplate reader (Thermo Scientific, Boston, USA) [14]. All tests were conducted in triplicate.

The experimental data were expressed as mean ± standard deviation ($\bar{x}\pm$ sd) and analyzed by SPSS 26.0. Comparisons between groups were performed with One-Way Anova. P<0.05 means a significant difference and P<0.01 means a very significant difference.

2.5. ECD Calculations

Conformational analyses of compound **1** was determined by GMMX software using the MMFF94 force field. Then conformers with a Boltzmann distribution ⩾ 1% were imported into Gaussian 16 software before they were optimized at the B3LYP/6-31G (d,p) level. The calculations of ECD curves were performed by the TDDFT method at the B3LYP/6-311G (d,p) level in CH_3OH solution. The final ECD spectrum was simulated by SpecDis software based on Boltzmann weighting of each conformer with a half-band width of 0.25 eV [15].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as a pale-yellow powder with the molecular formula of $C_{22}H_{28}O_7$ established by the HRESIMS (*m/z* 427.1736 $[M+Na]^+$, calcd. for 427.1727) and NMR data. The IR absorption bands at 3389, 1652 cm^{-1} suggested the presence of hydroxyl and carbonylic groups. The ^{1}H-NMR data of **1** (Table 1) revealed the presence of six aromatic protons at δ_H 6.66 (1H, d, *J* = 7.9 Hz, H-5'), 6.65 (1H, d, *J* = 8.0 Hz, H-5), 6.58 (1H, d, *J* = 1.8 Hz, H-2), 6.53 (1H, d, *J* = 1.9 Hz, H-2'), 6.52 (1H, dd, *J* = 7.9, 1.9 Hz, H-6'), 6.51 (1H, dd, *J* = 8.0, 1.8 Hz, H-6), two oxygenated methylenes at δ_H 4.20 (1H, dd, *J* = 11.2, 6.0 Hz, H-9'a), 3.98 (1H, dd, *J* = 11.2, 6.5 Hz, H-9'b), 3.66 (1H, dd, *J* = 10.9, 5.9 Hz, H-9a), 3.50 (1H, dd, *J* = 10.9, 6.5 Hz, H-9b), and two methoxy groups at δ_H 3.73 (3H, s, 3-OCH_3), 3.72 (3H, s, 3'-OCH_3). Its ^{13}C-NMR and DEPT spectra exhibited 22 carbon resonances consist of a carbonylic carbon at δ_C 173.0 (C-10), two benzene rings carbons at δ_C 148.9 (C-3), 148.8 (C-3'), 145.6 (C-4, C-4'), 133.6 (C-1), 133.1 (C-1'), 122.7 (C-6), 122.6 (C-6'), 115.8 (C-5, 5'), 113.4 (C-2), 113.2 (C-2'), two oxygenated methylene carbons at δ_C 66.0 (C-9), 62.6 (C-9'), two methoxy groups at δ_C 56.1 (C-3, 3'-OCH_3). These data were similar to those of 3,3'-dimethoxy-4,4'-dihydroxyligan-9-ylacetate [16], expect for the methyl group (C-9') replaced by a hydroxymethyl group. The absolute configuration of **1** was established as (8*R*, 8'*S*) by comparing the experimental and calculated ECD spectra. Therefore, the structure of **1** was elucidated and named as eplignan A.

The structures of known compounds **2**–**7** were isolated as syringaresinol (**2**) [17], (+)-pinoresinol (**3**) [18], 9-*O*-methylcubebin (**4**) [19], isolariciresinol (**5**) [20], rac-(8α,8'β)-4,4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyldiacetate (**6**) [21], 9'-*O*-acetylisolariciresinol (**7**) [22] by comparison of experimental data with those reported in the literatures.

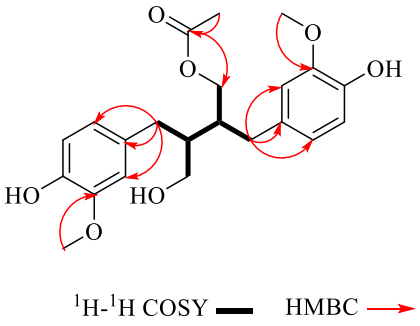

Figure 2. The key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

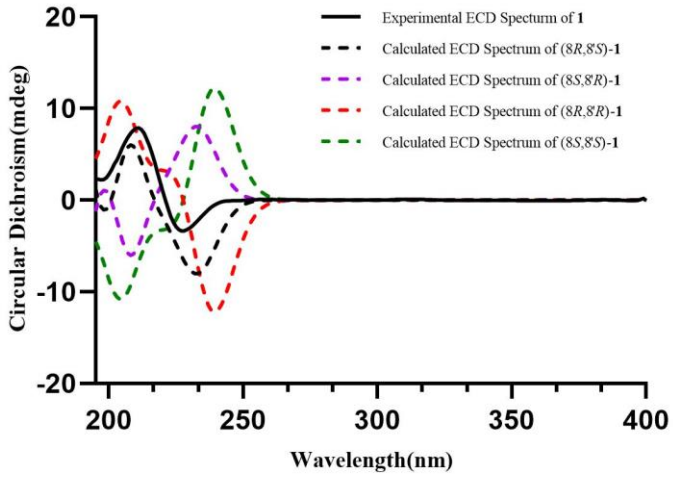

Figure 3. Experimental and calculated ECD spectra of compound **1**.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **1** (CD_3OD, δ in ppm, J in Hz)

Position	δ_C	δ_H
1	133.6	
2	113.4	6.58 (1H, *d*, 1.8)
3	148.9	
4	145.6	
5	115.8	6.65 (1H, *d*, 8.0)
6	122.7	6.51 (1H, *dd*, 8.0, 1.8)
7	35.8	2.64 (1H, *dd*, 13.8, 7.4) 2.62 (1H, *dd*, 13.8, 7.4)
8	44.3	1.90 (1H, *m*)
9	66.0	3.66 (1H, *dd*, 10.9, 5.9) 3.50 (1H, *dd*, 10.9, 6.5)
10	173.0	
11	20.9	2.02 (3H, *s*)
1'	133.1	
2'	113.2	6.53 (1H, *d*, 1.9)
3'	148.8	
4'	145.6	
5'	115.8	6.66 (1H, *d*, 7.9)
6'	122.6	6.52 (1H, *dd*, 7.9, 1.9)
7'	35.5	2.56 (1H, *dd*, 11.4, 5.6) 2.54 (1H, *dd*, 11.0, 6.0)
8'	40.4	2.14 (1H, *m*)
9'	62.6	4.20 (1H, *dd*, 11.2, 6.0) 3.98 (1H, *dd*, 11.2, 6.5)
3-OCH_3	56.1	3.73 (3H, *s*)
3'-OCH_3	56.1	3.72 (3H, *s*)

*3.2. Effects of Compounds **1**–**7** on TGF-β1-Induced BEAS-2B Cells Injury*

The effects of compounds **1**–**7** on TGF-β1-induced BEAS-2B cells injury were evaluated by MTT assay. As shown in Table 2, compared with the CON group, the cell viability of BEAS-2B cells in the TGF-β1 group was significantly decreased (P<0.01), and the cell viability of BEAS-2B induced TGF-β1 were significantly increased by compounds **1**, **2**, **4**, and **6** (P<0.01). It can be inferred from the above results, compounds **1**, **2**, **4**, **6** may be the pharmacological basis of *E. intermedia* against pulmonary fibrosis.

Table 2. The effects of compounds **1**–**7** on BEAS-2B cell by TGF-β1

Group	Does (μM)	Cell viability (%)
CON	---	100.0 ± 3.6**
TGF-β1	1ng/mL	90.7 ± 1.9
1	10	97.9 ± 3.6**
2	10	98.4 ± 1.0**
3	10	94.4 ± 2.3
4	10	96.0 ± 1.1**
5	10	94.2 ± 4.4
6	10	99.6 ± 1.9**
7	10	94.4 ± 2.2

(** P<0.001 compared with the TGF-β1 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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