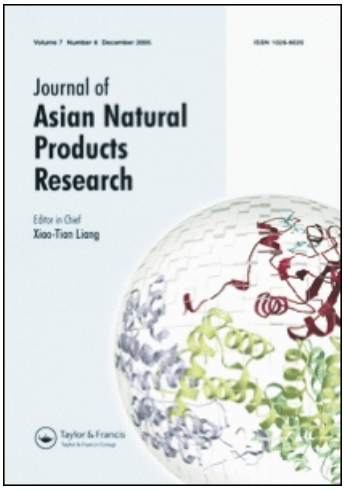

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Wen Xu[ab]; Hui Wang[c]; Guang-Xiong Zhou[a]; Xin-Sheng Yao[a]
[a] Institute of Traditional Chinese Medicine & Natural Products, Guangdong Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou, China [b] The Second College of Clinic Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China [c] College of Medicine, Jinan University, Guangzhou, China

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ORIGINAL ARTICLE

Two new 8-*O*-4′-type lignans from the stem of *Schima superba* and their cell growth inhibitory activities against human cancer cell lines

Wen Xu[ab], Hui Wang[c], Guang-Xiong Zhou[a]* and Xin-Sheng Yao[a]

[a]*Institute of Traditional Chinese Medicine & Natural Products, Guangdong Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, China;* [b]*The Second College of Clinic Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510120, China;* [c]*College of Medicine, Jinan University, Guangzhou 510632, China*

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Two new lignans (**1** and **2**) were isolated from the EtOH extracts of the stem of *Schima superba*, and elucidated as (7*R*,8*S*)-1-(3,4-dimethoxyphenyl)-2-*O*-(2-methoxy-4-omegahydroxypropylphenyl)propane-1,3-diol (**1**) and *threo*-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy-2-{4-[1-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-3-propanol (**2**) by spectral analysis. Compounds **1** and **2** showed cell growth inhibitory activity against HeLa, CNE, HepG-2, and HEp-2 cell lines. Compound **1** exhibited significant cytotoxicities with IC_{50} values of less than 4 μg/ml against all the tested cell lines.

Keywords: *Schima superba*; 8-*O*-4′-type lignan; cell growth inhibitory activity

1. Introduction

Schima superba Gardn. et Champ. (Theaceae), commonly known as 'Mu He' in China, is widely distributed in southern and eastern provinces of China. This plant is an important timber resource and an available species for fast-growing and fire-preventing of forest. Its root bark has been used for heat-clearing, detoxicating, and as an insecticide agent in traditional Chinese medicine and for the treatment of furuncle and some kind of sores by folk herbalists [1,2]. Although previous chemical investigation carried out on this genus (*Schima*) has led to the isolation of triterpenoid saponins, hydrolyzable tannins, and volatile components [3–7], little chemical work of this species has been done so far.

Based on the cytotoxicity of its 95% alcoholic extract of the stem and the $CHCl_3$ fraction from the extract against a few cancer cell lines in our screening assay, we had investigated the chemical constituents of the active fraction from *S. superba* and obtained 25 compounds, most of which are lignans and triterpenoids [8,9]. In this paper, we present the structural elucidations of two new 8-*O*-4′-type lignans **1** and **2** (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless sticky oil with an $[\alpha]_D^{18}$ value of 7.7 ($c = 1.9$, MeOH). ESI-MS (positive) showed $[M + Na]^+$ and $[2M + Na]^+$ ion peaks at *m/z* 415.4 and 807.3, respectively. Compound **1** has the molecular formula $C_{21}H_{28}O_7$ as determined from its

*Corresponding author. Email: guangxzh@sina.com

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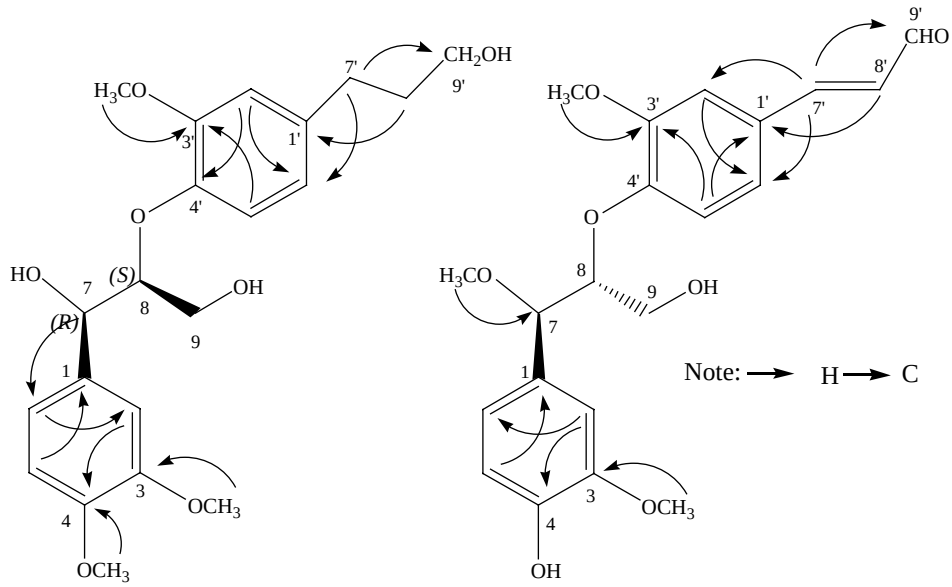

1 **2**

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

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2**.

	1		**2**	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		132.9		130.7
2	6.92 br s	109.4	6.97 d ($J = 1.7$)	112.2
3		148.6		149.2
4		148.1		147.7
5	6.84 d ($J = 8.0$)	110.8	6.77 d ($J = 8.0$)	116.0
6	6.75 d ($J = 8.0$)	118.4	6.82 dd ($J = 8.0, 1.7$)	121.6
7	4.90 d ($J = 3.5$)	72.5	4.43 d ($J = 6.0$)	84.3
8	4.07 m	86.3	4.51 m	85.1
9	3.82 m, 3.64 m	60.6	3.50 dd ($J = 11.8, 5.9$), 3.68 dd ($J = 11.8, 3.9$)	62.2
1′		137.4		129.1
2′	6.66 br s	112.2	7.27 d ($J = 2.0$)	112.8
3′		150.6		151.7
4′		144.7		153.4
5′	6.72 d ($J = 8.0$)	119.6	7.04 d ($J = 8.4$)	117.0
6′	6.60 d ($J = 8.0$)	120.9	7.18 dd ($J = 8.4, 2.0$)	124.5
7′	2.55 t ($J = 7.2$)	31.5	7.58 d ($J = 15.6$)	155.5
8′	1.76 m	33.9	6.67 dd ($J = 15.6, 7.6$)	127.6
9′	3.55 m	61.6	9.59 d ($J = 7.6$)	196.1
3-OMe	3.77 s	55.6	3.81 s	56.4
4-OMe (7-OMe)	3.74 s	55.6	3.22 s	57.2
3′-OMe	3.77 s	55.6	3.90 s	56.7

high-resolution positive ion ESI-MS (*m/z* 415.1731 $[M + Na]^+$). In the ^{13}C NMR spectrum (Table 1), signals arising from two aromatic rings and six aliphatic carbons along with three methoxyl groups were observed, suggesting that **1** is a lignan. The ^1H NMR spectrum of **1** showed signals due to three methoxyl groups [δ_H 3.77 (6H, s), 3.74 (3H, s)] and two 1,3,4-trisubstituted phenyl groups [δ_H 6.92 (1H, br s), 6.84 (1H, d, $J = 8.0$ Hz), and 6.75 (1H, d, $J = 8.0$ Hz)/6.66 (1H, br s), 6.60 (1H, d, $J = 8.0$ Hz), and 6.72 (1H, d, $J = 8.0$ Hz)]. The two propanyl groups were easily defined in the $^1H-^1H$ COSY spectrum. The phenylpropanoid unit was confirmed by HMBC correlations of H-7 resonating at δ_H 4.90 with C-2 (δ_C 109.4) and C-6 (δ_C 118.4) (Figure 1). In the same way, the omegahydroxypropylphenyl group was proved by HMBC correlations of H-7′ (δ_H 2.55) with C-2′ (δ_C 112.2) and C-6′ (δ_C 120.9). By comparing its ^1H and ^{13}C NMR spectral data with those in the literature [10], the plane structure of **1** was determined to be 1-(3,4-dimethoxyphenyl)-2-*O*-(2-methoxy-4-omegahydroxypropylphenyl)propane-1,3-diol. The *erythreo* configuration of **1** was predicted by the coupling constant $J_{7,8} = 3.5$ Hz [11,12]. The 8*S* configuration of **1** was confirmed with positive signs at 210–250 nm in the CD spectrum [10]. Thus, compound **1** is deduced to have a (7*R*,8*S*) configuration, and the structure was elucidated to be (7*R*,8*S*)-1-(3,4-dimethoxyphenyl)-2-*O*-(2-methoxy-4-omegahydroxypropylphenyl)propane-1,3-diol.

Compound **2** was obtained as a light yellow sticky oil with an $[\alpha]_D^{18}$ value of 10.0 ($c = 1.1$, MeOH). ESI-MS showed an ion peak $[M + Na]^+$ at *m/z* 411.2. Compound **2** has the molecular formula of $C_{21}H_{24}O_7$ with eight degrees of unsaturation as deduced from HR-ESI-MS (*m/z* 388.1522), suggesting the presence of two aromatic rings. The ^1H and ^{13}C NMR spectral data (Table 1) of **2** showed signals assignable to three methoxy groups [δ_H 3.90 (3H, s), 3.81 (3H, s), and 3.22 (3H, s)], a methylene bearing a hydroxyl group [δ_H 3.50 (1H, dd, $J = 11.8$, 5.9 Hz) and 3.68 (1H, dd, $J = 11.8$, 3.9 Hz)], two methine groups bearing an oxygen atom [δ_H 4.43 (1H, d, $J = 6.0$ Hz) and 4.51 (1H, m, H-8)], and two 1,3,4-trisubstituted phenyl groups [δ_H 7.27 (1H, d, $J = 2.0$ Hz, H-2′), 7.04 (1H, d, $J = 8.4$ Hz, H-5′), and 7.18 (1H, dd, $J = 8.4$, 2.0 Hz, H-6′)/6.97 (1H, d, $J = 1.7$ Hz, H-2), 6.77 (1H, d, $J = 8.0$ Hz, H-5), and 6.82 (1H, d, $J = 8.0$, 1.7 Hz, H-6)]. In the $^1H-^1H$ COSY spectrum, an α,β-unsaturated aldehyde group was proved by the connectivity from H-7′ to H-9′, and the —CH(O)CH(O)CH_2O— moiety can be defined by the correlations among H-7, H-8, and H-9. Thus, the facts suggested that **2** is a lignan. The α,β-unsaturated aldehyde group was attached to the C-1′ position, because the H-7′ proton resonating at δ 7.58 displayed HMBC correlations with C-2′ and C-6′. The HMBC correlations from H-7 to C-2 and C-6 were observed, suggesting that the propanolic group (—CH(O)CH(O)CH_2O—) was linked to the phenyl group at the C-1 position. Three methoxy groups can be easily assigned because each of them was correlated with C-3, C-3′, and C-7, respectively, in the HMBC spectrum. The correlation between H-5′ and H-8 observed in the ROESY spectrum suggested that the propanol group was attached at the C-8 position. The *threo* configuration of **2** was predicted by the coupling constant $J_{7,8} = 6.0$ Hz [11]. Unexpectedly, there was no Cotton effect at 210–250 nm in the CD spectrum. So, the absolute configuration of **2** is undetermined. Therefore, compound **2** was elucidated as *threo*-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy-2-{4-[1-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-3-propanol.

The *in vitro* cell growth inhibitory activities of the isolated compounds against tumor cell lines were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in Table 2, compounds **1** and **2** exhibited tumor cell inhibitory effects against HeLa, CNE, HepG-2, and HEp-2 cell lines. Compound **1**

Table 2. *In vitro* cell growth inhibitory activities of compounds **1** and **2**.

	IC_{50} (μg/ml)		
Cell lines	**1**	**2**	Adriamycin
HeLa	3.61	34.45	3.16
CNE	<3.13	38.90	8.12
HepG-2	3.13	11.22	3.20
HEp-2	<3.13	45.57	8.22

showed potent cell inhibitory activities with IC_{50} values of less than 4 μg/ml against these four cancer cell lines, while compound **2** showed weaker effects (IC_{50}: 11.22–45.57 μg/ml). Moreover, the activity of **1** was nearly equal to that of the positive control, adriamycin.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained using a JASCO P-1020 polarimeter. UV spectra were measured on a JASCO V-550 spectrophotometer. CD spectra were obtained with a JASCO 715 spectropolarimeter. The ^{1}H and ^{13}C NMR DEPT-135 experiments were conducted on a Bruker (AV-400) FT-NMR spectrometer with tetramethylsilane as the internal standard. 2D NMR experiments included HSQC, HMBC, ^{1}H–^{1}H COSY, and ROESY. ESI-MS were recorded on a Finnigan LCQ Advantage MAX instrument. The HR-ESI-MS experiment was performed on a Thermo Scientific LTQ Orbitrap XL hybrid FT-MS (for compound **1**), and an Agilent 6210 LC/MSD TOF mass spectrometer (for compound **2**). Sephadex LH-20 (Pharmacia Biotech, Zurich, Switzerland) and silica gel (200–300 mesh; Qingdao Ocean Chemical Factory, Qingdao, China) were used for column chromatography. Silica gel F_{254} (Qingdao Ocean Chemical Factory) was used for TLC.

3.2 Plant material

The plant material was collected from Jixi County, Anhui Province, China. The original plant was identified as *S. superba* Gardn. et Champ. (Theaceae) by Prof. Guang-Xiong Zhou, Department of Pharmacognosy, College of Pharmacy, Jinan University. An authenticated voucher specimen (No. THE-1) of the plant has been deposited at the Department of Pharmacognosy, College of Pharmacy, Jinan University.

3.3 Extraction and isolation

The air-dried stems of the plants (4.5 kg) were cut into small pieces and ground, and then extracted with 95% EtOH (30 liters × 3). The solvent was removed by rotary evaporation and the dark brown extract obtained was suspended in H_2O and extracted successively with petroleum ether, $CHCl_3$, and ethyl acetate to yield petroleum ether (55 g), $CHCl_3$ (35 g), EtOAc (22 g) fractions, respectively. The $CHCl_3$ extract was subjected to silica gel chromatography with a gradient EtOAc–hexamethylene (0:100 → 100:0) system to give 108 fractions (F1–F108). F79–85 (A) were permeated through Sephadex LH-20 using a $CHCl_3$–MeOH (1:1) system to give 36 subfractions A1–A36. Fractions A12–A19 were further purified with silica gel column chromatography eluted with $CHCl_3$–MeOH (95:5 → 1:1) to afford **1** (24 mg). Fractions A20–A28 were purified with silica gel column chromatography eluted with $CHCl_3$–MeOH (95:5 → 1:1) and Sephadex LH-20 eluted with MeOH–$CHCl_3$ (1:1) to afford **2** (4.2 mg).

3.3.1 (7R,8S)-1-(3,4-Dimethoxyphenyl)-2-O-(2-methoxy-4-omegahydroxypropyl phenyl)propane-1,3-diol (**1**)

Colorless sticky oil; $[\alpha]_D^{18}$ 7.7 ($c = 1.9$, MeOH); UV λ_{max} (MeOH) nm: 279, 230; CD (MeOH): $\Delta\varepsilon_{225\,nm}$ −0.59, $\Delta\varepsilon_{245\,nm}$ +0.98; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data: see Table 1. Positive mode ESI-MS: (*m/z*) 415.4 $[M + Na]^+$, 807.3

$[2M + Na]^+$; HR-ESI-MS: *m/z* 415.1731 (calcd for $C_{21}H_{28}O_7Na$, 415.1733).

*3.3.2 Threo-1-(4-hydroxy-3-methoxy phenyl)-1-methoxy-2-{4-[1-formyl-(E)-vinyl]-2-methoxyphenoxy}-3-propanol (**2**)*

Light yellow sticky oil; $[\alpha]_D^{22}$ -10.0 ($c = 1.1$, MeOH); UV λ_{max} (MeOH) nm: 230, 288; ^1H and ^{13}C NMR (CD_3OD) spectral data: see Table 1. Positive mode ESI-MS: *m/z* 411.2 $[M + Na]^+$, 799.4 $[2M + Na]^+$; HR-ESI-MS: *m/z* 388.1553 (calcd for $C_{21}H_{24}O_7Na$, 388.1522).

3.4 MTT assay

Four human cancer cell lines including HeLa, CNE, HepG-2, and HEp-2 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cell viability was determined by measuring the ability of cells to transform MTT to a purple formazan dye. Cells were seeded at 8000 cells/100 μl/well in 96-well plates for 24 h, and then treated with the two natural compounds and adriamycin at different concentrations for 72 h. Subsequently, 12 μl of the MTT dye (5 mg/ml in phosphate buffered saline) was added to each well, and the plates were incubated at 37°C for 4 h. The surviving cells converted MTT to formazan crystals which are dissolved in 100 μl/well of DMSO, generating a blue-purple color. The absorbance was monitored at 570 nm using a microplate spectrophotometer (SpectroAmax^{TM} 250).

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